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Land cover correlates of Tsetse distribution and its implications for cattle movement and Trypanosomiasis control in the Maasai steppe

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LAND COVER CORRELATES OF TSETSE DISTRIBUTION AND ITS IMPLICATIONS FOR CATTLE MOVEMENT AND TRYPANOSOMIASIS CONTROL IN THE MAASAI STEPPE

Anibariki Ngonyoka

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ABSTRACT

Anthropogenic activities changes ecosystem structure, and alter the vital rates of vectors, host-vector interaction and consequently disease transmission dynamics across the landscape. This research examined the participatory epidemiology to elicit Maasai pastoralist knowledge on land use changes, cattle grazing patterns and trypanosomiasis control. Furthermore, abundance and infection rates were determined in relation to age of tsetse flies, habitat types, host presence and predicted potential spatial distribution patterns in the Maasai Steppe of northern Tanzania. Tsetse abundance was obtained through geo-referenced straps, host counts around trap sites, between July 2014 and November 2015 in selected habitats across four villages: Emboreet, Loiborsireet, Kimotorok and Oltukai adjacent to protected areas. PCR identification of trypanosome species performed an to establish the infection rates. Extraction of monitored Normalized Difference Vegetation Index (NDVI) data derived from Moderate Resolution Imaging Spectrometer (MODIS) were performed asses vegetation cover changes. Presence only niche modelling approach used to predict spatial distribution of tsetse species through integration of heterogeneous biophysical factors across the entire landscape with tsetse presence data. Our findings show the variation of tsetse fly species abundance and infection rates among habitats in surveyed villages in relation to NDVI and host abundance. Results show higher tsetse fly abundance in Acacia-swampy ecotone, open woodland and riverine habitats. Tsetse species abundance was inconsistent among habitats in different villages. Emboreet was highly infested with *Glossina swynnertoni* (68%) in ecotone and swampy habitats followed by *G. morsitans* (28%) and *G. pallidipes* (4%) in riverine habitat. In the remaining villages, the dominant tsetse fly species by 95% was *G. pallidipes* in all habitats. *Trypanosoma vivax* was the most prevalent species in all infected flies (95%) with few observations of co-infections (with *T. congolense* or *T. brucei*). The relationship of tsetse with abundance of wildlife and livestock was more complex, as we found positive and negative associations depending on the host and fly species. Furthermore, niche modelling of tsetse species provides the hotspots for tsetse infestations and infections and hence plan for cost effective eco-health intervention approaches to increase the resilience of pastoral communities against Trypanosomiasis.

Keywords: Tsetse, Host, Participatory epidemiology, Trypanosomiasis, Species distribution modeling

DECLARATION

I, **Anibariki Ngonyoka** do hereby declare to the Senate of Nelson Mandela African Institute of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Anibariki Ngonyoka

18 December 2017

Name and signature of candidate	**Date**

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CERTIFICATION

The undersigned certify that have read and hereby accept the dissertation titled "**Land cover change correlates of tsetse distribution and its implication to cattle movement and trypanosomiasis control in Maasai steppe",** in fulfilment of the requirements for the Degree of Doctor of Philosophy in Life Science and Bioengineering (LSBE) at the Nelson Mandela African Institution of Science and Technology (NM-AIST)

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LIST OF ABBREVIATIONS AND SYMBOLS

FAO	Food and Agriculture Organization
GIS	Geographical Information Systems
R_0	Basic Reproductive number
NDVI	Normalized Difference Vegetation Index
TNP	Tarangire National Park
GPS	Global Positioning systems
MODIS	Moderate Resolution Imaging Spectrometer
LME	Linear Mixed Effect Model
S.D	Standard Deviation
WHO	World Health Organization
TDR	Programme for Research and Training in Tropical Diseases
IDRC	Canadian International Development Research Centre
EROS	The Earth Resource Observation and Science Centre
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic acid
MWFV	Mean Wing Frey Value
DALY	Disability Life Adjusted Year
MRC	Manyara Ranch Conservancy
MNP	Manyara National Park
AAT	Animal African Trypanosomiasis
ENM	Ecological Niche Modelling

CHAPTER ONE

1.1 Background Information

The emergence, transmission and re-emergence of vector-borne diseases in addition to other factors is influenced by ecosystem changes (Lambin *et al*., 2010). Various forms of landscape changes such as construction projects, agriculture, mining and expansion of settlements change disease infection dynamics through land cover fragmentation, pathogen introduction, pollution and human migration (Patz *et al*., 2004). Human induced transformation of forest and savannah landscapes takes place at rampart, influencing the dynamics of infectious diseases including trypanosomiasis (Wilcox and Gubler, 2005). In Africa, changes in trypanosomiasis vector distribution is influenced by human activities especially expansion of agricultural activities (Malele 2011). Therefore, understanding the interaction of various components in the ecosystem of epidemiological importance, such as vector biology, parasites, hosts, land cover and use needs an integrative analysis (Keesing *et al*., 2010).

Tsetse transmitted trypanosomiasis is restricted to the African continent with distinct geographical boundaries, the Sahara and Somali deserts in the North and Kalahari and Namibian deserts in the South (Leak, 1999; Magez and Radwanska, 2014). Trypanosomiasis is caused by multiple species of trypanosomes, which results into two major forms of the disease: sleeping sickness and Nagana to human and livestock, respectively. In sub-Saharan Africa there are 250 foci of the disease, 60 million people are at risk with 70 000 cases of human African trypanosomiasis are reported each year (Moore *et al*., 2012).Trypanosomiasis draws back the efforts to increase agricultural production in tropical African countries (Cecchi *et al*., 2008).

In the literature, It is documented that, 45-65 million cattle are susceptible to Trypanosomiasis (Fieldman *et al*., 2005), 3 million cattle die each year (Catand *et al*., 2006). Animal African Trypanosomiasis constrains livestock benefits and land productivity through reduction of meat, milk production, drought animals, nutrient cycling and liquid capital (Kristjanson *et al*., 1999). In addition, Nagana restricts grazing patterns of livestock to avoid infected landscapes, reduces livestock productivity by reducing birth rates by 10-40% and increasing mortality rates (Swallow, 1999). Trypanosomiasis which undermine the livestock development and barrier to land use in tsetse infested areas as a strategy to avoid mechanical transmission of trypanosome through tsetse biting. and lowered productivity in form of draft

power, milk, meat, growth and lowered natality rate (Swallow, 2000). Furthermore, Trypanosomiasis is a zoonotic disease, where out of 250 foci, 60 million people are susceptible to the disease and 300 000 cases contact the disease (Cattand *et al*., 2006).

Habitat plays important role in vector fitness, sustaining various stages of vector development and finding suitable hosts. It is estimated that one third of land in Africa provides favourable climate and vegetation cover suitable for tsetse (Kristjanson *et al*., 1999). Nonetheless, increase of human and livestock population growth dramatically shift land use and cover patterns which change the spatial distribution of the vector and trypanosomiasis. In addition, arthropod vectors are also sensitive to variation of temperature and moisture (Ostfeld *et al*., 2005). For that reason, strategies to control the disease inevitably needs eco-health approach to increase efficiency of disease control programmes (Veldkamp and Verburg, 2004).

There are biotic and abiotic factors that drive the abundance and distribution of vector causing Trypanosomiasis disease. The association of tsetse species and vegetation started early in 1900's. In 1960's the efforts were concerted to population dynamics of tsetse and mapping 1970's where tsetse maps were produced through use of previous year data without ground-truthing (Malele, 2011). Since I990's to data the integration of available satellite data consolidated with biophysical layers have enabled to capture broader understanding of the distribution through species distribution models. The biophysical layers frequently used in the mapping include NDVI, soil moisture, land cover, ground temperature, cattle density layer, wild animals, Digital elevation model (Rogers, 2006; Peterson, 2006; Moore and Messina, 2010).

Efforts to map tsetse distribution in Tanzania can be traced before and after independence (Kjekshus, 1977). For many years the information on tsetse distribution to date is based on extensively referred maps reported Ford and Katondo (1977). However, its production is based on previous maps without ground truthing (Malele, 2011). Unfortunately, much of landscape changes which took place in relation to tsetse abundance are not extensively documented. The land cover changes may probably affect the distribution of tsetse because elsewhere in East Africa half of the wildlife areas; the main habitat for *Glossina spp* has been lost due to human activities (Stoner *et al*., 2006). In Maasai steppe, expansion of large scale agriculture, settlement, ranching and other human activities to the wild lands (Msoffe *et al*.,

2011) push humans towards tsetse infested areas, encroaching the wild protected areas and hence becoming vulnerable to Nagana and Human African Trypanosomiasis (HAT).

In recent years, the focus of mapping and modelling of land cover and tsetse fly distribution has shifted from traditional tedious and labour intensive field oriented data collection to inexpensive, efficient remote sensing and Geographical Information Systems (GIS). Incorporation of tsetse field data with GIS provides more precise prediction and mapping. This study hypothesized that tsetse fly distribution and prevalence of trypanosomes in the study area is determined by land cover characteristics and host availability. Also, the land use changes of tsetse infested landscapes have impact to livestock grazing patterns and disease control strategies.

1.2 Research problem and justification of study

Maasai steppe is the refuge of rich biodiversity with suitable habitats for tsetse flies, where human and wildlife coexisted for many years. Studies conducted in parts of the steppe at different temporal scales indicate both alarming and non-uniform land cover transformation. For example, in Babati and Mbulu districts, crop cultivation expanded by 118% from 1960 to 1987 (Mwalyosi and Mohamed, 1992), whereas in Monduli and Simanjiro districts, farming area expanded five-fold and three-fold, respectively from 1984 to 2000 (Msoffe *et al*., 2011). These human activities probably have altered both tsetse spatial limits and abundance and expose communities to areas with high levels of tsetse infestation and transmission of Nagana and Human African Trypanosomiasis (HAT).

In addition most of distribution maps found in the public domain are outdated and entomological research survey data availability is inadequate. Nevertheless, the direct use of recently produced tsetse distribution maps for Africa (Cecchi *et al*., 2008), is limited given that its model assumptions are based on continent variables which varies with local regions such as Maasai steppe. This necessitates the need of generating new geospatial information on tsetse abundance and distribution. Furthermore, there is scanty information on the influence of tsetse distribution in relation to seasonal movements of livestock and wildlife.

This research study hinges on determining temporal and spatial correlates of changes of land land cover to tsetse distribution and abundance through use of GIS and satellite imagery and their implication to cattle movement and Trypanosomiasis control. The output of this

research study will provide baseline epidemiological information of hotspots of trypanosome infections to curb the vulnerability of Maasai communities against Trypanosomiasis through use of Eco-health approaches.

1.3 Objectives

1.3.1 General objective

To assess the impact of land cover changes on spatial and temporal distribution of tsetse flies and its implication to livestock movement and disease control.

1.3.2 Specific objectives

This research study specifically sought,

i. To determine the longitudinal variation of tsetse abundance in relation to habitat and host presence in Emboreet village of the Maasai steppe.
ii. To determine the patterns of tsetse abundance and trypanosome infection rates among habitats of three surveyed villages in Maasai Steppe.
iii. To conduct participatory epidemiology in order to generate community knowledge on land use changes, cattle grazing patterns and trypanosomiasis control.
iv. To predict the spatial distribution of tsetse species using niche modeling approach in the Maasai steppe.

1.4 Hypotheses/Research questions

Hypothesis 1: The abundance of tsetse species is associated with land cover characteristics and host availability.

Hypothesis 2: The pattern of tsetse abundance and trypanosome infection rates are the same in all villages.

Hypothesis 3: The understanding level of trypanosomiasis, cattle grazing patterns and control strategies are the same among pastoralist Maasai communities.

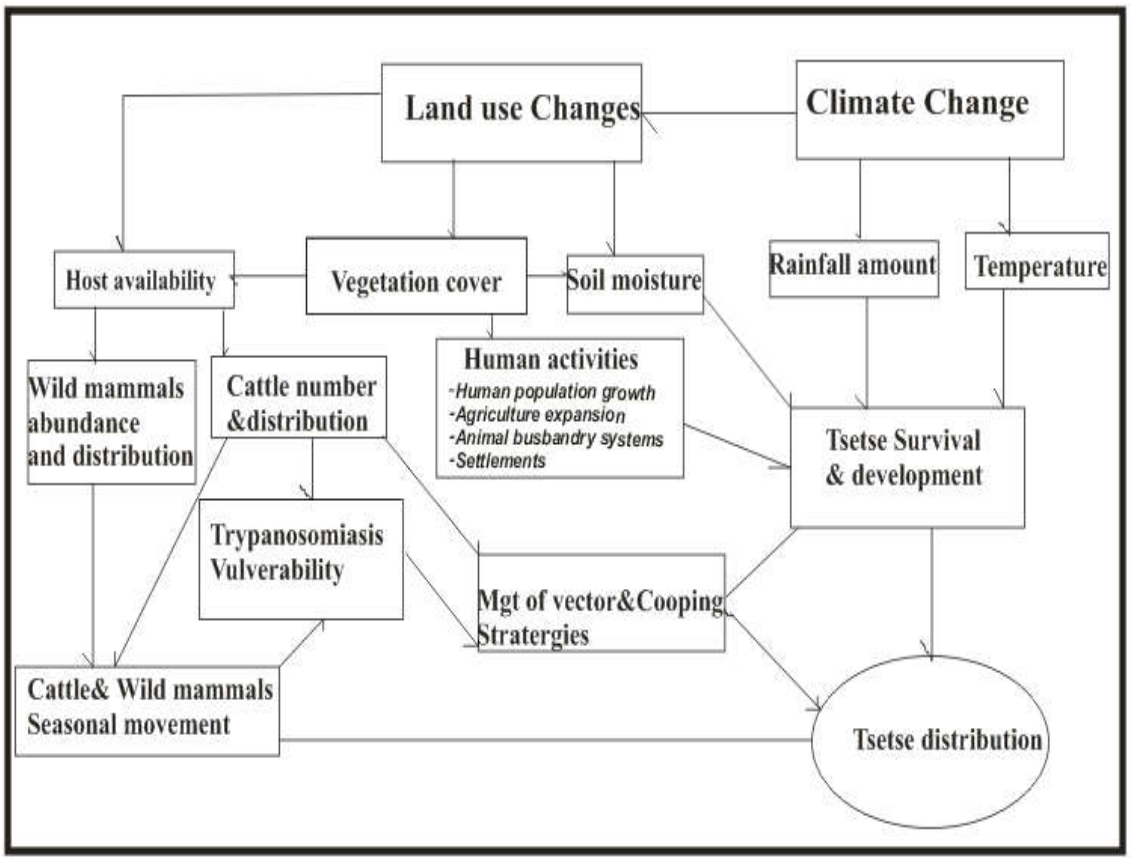
Hypothesis 4: There is an even spatial distribution of tsetse fly species among villages of the Maasai steppe.

1.5 Significance of the research

This study will provide baseline information for hotspot maps of tsetse abundance useful to livestock keepers, wildlife managers, and medical specialists towards understanding the magnitude of the problem in spatial and temporal scales. Unveiling the hotspots of high

infection will help in planning of eco-health coping strategies against environmental changes and effective disease control to improve resilience of the Maasai communities against Trypanosomiasis. The eradication of the disease will thrice the production of cattle and consequently improve human economy (Hendrix *et al*., 1999). Additionally, it will provide useful information for environmental and land use management of the Maasai steppe.

1.6 Conceptual Framework



CHAPTER TWO

Variation of tsetse fly abundance in relation to habitat and host presence in the Maasai Steppe, Tanzania[1]

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Abstract

Human activities modify ecosystem structure and functioning, and can also alter the vital rates of vectors and thus the risk of infection with vector borne diseases. In the Maasai Steppe ecosystem of northern Tanzania, local communities depend on livestock and suitable pasture that is shared with wildlife, which can increase tsetse abundance and the risk of trypanosomiasis. We monitored the monthly tsetse fly abundance adjacent to Tarangire National Park in 2014-2015, using geo-referenced, baited epsilon traps. We examined the contribution of habitat types and vegetation greenness (NDVI) on the relative abundance of tsetse fly species. Host availability (livestock and wildlife) was also recorded within 100 m x 100 m of each trap site. The highest tsetse abundance was found in the ecotone between *Acacia-Commiphora* woodland and grassland, and the lowest in riverine woodland. *Glossina swynnertoni* was the most abundant species (68%) trapped throughout the entire study, while *G. pallidipes* was the least common (4%). Relative species abundance was negatively associated with NDVI, with greatest abundance observed in the dry season. The relationship with the abundance of wildlife and livestock was more complex, we found positive and

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negative associations depending on the host and fly species. While habitat is important for tsetse distribution, hosts also play a critical role in affecting fly abundance and, potentially, trypanosomiasis risk.

Keywords

Habitat, livestock, wildlife, Tsetse fly, Maasai Steppe

2.1 INTRODUCTION

Vector fitness depends on selecting a suitable habitat that is good for development and also provides the opportunity of finding a suitable host for a blood meal. The blood meal, coupled with the competence of the host, then shape the spatial and temporal dynamics of the vector and associated vector-borne infections. Clarifying the relative contribution of habitat and competent hosts is important for understanding the risk of infection and disease transmission.

Tsetse flies (*Glossina* spp.) are distributed over a large part of sub-Saharan Africa (about 10^7 km^2) and are the common vector of the protozoa trypanosome that causes serious disease in both livestock and humans (Kristjanson *et al*., 1999). Most of the sub-Saharan countries infested with tsetse flies are poor with food insecurity (Muriuki *et al*., 2005). Trypanosomiasis is considered a neglected tropical disease that has caused agriculture loss by up to $4.5 billion US dollars per year, with as many as 3 million cattle dying each year worldwide (Muturi *et al*., 2011; Schofield *et al*., 2008). Furthermore, about 60 million humans are at risk of contracting the disease and more than 10,000 cases are reported each year across Africa (Funk *et al*., 2013; Ruiz-Postigo *et al*., 2012). A recent survey in Tanzania showed that communities close to wildlife protected areas are at risk and will remain the hotspots of tsetse infestation and trypanosome infections since the livestock associated with humans is also targeted as alternative host species (Muturi *et al*., 2011). Maasai pastoralist communities are particularly vulnerable because of they live and graze their cattle alongside wildlife and are dependent on the products from their cattle for their livelihoods.

Tsetse flies exhibit adentrophic vivaparity whereby the mother fly retains the fertilized egg in her uterus and the larvae develops and grows within the uterus by utilizing a maternal supplement of nutrients, before passing into the environment and pupating (Hargrove, 2004; Muzari and Hargrove, 2007). Subsequent puparial development into an adult is dependent on finding suitable sandy conditions to pupate and the period of pupation is determined by temperature (Hargrove, 2004). Both the availability of hosts to the female *Glossina* and the

suitability of habitat are therefore essential. Tsetse flies are haematophagus vectors known to feed on a wide range of available host species (Kaare *et al*., 2007; Muturi *et al*., 2011). The males often sit in low vegetation close to moving hosts, to obtain access to recently emerged females. Females need up to 3 blood meals to produce a pupa and failure to successfully obtain a blood meal can lead to abortion (Hargrove and Williams, 1995). Suitable vegetation cover is important since it provides appropriate breeding areas, shelter in adverse climate conditions and resting sites (Pollock, 1982).

In this paper we investigated the distribution of tsetse fly (*Glossina*) species in the Maasai Steppe of Tanzania in relation to habitat characteristics and relative abundance of host species. We selected areas where there is interaction between livestock and wildlife, at the edge of the Tarangire National Park in northern Tanzania, since this provides an insight into the relative importance and interaction between habitat and host abundance. We addressed three fundamental questions: 1. Do *Glossina* species and abundance vary between habitats and if so, in which habitats are they most common, which could lead to higher risk of trypanosome exposure? 2. How do the effects of vegetation in terms of NDVI (degree of vegetation greenness) and ground cover account for fly distribution? 3. How is host availability and its interaction with vegetation associated with the presence of tsetse flies?

2.2 MATERIALS AND METHODS

2.2.1 Study Area

The Maasai Steppe (3°40’ and 4°35’ South, 35°50’ and 36°20’E) is well known for the presence of tsetse fly species and associated cases of both human and bovine trypanosomiasis (Salekwa *et al*., 2014) We sampled tsetse flies close to the village of Emboreet, in proximity to Tarangire National Park (TNP) (Fig.

1). The local community has set aside an area of 1200 km^2 for livestock grazing that also provides refuge for abundant wildlife including buffalo, wildebeest, impala, giraffe, wild pigs and warthogs. Both livestock and wild animals have distinct grazing patterns following their seasonal migration which is associated with the availability of food, water and breeding grounds; however, they often share common areas especially when resources are limited (Msoffe *et al*., 2010). The steppe is dominated by arid scrubland and Acacia-Commiphora woodland, interspersed with open grassland and seasonal swamps. Other common tree species include *Erythrina burtii*, *Combretum spp.*, *Albizia spp.* and *Cordia spp*. The climate is

semi-arid and characterized by a long dry season in the months of July to October followed by a short rain season from November to January and a long rainy season in March to May (Miller *et al*., 2014).

2.2.2 Entomological survey

Tsetse flies were collected once a month between July 2014 and November 2015 using Epsilon traps baited with three types of odour: 4-methyl phenol (1g/h), 3-n-propylphenol (0.1 mg/h) and 1-octen-3-ol (0.5 mg/h) and an additional component of acetone (100 mg/h) (Torr *et al*., 1997). Trap sites were located in four habitat types: the ecotone between open *Acacia* woodland and seasonally swampy habitat, open woodland (characterized by < 30% canopy cover and a continuous herbaceous layer), swampy habitat (defined by land with scattered trees and soil permanently saturated with water in wet season and moist during the dry season) and riverine woodland. Three sites were located at random within these habitats, at different distances from the national park boundary, and each site had three traps 200 meters apart from each other (Pollock, 1982; Malele *et al*., 2011;). Trap locations were recorded using a Global Positioning System (GPS) and revisited once a month. Flies were removed from the traps each day for six consecutive days every month and preserved in alcohol for future analysis. Tsetse species were identified following the training manual for tsetse control personnel (Pollock, 1982).

2.2.3 Ground cover

Three plots of 20-m radius were selected at random in each site to estimate the percent of ground cover (grass and herbs cover) associated with each trap. The ground cover was recorded once in each month of trapping by estimating the percentage of cover per plot and then taking average of the three plots to estimate the site cover.

