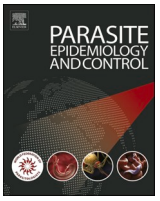
Parasite Epidemiology and Control 27 (2024) e00389

Contents lists available at ScienceDirect

Parasite Epidemiology and Control

journal homepage: www.elsevier.com/locate/parepi



Species richness and abundance of wild tsetse flies collected from selected human-wildlife-livestock interface in Tanzania

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ARTICLE INFO

Keywords:
Tsetse fly
Human-wildlife-livestock interface
NZI
Pyramidal
Abundance
Diversity

ABSTRACT

The successful control of tsetse flies largely depends on understanding of the species available and abundance. This study assessed the species richness, abundance and apparent density of wild collected tsetse flies from selected human-wildlife-livestock interface in Tanzania. Seasonal trapping using baited NZI, Pyramidal and Biconical traps was done across selected wards. Traps were set at 200 m apart, emptied after every 24 h then rotated to the next sites after 72 h. Collected flies were identified morphologically and letter confirmed using the Polymerase Chain Reaction (PCR). Only two *Glossina* species; *Glossina pallidipes* (n = 371; 47.32 %) and *Glossina morsitans morsitans* (n = 413; 52.68 %) were identified. Among them, 96 flies (80 Female, 16 Male) were blood fed; 57(48 Female and 9 Male) *G. pallidipes* and 39(32 Female and 7 Male) *G.m. morsitans*. Tsetse fly abundance varied across wards ($\chi^2 = 4.597$, df = 1, $p = 0.032$), villages ($\chi^2 = 9.491$, df = 3, $p = 0.023$), habitats ($\chi^2 = 17.239$, df = 2, $p < 0.001$), months ($\chi^2 = 13.507$, df = 3, $p = 0.004$) and deployed traps ($\chi^2 = 6.348$, df = 2, $p = 0.04$). About 78.82 % of the total catch occurred in Kisaki ward ($n = 618$; $p < 0.001$) and 21.17 % ($n = 166$; $p = 0.032$) in Bwakila chini. Similarly, 62.37 % of the catch occurred in Mbojoge village. NZI traps ($n = 422$; 54 %; 4.98 FTD) were most successful traps. Moreover, 78.06 % of the catch occurred in bushed grassland habitat ($n = 612$; 55.41 FTD) while 5.48 % in farmland ($n = 43$; 7.17 FTD). This study recommends NZI and Pyramidal traps for tsetse flies control at the interface and proposes wet season as appropriate time for successful trapping of the flies. Finally, it attracts a need for assessing tsetse flies' blood meal sources and the infection status to establish the prevalence to inform existing trypanosome control programs.

1. Introduction

Tsetse flies are bloodsucking flies of the order Diptera, family Glossinidae and genus *Glossina*. They are cyclical vectors of

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https://doi.org/10.1016/j.parepi.2024.e00389
Received 13 January 2024; Received in revised form 2 September 2024; Accepted 24 October 2024
Available online 25 October 2024

flagellated protozoa parasites that cause sleeping sickness in humans (HAT) and nagana (AAT) in domestic animals (Vreysen et al., 2013; Gashururu et al., 2021). Tsetse flies are confined between latitudes 14° N and 20° S (Bouteille et al., 2014), hence inhabiting only sub-Saharan Africa (World Health Organization, 2013) from Sahara to Somali desert in the Northern part and Kalahari to Namibian deserts in the Southern part (Vreysen et al., 2013). There are about 33 known species and subspecies of tsetse flies(Gooding and Krafsur, 2005) which are sub-divided into three subgenera basing on their morphological and ecological characteristics: Austenina (Fusca group), Nemorhina (Palpalis group), and Glossina (Morsitans groups) (Cecchi et al., 2008). Tsetse fly from the three subgenera occupy different habitats. The Nemorhina species occupies the vegetation close to watercourses such as: riverine forest, protected forests, vegetation along lakes and mangroves. *Glossina* species occupy the dry savannah woodland where by Austenina species inhabit dense forest belts (Van Den Bossche et al., 2010).

The dominant tsetse fly species in East Africa including Tanzania are savannah species, which among others include: *G. morsitans, G. pallidipes and G. swynnertoni* (Bouteille et al., 2014). Other species such as; *Glossina austeni*, *Glossina brevipalpis*, *Glossina longipennis, Glossina fuscipes martinii* and *G. fuscipes fuscipes* exist but in limited distribution (Moloo, 1993; Kweka et al., 2017). This limited distribution is generally determined by climatic and demographic pressure (Dicko et al., 2015: Nnko et al., 2021). In general, tsetse occurrence and dispersal is affected by factors such as humidity, altitude, vegetation/shades availability and host density (Soberon and Peterson, 2005). Of the mentioned factors, temperature is considered as the key driver influencing all others factors that determine tsetse occurrence in sub-Saharan Africa (Nnko et al., 2021). All tsetse flies can transmit pathogenic trypanosome parasites, but, only 6–10 species have public health and veterinary significance (Gooding and Krafsur, 2005; Vreysen et al., 2013; Kweka et al., 2017). Notable examples of such species include *G. pallidipes*, *G. brevipalpis*, *G. m. moristans* and *G. swynertoni*, *G.m. centralis*, *G.p. palpalis*, *G. fuscipes fuscipes*, *G. tachinoides*, and *Glossina palpalis gambiensis* (Malele et al., 2003; Malele et al., 2011; Auty et al., 2012).

Tsetse fly infests an area of about 8–11 million km^2 across 38 sub-Saharan countries (Holmes, 2013; Vreysen et al., 2013). Thus, posing the risk of tsetse-borne trypanosome infection to approximately 60 to 70 million people (Holmes, 2013) and 50 to 60 million cattle (Cecchi and Mattioli, 2009; Cecchi et al., 2014; Holmes, 2013) in the entire tsetse-endemic region. Moreover, tsetse-borne trypanosome infection risk sometimes affects other non-endemic countries whose citizen visits tsetse-endemic foci for several reasons (Simarro et al., 2012). In Tanzania, tsetse flies infest about 32.83 % (30,931,957 ha) of the total area. The tsetse infested area comprises of high-risk infested area (17.44 %, 16,431,622.63 ha) and medium and low-risk infested areas (15.39 %, 14,500,335 ha) (Matthew et al., 2016). This kind of infestation pose risk to approximately 4 million people and 4.4 million livestock (Malele et al., 2011). This high risk is arguably contributed by an exclusive hematophagous nature of both the male and female tsetse flies and their tendency to blood feed on several vertebrate disease reservoirs (Holmes, 2013; Malele et al., 2011; Vale et al., 2014).

Poverty in sub-Saharan Africa is attributed to many factors, among them includes diseases caused by tsetse-transmitted trypanosomes infection which result to livestock death, decreasing in animal productivity and loss of work force. All these tsetse-related impacts hinders the invested efforts that aim at attaining food self-sufficiency in most rural areas. The approximated losses due to AAT in sub-Saharan Africa are over USD 4 billion (FAO, 2016). In Tanzania, AAT vectored by tsetse flies alone leads to about 7.98 million USD annual loss (Malele, 2012). Therefore, to attain maximum agricultural productivity and minimizing tsetse flies related risks in public health, the control methods which minimizes tsetse vectors are highly encouraged (Vreysen et al., 2013). The methods which targets vectors have proven their ability to reduce disease risk both in humans and animals (Hordofa and Haile, 2017). Another control approach targets trypanosomes by trypanocidal drugs such as suramin, pentamidine, melarsoprol, diminazene diaceturate (DA), Isometamidium chloride hydrochloride (ISM) and homidium bromide (Bengaly et al., 2018).