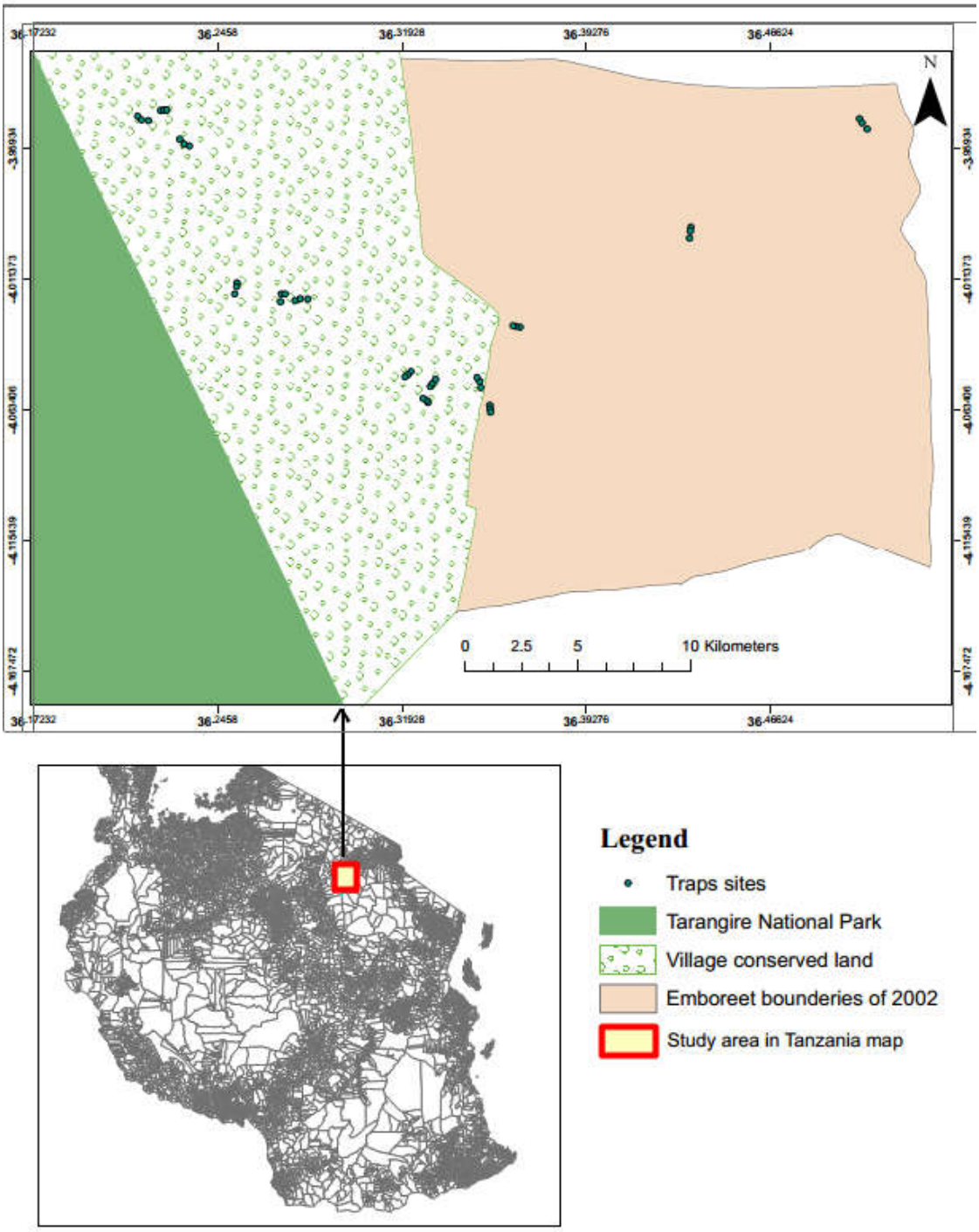

Fig. 1: Location of the main study area in Tanzania showing the trapping sites (dark dots) in the Maasai Steppe

2.2.4 Satellite-derived vegetation indices

We looked for relationships between the Normalized Difference Vegetation Index (NDVI), an index of habitat quality or greenness (Pettorelli *et al*., 2011), and patterns of tsetse fly

abundance. NDVI has previously been shown to be correlated with both tsetse population dynamics and trypanosome prevalence (Rogers, 1991). We used 16-day composite NDVI images from the Moderate Resolution Imaging Spectroradiometer (MODIS) aboard NASA's Terra satellite (https://lpdaac.usgs.gov/). MODIS 16-day NDVI composites are produced from daily 250-meter resolution images, where NDVI is a ratio of the red and the near infrared reflectance, the spectral bands that most strongly reflect green, photo-synthetically active vegetation (Rogers *et al*., 1996a; Wardlow *et al*., 2007). We extracted NDVI values matching each trap and the time of tsetse sampling, and used these for the analyses. We scaled NDVI values between 0 and 1, with higher values indicating greener vegetation.

2.2.5 Host data

Data on livestock and wildlife host abundance were collected during every visit to the traps and recorded on field cards. This included relative abundance of species observed as well as GPS location and habitat for every animal within an area of 100 m x100 m for every trap. Relative abundance was expressed as the percent of a particular animal species to the total number of animals of all species in the area. Total number of individual animal species recorded in every site was calculated by combining all the observations during a 6-day trapping session.

2.2.6 Data analysis

Data were analysed using the R software package (R Development Core Team, 2011). In this paper we compared relative abundance of tsetse flies, based on the numbers of individuals of each tsetse fly species per trap per day in the entire study period across habitats. The relationship between log-transformed tsetse species relative abundance and habitat, NDVI, ground cover, season and host species was examined using Linear Mixed Effect Models (LMEs, fit by maximum likelihood). Sampling site, or habitat when necessary, was included as a random factor to take into account variability among sites and the sampling of the same site every month. Changes in host species relative abundance by habitat and sampling month was also examined using LME. Linear mixed effect models described above in analysis of tsetse data among habitats, NDVI and host data are an extension of linear models in which random effects are added to the linear predictor (Rabe-Hesketh and Skrondal, 2008). We used this method because it allows the modeling of correlated or pseudo-replicated data while maintaining a normal distribution of the errors (Crawley, 2007). Independent variables (i.e. fixed effects) are unknown constants to be estimated from the data while random variables

(here included as a random effect of the fixed effect intercepts) govern the variance-covariance structure of the response variable. The general equation (matrix notation) is:

$$y = X\beta + Z\gamma + \varepsilon$$

Where y is a $N\ x\ 1$ column vector of observation ; X is a $N\ x\ p$ design matrix for fixed effects relating observation y to β; β is a $p\ x\ 1$ column vector of the fixed-effects; Z is the $N\ x\ q$ design matrix for the q random effects relating observation y to γ; γ is a $q\ x\ 1$ vector of the random effects; and ε is a $N\ x\ 1$ column vector of the residuals, that part of y that is not explained by the model, Xβ+Zγ (Rabel-Hesketh *et al.,* 2008).

2.3 RESULTS

2.3.1 Tsetse fly species abundance by habitat

Over the study period, from July 2014 to November 2015, three tsetse fly species were collected: overall *Glossina swynnertoni* (68%) was the most abundant, followed by *Glossina morsitans* (28 %) and *Glossina pallidipes* (4%). The relative abundance of *G. morsitans* and *G. swynnertoni* exhibited similar patterns across habitats and were significantly more abundant in the woodland-swampy ecotone and less common in the riverine habitat (Fig. 2; Appendix 1). *Glossina pallidipes* was consistently the least abundant species across time and habitats and only showed significant higher relative abundance in riverine habitats (Fig. 2; Appendix1).

2.3.2 Tsetse fly species abundance by NDVI

We observed large seasonal variation in vegetation greenness (NDVI) from the long dry season, between July and November (NDVI: 2-3.5), to the wet season, from January to May (NDVI>3.5), and this pattern was consistent for all the habitats (Fig. 3). NDVI varied significantly among all the habitats and the two seasons described above but there was no effect of any two-way interaction (Appendix 2).

We found significant negative relationships between the relative abundance of the three tsetse fly species and NDVI values (Table 1a), with all the tsetse species were more abundant in the dry compared to the wet season (Table 1b).

2.3.3 Tsetse fly species abundance by ground cover

The percent of ground cover was positively associated with NDVI (Coeff.±SE = 0.006±0.0003, DF=1836, P <0.0001), indicating that the ground cover was thicker with

vegetation greenness, and thus the increase in rainfall. However, it is important to note that it is possible to have high values of ground cover related to low values of NDVI, because dead grasses do not reflect photosynthetic activity (i.e. low greenness) and yet provide ground cover for shade, which is important for fly developmental stages and as food for the animal hosts. Typically, though, ground cover decreases in the dry season when grass is no longer growing, as a result of grazing pressure.

Relative abundance of *G. pallidipes* was positively associated with the two-way interaction between ground cover and riverine habitat (Coeff.±SE=0.004±0.0009, DF=1834, P=0.0002). *G. swynnertoni* and *G. morsitans* showed no significant relationships with either ground cover or any interaction between ground cover and habitat.

2.3.4 Tsetse fly species abundance by host species

In addition to cattle, a number of wildlife species were also recorded at the study sites (Fig. 4a). Cattle, impala, giraffe and wildebeest relative abundances were significantly different among habitats and were higher in the ecotone than the other habitats (Table 2).

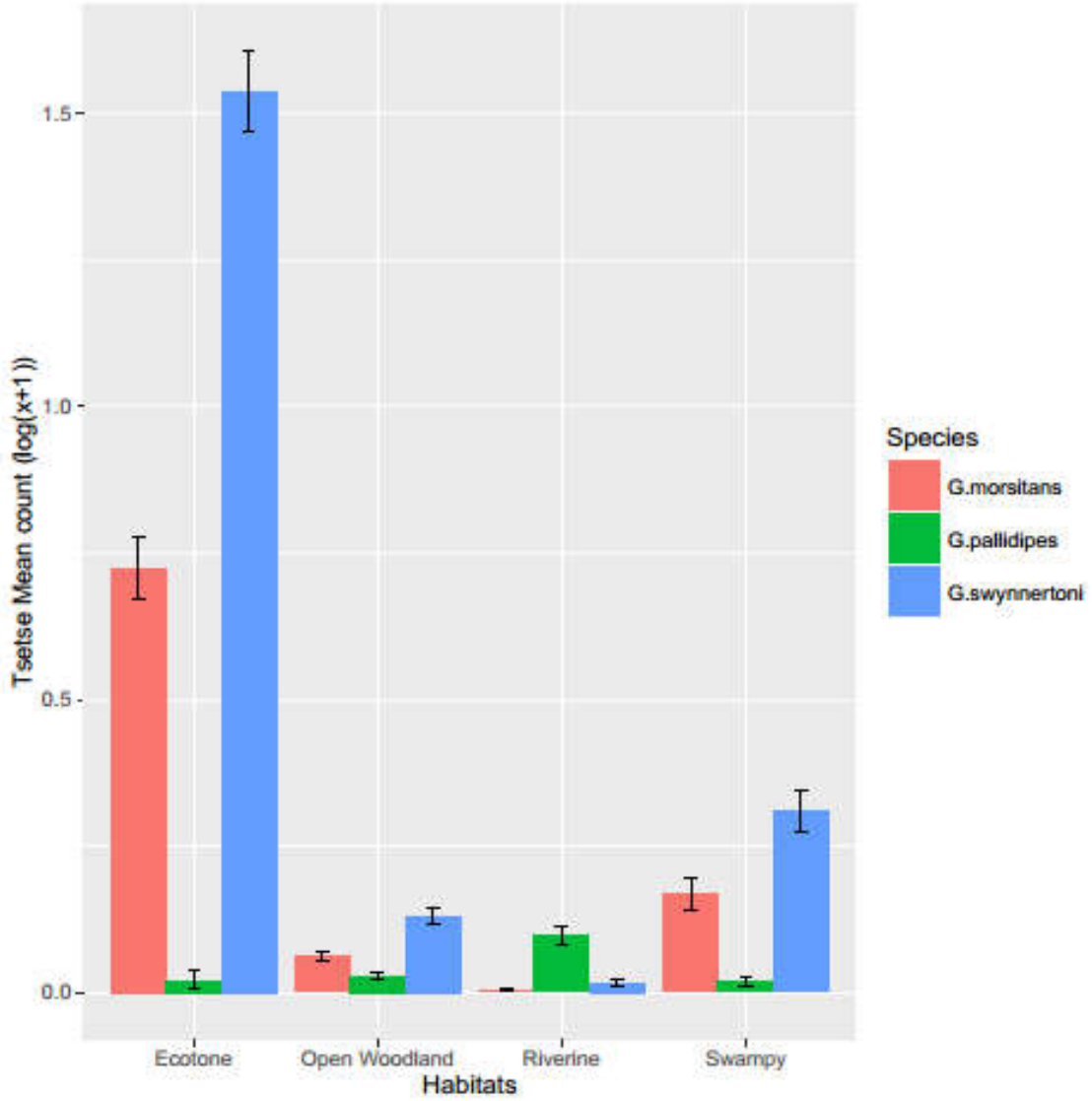

Fig. 2. Variation in geometric mean catches of *Glossina* species by habitat in the Maasai Steppe over a period of 15 months. Both *G. swynertoni* and *G. morsitans* were most abundant in the ecotone habitat while *G. pallidipes* was most abundant in riverine habitats.

Temporally, impala and to a lesser extent, giraffe and elephant, were present at all habitats throughout the year although relative abundance changed between months (Fig. 4b, Table 3). Cattle abundance increased from May to November (Fig. 4b, Table 3) while more buffalo were found in the wet season from February to May (Fig. 4b and Table 3). The relative abundance of giraffe was significantly higher in February, March, May, June and August

while Kudu were significantly higher on February, May, July, September, October to November (Fig. 4b and Table 3).

G. swynnertoni relative abundance was positively related to impala and kudu, while a negative association was observed with buffalo and wildebeest (Table 4). *G. pallidipes* was positively related with cattle and buffalo relative abundance, while no significant relationships with host species were detected for *G. morsitans*.

2.4 DISCUSSION

We investigated the role of habitat type, vegetation characteristics and host species abundance on tsetse fly abundance and distribution in the Maasai Steppe of Tanzania, with the aim of detecting associations that could inform the assessment of vector biting risk and so trypanosome infection. We showed that tsetse abundance was strongly affected by the habitat type and different fly species were more common in some habitats than others.

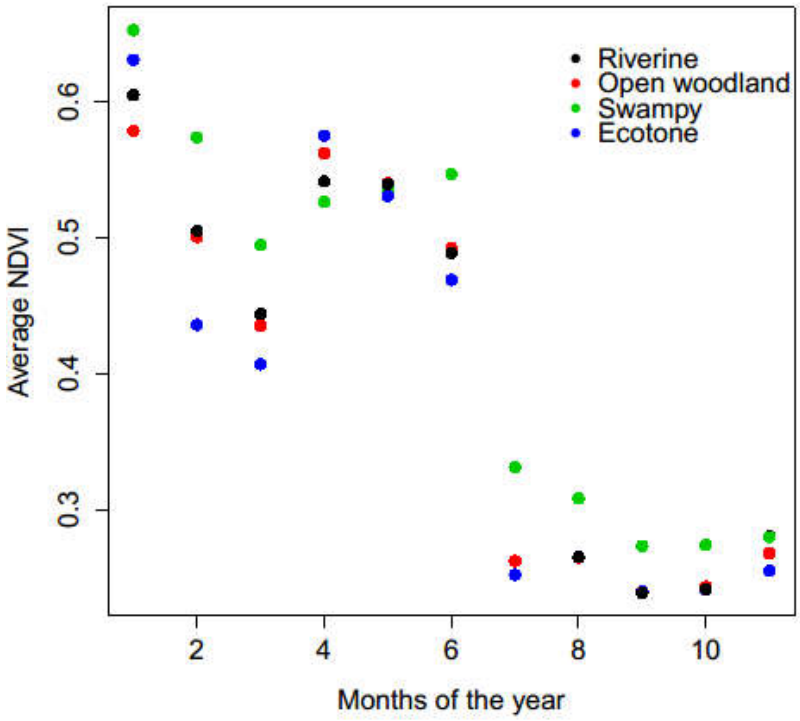

Fig. 3: Mean variation in NDVI among months and habitats over the study period .

These differences were associated with ground cover and vegetation greenness (NDVI). Abundance and distribution of host species also played an important role but was not consistent among the three fly species.

The first objective was to determine if habitat type was related to the abundance and distribution of tsetse flies. Study findings show that *G. swynnertoni* and *G. morsitans* are the most common species in the study area, and coexist in all habitats except riverine woodland. *G.swynnertoni* is endemic in northern Tanzania (Cecchi *et al*., 2008) and appears well adapted to a variety of habitats from open woodlands to swampy ecotone. Most of *G. pallidipes* were collected in the riverine woodland. This is similar to what has been observed in the Nguruman valley (Brightwell *et al*., 1992), but contrasts with previous work in Maasai Mara (Hargrove and Vale, 1980; Woolhouse *et al*., 1996), where the habitat was not significantly related with relative abundance of *G. pallidipes.* Overall, our findings on abundance of tsetse flies across habitats agree with previous work in Zambia, Kenya and Zimbabwe (Vale, 1977; Brightwell *et al*., 1992; Vale, 1998; Bossche and Deken, 2002).

Vegetation greenness (NDVI) was negatively related to fly abundance for all the three species, indicating that dry conditions have positive effects on fly relative abundance. A possible explanation for this trend is that during the dry seasons, flies tend to move less and aggregate in the available vegetated areas, which provide suitable microclimate and breeding sites. In addition to vegetation greenness, we found that ground cover affected the fly abundance but the trend was not consistent across the species. For example, both *G. swynnertonii* and *G. morsitans* showed no significant differences in abundance with ground cover, suggesting that they tend to occupy habitats in which the cover remains relatively constant throughout the year, like the ecotone or swampy areas, compared to the more variable and open woodlands. This contrast with *G. pallidipes* that tends to select the more consistent and denser ground cover of riverine habitats and avoid the less shaded cover of the more open habitats. These findings are consistent with previous studies that found that *G. swynnertonii* prefers thickets and *G. pallidipes* riverine habitat (Leak, 1999). Overall, the combination of ground greenness and cover is strategic in affecting fly abundance and species distribution with increased abundance during dry season. These results contrast with previous studies in the Maasai Mara where higher abundance was found during the wet than the dry season (Ndegwa *et al*., 2001; Sindato *et al*., 2007). Previous findings also suggest that NDVI values act as a proxy for vegetation cover (Wardlow *et al*., 2007) and are a robust

predictor variable in tsetse distribution, in particular when combined with other climatic variables and models that include elevation data (Rogers and Randolph, 1991; Rogers *et al*., 1996; Rogers *et al*., 1997), as well as when are included as a covariate to predict tsetse distribution in Kenya (Moore and Messina, 2010).

While habitat structure and composition are critical, host distribution is also an important predictor of tsetse abundance, and may explain why the seasonal patterns we observed differed than the above-mentioned studies. We showed that habitats with high tsetse fly abundance also correspond to areas with greater abundance of hosts, which provide blood meals for females and thus pupation for their offspring. Indeed, the woodland-swampy ecotone and swampy habitat were recorded to have the highest abundance of wildlife and cattle hosts. These habitats provide more consistent year-round food availability for the animal hosts compared to drier open woodlands, in which the grass layer dries out more quickly, or riverine areas that are limited in size. Seasonal grazing patterns further contribute to variation in cattle relative abundance and wild animals by directly affecting animal distribution and indirectly availability of resources to tsetse flies. The positive association between cattle and buffalo and *G. pallidipes*, which was found to select riverine habitat, is probably related to the fact that both of these species are highly water dependent, specifically cattle graze and rest along riverine habitats during the dry season, and buffalo have the tendency of hiding in wooded habitats. *G. swynnertoni* appeared to be related to a broader range of wild host species but not to cattle. This trend is consistent with previous work where this tsetse species was found to be abundant in *Acacia* woodland thickets and open woodland, alongside wild hosts, where thickets provide breeding areas and open woodlands feeding grounds (Swynnerton, 1936; Ndegwa *et al*., 2001). The lack of association of *G. morsitans* with cattle or wildlife supports the generalist preferences of this fly, irrespective of the habitat they use. The range of hosts identified in this study are consistent with previous work undertaken through ground surveys and blood meal analysis (Brightwell *et al*., 1992; Moloo, 1993; Bett *et al*., 2008; Okoth *et al*., 2007; Muturi *et al*., 2011). Other results (Bett *et al*., 2008) indicated that up to 80% of blood meals could be traced back to large to medium warm blooded animals similar to those seen in this study. This suggests the tsetse-host patterns identified in this paper may play a fundamental role in the epidemiology of trypanosomiasis.

The lack of a consistent relationship between habitat and host species for all three species of tsetse fly might have been affected by the sampling methods and the fact that we limited the study to a period of 15 months. However, since our findings were also in agreement with previous work, this does indicate that the significant patterns observed are robust and add new insights for the Maasai Steppe area.

Table 1a. Linear mixed effect models between the tsetse relative abundance (log x+1) by species, as dependent variables, and NDVI as an independent variable; habitat is included as a random factor and the standard deviation (S.D) is reported.

	Glossina pallidipes			*Glossina morsitans*			*Glossina swynnertoni*		
	Coeff.±SE	DF	P	Coeff.±SE	DF	P	Coeff.±SE	DF	P
Intercept	0.07±0.02	1836	0.0002	0.32±0.0821	1836	<0.001	0.59±0.17	1836	6E-04
NDVI	-0.08±0.03	1836	0.0149	-4.53±0.06	1836	<0.001	-0.75±0.083	1836	<0.001
Random effect S.D: Habitat	0.04			0.22			0.47		
AIC	-456.7			1721.36			-458.13		

Table 1b. Linear mixed effect models between the tsetse relative abundance (log x+1) by species, as dependent variables, and the seasonal category as independent variable; site is included as a random factor and the standard deviation (S.D). is reported. Tsetse species abundance in wet season is compared with the abundance in dry season.

	Glossina Pallidipes			*Glossina morsitans*			*Glossina swynnertoni*		
	Coeff±SE	DF	P	Coeff.±SE	DF	P	Coeff.±SE	DF	P
Intercept	0.05±0.01	1836	0.0009	0.21±0.07	1836	0.0087	0.40±0.16	1836	0.0172
Wet Season	-0.02±0.01	1836	0.0381	-0.14±0.02	1836	0.0000	-0.21±0.02	1836	<0.0001
Random effect S.D: Site	0.04			0.22			0.48		
AIC	-455.07			1718.8			2867.7		

Table 2. Linear mixed effect models between the host relative abundance (log x+1) by species, as dependent variable and habitat as independent variable. Host abundance in other habitats is compared with abundance recorded in ecotone habitat. Only the host species that showed significant differences among habitats are reported

Host	Habitat	Coefficient	SE	DF	P
Cattle	Intercept	-1.74	0.15	1589	0.0000
	Open woodland	-1.49	0.17	3	0.0033
	Riverine	-1.48	0.18	3	0.0043
	Swampy	0.018	0.21	3	0.9368
Impala	Intercept	1.42	0.291	1589	0.0001
	Open woodland	-1.249	0.376	3	0.0309
	Riverine	-0.877	0.357	3	0.0914
	Swampy	-0.73	0.411	3	0.1727
Wildbeest	Intercept	0.338	0.029	1589	0.0001
	Open woodland	-0.329	0.034	3	0.0331
	Riverine	-0.338	0.038	3	0.0273
	Swampy	-0.203	0.041	3	0.1226
Giraffe	Intercept	0.269	0.048	1589	0.0001
	Open woodland	0.23	0.056	3	0.0249
	Riverine	0.18	0.060	3	0.0572
	Swampy	0.41	0.068	3	0.0094

Table 3. Changes in host species relative abundance by month. Only the species with consistent differences among the months are reported; site is included as a random factor and the standard deviation (S.D). is reported. Host abundance in other months is compared with abundance recorded in January.