The increased frequency of tsetse-reservoirs-human interactions due to human encroachment and pressure in protected areas (PA) tend to maximize the risk of trypanosome transmission in many adjacent areas (Thompson, 2013). And, since many new Protected Areas have been established in Tanzania in recent years, the number of disease reservoirs in those areas also tend to increase. Also, considering that the transmission risk is largely driven by the escalating interaction between tsetse vector species, animals and human being, acquiring in-depth understanding of the tsetse fly species richness, abundance and apparent density is vital in enhancing the desired improvements and informing the decision makers while making rational decision that will guide the control of tsetse flies and trypanosomiasis (Muturi et al., 2011). Therefore, this study assessed species richness, and abundance of tsetse flies in selected human-wildlife-livestock interface in Tanzania.

2. Materials and methods

2.1. Study site

The study was conducted in Morogoro rural district in Morogoro region, Tanzania. The district is located between latitude 6° 54′ 0" S, and longitude 37° 53′ 59″ E, at an altitude of 509 m a.s.l and covers an area of about 12,457.44 km^2. Morogoro rural district is bordered to Pwani region in the north and east, Kilombero district to the south, Kilosa district to the southwest, Mvomero and Morogoro urban district to the west (Ng'ida et al., 2019). The district contain several wildlife protected areas, among which includes; Mikumi National Park and Selous game reserve. Selous game reserve is bordered to Mikumi National Park to the north and extending to the south to several other regions. The two protected areas prohibits human economic activities to be conducted within the areas. The only activity allowed within the areas are tourism, and research but under special permits. Outside of these protected area, several economic activities such as, crop cultivation and pastoralism are conducted. The annual rainfall of the district ranges from 700 to 1000 mm. The short-wet season occurs from mid of October to the end of December, long wet season from February to the mid-May and the dry season occurs from June to October. The temperature ranges from 21 °C to 26 °C. Crop cultivation is the main economic activity, where the main crops that are cultivated includes; rice, maize, banana, beans, soybeans and horticultural crop (Mgode et al., 2014).

2.2. Tsetse fly trapping

Trapping was done across season (wet and dry). The NZI, Pyramidal, and Biconical traps baited with acetone (100 mg/h), 1-octen-3-ol (0.5 mg/h), 4-methyl phenol (1 mg/h), and 3-n-propyphenol (0.1 mg/h) were deployed following the procedures described by (Auty et al., 2016). Four villages (two in each ward) were purposively selected as trapping sites targeting the area bordering protected areas but with human–wildlife-livestock interaction. Prior to setting traps, grass was slashed to ground level then traps poles were greased to avoid ant's predation on caught flies. The individual traps were set at 200 m distance apart from each other and maintained in one trapping site for three days (72 h) in each trapping site. The deployed traps were revisited after every 24 h from 10:00 am to 11:00 am where collection cages were emptied (Fig. 2). The GPS coordinates of all trapping sites and points were recorded using GPS device (Garmin GPSMAP 60CSx). (See Fig. 1.)

2.3. Morphological identification and storage of tsetse flies

Collected tsetse flies were morphologically identified using the taxonomic keys developed by Leak et al. (2008). By the aid of hand lens, trapped tsetse flies were firstly identified in the field and later confirmed in the laboratory using stereomicroscope. Before identification, all live tsetse flies were sacrificed by placing them in the cool box for 2–3 min then categorized according sex and feeding status. Individual fly were stored in a well labelled 1.5 ml Eppendorf tubes containing silica gel for Molecular identification following the developed Standard Operating Procedures (SOP) for identification of tsetse species from the wild populations (FAO/IAEA, 2018).