	Cattle		Impala		Giraffe		Buffalo		Kudu	
Month	Coeff.±SE	P	Coeff.±SE	P	Coeff.±SE	P	Coeff.±SE	P	Coeff.±SE	P
Intercept	0.41±0.36	0.2612	1.13±0.39	0.0039	0.09±0.12	0.5059	0.01±0.05	0.8905	0.01±0.03	1.0000
February	-0.11±0.15	0.4483	-0.44±0.12	0.0004	0.18±0.04	<0.001	0.24±0.04	<0.001	0.09±0.02	0.0001
March	-0.11±0.15	0.4483	-0.56±0.12	<0.001	0.44±0.04	<0.001	0.31±0.04	<0.001	0.01±0.02	0.7210
April	-0.11±0.15	0.4483	-0.07±0.12	0.5527	0.16±0.04	0.001	0.48±0.04	<0.001	0.02±0.02	1.0000
May	0.17±0.15	0.2432	-0.16±0.12	0.1808	0.12±0.04	0.0058	0.59±0.04	<0.001	0.08±0.02	0.0005
June	0.22±0.15	0.1332	-0.58±0.12	<0.001	0.20±0.04	<0.001	0.00±0.04	1.0000	0.01±0.02	1.0000
July	0.37±0.16	0.0260	-0.30 ±0.12	0.0260	0.13±0.04	0.0039	0.04±0.04	0.3156	0.14±0.03	<0.001
August	0.67±0.13	<0.0001	-0.30±0.13	0.0075	0.20±0.03	<0.001	0.03±0.03	0.3521	0.01±0.02	0.7799
September	1.16±0.13	<0.0001	0.38±0.11	0.0004	0.17±0.03	<0.001	0.01±0.03	0.6047	0.07±0.02	0.0022
October	1.20±0.13	<0.0001	0.10±0.10	0.3371	0.16±0.03	<0.001	0.001±0.03	0.9633	0.05±0.02	0.0122
November	2.02±0.15	<0.0001	0.09±0.12	0.4930	0.10±0.03	0.0148	0.003±0.02	0.9633	0.05±0.02	0.0299
Random effect S.D: Site	0.69		0.67		0.24		0.061		0.07	
DF	1579		1579		1579		1579		1579	
AIC	5138		4513.65		1128.28		909.3		2041.1	

Table 4. Relationships between tsetse species abundance as a response variable and host species as explanatory variables; site is included as a random factor and the standard deviation (S.D) is reported. Only significant results are presented in this table.

	Glossina swynnertoni			*Glossina pallidipes*		
	Coeff.±SE	DF	P	Coeff.±SE	DF	P
Intercept	0.333±0.189	1585	0.0789	0.0337±0.016	1579	0.0400
Cattle				0.0004±0.0002	1579	0.0330
Impala	0.003±0.001	1585	0.0022			
Buffalo	-0.051±0.017	1585	0.0030	0.0149±0.006	1579	0.0245
Wildebeest	-0.002±0.001	1585	0.0257			
Kudu	0.078±0.034	1585	0.0216			
Random effect S.D: Site	0.50			0.04		
AIC	2746.45			-301.46		

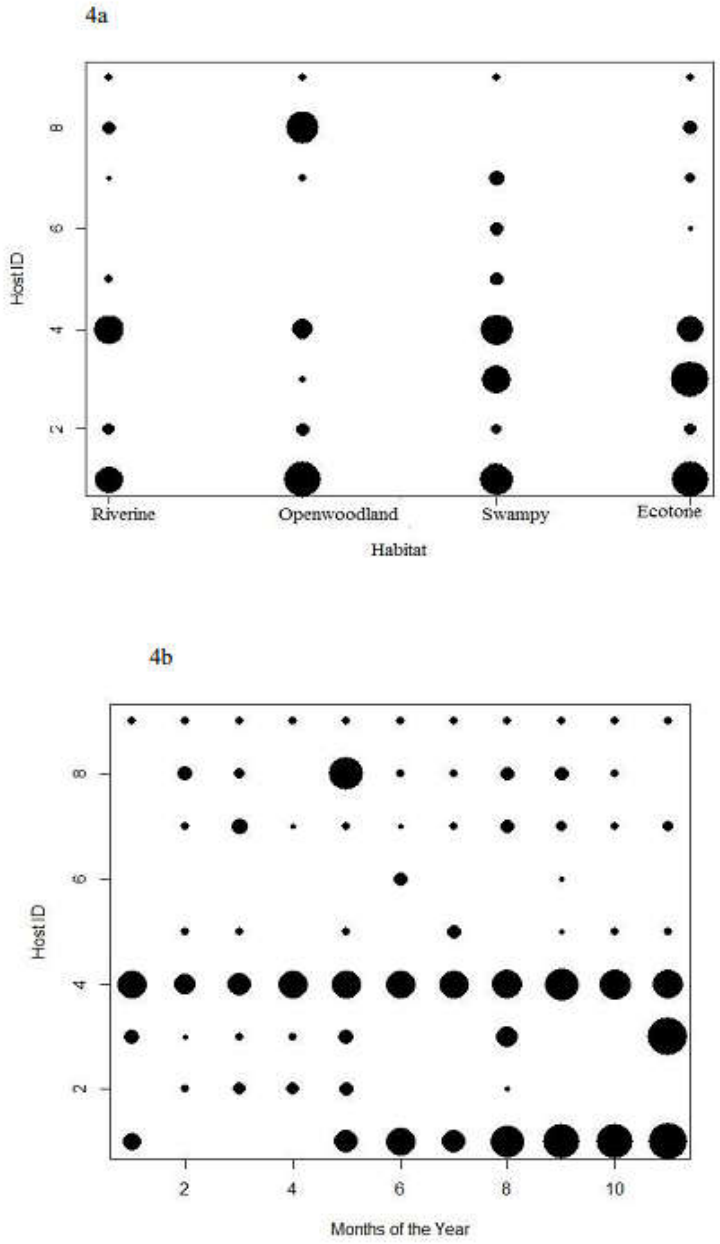

Fig. 4: Changes in host species abundance by: a) Habitats and b) Months. Circle size is proportional to the number of animals detected in a square area of 1hectare: smallest circle = host, largest circle 200 hosts. Host ID is the type of host species: 1= Cattle, 2=Buffalo, 4=Impala, 5=Greater Kudu, 6=Warthog, 7=Giraffe, 8=Elephant and 9=Dikdik

Further research on this topic should be undertaken by focusing on the role of microclimate in each habitat and how this affects abundance of tsetse flies and population dynamics. In addition to that, the role of wildlife hosts on the epidemiology of trypanosomiasis through the use of blood meal analysis could improve our understanding of host species' relative contribution, as well as provide critical parameters on disease transmission.

While we did not examine our results in relation to the spatial distribution of trypanosome prevalence, our study offers fundamental knowledge on the understanding of factors that can drive vector species abundance and host availability in an ecosystem where wildlife and livestock overlap. This study suggests that the magnitude of tsetse fly abundance varies among habitats and is strongly associated with vegetation condition in the Maasai Steppe. Host abundance and distribution is also associated with habitat features and contributes to the habitat differences observed. Therefore, this work provides a framework for habitat suitability mapping through use of NDVI and hosts to determine the abundance and distribution of tsetse flies and hence hotspots of tsetse infestation. Ultimately, our study provides the ecological information necessary to design sustainable control methods of tsetse populations in the Maasai Steppe.

2.5 Acknowledgements

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CHAPTER THREE

Patterns of tsetse abundance and trypanosome infection rates among habitats of surveyed villages in Maasai Steppe of northern Tanzania[2]

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Abstract

Background

Changes of land cover modify the characteristics of habitat, host-vector interaction and consequently infection rates of disease causing agents. In this paper, we report variations in tsetse distribution patterns, abundance and infection rates in relation to habitat types and age in the Maasai Steppe of northern Tanzania. In Africa, Tsetse-transmitted trypanosomiasis negatively impacted human life where about 40 million people are at risk of contracting the disease with dramatic socio-economical consequences, for instance, loss of livestock, animal productivity, and manpower.

Methods

We trapped tsetse flies in dry and wet seasons between October 2014 and May 2015 in selected habitats across four villages: Emboreet, Loiborsireet, Kimotorok and Oltukai

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adjacent to protected areas. Data collected include number and species of tsetse flies caught in baited traps, PCR identification of trypanosome species and extraction of monitored Normalized Difference Vegetation Index (NDVI) data from Moderate Resolution Imaging Spectrometer (MODIS).

Results

Our findings demonstrate the variation of tsetse fly species abundance and infection rates among habitats in surveyed villages in relation to NDVI and host abundance. Results have shown higher tsetse fly abundance in Acacia-swampy ecotone and riverine habitats for Emboreet and other villages, respectively. Tsetse abundance was inconsistent among habitats in different villages. Emboreet was highly infested with *Glossina swynnertoni* (68%) in ecotone and swampy habitats followed by *G. morsitans* (28%) and *G. pallidipes* (4%) in riverine habitat. In the remaining villages, the dominant tsetse fly species by 95% was *G. pallidipes* in all habitats. *Trypanosoma vivax* was the most prevalent species in all infected flies (95%) with few observations of co-infections (with *T. congolense* or *T. brucei*).

Conclusion

The findings of this study provide a framework to mapping hotspots of tsetse infestation and trypanosomiasis infection and enhance the communities to plan for effective control of trypanosomiasis.

Keywords: Habitat variability, Tsetse fly, Host availability, Infection rate, Trypanosomes

3.1 Background

The epidemiology of trypanosomiasis is driven by the transmission of multiple species of protozoa of the genus *Trypanosoma* by various species of vectors, human and livestock hosts, and a large number of wild animal species that act as the major reservoir hosts (Muriuki *et al*., 2005; Reid *et al*., 2000). In Africa, tsetse-transmitted trypanosomiasis negatively impacted human life where about 40 million people are at risk of contracting the disease with dramatic socio-economical consequences, for instance, loss of livestock, animal productivity and manpower (Maudlin, 2006; Swallow, 1999).

The important pre-requisite towards the understanding of the transmission dynamics of the disease is the quantification of trypanosome infection rate and abundance in the vectors and

susceptible hosts (Milligan and Baker, 1988; Moore *et al*., 2012). Multiple trypanosome species are found to infect animals with varying degrees of adaptation among host species (Malele *et al*., 2003; Geiger *et al*., 2015). Geospatial data in sub-Saharan Africa shows that it is extensively infested by various species of tsetse and hence potentially transmit the disease to both humans and animals (Cecchi *et al*., 2015). Literature shows distinct patterns of spatial prevalence in humans caused by *Trypanosoma brucei rhodensiense* and *Trypanosoma brucei gambiense* for East and West Africa (Simarro *et al*., 2014). *T. b. rhodesiense* is known to cause acute and rapidly progressive disease, and widely affecting livestock and wildlife but occasionally humans (Funk *et al*., 2013). Gambian trypanosomiasis is a chronic infection which accounts for 98% human reported cases, whereas, *T. b. rhodesiense* has the potential for epidemic outbreaks in humans (Kaare *et al*., 2007; MacLean *et al*., 2010).

The distribution and abundance of vectors and the availability of hosts invariably influence the prevalence of *Trypanosoma* species among habitats (Cecchi *et al*., 2008; Lambin *et al*., 2010). These components play a critical role in the identification of the "*pathogenic landscapes*", which takes into account the pathogenicity of the parasite, the susceptibility of the host, both livestock and human, and how they vary over time to affect disease persistence (Lambin *et al*., 2010).

Tsetse flies are haematophagous vectors that depend on host species availability to feed pregnant female as well as the adult tsetse (Bossche and Staak, 1997). This provides a chance for circulation of trypanosomes between wild animals, human, livestock, and tsetse flies and hence transmission of the disease (Wamwiri and Changasi, 2016). Habitat plays a key role in supporting the parasite through the provision of microclimatic conditions important during the incubation period, before becoming infective (Hargrove, 2004). Habitat is also important for vector breeding, resting and refuge during adverse climatic conditions as it provides suitable microclimatic settings for pupal development and mature fly survival (Bossche *et al*., 2010).

The Maasai steppe is reported to be infested by tsetse flies and trypanosomiasis since colonial time (Potts, 1937). Maasai's livestock often co-exist with wild animals through sharing grazing areas. In recent years the Maasai people have integrated crop cultivation as a means of their livelihood and become sedentary (Lynn, 2009). This has restricted the areas for grazing and increased interaction with wildlife, especially towards the edge of protected areas, with the potential for an increase of susceptible cases, and infection risk. Given the

increased sharing of the habitat for grazing, it is important to determine the spatial and temporal dynamics of both vector and parasite in relation to habitat types in order to identify the infected landscape at higher risk of disease transmission and plan for both control and grazing which aims to reduce prevalence as well as transmission.

The scope of this paper was to examine the spatial abundance and seasonal changes in tsetse flies and trypanosomes prevalence in relation to the habitats present in the Maasai steppe of Northern Tanzania. Specifically, three questions were considered: How tsetse fly abundance varies with seasons across habitats of the surveyed villages? Do infection rates of various species of trypanosomes vary with habitats across seasons in the surveyed villages? What is the average age of vector infection by species and habitat?

3.2 Methods

3.2.1 Study Area

Maasai steppe (3°40' and 4°35' South, 35°50' and 36°20'E) includes protected areas; Tarangire National Park (TNP), Manyara National Park (MNP) and Simanjiro plains with semi arid vast open wooded savannah and seasonal swampy areas in northern part of Tanzania. The area is inhabited by livestock keepers dominated by Maasai people. The livelihood of the Maasai is centered around livestock management while crop cultivation is used as means to supplement the basic needs. The study area is characterized by bimodal rainfall pattern with short rain between October and December and long rain between March and April (Miller *et al*., 2014). The Maasai are known to co-exist with abundant wild animals which exhibit seasonal migrations to and from the community grazing area(Msoffe *et al*., 2010). The steppe is documented to be regularly infested by various species of tsetse and trypanosomiasis cases from the colonial time (Potts, 1937) until recent years (Cecchi *et al*., 2008; Malele, 2011; Magwisha, *et al*., 2011).

In this study, we sampled four main habitats across the villages close to TNP, MNP and Manyara ranch. The habitats include open-woodland swampy ecotone habitat, Swampy habitats (The wooded grassland area which retains waters above the surface during the rainy season and remains moist with green grass throughout the dry season), Open woodland (scattered trees with grassland undercover) and riverine habitat.

3.2.2 Host counts

Data on host availability for both wild animals and livestock were collected by recording the species and number of animals in each habitat detected up to a plot of 100 m ×100 m around the geo-referenced fly traps every sampling day. The mean number of each host species was calculated and hence compared with its corresponding tsetse relative abundance (proportions) in each trap in a habitat. The relative abundance of the collected host species were compared with corresponding tsetse catches from traps.

3.2.3 Acquisition of land cover satellite data

The extracted normalized difference vegetation index (NDVI) values from Moderate Resolution Imaging Spectrometer (MODIS) from the Terra and Aqua satellites, The Earth Resources Observation and Science Center (EROS) of the United States Geological Survey (http://glovis.usgs.gov/) for different sites that match with the time of tsetse sampling was used for the analysis. NDVI are derived from the visible red wavelength (620 - 670 nm) and near infrared wavelength (841 - 876 nm) of the electromagnetic spectrum (Rogers *et al*., 1996a; Wardlow *et al*., 2007). The spatial and temporal resolution of downloaded images was 250 m and in a composite of a 16 day period.

3.2.4 Entomological survey

The entomological survey was conducted by sampling tsetse flies in different habitats between July 2014 and November 2015. Four villages along the edge of protected areas; the Tarangire National Park and Manyara Ranch boundary were selected for sampling. Simple random selection was used to choose the four villages from other villages surrounding the protected areas. These were namely, Emboreet, Loiborsireet, Kimotorok, and Oltukai (Fig. 5). The minimum number of traps used per village was 24 and at least three traps were set per habitat. Traps were baited with 4-methyl phenol (1g/h) 3-n-propylphenol (0.1 mg/h) 1-octen-3-ol (0.5 mg/h) and acetone (100 mg/h) (Torr *et al*., 1997) were placed in the same geo-referenced points in each habitat for six days of one month during the dry and wet season. Tsetse flies were collected daily from the geo-referenced traps and were identified according to their species, sex, and age (Pollock, 1982) and preserved in collection tubes with pure ethanol for the molecular identification of trypanosome species.

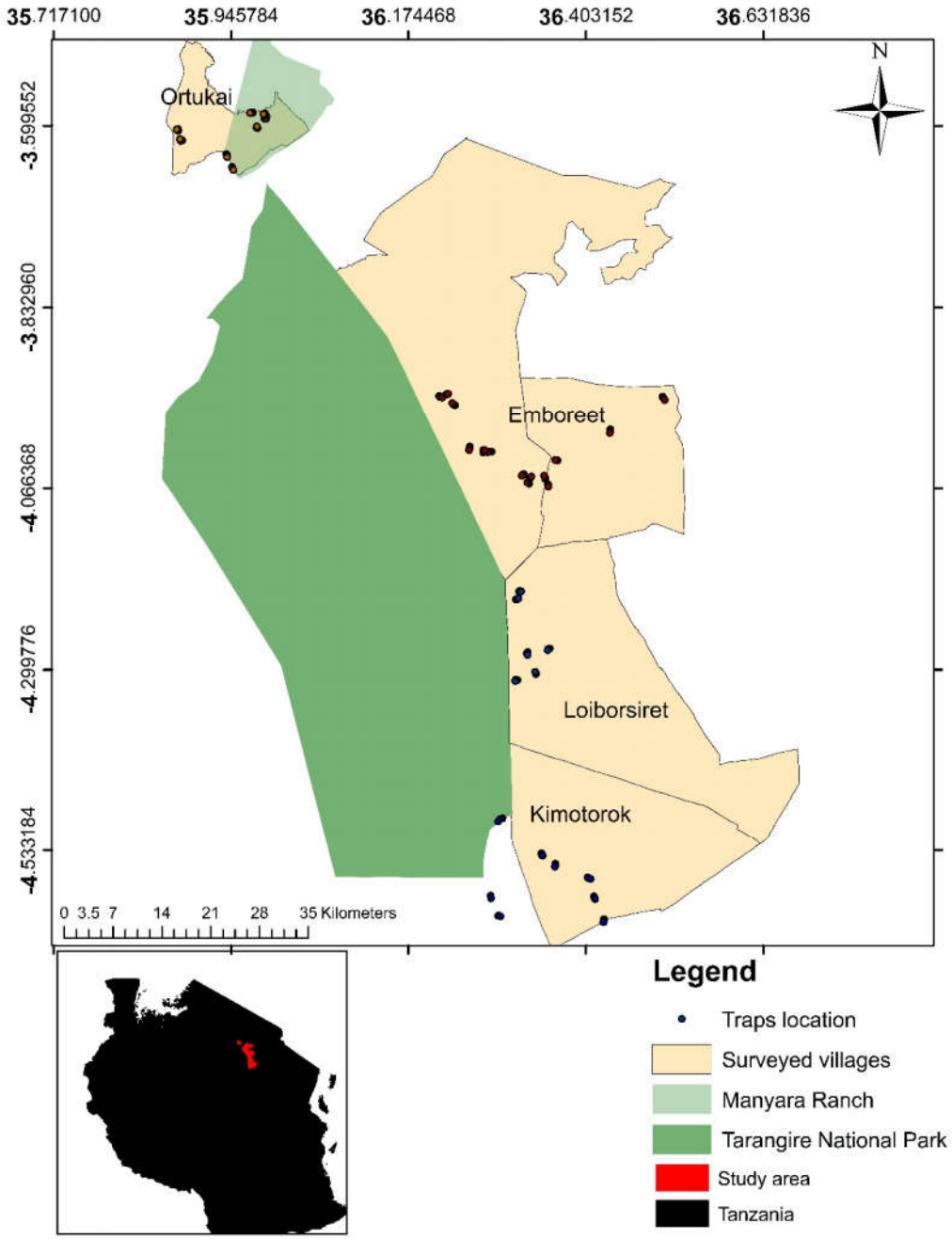

Fig. 5: The map showing location of traps and villages during extensive field entomological survey in the Maasai Steppe, Nothern Tanzania

3.2.5 DNA extraction and Identification of Trypanosomes

The collected samples of individual flies were dried and then crushed in the laboratory using hand pestle. The DNA extraction procedures followed the Ammonium Acetate Precipitation protocol (Bruford *et al.*, 1992; Salekwa *et al.*, 2014). The DNA samples were stored at -20℃

for further analysis. DNA extraction was followed by convectional PCR for identification of trypanosome species circulating in tsetse flies collected in the study area. Primers targeting the Internal Transcribed Spacer 1 (ITS1) gene of trypanosomes were used for screening the tsetse flies DNA. Tsetse DNA was analyzed in pools constituted of 10 DNA samples each and later individual samples of positive pools. The reaction was performed in a total volume of 15 µl containing 7.5 µl Dream Taq master mix, 200 nM of forward and reverse primers and 3.9 µl of nuclease free water. The ITS 1 primer sequences used were CF 5'-CCG GAA GTT CAC CGA TAT TG-3' and BR 5'-TTG CTG CGT TCT TCA ACG AA-3'(Njiru *et al*., 2005). The cycling conditions were: initial denaturation at 94℃ for 3 min followed by 30 cycles of denaturation at 94℃ for 30 sec, annealing at 55℃ for 30 sec, extension at 70℃ for 30 sec and lastly final extension at 72℃ for 10 min. The PCR products were separated on 2% GR- green stained agarose gels and positive results were identified based on the size of the PCR amplicons. The amplicon sizes differ between species of trypanosomes whereby, *Trypanosoma brucei gives* 480 bp and *Trypanosoma congolense savannah* 700 bp (Fig. 6) while *Trypanosoma vivax* 250 bp .

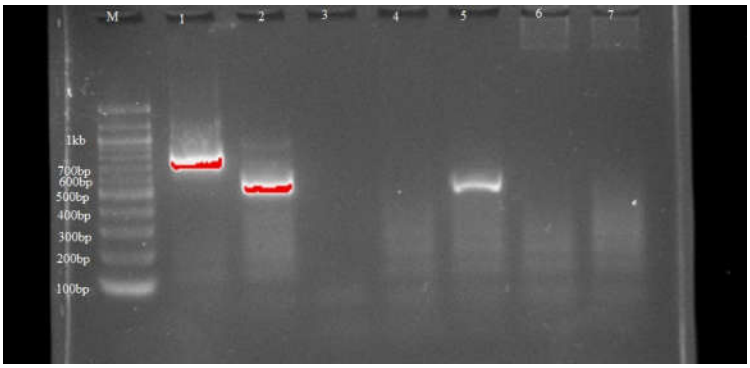

Fig. 6. Trypanosome DNA product by ITS - PCR from Maasai steppe tsetse samples**.** Number 1 is *T. congolense* ~ 700 and number 5 is a *Typanozoon* members (*T. brucei*)~480 bp. Number No. 2 is positive control for *T. brucei* and number 3 is negative control. M = 1 kb DNA ladder.

3.3 Data analysis

The data were analyzed using the R statistical software (Team, 2011) for analytical and descriptive statistics. In this work, we showed variation of three tsetse species among habitats and season for each village, presented using lattice and grid extra of ggplot2 of R libraries. The same libraries have been used to present the relationship between the infection rates and tsetse species and among habitats. Linear Mixed Effect Models (LME, fit by maximum

likelihood) were used to examine the relationship between tsetse species abundance, as a response, and habitat and season, included as independent variables. The abundance of tsetse species is considered as log transformed mean number of tsetse caught per trap per day in a habitat.

Sampling site or habitat, when necessary, was included as a random factor to account for variability among sites and the sampling of the same site every month. In this paper, we used linear mixed models because it models associated variables while retaining a normal distribution of the errors (Crawley, 2007). In addition, the random variables are added to the linear predictor as the extension of linear models.

However, infection among seasons was analysed using the R software. The mean age of fly survival and the mean age at which the fly become infective were calculated using the wingfrey technique described in the training manual for tsetse control personnel (Pollock, 1982). Kimotorok village was excluded from the analysis because of very low fly abundances.

3.4 Results

3.4.1 Abundance of tsetse among habitats and across villages during extensive survey

A total of 1483 tsetse flies were caught in all surveyed villages, of which 1213 were *Glossina pallidipes*, 150 were *G. morsitans,* and 124 were *G. swynnertoni*. Generally, *G. pallidipes* was the most abundant species in riverine habitat in all surveyed villages while *G. swynnertoni* was the most abundant in the ecotone habitat (Fig. 7, Table 5). However, *G. morsitans* was the second most abundant and significant in all habitats across villages (Fig. 7, Table 5). The variation of three tsetse abundance patterns among habitats may be influenced by the quality of vegetation NDVI and the abundance of hosts (Appendix 3 and Appendix 4)

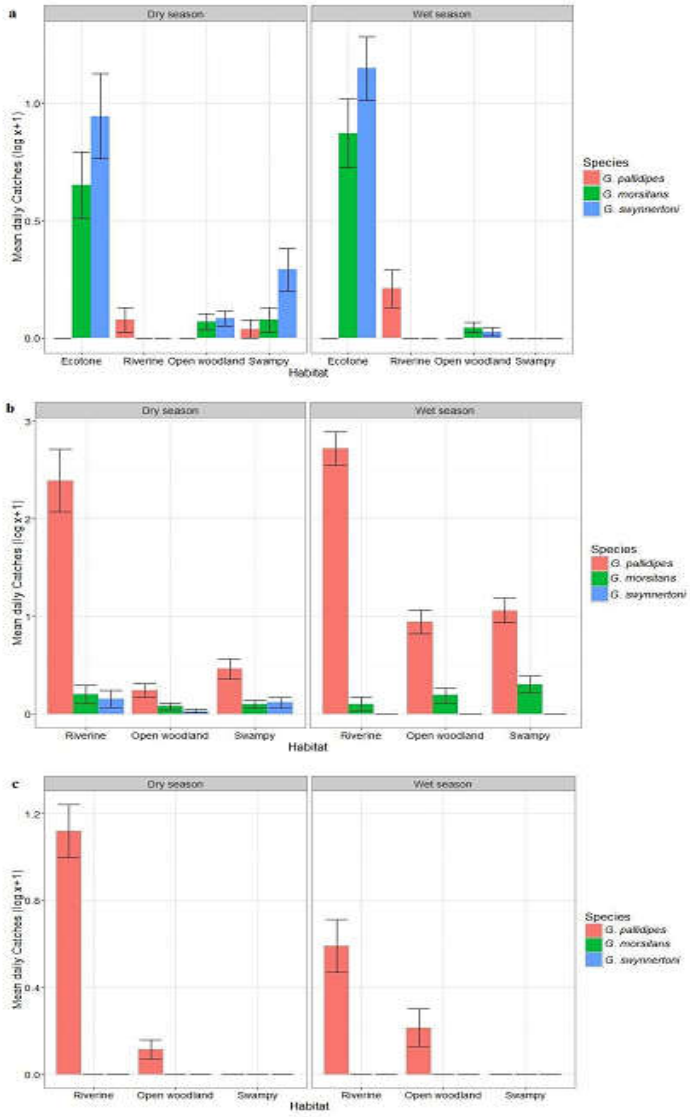

Fig. 7. Geometric mean daily catches of tsetse species by habitat in a) Emboreet b) Loiborsireet and c) Oltukai village

There was an inconsistent variation in tsetse species abundance pattern in open woodland and swampy habitats across villages (Figs. 7a and 7b) for Emboreet and Loiborsireet villages (Tables 5a and 5b). However, only *G. pallidipes* species was found to infest Oltukai village habitats (Fig. 7c). There was incoherent relationship between tsetse species and seasons across villages. *G. pallidipes* had a significantly higher abundance in wet season for Loiborsireet and Oltukai villages (Tables 5b and 5c). Nevertheless, *G. swynnertoni* only

significantly had lower abundance in the wet season than dry season in Loiborsireet (Table 5b) while *G. morsitans* showed no significant variation between seasons across all villages.

Table 5: Linear mixed effect models between the tsetse abundance (log x+1) by species, as dependent variables, and habitats, as independent variables; the site is included as a random factor. a) Emboreet village b) Loiborsireet village and c) Oltukai village

a)

	G. pallidipes		***G. swynnertoni***		***G. morsitans***	
Habitats	Coeff.±SE	*P*-value	Coeff.±SE	*P*-value	Coeff.±SE	*P*-value
Intercept	-0.01±0.03	0.7690	0.168±0.06	0.0028	0.04±0.05	0.4946
Ecotone	0.02±0.03	0.6855	0.90±0.07	0.0003	0.72±0.06	0.0005
Open woodland	0.14±0.04	0.6109	-0.09±0.05	0.1925	0.01±0.05	0.7561
Riverine	0.02±0.02	0.0241	-0.14±0.06	0.1102	-0.34±0.06	0.5646
Wet season	0.02+0.02	0.4128	-0.04±0.04	0.2512	0.006±0.04	0.8642
Random effect SD: site	3.9E-06		2.4E-05		7.2E-0.6	
DF	261		261		261	
AIC	-128.84		148.77		109.76	

b)

	G. pallidipes		***G. swynnertoni***		***G. morsitans***	
Habitats	Coeff.±SE	*P*-value	Coeff.±SE	*P*-value	Coeff.±SE	*P*-value
Intercept	0.46±0.17	0.0154	0.1±0.01	0.0001	0.14±0.08	0.0604
Open woodland	-0.17±0.23	0.5231	-0.04±0.02	0.2001	-0.06±0.09	0.5289
Riverine	1.79±0.31	0.0103	0.02±0.05	0.6371	-0.04±0.12	0.7163
Wet season	0.61+0.1	< 0.0001	-0.07±0.02	0.0019	0.11±0.06	0.0562
Random effect SD: site	0.22		2.6E-06		0.07	
DF	209		209		209	
AIC	507.86		- 119.01		209.5	

c)

	G. pallidipes	
Habitats	Coeff.±SE	*P*-value
Intercept	0.07±0.19	0.7041
Open woodland	0.16±0.26	0.5756
Riverine	0.85±0.26	0.0477
Wet season	-0.14±0.06	0.0162
Random effect SD: site	0.25	
DF	209	
AIC	276.5	

The abundance of tsetse species in other habitats is compared to swampy habitat

There was significant variation in tsetse species among villages (Table 6). *G. pallidipes* was more abundant in Loiborsireet and Oltukai villages whilst *G. swynnertoni* dominated in Emboreet village (Table 6). Surprisingly, the two closely located villages, Emboreet and Loiborsireet were dominated by different tsetse species: *G. swynnertoni* and *G. pallidipes,* respectively (Table 6).

Table 6: Linear mixed effect models between the tsetse abundance (log x+1) by species, as dependent variables, and village, as independent variables; the site is included as a random factor.

	G. pallidipes		***G. swynnertoni***		***G. morsitans***	
Village	Coeff.±SE	*P*-value	Coeff.±SE	*P*-value	Coeff.±SE	*P*-value
Intercept	0.061±0.103	0.5515	0.176±0.051	6E-04	0.12±0.04	0.0027
Loiborsireet	0.991±0.064	<0.0001	-0.105±0.026	1E-04	0.059±0.031	0.0561
Oltukai	0.187±0.057	0.0010	-0.196±0.023	<0.0001	-0.138±0.027	<0.0001
Random effect: site	0.301		0.15			
DF	762		762		0.11	
AIC	1485		102.96		367.2	

Tsetse abundance of other villages is compared to Emboreet village

3.4.2 Trypanosome infection rates in caught tsetse flies among habitats and villages

Out of 1483 flies collected 130 flies resulted positive to trypanosome infection with PCR analysis. The overall infection rate was 8.8%; the highest was found in *G. swynnertoni* species and the lowest was detected in *G. pallidipes* (Fig. 8). *Trypanosoma vivax* was the most common infection among the tsetse fly species (Fig. 8). The infection rates by trypanosome species showed spatial variation among habitats and villages. Specifically, ecotone 21.1%, open woodland 9.0%, riverine 6.8% and swampy habitat 7.7% and variation in the fly infection rates across villages: Emboreet 15.6%, Loiborsireet 5.5%, and Oltukai was 1.7 %. Most of the fly infections were found during the dry season across all habitats, (χ^2 =0.421, DF = 3, *P*<0.0001). *Trypanosoma vivax* was the most prevalent trypanosome species (92 %) found in tsetse flies from all the habitats while *Trypanosoma congolense* (2.2%) and *Trypanosoma brucei* (4.4%) were less prevelent, few cases of co-infection (1.1%) were also observed in riverine habitat specifically from *G. pallidipes* (Figs. 8 and 9).

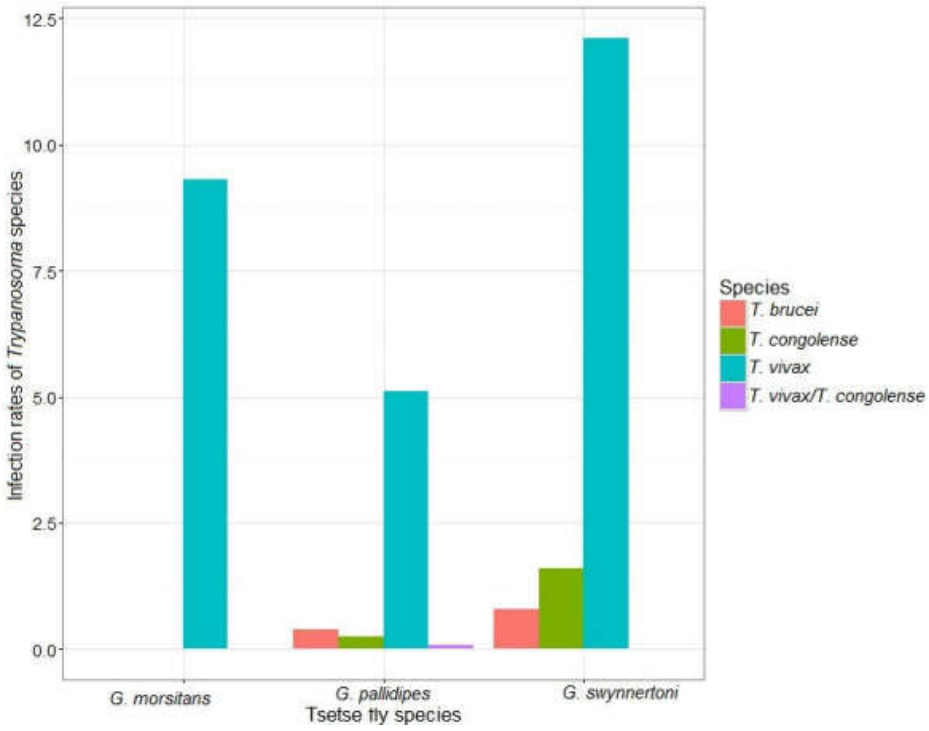

Fig. 8:Infection rates of Trypanosoma species across various habitats of Maasai steppe

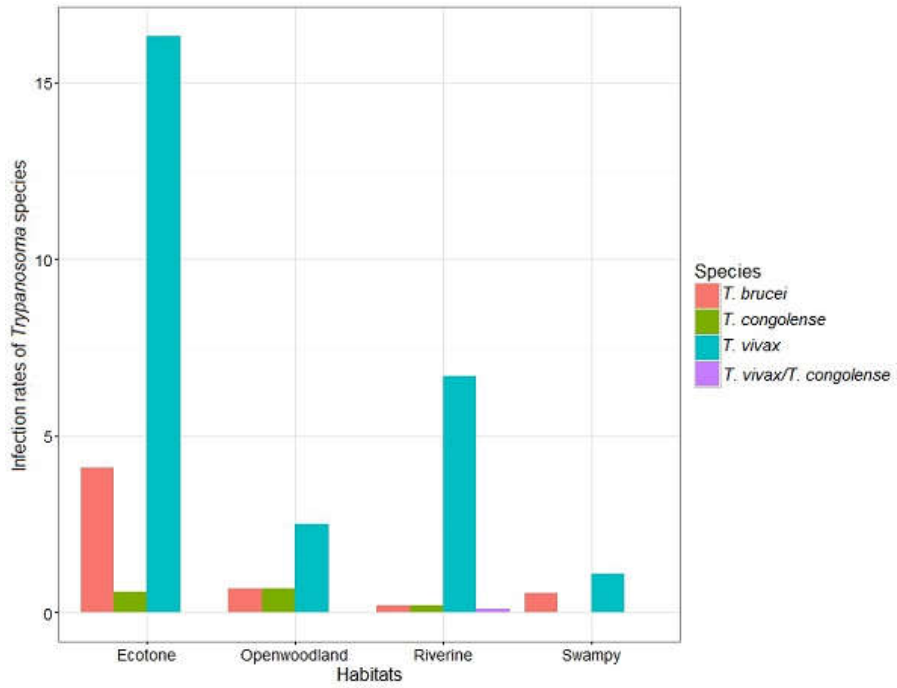

Fig. 9. Infection rates of various Trypanosoma species detected among various species of tsetse flies.

Age structure of trapped tsetse flies

The estimated mean age of all tsetse caught was below 11 days. Based on the mean wing fray value of the age structure of caught flies during sampling, vector longevity in riverine habitat was the highest than flies caught from other habitats and mostly was *G.pallidipes* (Tables 6 and 8. The number and percentage of each Wingfrey category in relation to habitats and species are summarised in Tables 7 and 8. The overall mean wing fray value at which tsetse flies were detected with the infection was 1.6 which is equivalent to 11 days. However, there were differences in the wingfrey categories of infected flies among habitats and species (Tables 7 and 8).

Table 7: Age structure of trapped and infected tsetse species sampled during the dry and wet season collectively.

Species	**Wing fray categories, *n* (%)**						**Total**	**Age MWFV**	**Estimated age (days)**
Trapped flies	**1**	**2**	**3**	**4**	**5**	**6**			
G. pallidipes	1060(80)	71(99)	35(85)	25(80)	13(100)	6(100)	1 210	1.3	Under 11
G. morsitans	142(11)	0(0)	3(7)	4(12)	0(0)	0(0)	149	1.2	Under 11
G. swynnertoni	117(9)	1(1)	3(7)	3(8)	0(0)	0(0)	124	1.0	Under 11
Total	1 319	72	41	32	13	6	1 483		
Infected flies									
G. pallidipes	52(61)	6(100)	6(100)	3(100)	4(100)	3(100)	74	1.9	13 days
G. morsitans	14(15)	0(0)	0(0)	0(0)	0(0)	0(0)	14	1.0	Under 11
G. swynnertoni	19(24)	0(0)	0(0)	0(0)	0(0)	0(0)	19	1.0	Under 11
Total	85	6	6	3	4	3	107		

The numbers in brackets present percentage in the wingfrey category while the numbers outside the brackets present the number of tsetse flies in the wing free category. MWFV means mean wing fray value

Mean age of infected flies caught from riverine habitat was higher than others from the other habitats. Furthermore, our results show that only tsetse flies caught from ecotone had lower mean infective age compared to mean age of survival (Table 8).

Table 8: Age structure of trapped and infected tsetse sampled in different habitats during the dry and wet season.

Habitats	Wing fray categories, *n* (%)						Total	Age MWFV	Estimated age (days)
Trapped flies	**1**	**2**	**3**	**4**	**5**	**6**			
Ecotone	140(10)	3(5)	1(3)	2(8)	1(7)	0(0)	147	1.1	Under 11
Riverine	139(55)	64(96)	30(97)	24(92)	14(93)	7(100)	878	1.4	Under 11
Open woodland	277(21)	0(0)	0(0)	0(0)	0(0)	0(0)	277	1.0	Under 11
Swampy	181(14)	0(0)	0(0)	0(0)	0(0)	0(0)	181	1.0	Under 11
Total	1337	67	31	26	15	7	1 483		
Infected flies									
Ecotone	29(34)	0(0)	0(0)	0(0)	0(0)	0(0)	29	1.0	13 days
Riverine	43(50)	6(100)	5(80)	3(100)	4(100)	3(100)	64	2.0	Under 11
Open woodland	10(12)	0(0)	1(20)	0(0)	0(0)	0(0)	11	1.2	Under 11
Swampy	3(4)	0(0)	0(0)	0(0)	0(0)	0(0)	3	1.0	Under 11
Total	85	6	6	3	4	3	107		

The numbers in brackets present percentage in the wingfrey category while the numbers outside the brackets present the number of tsetse flies in the wing free category. MWFV means mean wing fray Value.

We observed contrasting results for tsetse flies in riverine habitat, where mean wing fray value for infected flies was 2.0 equivalent to 14 days compared to mean longevity age of 1.4 which is below 11 days (Table 8). Similarly, findings were observed in age structure by species where mean age of *G. pallidipes* was higher compared to the same of the infected flies (Table 7). This suggests that many flies particularly *G. pallidipes*; the most abundant fly in riverine habitat dies without being infected by trypanosomes or reaching the infective stage.

3.5 Discussion

The main objective of this study was to investigate the relative abundance and infection rates in various species of tsetse among habitats across villages of the Maasai steppe. In general, there were variations of tsetse species distribution patterns, abundance and infection rates in relation to habitat types and age.

On the question of influence of habitat on tsetse species abundance, we found a significant variation in habitat use by tsetse species across surveyed villages. *G. swynnertoni* which is endemic in northern Tanzania was observed to infest woodland-swampy ecotone

characterized by frequent movement of wild animals, what suits mobile behaviour of this species of following moving objects (Leak, 1999). On the other hand, the *G. pallidipes* infested riverine areas and higher vegetation areas of open woodland and swampy areas. These findings are in agreement with findings from other studies with similar savanna landscape in Africa (Brightwell *et al*., 1992; Woolhouse *et al*., 1996; Bossche and Deken, 2002). However, two closely bordered villages were dominated by two different species of tsetse flies; Emboreet and Loiborsireet had higher proportions of two caught tsetse species; *G. swynnertoni* and *G. pallidipes,* respectively. It is probably because Loiborsireet had higher vegetation cover with many trees and tall grasses which provide suitable vegetation cover favouring pallidipes during wet season while Emboreet was dominated by open woodland and grassland. *G. pallidipes* catches in Oltukai village were caught in riverine areas.

The abundant patterns of different species of tsetse flies were significantly associated with NDVI except for *G. morsitans*. *G. swynnertoni* had a negative relation with NDVI indicating that there were low catches as vegetation cover increases which is in agreement with our previous work in press (Ngonyoka *et al*., 2017). Nevertheless, *G. pallidipes* patterns increased with an increase in NDVI. This is probably because of high abundance of pallidipes in all habitats of Loiborsireet and Oltukai in riverine habitats where its abundance increased with increase of vegetation cover. This observation is in agreement with other studies which reported higher pallidipes abundance in the wet season with higher NDVI compared to dry season (Ndegwa *et al*., 2001; Sindato *et al*., 2007). Although, this study did not show a statistical significance between *G. morsitans* and NDVI, our findings may still have epidemiological importance.

We found that habitats with the highest number of host animals also had higher abundance of tsetse flies. Cattle play an important role in sustaining female tsetse to feed and pupate. This could be probably because of tsetse preference to feed on cattle (Bossche and Staak, 1997; Krafsur, 2009), increase in a number of cattle in the area and expansion in small-scale agriculture (Lynn, 2009). These factors consequently reduce both cattle grazing area which push grazing towards the shrinking wild-land and seasonal grazing patterns of wildlife (Msoffe *et al*., 2010). In addition, other studies have shown changes of tsetse habitats through cultivation and other human activities leading to their elimination or tsetse appearing in what may be considered to be inappropriate habitats (Potts, 1937; Sindato *et al*., 2007). This may be the case for Loiborsireet and Oltukai villages. However, the latter village has limited

grazing area, thus farming and overgrazing have pushed livestock keepers to graze their animals in the permitted area of Manyara ranch, where we also documented most catches of tsetse. This is inconsistent with (Sindato *et al*., 2007), that, land use changes in community land reduce habitat for host and tsetse and hence the conserved areas will remain to be the hotspot of trypanosome infection.

Trypanosome infection rates of various trypanosome species vary among habitats, village, tsetse species, and age. Ecotone and riverine habitats, which are dominated by *G. swynnertoni* and *G. pallidipes* in Emboreet and Loiborsireet villages, respectively were the most infected habitats in Maasai steppe. This is probably because of the abundant hosts in spite of seasonal wild animal migration inside the park especially during dry season, the time when livestock grazing patterns move towards the park boundary. This provides constant development for tsetse and circulation of trypanosomes in vector and vertebrate hosts. Lower trypanosome infection rates in swampy areas were limited by host availability especially during the wet season where animals move to the plains and during late dry season tsetse have limited shade to avoid desiccation. In spite of its highest abundance, *G. pallidipes* had lowest infection rates that could be contributed by variation in transmission of trypanosomes and vectorial capacity among tsetse species (Reifenberg *et al*., 1997; Leak, 1999; Haines, 2013). In addition, the infective age of flies based on mean wing fray value was higher compared to mean age of tsetse longevity in riverine habitats which were dominated by *G. pallidipes*.

Trypanosoma vivax was the most prevalent species in all infected tsetse flies compared to *T. congolense*, *T. brucei* and few observations of co-infections. This is probably because of biological differences of this species in development in tsetse flies. The midgut establishment of ingested trypomastigotes of *T. congolense* and *T. brucei* are notably limited by anti-trypanosomal factors (midgut trypanolysin and trypanoaglutinin) (Molyneux and Stiles, 1991). These factors fluctuate with normal digestive cycles and feeding patterns hence feeding at short interval stimulates their release and renders the fly refraction to infection. In addition, the longer periods of starvation renders the flies more susceptible to infection as agglutinin and lytic activity decrease with time. Furthermore, the migration of cyclic trypomastigotes from midgut loose ability to invade hypopharynx (Dipeolu and Adam, 1974; Aksoy, 2003). Previous experiments showed up to 50 – 60% of *T. congolense* in morsitans persisted up to 30 days while they take 19 - 23 days to develop (Dipeolu, 1975).

Trypanosoma vivax development takes place in the proboscis of the tsetse flies and persists up to 58 days (Soltys and Woo, 1977). *Trypanosoma vivax* also has unique differentiation in tsetse through loss of surface coat and 100% of its infection rate could be achieved with repeated feeding flies on an infected host.