2.4. Molecular identification of tsetse flies

2.4.1. Sample preparation

Fifteen (15) tsetse flies individually stored in Eppendorf tube contain silica gel were selected for molecular identification. These samples were washed in a phosphate-buffered saline, air dried for 5 min using tissue paper and separately grinded by a sterile motor

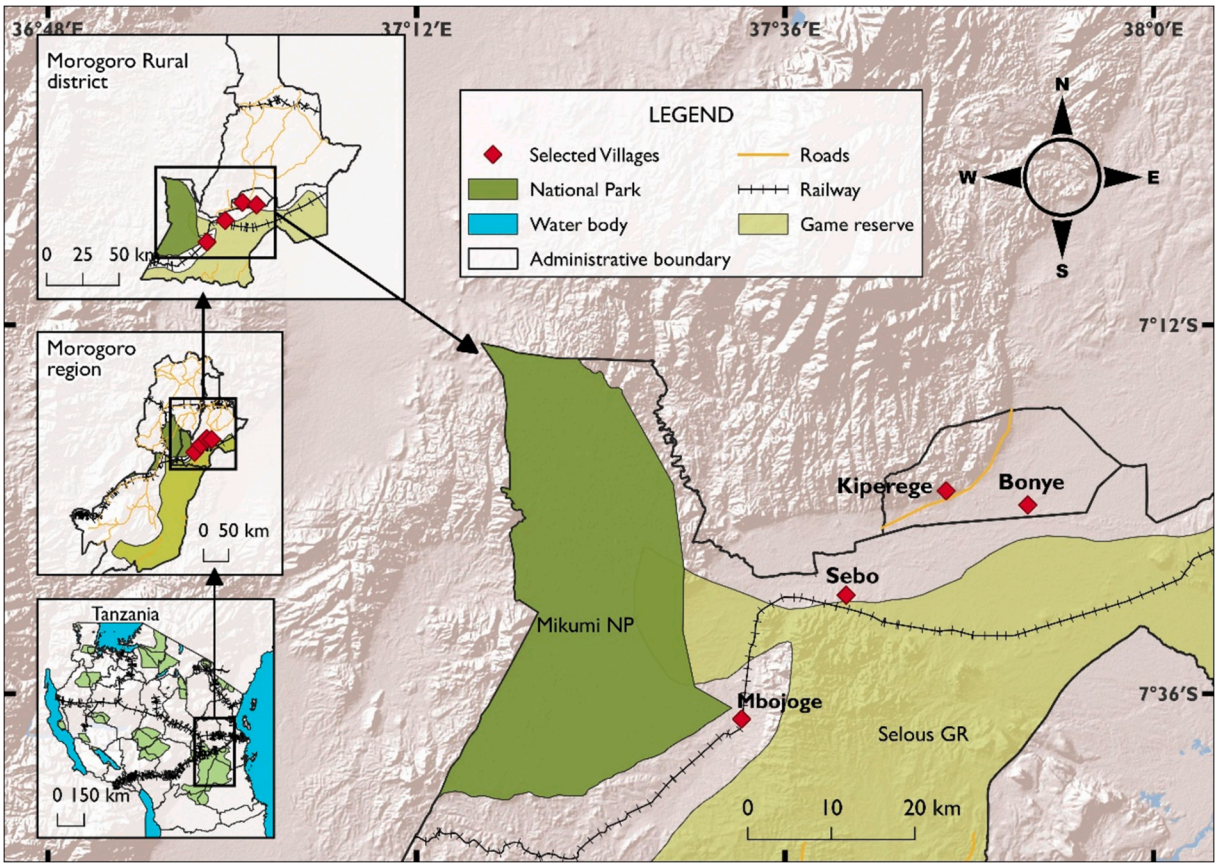

Fig. 1. A map showing trapping sites. Tsetse flies were collected from Kisaki ward (Bonye and Kiperege) and Bwakila chini wards (Mbojoge and sebo) in Morogoro region. The map was developed using QGIS software version 3.26.1 and data from DIVA-GIS and The Humanitarian Data Exchange (HDX), freely available at https://www.diva-gis.org/datadown and https://data.humdata.org/dataset/cod-ab-tza respectively.