The average age of the caught tsetse flies was below 11 days what provides a chance for *T. vivax* to circulate as its maturation takes 5 days at a temperature of 26°C. This can also be used to explain the higher infections during dry season compared to wet season (Desowitz and Fairbain, 1955). The longevity of the vector may be a limiting factor for the development of some trypanosomes requiring a longer time for maturation for example, *T. congolense* at 24°C takes up to 15 - 20 days (Leak, 1999). On the other hand, fly species differ in their infection rates and age at which tsetse become infective. In this study, there are clear differences in infection rates in *G. pallidipes* and *G. swynnertoni* which are mostly abundant in riverine and ecotone habitats respectively. This is probably because its mean infective age is higher than mean age of its survival and lower abundance levels of hosts, whereas, *G. swynnertoni* had low infective age to mean age of survival hence higher infection rates.

Although this study was limited to two seasons, the similarity of our findings with other studies suggests that observed patterns are significant to provide insight into the epidemiology of Trypanosomiasis in the area. The outcome of this study will provide insights in mapping hotspots of tsetse infestation and trypanosome infections. Since *T. vivax* is responsible for sylvatic transmission of trypanosomiasis, it opens avenues for further research on the role played by *Stomoxys* spp. and Tabanids in mechanical transmission of the infections.

3.6 Conclusion

In summary, the study examined the spatial variation of abundance of tsetse flies and infection rates with trypanosomes among habitats, villages, tsetse species and age structure. The data suggests that the abundance of tsetse in various habitats was influenced by vegetation cover and host availability, while infection rates varied with the composition of tsetse species, species of trypanosome species, the age of fly to become infective in relation to the longevity of the tsetse species. The study lays groundwork for modelling tsetse spatial distribution patterns, the potential risk of trypanosomiasis transmission and plan for control of vector and disease.

CHAPTER FOUR

Participatory epidemiology of bovine trypanosomiasis, livestock grazing patterns and disease control measures: The case of livestock keepers in the Maasai Steppe.[3]

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Abstract

Participatory Epidemiology (PE) has proved useful in generating epidemiological information crucial for disease control, ways to enhance community engagement and addressing policy gaps towards public-private partnership for effective disease control under eco-health approach. In this paper PE was used to study incidence and mortality of infectious diseases, seasonality and land cover changes in relation to vectors, wild animals as the reservoir host, disease control options and sustainability, and needs assessment in the Maasai steppe. The Maasai communities live adjacent to protected areas close to wild animals hence are under increased risk of tsetse infestation and trypanosomiasis infection. The study was conducted in four villages from July to August 2015. Results show a spatial variation of disease incidence and mortality rates within and among villages. Furthermore, the Maasai calendar has five seasons which are associated with land cover changes, disease occurrence, and contact with vectors and wild animals. The Maasai have integrated vector and parasite

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control measures with dosage and optimization concerns. Regarding sustainability criteria to disease control issues, the Maasai prefer new drug projects against tsetse and trypanosomes. There was a variation of in communities response on understanding of the vector, control methods and need assessment among villages. However geospatial modelling is powerful in determining the vector distribution across the space crucial for landscape epidemiology. This study opens the way for ecohealth cost effective intervention strategies against the disease and vector from the identified hotspots. The study provide potent identified a need for linkage between local government, national institutions which deal with vectors or diseases, policy makers, training and capacity building to enhance tsetse suppression and treatment.

Keywords

Participatory Epidemiology, Trypanosomiasis, Tsetse flies, Maasai Steppe

4.1 Introduction

Participatory epidemiology (PE) integrates participatory approaches such as mapping, ranking, and grouping making an epidemiological calendar for various uses by livestock keepers while researchers play a facilitation role to generate information about animal diseases, research plans and/or control disease programs (Catley *et al*., 2014). In recent years, participatory epidemiology has been explored for its integration potential in modeling which increasingly becomes more important under eco-health approach (Grant *et al*., 2016). Its integration has potential in mapping disease risk areas using participatory expert opinion (Fuller *et al*., 2014), used in structuring the models, identify parameters, prioritize the alternatives (Grant *et al*., 2016b). It enhances "bottom-up" community involvement in generating information related to the livestock disease problem identification and priorities, criteria selection and weighing alternatives and mapping (Grace, 2003; Jibril *et al*., 2015).

Trypanosomiasis belongs to the group of diseases caused by protozoa of the genus Trypanosoma with both public health and socio-economic consequences. The major veterinary species are *Trypanosome congolese*, *T. vivax*, *T.brucei* and *T. simiae* (Kaare *et al*., 2007). The former three affect cattle, goats and sheep. In animals, trypanosomiasis is transmitted by multiple species of vectors of Genera Glossina. Its impacts range from livestock death, loss of production, changes in livestock grazing patterns to avoid disease transmission, human death and Diability-adjusted life year (DALY) (Swallow, 1999). In Africa, trypanosomiasis disease transmission dynamics, and control methods for vectors and

parasites remain to be a challenge due to uncoordinated research and monitoring activities (Malele, 2012). With the paucity of epidemiological information in Africa, participatory epidemiology provides tools among veterinarians, epidemiologists and practitioners in generating and sharing of qualitative data about infectious disease to supplement and fill the gap and thereby to provide the necessary information for the public (Catley *et al*., 2004; Jost *et al*., 2007; Mariner *et al*., 2011).

In recent years the Maasai people have integrated agriculture which converts grazing land to crop cultivation land as part of their livelihood strategies in the Maasai steppe (Msoffe, 2010). In spite of benefits accrued from agriculture, the same appears to push grazing activities close to wild land hence increasing exposure to trypanosomiasis vectors (Malele, 2011). Although livestock keepers have used trypanocides for three decades, trypanosomiasis remains to be a problem. Little has been done to assess the impact of disease control measures adopted by farmers.

This paper describes a study on participatory mapping of livestock seasonal grazing patterns and trypanosomiasis control under changing land use in the Maasai Steppe. Specifically, the study answered three main questions: 1) to what extent do the seasonal livestock grazing patterns expose cattle to trypanosomiasis infection? 2) What is the understanding among the Maasai on land cover changes (vegetation), local classification of seasons related to disease vector abundance and incidence? and, 3) are control options adopted by livestock keepers for different seasonal grazing patterns efficient and sustainable?

4.2 Methodology

4.2.1 Study area

The Maasai steppe is located between 3°40’ and 4°35’ South, 35°50’ and 36°20’E. The Maasai Steppe landscape stretches over 8 districts in Arusha and Manyara regions and covers approximately 40 000 km^2. The area is semi-arid grassland with predominantly acacia woodlands in the low-lying areas and Miombo woodlands in the hills rising in the southwest. It is known to have trypanosomiasis cases transmitted by tsetse to human and livestock. Four villages were purposively selected for the study namely, Emboreet, Loibor-siret, Kimotorok and Ortukai. The villages border with wildlife protected areas, Tarangire National Park (TNP) in case of Loibor-siret and Emboreet villages, while Ortukai village borders with

Manyara Ranch Conservancy (MRC) and Manyara National Park (MNP) (Fig.10). The area is characterized by abundant flora and fauna that are hospitable to many disease vectors including tsetse flies. There are diverse wild mammals such as bovids, giraffes, suids and elephants which act as the reservoir of parasites. The area is inhabited predominantly by the Maasai people whose livelihood depends entirely on livestock especially cattle, goat, and sheep although crop cultivation supplements both dietary and additional income. The area is semi-arid receiving about 500mm of rainfall in a year. The study area is characterized by seasonal grazing pattern, pushing grazing activities close to tsetse habitats near protected areas especially during the dry season (Fig. 10).

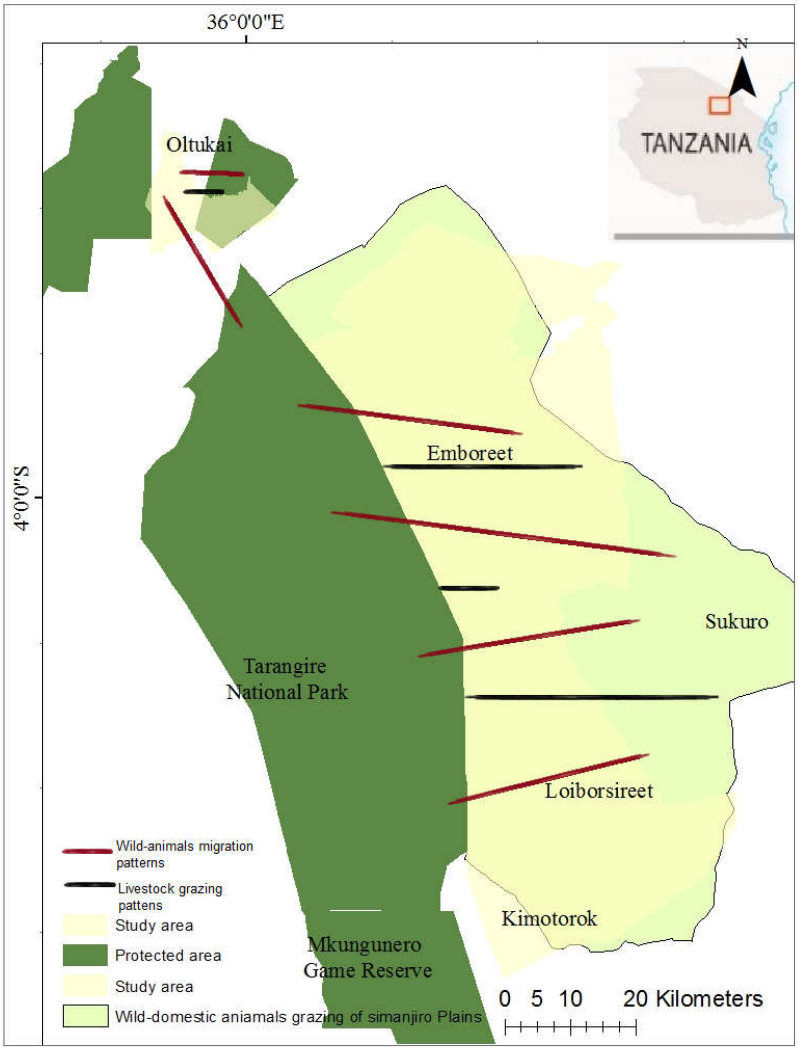


Fig. 10: Map showing wildlife and cattle grazing patterns between home range and inter phase areas captured during participatory epidemiology.

Wild-animals migration patterns from the park to community grazing field in the plain at the end of november and move back to the park in may. In January-February and July-November Livestock grazing patterns towards the park boundary to avoid Malignant Catarrhal fever (MCF) infection in wildbeast calving field and search for good pasture respectively.

4.3 Data Collection

4.3.1 Secondary data

Information about trypanosomiasis and other related data such as maps and grey materials were obtained from village and district offices, which were used to map and track the background of the study area.

4.3.2 Interviews

Survey data were collected using a questionnaire between August and September 2015. A total of 125 respondents were recruited through random selection of the households picked from the list generated from village offices. In this study the entire boma was treated as a single unit and one person was picked for an interview. The questionnaire was translated from English to Swahili and the interviews were conducted in Swahili language. The interviewers were trained Maasai individuals conversant in both Swahili and Maasai, who could explain in the Maasai language to respondents who neither knew Swahili nor English. The interviews were trained by social scientists on the methods of probing and record taking from the respondents. The questionnaires had both structured, and semi-structured questions. The questions sought information about respondents demographic characteristics (name, age group, sex and occupation), trypanosomiasis disease signs, vector control measures in the past year and treatment options in practice.

4.3.3 Focus Group Discussion (FGD)

A total of 8 focus group discussions were conducted in the 4 study villages. The FGDs were conducted at appropriately designated areas, as directed by the village chairman. Participants were well informed of the process and were all identified during the extensive questionnaire survey. All participants involved in FGDs were herders, who participate in decision making about grazing patterns, animal treatment and production. The number of discussants in the groups ranged from 5 to 9 people comprising of elders, tribal leaders, sub-village chairmen and young men aged 25 to 40 years. The FGD focused on eliciting information about potential control options against trypanosomiasis and diseases in general, drug administration

and dose preparation, change of control methods by seasons while sustainability of control options against indicators was adopted from Catley *et al*. (2011).

4.3.4 Mapping

During the focus group discussions, five key members were selected as analysts for mapping. The selection of these analysts was based on their understanding of village boundaries and grazing areas. Participants showed the major grazing routes in existing maps. Mapping of the important grazing routes was conducted through either transect walking or driving for short and long distances, respectively each time taking GPS points and route tracking. The group mapped grazing areas in seasons of the year, grazing routes, tsetse infested areas and water points.

4.3.5 Proportional Piling

Livestock keepers generated information about the incidence of trypanosomiasis and other diseases in each sub-village. Three groups each comprising of 5 individuals were involved in generating information about incidence of trypanosomiasis and other most affecting livestock diseases. The participants used 100 stones to allocate the proportion of healthy and sick cattle then re-allocated the stones to illustrate magnitude of individual livestock diseases for the past year. For each disease they further divided between dead and recovered animals upon receiving treatment. The same approach was used to estimate disease incidence based on age groups according to Maasai criteria.

4.3.5 Seasonal calendar and Matrix Scoring

Seasonal calendars were generated by individuals with indigenous knowledge about both Maasai year calendar and Gregorian calendar. In each village participants listed the five seasons of the Maasai calendar and matched it to the months of Gregorian calendar. Various parameters including rainfall, land cover, disease incidences, fluctuation of disease vectors and presence of host animals were independently ranked across seasons. Each parameter was plotted along y-axis against seasons plotted on the x-axis. Matrix scoring was used by participants through dividing 20 stones according to the season weight for the parameter.

4.4 Data analysis

Data gathered from semi-structured questionnaire were cleaned and analyzed using Statistical Package for Social Sciences (SPSS) version 21. Data collected through proportional pilling were entered into Excel spreadsheet for descriptive statistics in form of tables, charts and

percentages. Non-parametric tests, Kendall coefficient, were used to analyze the agreement of group rankings against disease incidence, land cover changes, contact with vectors and wild animals and Friedman's test was use to analyze control alternatives against sustainability indicators.

The data from FGDs was carefully checked several times to gain a solid overview, then sorted and analysed manually through a template approach. Codes for ordering and analyzing the data were identified inductively from the data. Based on the codes, the data material was deconstructed and re-assembled in codes, then themes and re-read to identify trends and variations. Positionality, conditions and situations under which data was collected were reflexively considered in the process of analysis.

4.5 RESULTS

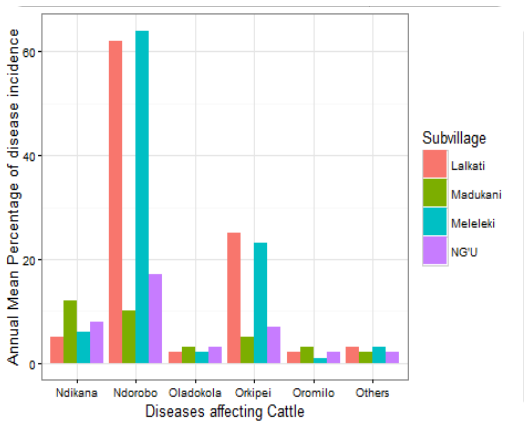
A total of 125 respondents participated in interviews and participatory epidemiology surveys. Majority of the participants (92%) were aged 20 to 45 years, which corresponded to Maasai age groups:*Nyangulo* (teenagers) 15.2%, *Korianga* (20–39 years) 44% and *Irrikidotu* (40-55) 32.8%. Elders comprised 8% of the respondents with specific age groups (*Nyangus* (55-60) 0.8%, *Seuri* (61-70) 2.4% and *Makaa* (above 71 years) 4.8%. Regarding literacy level, our findings indicated that 44.8% (n=56) of respondents never attended formal education, 8% (n=10) could neither read nor write, 44.8% (n=56) had primary school education, 8.8% (n=11) had secondary education and 1.6% (n=2) had certificate level education. Ninety six percent of participants were livestock keepers as their primary economic activity but also engaged in crop cultivation and only 4% were peasants. All individuals who participated in focus group discussion and transect walk or drive across sub-villages were livestock herders (100%).

4.5.1 Incidence of most prevalent diseases and death rate

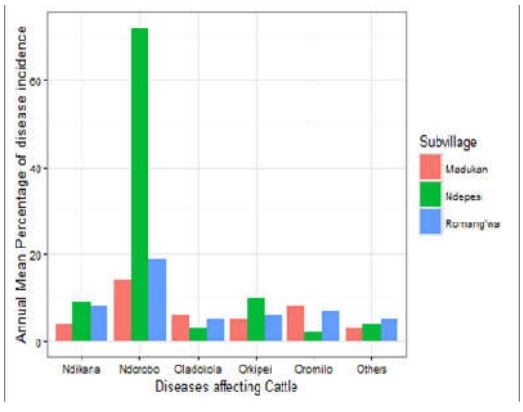
Respondents across all villages mentioned East Coast Fever (ECF) (*Ndigana*), African Animal Trypanosomiasis (*Ndorobo*), Babesiosis (*Oladokola*), Cerebral Theileriosis (*Oromilo*), and contagious bovine pleuropneumonia (CBPP) (*Orikipei)* as the most prevalent diseases affecting their livestock. Other diseases were Anthrax, Q-fever and Tuberculosis. During proportional piling exercise Trypanosomiasis accounted for the highest disease incidence in all villages ranging from 15% to 100% except in Ortukai Village, ECF ranged from 5% to 18%, CBPP ranged from 3% to 26% , Babesiosis 1% to 8% and others were

below 5%. Only Ortukai village had very low Trypanosomiasis incidence estimates from 7% to 10% among its sub-villages (Fig. 11d). There was a difference in disease incidence between villages and within sub-villages (Fig. 11). In Emboreet village, Meleleki and Lalkati sub-villages which are located adjacent to reserved grazing areas which extend to the park boundary had trypanosomiasis incidence above 60% while Ng'u and Madukani sub-villages, located adjacent to human settlements and farms had trypanosomiasis incidence below 20% (Fig. 11a). Similarly, in Loiborsireet village, the Ndepesi sub-village which has high vegetation cover had a 70% incidence of AAT while Romang'wa had estimated AAT incidence to be below 20%. Kimotorok had fairly higher AAT incidences across its sub-villages ranging from 40% to 75%.

When incidence of Trypanosomiasis was compared between cattle age groups, *lahokwinyinyiki* age group (1 to < 2 years old age group) was indicated with lowest incidence (30%), while *Alaram* age group (2-3 years old) had a 50% incidence and *Ngehu* age group (> 3 years old) had 70% incidence of AAT. Furthermore, the participants described two patterns of clinical signs of trypanosomiasis, on one side, respondents characterized the disease with a rapid onset, diarrhoea and short duration fever followed by death. On the other hand they distinguished the disease with signs of reduced appetite, rough and standing hair coat, diarrhoea, swelling of lymph nodes and longer time suffering to death. Participants further acknowledged that, sometimes inexperienced herders could confuse AAT with ECF which often leads to mistreatment and misuse of drugs.



(a).



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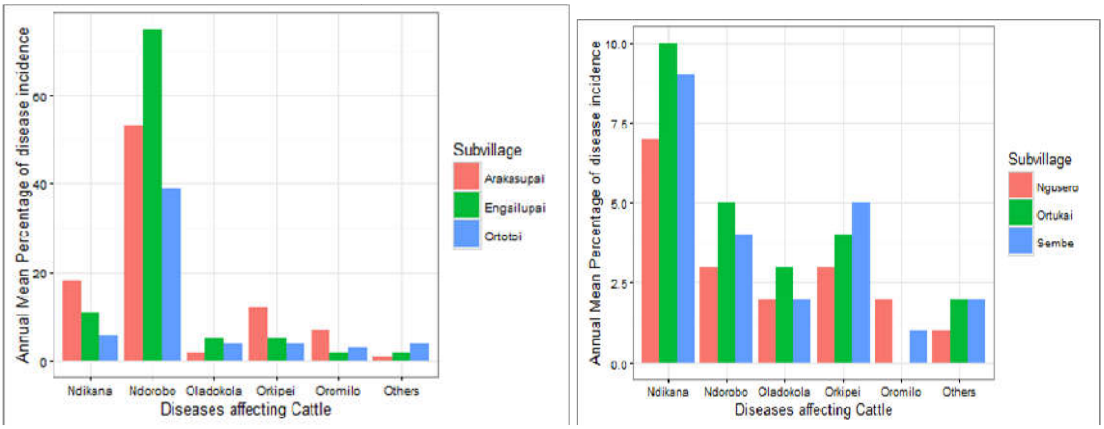

(c) **(d)**

Fig. 11: Mean incidence percentages of trypanosomiasis and other most prevalent diseases generated during in-depth group discussion across villages and sub-villages in an entire year: **(a)** Disease incidence at Emboreet **(b)** Disease incidence at Loiborsiret **(c)** Disease incidence at Kimotorok **(d)** Disease incidence at Ortukai

Trypanosomiasis was reported to cause the least mortality if animals were treated. In most villages respondents reported that all cattle recovered after treatment except in Kimotorok village where herders reported mortality rate of 5% even if the animals were treated. Respondents claimed that all animals with cerebral theileriosis usually died, whereas ECF caused 1.6% to 3.3% mortality, Babesiosis up to 1.3% and CBPP caused mortality up to 2%. Mortality from other diseases was estimated to be below 1% (Fig. 12).

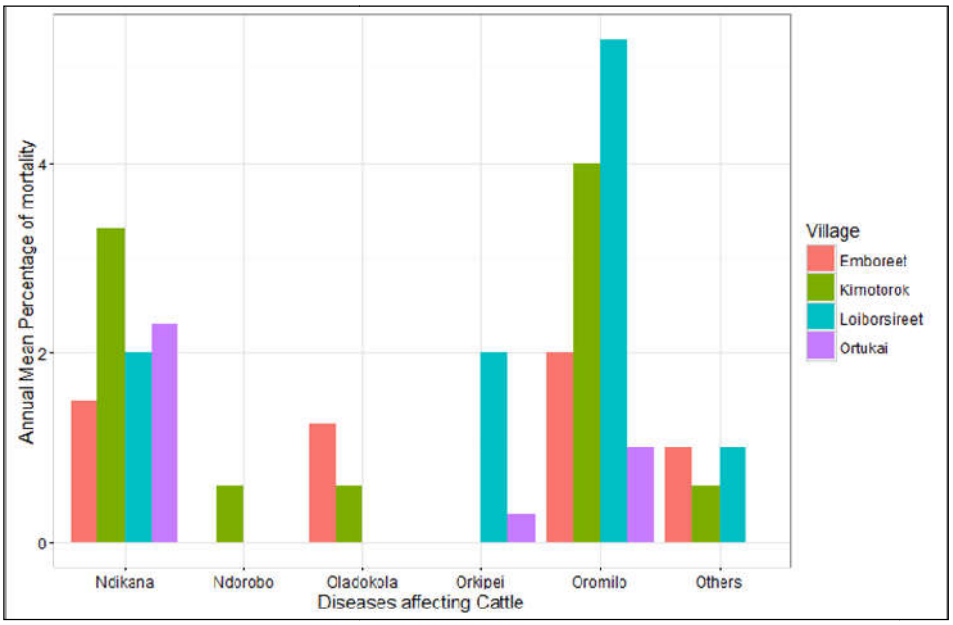

Fig. 12. Estimated mean death rate of cattle due to diseases prevalent in surveyed villages as perceived by Maasai pastoralists.