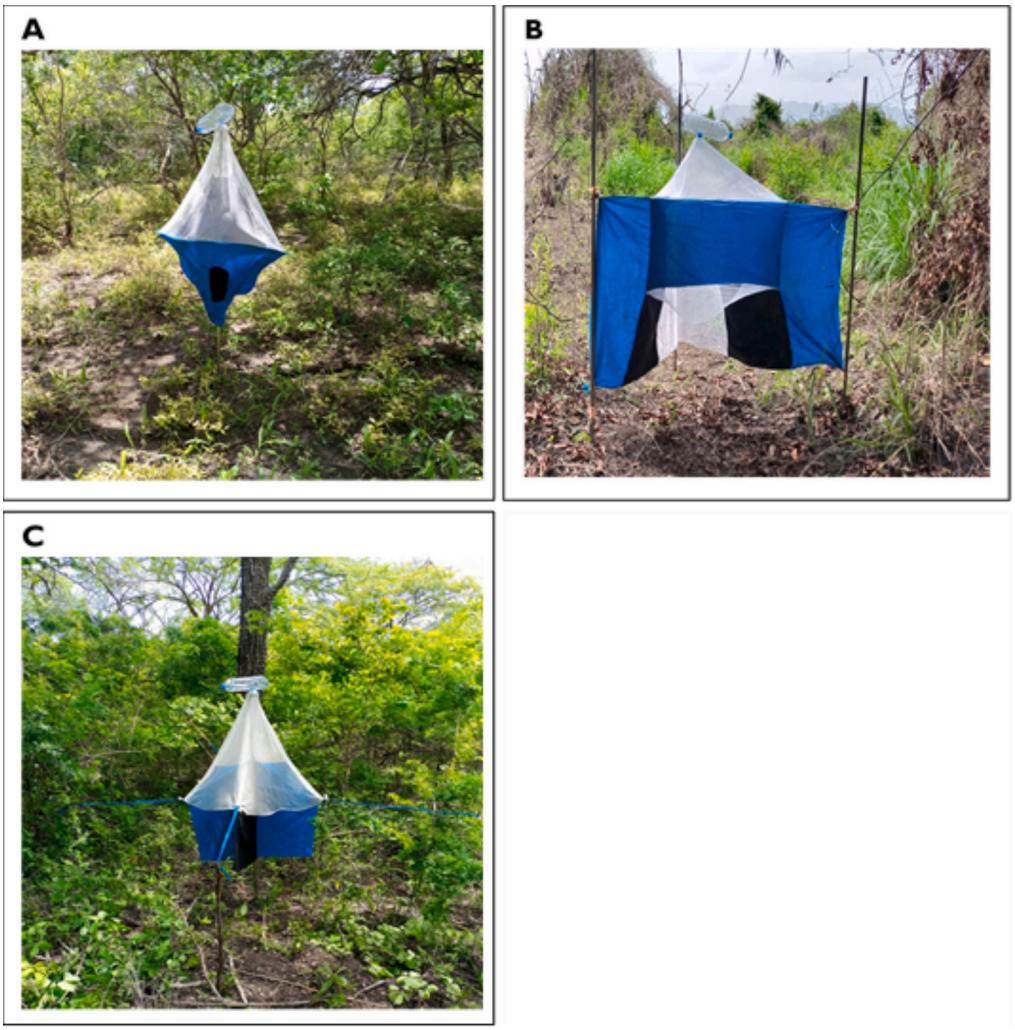

Fig. 2. Baited traps deployed in study area, A)Biconical trap, B)Nzi trap, and C) Pyramidal trap.

and pestle. Resulting samples were re-suspended in 100 µl of PBS in 1.5 ml micro-centrifuge tube, and homogenized manually with a sterile micropipette tip.