4.5.2 Perceptions of Maasai on land cover characteristics (vegetation) and seasons based on local classification in relation to disease vector abundance, disease signs and incidence

Table 9: Seasonal patterns of disease incidences in relation to amount of rainfall and land cover.

	Maasai steppe seasons											
	Orekiserati		*Aladalo*		*Halari*		*Korumale*		*Alamei*			
	Gregorian Calendar											
	N	D	J	F	M	A	M	J	J	A	S	O
Rainfall *Enchan* W=0.9385**	●● ●●● 5(4-7.5)		0(0-0.5)		●●●●● ● ●● ●● 10(10-13.5)		●● ● 3(1-5)		0(0-0)			
Land cover W=0.8625**	●● ●● 4(2.5-5)		●● ● 3(1.5-4.5)		●●● ●● 5(3.5-7.5)		●●● ● ● ● 6(4.5-6)		●● 2(1-4)			
Trypanosomiasis *Endorobo* W=0.6814**	● ● ● ●●●● 7(2.5-8)		●●● 3(1.5-7)		●● 2(0-3.5)		●● 2(0-4.5)		● ● ●● ● ● ●● 8(6-10.5)			
East Coast Fever *Ndikana* W=0.6172**	●● ● 3(2.5-4)		●● ● 3(2-4.5)		●●● ●● 5(3.5-6)		●●● ●●● 6(4.5-7.5)		● ● ● 3(2-3.5)			
(CBPP) *Orikipei* W=0.3076*	0(2-11)		0(0-2.5)		●●●●●● ● ● ● ●●● 12(0-17.5)		● 1(0-3)		● ● ● ● 4(0-11)			
Babesiosis *Oladokola* W=0.3827**	0(0-5)		● ● ● ● 4(0-12.5)		0(0-0)		● ● ● ● ● ● ●● ● ● 10(0-15)		0(0-17.5)			
(Cerebral Theileriosis) *Oromilo* W=0.4021**	● ● ● 3(0-4)		● ● ● ● 4(2-10.5)		● ● ● ● 4(0-4)		● ● ● ● 4(2-5)		● ● ● 3(0-4)			

Number of informant groups =7; W=Kendall's Coefficient of Concordance (*P<0.05, **P<0.01). Black dots represent median scores. In the brackets are the 95 % confidence limits of the median; N, D, J, F, M, A, M, J, J, A, S, O represent

November, December, January, February, March, April, May, June, July, August, September, and October months of the year.

The highest incidence of trypanosomiasis was reported between July and December. This period corresponds with the Maasai seasons of *Alamei* (July - October) and *Orekiserati* (November-December). Disease incidence remained low during the remaining seasons when rainfall was high (Table 9). Conversely, participants associated high ground cover with low AAT incidence. Incidence of ECF was constantly high from January to October with a peak during July – October (*Alamei).* Babesiosis incidence was reportedly high during May-June months (*Korumale*) while Cerebral Theileriosis (*Oromilo*) cases were not related to seasons, rainfall or land cover (Table 9).

Further, it was reported that livestock are most heavily infested with tsetse during July-October months (*Alamei*) but infestation is lower during November-December months (*Orekiserati*), the infection risk being highest during *aladalo* (Jan – Feb) but relatively lower during rainy season *Halari* and *Korumale* (March – June) (Table 10). In addition the participants identified seasons *Orekiserati* (November – December), *Aladalo* (January – February) and *Halari* (March – April) during which there is high interaction with wild animals, which are potential reservoir hosts of trypanosome infections (Table 10).

Table 10: Seasonal dynamics of contact between livestock, disease vectors and wild animals.

Vectors	Maasai steppe seasons											
	Orekiserati		*Aladalo*		*Halari*		*Korumale*		*Alamei*			
	Gregorian Calendar											
	N	D	J	F	M	A	M	J	J	A	S	O
Contact with tsetse *Ndorobo* W=0.6439**	● ● ● ● ● 5(4-7.5)		● ● ● ● 4(1.5-5)		● ● 2(0.5-3)		● ● 2(1.5-5.5)		● ● ● ● ● ● 6(4.5-8.5)			
Ticks *Irimaheri* W=0.5593**	● ● ● ● 4(1.5-5.5)		● ● ● 3(1-4.5)		● ● ● 3(1.5-5)		● ● 2(1-3.5)		● ● ● ● ● ● ● ● 9(7.5-10)			
Stomoxy flies *Endusi* W=0.801**	0(0-0)		0(0-16.5)		0(0-7)		● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● 20(15-20)		0(0-0)			
Tabanus Orekimbai W=0.8857**	0(0-0)		0(0-5.5)		0(0-0)		● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● 20(16-20)		0(0-0)			
Wild animals W=0.8348**	● ● ● ● ● ● 6(4.5-7.5)		● ● ● ● ● ● ● ● 8(7-9.5)		● ● ● 3(1.5-4.5)		● 1(0.5-2.5)		● 1(1-2)			

Number of informant groups = 7; W = Kendall's Coefficient of Concordance (*P<0.05, **P<0.01). Black dots represent median scores. In the brackets are the 95% confidence limits of the median; N, D, J, F, M, A, M, J, J, A, S, O represent November, December, January, February, March, April, May, June, July, August, September, and October months of the year.

4.5.3 Control options adopted by livestock keepers during different seasonal grazing regimes.

The use of trypanocidal drugs to treat cattle was the most favoured control option as reported by 96% of respondents. Different brand names of trypanocides were mentioned as being used by the Maasai livestock keepers, including Isometamidium salts; Samorin® (7.2%), Saridium® (21.6%), Homidium salts, Novidium® (13.6%), and Diminazene® salts; Berenil® (48.8%) and Diminazin® (13.6%).

Further, in-depth interviews revealed that most community members did not use the drugs as per prescribed dosage by drug manufacturers. Some farmers considered that the prescribed

dosage for the drugs was not adequate to completely treat their animals. Table 11 summarizes the self-prescribed dosages used by Maasai herders in surveyed villages.

Table 11: Self-prescribed dosages of trypanocidal drugs used by Maasai herders to treat cattle against trypanosomiasis in different villages of the Maasai steppe.

Village	Option	Criteria	Dose (mls per injection)			Dilution of trypanocides in water	
			Calf	Cow	Bull	Amount of trypanocides	Amount of water
Emboreet	1	Age group	5	10	10	1 sachet Berenil®	10cc
	2	Age and type of drug	5	10	10	1 sachet Berenil®	10cc
						2 tablets Novidium®	10cc
						3 pcs Samorin®	10cc
	3	Weight	5	10	20	2 sachets Berenil® + 5cc OTC	20cc
	4	Unclear diagnosis	5	10	10	1 sachet of Diminazine® + OTC®	10 cc
Kimotorok	1	Diarrhoea	5	10	20	1 sachet of Samorin®	20cc
	2	Enlarged lymph nodes (Tezi)	5	10	20	1 sachet Berenil	20cc
	3	Large herd of cattle	5	10	20	8 sachets of Samorin®+ 30 sachets Berenil® for 50 cattle	500 cc
			5	10	20	8 sachets of Samorin® + 20 Diminazine® for 35 cattle	350 cc
Ortukai	1	Breed, Zebu	5	10	15	1 sachet Berenil®	10cc
	2	Breed, Sahiwal	5	10	40	2 sachets Berenil®	20 cc
Loiborsireet	1	Age group	5	10	10	1 sachet Berenil®	10cc

1 sachet of Berenil contains 2.36 gm of active compounds of 1.05 gm recommended to be diluted in 12.5mls of water. It is recommended to inject 1 ml per 10 kg of animal body weight. A cold water solution of 2.5% of Novidium Chloride is recommended to be adminstered at a dose rate of 1mill to 25 kg of animal body weight.

Table 12 shows the preferred vector control measures among surveyed villages. Two vector control methods mentioned by respondents were spraying (51% of respondents) and dipping (49% of respondents) using synthetic pyrethroids (Paranex® and Dominex®). The frequency of spraying or dipping was either once or twice per month depending on season or cattle grazing pattern. Frequency of spraying or dipping was increased during extended droughts when cattle were grazed in tsetse infested areas close to the park. Furthermore, herders in some villages of Simanjiro plains graze their cattle further into wilderness strategically away from pastures used by wildebeest during calving as a way to avoid other infections, such as malignant catarrhal fevel and Q fever. Besides spraying and dipping ranked top by Simanjiro herders, respondents in Ortukai village ranked targets as the second best vector control alternative (Table 12).

Table 12: Ranking of vector control methods against criteria defined by pastoralists in 4 surveyed villages. (1 refers to high preference whereas 4 is least preference)

Criteria	Emboreet village				Loiborsireet village			
	Dipping	Spraying	Targets	Local Herbs	Dipping	Spraying	Targets	Local herbs
Effectiveness	2	3	1	4	2	3	1	4
Availability	1	2	4	3	1	2	3	4
Easy to use	2	1	4	3	1	2	4	3
Cost	2	3	4	1	2	3	4	1
Total rank	**9**	**9**	**13**	**11**	**6**	**10**	**12**	**12**
Criteria	Kimotorok village				Ortukai village			
	Dipping	Spraying	Targets	Local Herbs	Dipping	Spraying	Targets	Local herbs
Effectiveness	2	1	3	4	2	3	1	4
Availability	4	1	3	2	1	3	2	4
Easy to use	1	3	2	4	1	3	2	4
Cost	4	2	3	1	1	2	3	4
Total rank	11	7	11	11	5	11	8	12

Ranking of potential trypanosomiasis control options against sustainability criteria adopted from Catley *et al*., (2011) and needs assessment by community members indicated that use of commercial trypanocides were scored highest followed by use of insecticides while use of targets was ranked last in terms of project sustainability (Table 13).

Table 13: Ranking the potential trypanosomiasis control interventions against sustainability indicators by community members

Indicator	Median rank for possible control intervention			
	Control using Target by community	Spraying	Use of improved trypanocides	Community based dips
Willingness to contribute				
Finance	2	3	1	3
Labour	4	3	1	2
Management	3	2	1	4
Low financial costs to end users	2	3	1	2
Build on existing knowledge and practices	4	3	1	2
Individual can benefit by acting alone	4	2	1	3
Not affected by crises	2	3	1	4
Avoid conflict with neighbours (Effect to the Environment)	1	2	1	3
Overall rank	**23**	**21**	**8**	**21**

4.6 Discussion

This study reports the participatory epidemiology of bovine trypanosomiasis and its control in pastoral cattle in the Maasai steppe. The results have shown that there is high occurrence of bovine trypanosomiasis with low mortality rates. Disease incidence correlated well with the Maasai calendar, comprising of five seasons, which significantly associated with rainfall, land cover changes, disease incidence, abundance of vectors and wild animals. Although Maasai employ different measures to control tsetse vectors and trypanosomiasis, but there are several concerns with their practices, such as dosage, quality and access to drugs procured from the unregulated market.

The results of this study showed spatial variation of disease incidence and mortality rates within and between villages. High trypanosomiasis incidence was observed in less cultivated areas with extensive vegetation as in Lalkati, Meleleki and Ortotoi villages. Villages which were located adjacent to village reserved grazing areas and national parks, for example

Lalkati, Meleleki, Ndepesi and Engailupai also had good vegetation cover and hence high abundance of tsetse flies and wild animals. Conversely, sub-villages located at village centers had more human settlements, low number of cattle and low number of AAT cases. Across villages, Ortukai village had low tsetse abundance and AAT incidences, what was a result of a district-wide campaign for bush clearing aiming at engagement of communities in crop cultivation and deployment of targets for tsetse control. High AAT incidence was observed in Kimotorok village. This observation fits well with the geographical positioning of this village adjacent to Tarangire national park and Mkungunero game reserve, which are protected areas known to be a high refuge of tsetse flies (Malele, 2011).

Temporal patterns of vector abundance and trypanosomiasis cases corresponded with the Maasai calendar of seasons. High abundance of tsetse flies and most incidences of animal trypanosomiasis were scored during *Alamei* (July-October) and *Orekiserati* (November-December) what also relates with changing grazing patterns. Our findings are partly in agreement with studies conducted in Kenya (Catley *et al*., 2012). During the dry season herders graze their livestock in reserved land close to the park boundary. Apparently, disease incidence is also low during dry months of the year and this is due to reduced contact with wild animals who during this period of the year migrate deeper into the park for water and pasture. The Maasai herders move their cattle close to home settlements during wet season, when most vegetation and tsetse habitats are torn down due to crop cultivation and overgrazing. Interestingly, despite intense contact between cattle and wildlife reservoirs, disease incidences were low during the wet season, partly due to low tsetse infestation. These findings further confirm that, tsetse flies are the main vectors of AAT because the presence of tabanids which are responsible for mechanical transmission of a disease (Mahama *et al*., 2004), did not change the disease incidence.

Our study found a considerable variation of vector and disease control methods among surveyed villages. Choice of which control method to adopt seemed to be influenced by cost, experience and availability of infrastructure., For instance use of targets ranked higher in Ortukai than in other villages because of the long experience acquired by livestock keepers in this village. Use of traditional (herbal) medicines scored lowest in all villages and most farmers aluded to alleged ineffectiveness and labour intensity during the preparation of local medicines. Similar observations were previously reported by Grace (Grace, 2003). The Maasai varied frequency of acaricide use on their livestock proportionate with variation of

tsetse flies during the dry and wet seasons. Moreover, the Maasai often treated their sick cattle themselves and with regards to animal trypanosomiasis, local practice included multiple ranges of drug dosage such as, use of trypanocides alone or in combination with oxytetracycline. This may probably caused by unregulated markets (Grace 2003, limited veterinary services (Grace *et al*., 2009), efforts to encounter drug resistance (Geerts *et al*., 2001; Clausen *et al*., 2010).

Sustainability of control activities by local communities is critical for the control of trypanosomiasis. In the present study, we found that herders ranked use of trypanocides, followed by dipping and spraying in that order of preference. Use of targets ranked lowest partly due to lack of training and lack of community-based approaches towards tsetse control (Catley *et al*., 2012). Our study emphasizes the importance of training of Maasai communities, including Masai para-vets on diagnosis, control and treatment of animal and human trypanosomiasis through mass education, practical demonstrations, and farm/field based schooling. Such efforts will contribute to reduction of risk of drug resistance development in pastoral areas (Grace *et al*., 2009) and help forging linkages between researchers and tsetse and trypanosomiasis programmes at the district and National levels (Malele, 2012).

4.7 Conclusion

The findings of this paper show that participatory epidemiology is a useful approach to generate important information crucial for trypanosomiasis control. Our data open ways to enhance community engagement in trypanosomiasis control activities as well as to address policy gaps towards public-private partnership for effective eco-health based disease control. Our results have also opened way for further research targeting reduction of drug resistance, new drug design and optimization of integrated control approaches for vector-borne diseases in the Maasai steppe.

CHAPTER FIVE

Niche modelling approach on spatial predictive mapping of tsetse species habitat suitability in Maasai Steppe of Northern Tanzania.[4]

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Abstract

Introduction

Predicting the current and potential distributions of tsetse species are critical for evaluating vector management options, disease occurrence and emergence and habitat suitability. In particular, there is uncertainty among tsetse field personnel about the magnitude of tsetse presence based on point tsetse catches to another point with no records. This study predicts the spatial distribution of tsetse and hence potential hotspots of trypanosomiasis infestations through niche modelling by bringing together complex interaction of environmental factors and tsetse presence sightings.

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Methodology

In this study, 50 presence sightings were recorded using epsilon traps and 5 predictor variables; cattle density, DEM, NDVI, soil moisture and soil type were used to map presence-only logistic habitat suitability model using Maxent. The output products from maxent were finally processed into ArcGIS 10.4.

Results

The three species show similar spatial distributions of habitat suitability and their models were largely contributed by cattle density. However, soil moisture contributed more in *G. pallidipes* than to soil type while the opposite was found for *G. swynnertoni* and *G. morsitans*. Our models show areas of highest tsetse presence probability were close to protected area or community grazing land with host availability.

Conclusion

This study enables tsetse field personnel, livestock officers and researchers to focus on hotspots of tsetse infestations on more cost effective basis to suppress tsetse populations and informed resource allocation for Trypanosomiasis control programs across the Maasai steppe.

Keywords

Niche modelling, tsetse flies, habitat suitability, Maasai steppe

5.1 Background information

Environmental and climatic factors are strong predictors of vector habitat covariates which influence the fitness disease vectors, hosts and parasite and hence disease incidence and dynamics of the disease. Anthropogenic activities vary in space and changes the ecosystem characteristics hence contribute to habitat heterogeneities hence its spatial epidemiology. Modelling suitable habitat for vector is essential towards identifying potential disease risk areas and its plan for both prevention and control of tsetse and disease to increase resilience of communities against disease burdens.

Trypanosomiasis is a tropical neglected diseases transmitted by tsetse flies reported to infest one-third of African continent. It is caused by various forms of trypanosome species costing both life of the people and livestock up to US$ 7.98 million annually. In Tanzania, only 60% of the grazing lands are free from tsetse infestation , loss of milk and production, death of livestock and addition cost for avoided grazing (Shaw *et al*., 2014). Human and livestock at risk of contracting disease in Tanzania are 4.4 and 4 million respectively (Malele, 2012).

Trypanosomiasis has economic implication spending more on treatment because of trypanosome resistance to livestock keepers due to lack of new drug and drug abuse by livestock communities.

Tanzania is infested by 7 species of tsetse flies and some have endemic and distinct geographical patterns depending on habitat type and characteristics. The species commonly found in forest patches and savannah are *G. brevipalpis* and *Glossina morstans sl*, *G. pallidipes* and *G.swynertoni*, *G. swynertonni* respectively (Hamilton *et al*., 2008). However, the distribution of *G.swynnetoni* limited to northern Tanzania and associated with human trypanosomiasis transmission in protected areas others are widely distributes throughout the country. While the distribution of *G.longipenis* is restricted to north western part of Serengeti, low abundance *G. fuscipes sl*. levels are recorded in prefer riverine habitats of Lake Victoria and Tanganyika. *G. austeni* species still infests the coastal belt and recently eradicated in Zanzibar in recent years through SIT technology (Kasilagila, 2003; Malele, 2012).

Extensive studies conducted are based on entomological survey in Tanzania are resource constrained characterized by one time at a point lacking both temporal trends and wide spatial coverage. Vector habitat modelling provide using environmental layers, offers opportunity for mapping the hotspots of vector infestations and hence set plans for effective disease control and prevention. Several modelling approaches have been used to map tsetse distribution are logistic regressions (Manel *et al*., 1999; Palma *et al*., 1999; Moore and Messina, 2010; Albert *et al*., 2015), Gaussian logistic regressions (Rogers and Randolph, 2006) , discriminant analyses (Rogers and Randolph, 1993; Rogers *et al*., 1996; Hay *et al*., 1997), Ma-halanobis distances (Rogers *et al*., 1996), and artificial neural networks (Manel *et al*., 1999; Hirzel *et al*., 2002). All these methods share largely similar principles.

In recent years the use of ecological niche modeling (ENM) is increasingly applied in mapping the spatial patterns of disease ecology and transmission. The ENM is pivoted on the known species occurrence data across landscapes which are related with other layers to explain the condition necessary for sustainably survival of the organism without immigration. The variations across the layers matching with the occurrence data provide generalization of quantitative picture of ecological niche distribution of a species. In epidemiological studies, ENM provides an inference robust predictions of species spatial distributions or phenomena (Costa and Peterson, 2012; Moore *et al*., 2012) and applied in determination of ecological

conditions for Ebola and Marburg diseases outbreak (Peterson *et al*., 2006). It identifies the potential areas for disease spread or invasion by vectors (Levine *et al*., 2004; Peterson, 2006). It may further be used to identify risk areas to climate land use change (Peterson and Shaw, 2003; Peterson, 2006).

In Maasai Steppe, expansion of land use changes exacerbated by expansion of small scale agriculture and overgrazing and hence grazing livestock along the inter-phase areas. This shift of grazing patterns adjacent to protected area and wild lands where there is high density of wild animals, the reservoir of trypanosomes, increase vulnerability to tsetse infestation and trypanosome infection. This paper hinges on modelling the spatial habitat suitability of tsetse fly species using presence data and predictor layers influencing its distribution. Factors included in this study includes, Cattle density, Normalized Vegetation Difference Index (NDVI), land use type, soil type, digital elevation model (DEM), availability of cattle and wild animals. Specifically tree important questions will be answered; (a) Do tsetse species distribution of differ across the Maasai steppe landscape? (b) What are the potential high risk areas for tsetse infestation and hence infection? (c) What are the relative contributions of tsetse predictor variables in tsetse distribution patterns?

5.2 Methodology

5.2.1 Study area

The study was carried out in four villages adjacent to Tarangire and Manyara ranch protected areas. The climate condition of the area is semi arid land savanna receiving average rainfall ranging from 500mm to 1000mm which concentrate on November to December for the short rain and in March-May for the long rainfall season (Kahurananga and Silkiluwasha, 1997). The temperatures are moderate of higher regions, but on the plains the average temperature is 30°C. The natural vegetation landscape have extensive grassland, bushed grassland and patched of forest in highlands. These vegetation cover are influenced by altitude, rainfall, temperature, basement rock and soils (Miller and Doyle, 2014; Miller, 2015). It is rich with diverse wild animals recognized to have the second largest migration of wild mammals from Serengeti-Maasai Mara migrations (Kahurananga and Silkiluwasha, 1997; Msoffe *et al*., 2010). The main sources of livelihood are centred around livestock grazing keeping and in recent years expansion of crop cultivation primarily maize and beans to supplement food requirements.

5.2.2 Tsetse presence data

Field entomological survey was conducted during dry and wet season of the 2014 an 2015 through geo-referenced baited traps along interface areas in four villages; namely Emboreet, Loiborsireet, Kimotorok and Oltukai. Traps were located in four habitat types namely open acacia woodland-swampy ecotone , open woodland, swampy and riverine between protected area and the home range grazing grounds . Tsetse flies were removed from traps each day for six consecutive days of the dry and wet season sampling month. Three sites were selected from each habitat and three traps being 200 meters apart were placed per site (Pollock, 1982; Kasilagila, 2003). We recorded we recorded number of tsetse's caught, fly species and age. Because the scope of habitat suitability is based on the probability of presence of tsetse flies, the location with tsetse catches were regarded as presence points. The files for each tsetse species were prepared separately and the replicates of presence points per season were removed season using ecological niche modelling tool (ENM tool) software version 1.4.3 (Sindato *et al*., 2016).

5.3 Predictors of presence of tsetse flies

In this paper, the environment layers were selected based on their relevance to tsetse survival (Elith *et al*., 2006; Moore and Messina, 2010). The qualities of data were determined by data providers accessed remotely from various depositories. In predictor layers related to abundance patterns are Normalized Difference Vegetation Index (NDVI), habitat type, density of livestock and wild animals, Land surface temperature, elevation.

5.3.1 Normalized Vegetation Difference Index (NDVI)

Tsetse flies abundance and distribution are influences with vegetation cover. Vegetation index layers form Terra and aqua satellites matching with our sampling period were downloaded from Moderate Resolution Imaging Spectrometer (MODIS), The Earth Resources Observation and Science Centre (EROS) of the United States Geological Survey (http://glovis.usgs.gov/). The imageries had spatial resolution of 250 m and temporal resolution of 16 day composites. The preparation of the NDVI layers considered seasonality, where entire wet and dry season were combined and averaged separately and changed to ASCI file for further analysis using Idris selva version 17.

5.3.2 Land cover type

There is strong relationship between tsetse abundance and the land use cover type. NDVI predict with regard to health of vegetation but land use type my eliminate human farms and trees planted by human which may have similar amount of greenness. The land cover layers of 2014 were accessed from Global land Use Network database though http://www.glcn.org/databases/lc_glcshare_downloads_en.jsp.

5.3.3 Elevation

It has been considered as a parameter of importance in various studies of tsetse abundance in Africa (Rogers *et al*., 2002; Albert *et al*., 2015). Elevation data layers were obtained from the USGS GTOPO30 digital elevation model (DEM).

5.3.4 Soil type and Soil wetness index

Soil moisture content was correlated with tsetse survival as band seven of land sat image correlates with tsetse abundance levels (Kitron *et al*., 1996). Soil wetness index is included our model as proxy of soil moisture content derived from International Research Institute available at ILRI http://192.156.137.110/gis/search.asp. However, the soil type data were also included in the model.

5.3.5 Density of livestock layers

Livestock density layer were created from national sample census obtained from the ministry of Agriculture Livestock and Fisheries (MALF) available at https://livestock.geo-wiki.org

5.3.6 Collinearity analysis modelling Procedure and Assessment

The environmental layers were tested for Collinearity (De Clercq *et al*., 2015; Sindato *et al*., 2016), because highly related variables make it difficulties for the model to determine relative contribution of each parameter on the distribution of tsetse (Phillips, 2008). The criteria used for inclusion of the correlated data were done through running separate niche models for each in Maxcent software with the same model settings. The one which best fit in the was selected based on the area under the curve (AUC).

Logistic regression modelling was used to model the tsetse habitat suitability using MaxEnt software version 3.3.3k (Elith *et al*., 2006). The probability of habitat suitability ranged from 0 to 1, meaning the higher the probability the suitable habitat. The maxcent software is a

machine leaning approach based on maximum entropy which performs better than other methods in modelling species using presence only data (Elith *et al*., 2006; Gormley *et al*., 2011). We used presence only data and 10,000 as our user defined randomly selected points with predictive layers to generate habitat suitability index. Since we assumed the un-sampled area could be suitable for tsetse, maxcent default settings were considered for equal distribution species on the space. Regularization multiplier set was 1 to avoid over fitting of the model through leaving out data points lose to noisy and hence make the model simple (Sindato *et al*., 2016). The model was run in ten replicates and 500 iteration at convergence threshold of 0.00001 with cross validation type. In addition the relative contribution of each variable to the model, and the relationship between each variable generated (Elith *et al*., 2006).

Model performance for presence or absence only is assessed by examining the extent of false positive and false negative errors (Fielding and Bell, 1997). However, with our presence only data, we cannot measure false positive because the Maxent produce fraction of predicted area (FPA) which is the fraction of cells predicted to have suitable habitat (Phillips, 2008). Our model assessed through plotting a receiver characteristic curve which compares true positive (sensitivity) against false positive (specificity) which is equal to 1-sensitivity over entire range of threshold (Fielding and Bell, 1997). Because we dealing with presence only modelling, the area under the curve (AUC) depict the probability of randomly selected site will be ranked highly suitable compared to randomly pseudo-absence site. A model with perfect discrimination must have AUC of 1 while AUC of 0.5 shows the model perform the same as that of random points.

Assessment of the relative contribution of each predictor variable, jack-knife test was employed through backward stepwise approach while eliminating the least in the subsequent model (Sindato *et al*., 2016). The final model which contain optimum combination of predictor variables were assessed using two variables of maxent output; the one with smallest standing deviation (S.D) and with largest AUC (Phillips, 2008). An additional measure of training gain of each variable, the jack-knife of regularized training to describe how much better the maxent distribution fits the presence data compared to uniform distribution (Phillips, 2008).

5.4 Results

The models for *G. morsitans, G. swynnertoni, G. pallidipes* were independently evaluated against predictor variables namely livestock density, Normalized Difference Vegetation Index, Digital Elevation Model, soil type and soil moisture. All models had consistently higher AUC (0.939 - 0.974) and lower standard deviation (0.011 - 0.025) (Table 14). The best models for each of three species had four variables; soil type variable was dropped models for and *G. pallidipes* (Table 14b) where soil moisture was dropped from *G. morsitans* and *G. swynnertoni* model (Tables 14a and 14c).

Table 14: Percentage contribution of predictor variables in five ecological niche models that describe habitat suitability for spatial distribution a) *Glossina morsitans* b) *Glossina pallidipes* and c) *Glossina swynnertoni* in the Maasai steppe Tanzania. The number in each model (i.e. 1 to 5) indicates the number of predictor variables that model contained.

a)

Predictors	Models				
	5	4*	3	2	1
Cattle density	71.0	79.2	84.9	83.5	100.0
Soil type	11.5	7.1	2.9		
DEM	10.5	10.7	13.1	16.5	
NDVI	4.9	3			
Soil moisture	2.2				
AUC	0.965	0.969	0.959	0.959	0.951
S.D	0.016	0.011	0.017	0.017	0.017

b)

Predictors	Models				
	5	4*	3	2	1
Cattle density	47.7	63.2	70.2	83.5	100.0
DEM	22.1	22.2	26.9	16.5	
Soil moisture	21.9	4.4	2.9		
NDVI	4.5	10.2			
Soil type	3.7				
AUC	0.969	0.974	0.966	0.966	0.939
S.D	0.025	0.013	0.011	0.001	0.020

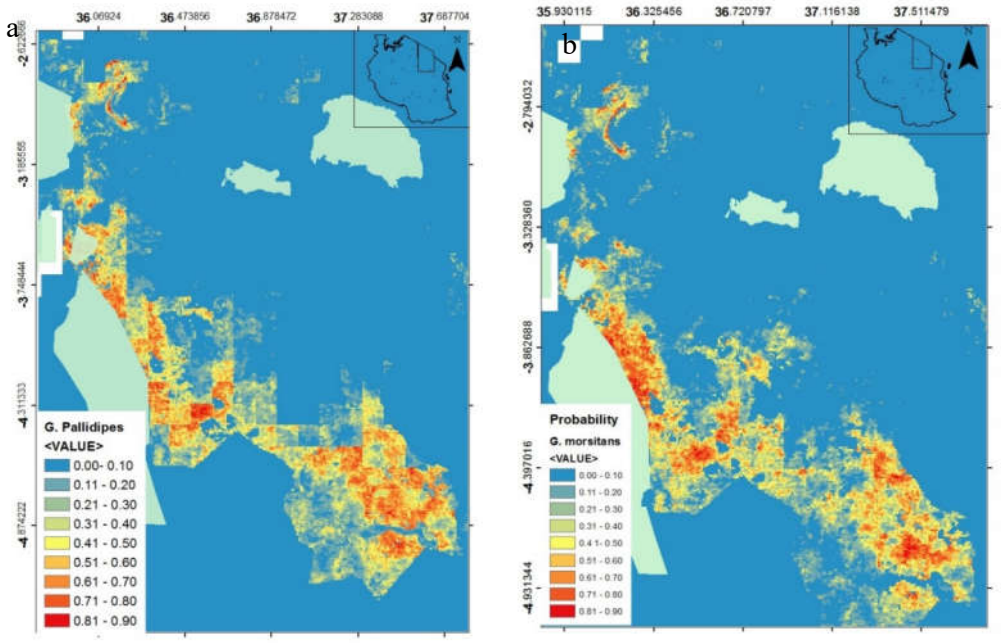
c)

Predictors	Models				
	5	4*	3	2	1
Cattle density	64.0	64.1	65.9	75.1	100.0
DEM	9.4	11.6	11.3		
Soil moisture	2.1				
NDVI	2.3	2.1			
Soil type	22.2	22.3	22.8	24.9	
AUC	0.971	0.971	0.966	0.960	0.958
S.D	0.015	0.015	0.018	0.024	0.036

*Best fit ecological niche model. Key: AUC: area under the curve, S.D: Standard deviation, NDVI: Normalized Difference Vegetation Index and DEM: Digital elevation model.

5.4.1 Distribution maps for tsetse species

The maps for three species show the similar probability of presence patterns in relation to the four factors of the best model (Fig. 13). Considering the permutation of importance for all factors still, cattle density had contributions in the mapping of all tsetse fly species. Variation observed in other factors, especially the importance of NDVI and soil type where *Glossina swynnertoni* differed with *Glossina morsitans* which accounted for more 69.9%, DEM scored 17.7% soil type 10.9% while NDVI 1.5 was the least for while the later had cattle density 79.2%, NDVI 10.7%, DEM 7.1% and soil type 3%. The *Glossina pallidipes* had a permutation of importance similar to the variable contribution.



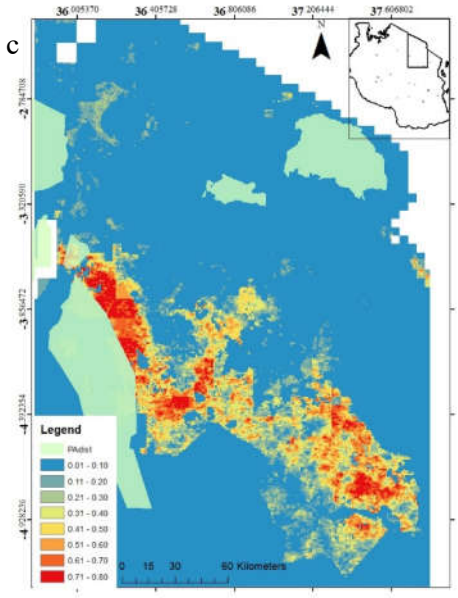
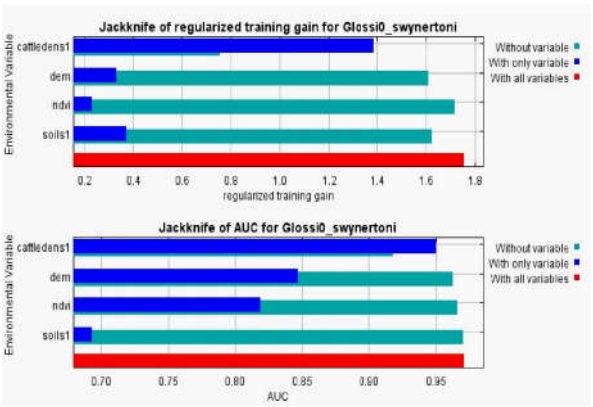
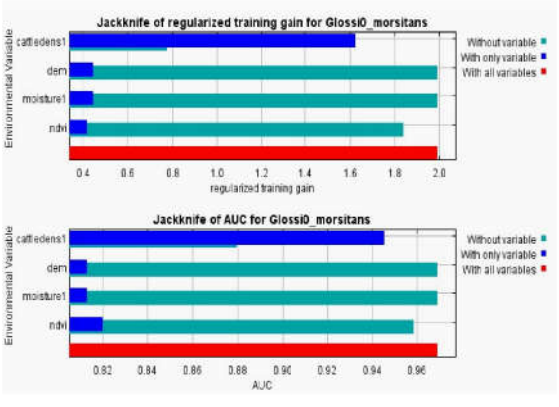


Fig. 13: Presence probability distribution of three tsetse species, a) *G. swynnertoni*, b) *G. morsitans* and c) *G. pallidipes* in the Maasai steppe, Northern Tanzania.

5.4.2 Jackknife of regularized training gain for tsetse presence habitat suitability

The results of the jackknife regularized training gain indicated that the predictor variable with the highest gain, when used in isolation, was livestock density, which therefore appears to have the most useful information by itself (Fig. 14). The environmental variable that decreases the gain the most when it is omitted is cattle density, which therefore appears to have the most information that isn't present in the other variables. Values shown are averages over replicate runs. Considering Jacknife of area under the curve (AUC) for DEM and NDVI variables increasingly gained for *G. swynnertoni* and *G. morsitans* (Fig. 14a and 14b).





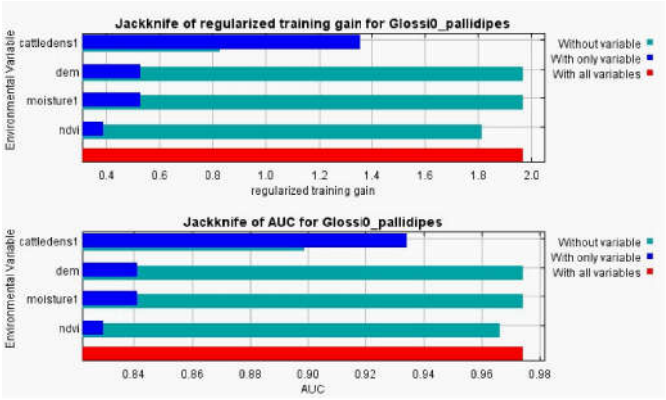


Fig. 14: Jacknife of regularised training gain and area under the curve (AUC) for three tsetse species; a) G. *swynnertoni* b) *G. morsitans* and c) *G.pallidipes.*

*Glossi0_pallidipes stands for *G. pallidipes*, Glossi0_swynertoni stands for *G. swynnertoni* and Glossi0_morsitans stands for *G. morsitans.*

On the other hand jackknife of AUC for soil moisture variable had *G. pallidipes* had higher gain after Digital elevation model and then NDVI (Fig. 14c). However the detailed logistic response curves of each variable used to map individual species of tsetse is depicted in relation to habitat suitability (Fig. 15). Apart from cattle density habitat suitability for DEM ranges 1200 to 200 with small standard deviation. NDVI associates with highest probability of tsetse presence at While soil type 16 is associated with the presence of *G. swynnertoni* and *G. morsitans*, suitable habitats of G. pallidipes are associated with higher soil moisture (Fig. 15a).

5.4.3 Response curves for habitat suitability of tsetse presence

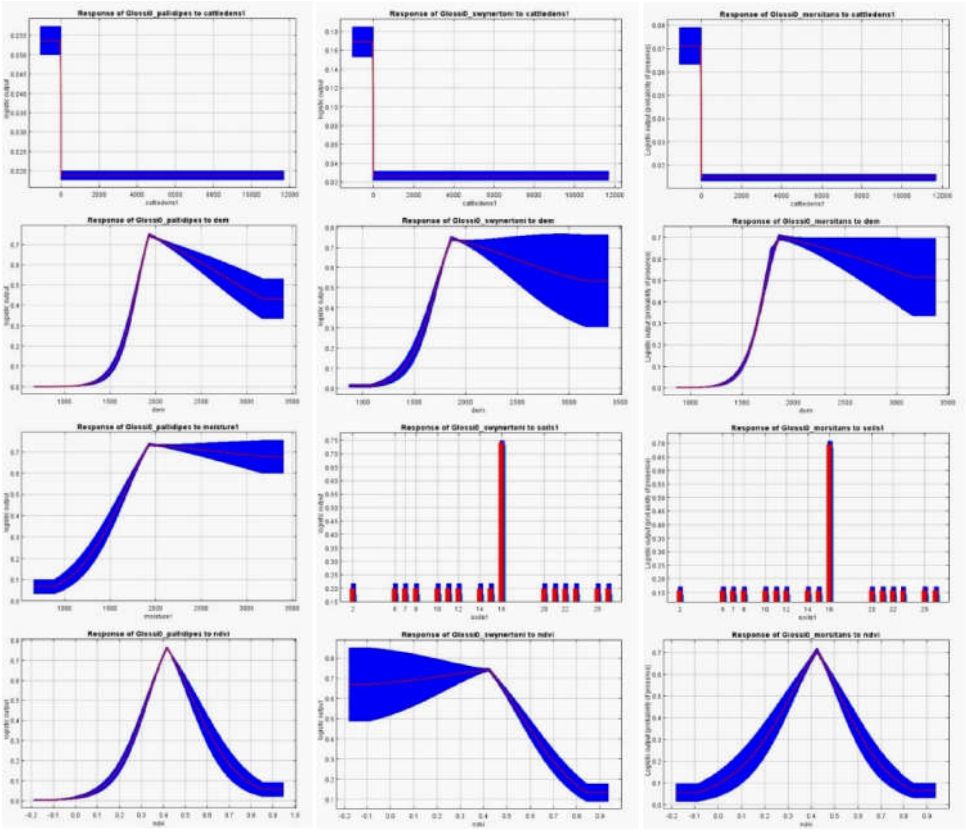


Fig. 15: Presence probability of tsetse presence all predictor variables for three tsetse species; a) *G. pallidipes* b) *G. swynnertoni* c) *G. morsitans*. The red curved present mean response of all 10 replicates of the model, while blue indicates the standard deviation of the mean.

*1. Glossi0_pallidipes stands for *G. pallidipes*, Glossi0_swynertoni stands for *G. swynnertoni* and Glossi0_morsitans stands for *G. morsitans*.

2. Cattle_dens1 stands for cattle density, dem stands for DEM, moisture1 stands for soil moisture, soils1 stand for soil type and ndvi stand for NDVI.

3. Soil types: 1= Haplic Ferrasols, 6 = Eutric Fluvisols, 7 = Chromic cambosol, 8 = Haplic Ferrasols, 10 = Fluvic Histosols, 12 Eutric vertisols, 14= Eutric Planasols, 16 = Luvic Phaeozems, 20 = Ferric Cambisol, 21 Eutric Vertisols, 22 = Chomic cambisols 25 = Haplic Ferrasols and 26 = Rodic Ferrasols.

5.5 Discussion

Mapping of tsetse flies is vital towards addressing eco-health challenges due to increased interaction between human livestock and wild animals. Quantifying the distribution and abundance of tsetse has been a problem of public health consequences impedes cost effective means of controlling the disease. Suppression of tsetse population needs an estimation of spatial risk assessment for effective intervention trypanosomiasis programs to increase livestock production.

This research study has shown the similar patterns contribution of predictor variables for all tsetse species. Our findings suggest that four predictor variables (livestock density, Digital elevation model, NDVI and soil type) in the model resulted in the best model fit. The resulting habitat suitability map of our model suggests that areas of high probability of presence are close to protected area and community grazing lands. The density of cattle largely contribute to the model probably because of tsetse feeding depends blood on hosts while other studies indicate cattle preference. The logistic model shows show curves show similar patterns for cattle density for all three species which could probably caused by lower resolution of the layer and which had a more combined large portion of the training presence data with the same density. Similar findings about the role of cattle density is found to have significant contribution in vector and disease occurrence distribution (Rogers and Randolph, 1993; Sindato *et al*., 2016).

Furthermore, the response curves for DEM shows increasingly probability of tsetse presence at arrange of 1300 m to 1900 m and gradually decrease above 2000 m. These changes may, in turn, be caused by a relative degree of hotness or coldness changes or other parameters affected by elevation. The degree of hotness in turn which affect puparial development and adult tsetse survival and hence tsetse presence. However, at higher altitudes, there is a higher standard deviation in tsetse presence probabilities for in the Maasai steppe which may be contributed by lower temperatures and scarcity of hosts. On the other hand, soil moisture influence the presence *G. pallidipes* while soil type is associated more with the presence of *G. swynnertoni* and *G. morsitans.* High probabilities in of *G. pallidipes* correspond with the seasonal swampy area and riverine areas. This finding is similar to other studies (Kasilagila, 2003; Sindato *et al*., 2016). Interestingly, soil type may play a role tsetse development its larva at third instars stage is buried in the soil. Therefore, soil conditions factors such as soil compactness, wetness or dryness, ground heat and trampling effect by animals may have probably played a big role with Luvic Phaeozems than other soil types. In addition, tsetse infested area are found in Simanjiro district with more than 80 percent of the luvic phaezems. Previous studies shows that properties of soil covering the Simanjiro plains are dark red sandy clay loam in the grasslands to black clay in the seasonal water logged areas (Miller and Doyle, 2014).

NDVI closely associated with tsetse distribution and higher probabilities of tsetse presence are observed at 4.2. This may be due to the fact that tsetse is well adapted to savana in

grazing areas where vegetation cover is not intact. This provides suitable habitat for tsetse because tsetse suck blood in from hosts and vegetation cover provides cover as well as microclimate conditions for tsetse survival. Other studies with suggesting the usefulness of NDVI as an important factor in predicting spatial patterns of tsetse distribution (De La Rocque *et al*., 2004; Rogers, 2006; Moore and Messina, 2010). However, a large area of the Maasai steppe with very low probability of occurrence may be associated with low NDVI as a result of the extensive expansion of agriculture and overgrazing.

Our work constrained by resolution differences among the predictor layers and unvailability of wild animals because that our study was conducted outside the protected areas where wild animal surveys are rare. This work still provides fundamental geospatial information of important for tsetse distribution and its hotspots in a heterogeneous composition of the biophysical components across the landscape. In addition, provide landscape epidemiological information vital for resource allocation for cost-effective means of area wide intervation strategies of vectors and guide grazing patterns to avoid infestation and trypanosomiasis infection.

5.6 Conclusion

Geospatial modelling of vector and disease data is a powerful tool towards understanding of the relative contribution of non-uniform multiple factors across space necessary for landscape epidemiology. This study enables tsetse field personnel, livestock officers and researchers to focus on hotspots of tsetse infestations and its spatial epidemiological consequences. Furthermore it shows the need of one-health approaches in allocating optimal resource allocation for Trypanosomiasis control programs across the Maasai steppe.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION

6.1 General discussion

This thesis focused on assessment of the impact of land cover on spatial and temporal distribution of tsetse flies, and its implications for movement of livestock and disease control strategies. Furthermore, the infection rates of trypanosome species were also assessed in relation to habitat, tsetse species and livestock grazing patterns in interface areas between humans, livestock and wild animals of the Maasai Steppe.

In this work, an integrative approach was used to unravel the relationship between habitat characteristics and tsetse relative abundance in both intensive monitoring sites (trapped every month for 15 months) and spatially-extensive sites (trapped once in the wet and dry seasons) across selected villages in the Maasai Steppe, chosen for their proximity to the wildlife interface. We found strong relationships between tsetse species and habitat in surveyed villages, and between certain host and tsetse species. We also showed that current grazing patterns practiced by herders in all village, increase risk of infection, particularly in the dry months. We also found that livestock keepers in the area had variation of knowledge on control and grazing patterns in relation to exposure to tsetse infestations and trypanosomiasis infections. In addition the mapping of potential distribution of tsetse correlate with suitable habitats close to protected areas and none in areas dominated by agricultural activities and high altitude areas which do not support the survival of tsetse.