2.4.2. DNA extraction

In a sample homogenate, 100 µl of autoclaved 0.5 % Tween-20 in 1× PBS solution were added then mixed by gentle momentary vortexing. Obtained mixtures, were incubated for 20 min at room temperature then centrifuged at 16,000 rpm for 2 min. To remain with pellets, supernatant was discarded. The pellets were re-suspended in 100 µl 1× PBS and centrifuged again at 16,000 rpm for 2 min and then the supernatant was discarded. By gentle vortexing for 5 s, the remaining pellets were re-suspended in 2:1 of sterile deionized water (PCR water) to 20 % *w/v* Chelex-100 resin suspension. Obtained sample suspensions were then incubated in water bath on floating rack for 15 min and centrifuged at 16,000 rpm, for 5 min then transferred the supernatant into pre-labelled 1.5 ml microfuge tube. The mixture was further centrifuged at 16,000 rpm for 10 min and resulting supernatant (extracted DNA template) transferred into a new well-labelled 1.5 ml Eppendorf tubes then stored at −20 °C while awaiting further laboratory analysis. Nano spectrophotometer was used to measure the amount of DNA concentration and contamination of an aliquots (1 µl) of each extracted DNA sample.

2.4.3. PCR amplification

The amplification of an Internal Transcribed Spacer (ITS) 1 DNA fragment was done using primers sequence; Glossina forward primer (GTGATCCACCGCTTAGAGTGA) and reverse primer (GCAAAAGTTGACCGAACTTGA) in a Polymerase chain reaction (PCR). The PCR was performed in a 20 µl mixture consisted of extracted DNA (2 µl), forward primer (1 µl), reverse primer (1 µl) and nuclease free water (16 µl) in micro-tube containing AccuPower® PCR PreMix concentrate. The thermo-cycling conditions were as follows: denaturation at 95 °C for 5 min, followed by 35 cycles of amplification at 95 °C for 1 min, 62 °C for 1 min and 72 °C for 1 min and

extension at 72 °C for 7 min.

2.4.4. Gel-electrophoresis of the PCR products

The PCR products were then subjected to electrophoresis in 1.5 % agarose gel (prepared by dissolving 1.5 g agarose into 100 ml of 1× Sodium borate then heated in microwave prior to staining using 4 µl of GelRed® Nucleic Acid Gel Stain). Then, 4 µl of the amplified PCR products was loaded into each wall of the gel after loading 4 µl of 100 bp DNA ladder into the first wall. 100 V was then subjected to the system and allowed electrophoresis to run for 35 min. The resulting DNA fragments were observed as grey bands against a black background. Identification of the species were done basing on the gel electrophoresis results following the Standard Operating Procedures for Identification of Tsetse species from Wild Populations (FAO/IAEA, 2018).

2.4.5. Data analysis

Data were entered, organized and cleaned in Microsoft Excel 2010 before analysis. The average apparent density (AD) or Fly trap density (FTD) was calculated using the formula: $AD = \sum F_t / (T \times D)$, where, $\sum F_t$ is the total number of tsetse flies caught, T is the number of trap deployed and D is the number of days the trap has been in place (Waiswa et al., 2006; Gashururu et al., 2021; Opiro et al., 2021).

The variation in tsetse fly abundance across different parameters such as; wards, villages, seasons, traps, species and habitat were analyzed using a Generalized Linear Mixed Models (GLMMs) in R statistical software version 4.2.2. Due to unequal variance and non-normal distribution of count data confirmed with Bartlett's test and Shapiro-Wilk test respectively, a negative binomial distribution (*glmer.nb* function of the lme4 package) was used to account for over-dispersion of the data. Collection date was set as random effect in all models. Plots were created using ggplot2 package. The association between number of collected flies and the distance from the protected Area as tested using Pearson correlation after transforming the data so as to attain normality.

The distance of the trapping points from the Protected Area boundary was measured in QGIS using "NNJoin plugin" which calculate the distance between the two points of vector layers.

3. Results

3.1. Tsetse flies abundance

A total of 784 tsetse flies (584 female and 200 males) were trapped using 16 traps deployed in four villages. Among them, 96 flies (12.24 % of the total catch; 16-Male, 80-Female) were blood fed, while 688 flies (87.76 % of the total catch, 184-Male, 504-Female) had not blood fed.

Only two glossina species were identified; *Glossina pallidipes* (1100 bp) and *Glossina morsitans morsitans* (900 bp) (Fig. 3). Proportionally, *Glossina morsitans morsitans* ($n = 413$, 52.68 %) were more abundant than *Glossina pallidipes* ($n = 371$, 47.32 %), but the difference in abundance between them was not significant ($\chi^2 = 1.577$, df = 1, $p = 0.209$). Of the non-blood fed proportion (87.76 % of the total fly catch, $n = 688$), 54.36 % were *G.m.morsitans* ($n = 374$) and 45.64 % were *G.pallidipes* ($n = 314$). The blood fed group (12.24 % of the total catch) comprised of: *G.m.morsitans* ($n = 39$, 40.62 % of blood fed flies; 32 Female and 7 Male) and *G.pallidipes* ($n = 57$, 59.38 % of blood fed flies; 48 Female and 9 Male). The variation in number of blood fed tsetse flies across species was significant ($\chi^2 = 5.678$, df = 1, $p = 0.017$).

The two wards surveyed recorded significant different tsetse flies abundance ($\chi^2 = 4.597$, df = 1, $p = 0.032$) with about three-times higher number of flies collected in Kisaki ($n = 618$; 78.82 %; p = 0.032) to those collected in Bwakila chini ($n = 166$; 21.17 %; $p < 0.001$). 82 % of *G.m.morsitans* ($n = 340$, $p = 0.07$) and 75 % of *G. pallidipes* ($n = 278$, $p = 0.822$) were collected from Kisaki ward, while 18 % ($n = 73$, $p = 0.004$) and 15 % ($n = 93$, p < 0.001) were collected in Bwakila chini ward respectively. The variation in the number of both *G.m.morsitans* ($\chi^2 = 3.404$, df = 1, p = 0.07) and *G.pallidipes* ($\chi^2 = 0.0502$, df = 1, $p = 0.823$) across wards was not significant.

The total tsetse flies abundance varied across villages ($\chi^2 = 9.491$, df = 3, $p = 0.023$) with significantly higher number recorded in Mbojoge ($n = 489$, 62.37 %, p < 0.001) and Kiperege (17.98 %; $n = 141$; $p = 0.15$) while Sebo (16.45 %; $n = 129$; $p = 0.12$) and Bonye ($n = 25$, 3.2 %, $p = 0.08$) recorded the least abundance (Fig. 4). Similarly, *G.m.morsitans* ($\chi^2 = 33.067$, df = 3, p < 0.001) and *G. pallidipes* ($\chi^2 = 18.392$; df = 3; p < 0.001) abundances significantly varied between villages (Table 1).

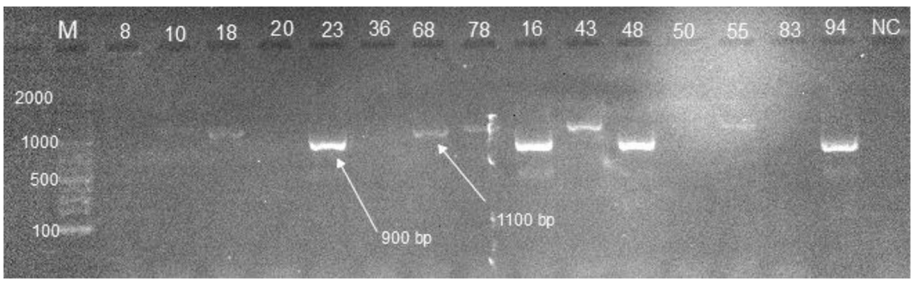

Fig. 3. Agarose gel electrophoresis showing ITS polymerase chain reaction product, amplified from the DNA extracted from wild collected adult tsetse flies.