In the intensive monitoring sites in Emboreet village, *G. swynnertoni* was the most dominant fly in ecotone habitats and *G. pallidipes* was dominant in riverine habitat. *G. morsitans* was generally found in all habitats. Other studies (Brightwell *et al*., 1992; Bossche and Deken, 2002) show similar findings with variation in seasonality where the higher abundance was recorded during wet season (Ndengwa *et al*., 2001). In this study, higher abundance of tsetse in the ecotone is likely associated with higher wildlife and cattle populations also found near these habitats during wet and dry season. The ecotone habitat was characterized with The grazing seasons and wildlife migration patterns makes the habitats along the interface filled with plenty and diverse moving hosts. This is probably the reason why the *G. swynnertoni* was the most abundant as tend to follows the moving hosts. Conversely, *G. pallidipes* was the most abundant tsetse species in all habitats in the other villages. This is probably because the health of the vegetation in the extensive study sites, as assessed by NDVI, appeared greater in

all habitats. Lower NDVI values were recorded in Emboreet, while higher NDVI was observed across all habitats of Loiborsireet, with higher ground cover. The dominance of *G. pallidipes* in all habitats in Oltukai village, may be related to the dense tall grass cover even in open woodland habitats in the Manyara Ranch area, where livestock grazing is controlled throughout much of the year. Whereas the communally owned grazing land outside the ranch is characterized by overgrazing, expansion of agriculture and bare, rocky soils which may restrict tsetse distribution.

However, groundcover had no significant effect on tsetse abundance. The ecotone habitat, swampy areas and open woodland dominated by *Acacia* species had higher abundance of *G. swynnertoni* while the riverine habitats had higher densities of *G. pallidipes*. This may suggest that, there is little or no overlap of the niches of *G. pallidipes* and *G. swynnertoni*. The suitable vegetation cover provide refuge for and pupating environment for tsetse. However, during dry season the distribution of tsetse is aggregated following the patchy vegetation cover which is suitable for grazing and expose cattle to higher risk of contraction the infection. This implies that pastoralists should increase frequency of control measures when grazing in contaminated habitats. Because of higher number of cattle in the villages, livestock keepers have to no choice of avoid highly infested habitats depending on the seasons.

Generally, the study showed that NDVI had negative and positive relationships with *G. swynnertoni* and *G. pallidipes* abundance, respectively. In other words, *G. swynnertoni* was more abundant in drier areas, whereas *G. pallidipes* was more abundant in greener areas with higher NDVI values. In addition to NDVI, distance from the park was a strong predictor of tsetse distribution. In particular, tsetse abundance in all species and all habitats was higher close to park boundaries in the intensively-sampled area. This is likely driven by the year-round availability of resident wildlife host species in these areas, and is backed up by our data on host availability, which shows impala, dikdik, giraffe and others present in every month in which we sampled.

Taking blood meals from multiple vertebrate hosts is important for maintaining the life and pseudo-pregnancy stage of tsetse flies, and hence determines the vital rates (Leak, 1999). Previous studies have shown the hosts based blood meals and ground survey but lack long term monitoring for the host presence (Brigtwell *et al*., 1992; Bett *et al.,* 2008; Muturi *et al*., 2011) however, this study shows the relative abundance of the hosts and whether their

variation in months and seasons of the year have significant contribution in abundance and distribution of tsetse flies.

The extensive surveys showed the abundance of tsetse flies does not drive their infection rates. For instance, although *G. pallidipes* was the most abundant species by three fold compared to the *G. swynnertoni*, *G. swynnertoni* had the highest trypanosome infection rate. The observed pattern may be attributed to lower relative abundance of hosts in the habitats where *G. pallidipes* catches were highest, indicating they may be exposed to fewer infected hosts. In addition, the pattern may be influenced by variation in transmission of trypanosome species and vector capacities of various species (Haines, 2013; Reinfenberg *et al.*,1997).

The molecular analysis shows the *Trypanosoma vivax* drives infection rates as it was detected to be the most common species circulating in tsetse. This may be caused by short life span of tsetse and hence allows its development as it may develop through proboscis in shorter time compared to *T. congolence* and *T. brucei* which develop in the mid-gut and mature in salivary gland (Asksoy, 2003; Molynex and Stiles, 1991) and consequently become infective after longer time than the average tsetse longevity established in the previous chapter. Other factors which may account for differences in infection rates in tsetse flies include the genotype of trypanosoma spp. and sex, where mid-gut development in male tsetse mature significantly more than female tsetse.

In spite of lower infection rates in Oltukai village, compared to the other villages, livestock and people may still be at risk of contracting trypanosome infections depending on the frequency and time spent grazing in highly infected habitats. Additionally, even low infection rates pose a threat, as one infected fly is capably of spreading the infection to multiple hosts. This shows the significance of extending effort on vector control even to areas with low tsetse densities and infection rates because they may potentially have high epidemiological significance.

The influence of human activities was observed as no tsetse flies were caught from traps set near settlements and agricultural lands. This was observed in Kimotorok village where farms and settlements start immediately after the park boundary and so few tsetse were caught that it was excluded from the analyses. This was likewise the case for traps that were set near settlements and crop fields in Emboreet villages. This is likely due to both removal of woody vegetation that constitutes suitable habitat for tsetse (no flies were caught in traps set in open

grassland), and exclusion of wildlife hosts in areas with higher levels of human disturbance. In fact, Malele (2011) shows that protected areas will remain as the hot spots for trypanosome infections in spite of decreases in tsetse in community grazing areas as a result of agricultural practices.

In Chapter 3 participatory approaches were used to elucidate the Maasai understanding of bovine trypanosomiasis, grazing patterns, vectors and their control strategies in relation to land use changes and seasons of the year. Trypanosomiasis was the disease of greatest importance to the study population, as it was reported to have higher incidences in cattle than other diseases such as East Coast Fever, which are controlled by vaccination. However, trypanosomiasis was reported to have the lowest mortality, because of intensive use of drugs to treat the disease.

The seasons of the year play a part in vector abundance and incidences of the disease. When integrated with land cover changes and grazing patterns may provide the best alternative for integrated disease control. This is because the current strategies and methods are constrained by lack of dipping infrastructure and specialized knowledge, as evidenced by the practice of treating animals for multiple diseases, which could lead to drug resistance. Furthermore, the livestock keepers demonstrated good understanding of vector control options and rational of their preferred choice. However, it was also found that the choice and ranking preferred vector control strategies was influenced by the respondents' experience, availability of infrastructure. This shows need for training and farmer field demonstrations in control options to enhance the understanding and reduce the gap of knowledge. The findings of this study are similar to others in the literature conducted in Kenya, Sudan and Ethiopia (Catley *et al*., 2004; Grace 2003).

Spatial predictive mapping of tsetse using five environmental predictor variables (list the predictors here) was used to determine potential distribution *Glossina morsitans*, *Glossina pallidipes* and *Glossina swynnertoni* in the Maasai steppe. The spatial predictive maps generated with species distribution modeling software, Maxent, are useful for identification of hotspots of tsetse and trypanosomiasis infestations. The species distribution model combines all predictor variables with no locations of occurrence to produce habitat suitability models. Of the environmental predictor variables used in the model, cattle density was the most important variable in predicting the distribution of all three tsetse species, accounting for more than 69% of the variation for all species. The other variables, elevation, soil type

and moisture, and NDVI were all also included in the best performing models, and were likewise important predictors of probability of presence of the tsetse species. Cattle density may be such an important predictor in the models, because regardless of other aspects of habitat, tsetse require blood meals to survive and reproduce. Due to limitations in the available data, other predictor variables that may be important to tsetse distribution were not able to be tested. In particular the lack of spatially-extensive wildlife host abundance data outside very few surveys of populations in protected areas made it impossible to assess the importance of wildlife distribution on tsetse presence across the entire Steppe. However, it seems likely that the increasing consolidation of livestock grazing in wildlife habitats near protected areas may mean that there is a relationship between livestock and wildlife abundance, particularly in areas from which are occurrence data come. In this way, the importance of livestock density in our models, may actually be linked to wildlife density in area close to the park.

Similar modeling approaches have been used in Kenya (Messina and Moore, 2010), and for Africa (Cechi, 2008) to predict the spatial occurrence of different vector species. However, there are constraints of using large-scale maps of countries or continents to predict aspects of vector presence, which may be better measured by finer-scale, more local variables.

Predicting the current and potential distributions of tsetse species is critical for evaluating vector management options, disease occurrence and emergence and habitat suitability. This study predicts the spatial distribution of tsetse and hence potential hotspots of trypanosomiasis infestations through niche modelling by bringing together complex interacting environmental factors and presence records.

6.2 Limitations of the study

This study was based on 15 months of monitoring the abundance and distribution of tsetse flies and the variation between seasons was limited to project time range. In spite the fact that the extensive spatial coverage of tsetse sampling was limited by skilled labor and resources to the sampled area, data was enough to model the whole of the Maasai Steppe and generate the relative spatial probability of tsetse distribution and hence potential risk of trypanosomiasis infection.

6.3 Conclusion

Trypanosomiasis is a neglected disease of poverty and its burden is increased by inceases in interactions between human, livestock and wild animals, which in turn are exacerbated by changes of land cover. In this study we have demonstrated that NDVI, habitat type, abundance of hosts, density of wild animals, and distance from the protected areas are strong predictors of tsetse abundance and distribution. The abundance of tsetse fly species does not drive prevalence of trypanosome species in tsetse flies, however higher prevalence levels were recorded close to the park boundary. Variation in prevalence of trypanosome species was strongly influenced by the age of the fly and development stages of the parasite in tsetse, which is the likely reason that *Trypanosoma vivax* was the dominant species. Cattle grazing patterns and migration of wild animals are related to the abundance of tsetse flies and infection rates of tsetse flies, and hence their potential transmission of the disease. Furthermore, the findings of this study clearly show the need for proper treatment and vector control, through a ecohealth approach that integrates land use planning to reduce risk of Maasai people and their livestock from contracting trypanosomiasis.

6.4 Recommendations

The study recommends that communities and district councils use integrated vector control methods in the village grazing fields adjacent to protected areas, including and suppression of tsetse populations along the park boundaries. Also, the study recommends avoidance of infected habitats in seasons with high infection or improve vector control frequency or prophylactic measures when grazing in infected habitats. The improvement of infrastructure such as dips, water dams, spray and drug storage facilitates for effective tsetse control of tsetse flies is likewise recommended. Training the Maasai herders and paravets in Maasai communities on proper treatment and proper storage, transport and delivery of medicines and drugs to maintain their quality will also help reduce negative impacts of the disease. We also recommend optimal land use planning that will allow the adjustment or maintenance of the grazing systems that reduce pushing livestock to the highly tsetse infested areas in wildlife and livestock inter-phase areas adjacent to the park boundary.

We recommend further research on prediction of land use changes on tsetse fly distribution across the Maasai Steppe landscape, to guide future intervention. Further, we recommend more comprehensive survey to acquire tsetse ground based data to make more rigorous predictive models incorporating factors shown to be important predictors at the site level.

Since remote sensing data do not replace field collected data, we recommend their integration so as to have cost effective entomological survey and disease management.

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APPENDICES

Appendix 1: Linear mixed effect models between the tsetse species abundance, as dependent variable, and habitat categories as independent variables and site is included as a random factor and the standard deviation (S.D). Abundance of tsetse species in other habitats are compared to Ecotone habitat.

	Glossina Pallidipes			*Glossina morsitans*			*Glossina swynnertoni*		
	Coeff±SE	DF	P	Coeff.±SE	DF	P	Coeff.±SE	DF	P
Intercept	0.02±0.03	1837	0.4710	1.52±0.07	1834	0e+00	0.54±0.07	1834	0e+00
Open woodland	0.01±0.03	4	0.8061	-1.39±0.09	4	0e+00	-1.41±0.09	4	0e-04
Riverine	0.07±0.03	4	0.1143	-1.47±0.1	4	0e+00	-1.51±0.1	4	0e-04
Swampy	-0.03±0.04	4	0.9557	-1.21±0.12	4	2e-04	-1.2±0.12	4	4e-04
Random effect S.D: Site	0.02			0.017			0.07		
AIC	-452.08			1747.39			2619.68		

Appendix 2: Linear mixed model showing the variation in NDVI among habitat and season; site is included as a random factor and the standard deviation (S.D)

Habitat and Season	NDVI		
	Coeff.±SE	DF	P
Ecotone	0.248±0.02	4	0.0005
Open woodland	0.243±0.01	4	0.0000
Riverine	0.249±0.02	4	0.0001
Swampy	0.288±0.02	4	0.0003
Season	0.260±0.01	1834	0.0000
Random effect: Site	0.02		
AIC	-1842.45		

Appendix 3: Linear mixed effect models between the tsetse abundance (log x+1) by species, as dependent variables, and NDVI as an independent variable; the site is included as a random factor.

	G.pallidipes		***G.morsitans***		***G.swynnertoni***	
	Coeff.±SE	P	Coeff.±SE	P	Coeff.±SE	P
Intercept	0.167±0.126	0.0002	0.061±0.043	0.1597	0.134±0.054	0.0140
NDVI	5E-6±1.1E-5	0.0149	6.8E-6±7E-6	0.3456	-1.2E-5±6.1E-6	0.0378
DF	763		763		763	
Random effect: site	0.329		0.097		0.151	
AIC	1713.37		418.74		165.11	

Appendix 4: Relationships between tsetse species abundance as a response variable and host species as explanatory variables. Only significant results are presented in this table.

	G.pallidipes		*G.morsitans*		*G.swynnertoni*	
	Coeff.±SE	P	Coeff.±SE	P	Coeff.±SE	P
Intercept	0.35±0.08	0.0000	0.045± 0.031	0.1533	0.04±0.05	0.3485
Cattle	0.02±0.001	0.0091	0.001±0.00035	0.0002	0.001±0.00003	0.0012
Impala	-0.005±0.002	0.0293				
Dikdik	-0.08±0.035	0.0226	0.049± 0.016	0.025	0.07±0.013	0.0000
Elephant	-0.007±0.002	0.0004				
Wildbeast			0.0008±0.003	0.0296		
Random effect: Site	0.229			0.088	0.04	
DF	744			745	746	
AIC	1562.4			386.6	147.4	

5: Social-Ecological Perspective on land cover dynamics on grazing patterns and tsetse abundance and its implication to Trypanosomiasis control.

A. Demographic Characteristics

1.Name_respondent	2. Age	3. Sex	3. Village	4. Education	5. Location	6. Occupation

Code: **Age**: 1= Korianga,2= *Irrikidotu* 3=*Nyangusi* 4=*Seuri* 5=*Ilindareto* **Sex**: 1=Male 2=Female**; Education**: 1 = Never went to school; 2 = Primary education; 3 = Secondary education; 4 = Certificate education**; Occupation**:1= Livestock keeper; 2=peaseant;3=Mixed farmer; 4=gatekeeper; 5=Entrepreneur

B. Land use land cover and trypanosomiasis

7. Would you tell me, if you are cultivating crops? i) Yes [ ] ii) No [ ]

8. If Yes, Please would you mention top three crops cultivated in your farms?

i) [ ] ii) [ ] iii) [ ] Codes 1 = Maize; 2 = Beans; 3 = Sunflower 4 = Other crops

Please specify the level of engagement in crop cultivation by the following groups in your village

9. Maasai (i) 80% and above) [ ] (ii) 50 to <80% [ ] (iii) 20 to <50% [ ] (iv) <20% [ ]

10 Waarusha (i) 80% and above) [ ] (ii) 50 to <80% [ ] (iii) 20 to <50% [ ] (iv) <20% [ ]

11. Wairaki (i) 80% and above) [ ] (ii) 50 to <80% [ ] (iii) 20 to <50% [ ] (iv) <20% [ ]

12. Wachaga (i) 80% and above) [ ] (ii) 50 to <80% [ ] (iii) 20 to <50% [ ] (iv) <20% [ ]

13. Others (i) 80% and above) [ ] (ii) 50 to <80% [ ] (iii) 20 to <50% [ ] (iv) <20% [ ]

14.What is the price for renting a piece of land for the following crops

i)Maize.....ii) Beans.....iii) Sunflower.....

15. Would you specify , the level of land ownership your village by the following groups?

16. Maasai (i) 80% and above) [ ] (ii) 50 to <80% [ ] (iii) 20 to <50% [ ] (iv) <20% [ ]

17. Waarusha (i) 80% and above) [ ] (ii) 50 to <80% [ ] (iii) 20 to <50% [ ] (iv) <20% [ ]

18. Wairaki (i) 80% and above) [ ] (ii) 50 to <80% [ ] (iii) 20 to <50% [ ] (iv) <20% [ ]

19. Wachaga (i) 80% and above) [ ] (ii) 50 to <80% [ ] (iii) 20 to <50% [ ] (iv) <20% [ ]

20. Others (i) 80% and above) [ ] (ii) 50 to <80% [ ] (iii) 20 to <50% [ ] (iv) <20% [ ]

21. Is there any land use regulation on communal grazing land areas? 1) Yes [ ] 2) No [ ]

22. If yes is it (i)Very good [ ] (ii) Good [ ] (iii) Bad [ ] (iv) Very bad [ ]

23. At present do you practice fallowing? (i) Yes [ ] (ii) No [ ]

24. If yes how many years.....

25. Please, would tell how you perceive the expansion of agriculture? (i) Very fast [ ] (ii) Fast [ ] (iii) Slow [ ](iv) Normal[ ]

26.What are the causes of the agriculture expansion?

(i) Food security [ ] (ii) Additional source of income [ ] (iii) Way of protecting the land ownership [ ] (iv) Increasing the number of people v) Any other mention.....[ ]

27. If all the reasons you mentioned in the previous question are important, please rank them from the most important to the least by assigning number 1,2,3,4

(i) Food security [ ] (ii) Additional source of income [ ] (iii) Way of protecting the land ownership [ ] (iv) Increasing number of people (v) Others mention..... [ ]

28.Would you mention if you associate presence of tsetse with the following type of trees

(i) Accacia (ii) Comniphora (iii) Others mention.....

29. In the following habitats, rank the presence of tsetse abundance from highest assigned 1,2.....to the lowest in the brackets

(i) Woodland [ ] (ii) Grass land [ ] (iii) Wooded grassland (iv) Marsh areas [ ] (v) Riverine [ ]

30.Would you tell me, to what extent the expansion of agriculture is reducing the areas for grazing cattle

(i) Very fast [ ] (ii) Fast [ ] (iii) Slow [ ] (iv) Very slow [ ] (v) No impact [ ]

31. Would you tell me, how the expansion of agriculture is the important factor that reduce areas for tsetse

(i) More important [ ] (ii) Important [ ] (iii) less important [ ] (iv) Not important [ ]

32.Would you know anything about land use planning in your village (i) Yes[ ] (ii) No [ ]

33. Would you specify the level of villagers involvement in land use planning

(i) Actively involved in decision making [ ] (ii) little involvement [ ] (iii) Informed about the plan [ ] (iv) any other mention.....

34. In your own opinion, do you think land use plan for pastoral activities considered the distribution of tsetse and trypanosomiasis? i) Yes [ ] ii) No [ ]

35. If Yes, how pastoralists being are considered

(i) Given areas with low tsetse challenge [ ] (ii) Control tsetse infested areas using targets [ ] (iii) Creating Dipping system to reduce tsetse [ ] (iv) any other mention..... [ ]

Please specify the level of agreement or disagreement with the following statement about land use

36. Crop cultivation contributes to destruction of tsetse habitats and infection reduction

(i) Strongly agree [ ] (ii) Agree [ ] (iii) Neutral [ ] (iv) Disagree [ ] (v) Strongly disagree [ ]

37. Extensive grazing of sprayed cattle towards in the wild lands reduce tsetse abundance and trypanosome infection

(i) Strongly agree [ ] (ii) Agree [ ] (iii) Neutral [ ] (iv) Disagree [ ] (v) Strongly disagree [ ]

38. Grazing towards park boundary increase high risk to contracting trypanosomiasis

(i) Strongly agree [ ] (ii) Agree [ ] (iii) Neutral [ ] (iv) Disagree [ ] (v) Strongly disagree [ ]

39. Conserved land for wild animals have both high tsetse abundance and trypanosome infection

(i) Strongly agree [ ] (ii) Agree [ ] (iii) Neutral [ ] (iv) Disagree [ ] (v) Strongly disagree [ ]

40. Residential areas rarely have tsetse flies and trypanosome infection

(i) Strongly agree [ ] (ii) Agree [ ] (iii) Neutral [ ] (iv) Disagree [ ] (v) Strongly disagree [ ]

B. Migration/Grazing Patterns and trypanosomiasis

41. Do you shift your animals for grazing to different places in seasons of the year?

i) Yes [ ] ii) No [ ]

42. If yes, would you please rank the following are reasons for migration from 1 to 5 where 1 is assigned to the most important reason to 5 assigned to the least important factor

(i) Scarcity of folder [ ] (ii) Water [ ] (iii) Scarcity of both water and folder [ ] (iv) Disease [ ] (v) other .....[ ]

43. When do you start migration (tick)

May	June	July	August	October

44. Where do you stay with your animals.....

45. Do you know some livestock keepers who stay away (lonjo) throughout the year

i) Yes [ ] ii) No [ ]

46. If yes why .....

47. Are there places previously had known to have high tsetse but now have low abundance

.....

48. Would you tell the reason, please choose the options below

(i) Controlled by targets [ ] (ii) Heavily grazed [ ] (iii) Cultivated [ ] (iv) Grazing animals sprayed with insecticides [ ] (v) Cutting down of trees [ ] (vi) if any mention.....[ ]

49. Would you please tell me a place in your village, currently still abandoned because of high tsetse challenge.....

50. Would you tell, what you have done this year to prevent your cattle form contracting a disease? (i) Spraying [ ] ii) Dipping [ ] iii) Prophylactic measure[ ] iv) Do Nothing [ ]

51. What was the frequency during the wet season

(i) Once per month [ ] (ii) Twice per month [ ] (iii) At least once per two months [ ] iv) Any other.....[ ]

52. What was the frequency in the dry season (i) Once per month [ ] (ii) twice per month [ ] (iii) At least once per two months [ ] (iv) Any other..... [ ]

53. What is the product(s) of drug do you normally use

.....

54. How did you prepare the solution/ dose.....

55. Where did you buy the product (i) Livestock officer [ ] (ii) Order from town [ ] (iii) Phamacy [ ] (iv) Mnada [ ] (v) Others.....[ ]

56. When do you get back near to residential grazing land (Please tick the answer)

December	January	February	March	April

57. Where do you graze your animals.....

58. What is your migration route?

First stop	Second step	Third step

59. Would you please mention the grazing routes which are no longer in use because of expansion of crop cultivation.....

60. Would you tell me, which season of the year cattle have high incidences of contracting a disease i) Dry season [ ] ii) Wet season [ ]

61. Do you know the reasons for high incidences in the season you have selected?.....

62. How many cattle died because of trypanosomiasis over the period of last year

i) Calves.....ii) Weaners .....iii) Cows and bulls.....

63. How many shoats died because of trypanosomiasis over the period of last year

i) Kids .....ii) Weaners .....iii) Adult sheep and goat.....

64.How many cattle recovered after treatment for the past year

i) Calves.....ii) Weaners .....iii) Cows and bulls.....

65.How many shoats recovered after treatment for the past year

i) Kids .....ii) Weaners .....iii) Adult sheep and goat.....

66. What drug(medicine) did you use to treat your animals.....

67. How did you prepared it (dose).....

68. What kind of facilitation you need, so that you can fight better against trypanosomiasis

.....

.....

69. Would you kindly specify, who needs the facilitation at which age.....

Thanks very much for your participation