The difference in tsetse fly catches across seasons was not significant ($\chi^2 = 0.178$, df = 1, $p = 0.673$). But higher fly catch occurred during wet season ($n = 499$; 64 %; $p < 0.001$) compared to dry season ($n = 285$; 36 %; p = 0.67). Likewise, the number of collected *G.m. morsitans* ($\chi^2 = 0.3199$, df = 1, $p = 0.572$) and *G.pallidipes* ($\chi^2 = 0.212$, df = 1, $p = 0.645$) across seasons were not significantly different. Also, there was no significant variation in the number of blood fed flies by species across season ($\chi^2 = 0.068$, df = 1, $p = 0.794$). However, 68 % of the blood fed flies were trapped during wet season; *G.m.morsitans* ($n = 30$; 31.35 % of total blood fed) and *G. pallidipes* ($n = 36$; 37.5 % of total blood fed) while 31 % during dry season; *G.m.morsitans* (n = 9; 9.38 % of total blood fed) and *G. pallidipes* ($n = 21$; 21.88 % of total blood fed). Note the distribution of tsetse flies catch in each surveyed month (Table 1).

The traps success varied significantly across deployed traps ($\chi^2 = 6.348$, df = 2, $p = 0.042$). The overall trap success in descending order was as follows; NZI traps ($n = 422$; 54 %; 4.98 FTD), Pyramidal traps ($n = 281$; 36 %; 4.01 FTD) and Biconical traps ($n = 81$; 10 %; 1.87 FTD) (Table 2). The same order of traps performance occurred when assessing the number of blood fed flies collected using three kinds of traps, but the variation between them was not significant ($\chi^2 = 3.3044$, df = 2, $p = 0.1916$).

The distribution tsetse fly catches varied across habitats ($\chi^2 = 17.239$, df = 2, $p < 0.001$). The highest fly catch occurred in bushed grassland habitat ($n = 612$; 78.06 %; 55.41 FTD), while, the lowest occurred in farmland habitat ($n = 43$; 5.48 %; 7.17 FTD). Most *G.m. morsitans* ($n = 285$; 69 %; $p < 0.001$) and *G.pallidipes* ($n = 327$; 88 %; $p < 0.001$) were caught in bushed grassland (Table 2). Blood fed flies were highly caught in bushed grassland habitat compared to other habitats ($p = 0.01$) (Table 2). Fig. 5 shows that, most blood fed flies were collected from Mbojoge village, using NZI traps in bushed grassland during wet season (Table 3).

The individual trapping points recorded significantly different mean fly abundances ($t = 22.27$, df = 64, $p < 0.001$). Most trapping points that were at higher distance from the PA boundary recorded relatively small proportion of flies compared to those that were near to the PA boundary ($r = -0.158$, p-value = 0.208). The trapping points that are located in Kisaki ward (Mbojoge and Sebo villages) contributed to 78.83 % of the total fly catch, compared to 21.17 % of the flies that were collected in Bwakila chini ward (Bonye and kiperege Villages) (Table 4).

4. Discussion

Several studies have assessed tsetse flies abundance and related risks around and within Protected Areas in Tanzania (Muturi et al., 2011; Luziga et al., 2017; Salekwa et al., 2014), but most of them were conducted in the northern regions and very few in the southern and central regions of the country. This study assessed the species richness, and abundance of wild collected tsetse flies from selected human-wildlife-livestock interface bordering Nyerere National Park, Tanzania.

Two *Glossina* species such as *Glossina pallidipes* and *Glossina morsitans morsitans* were identified during the survey. These species dominate the Selous ecosystem (Auty et al., 2016) where the surveyed interface is located. Proportionally, *G.m.morsitans* were more

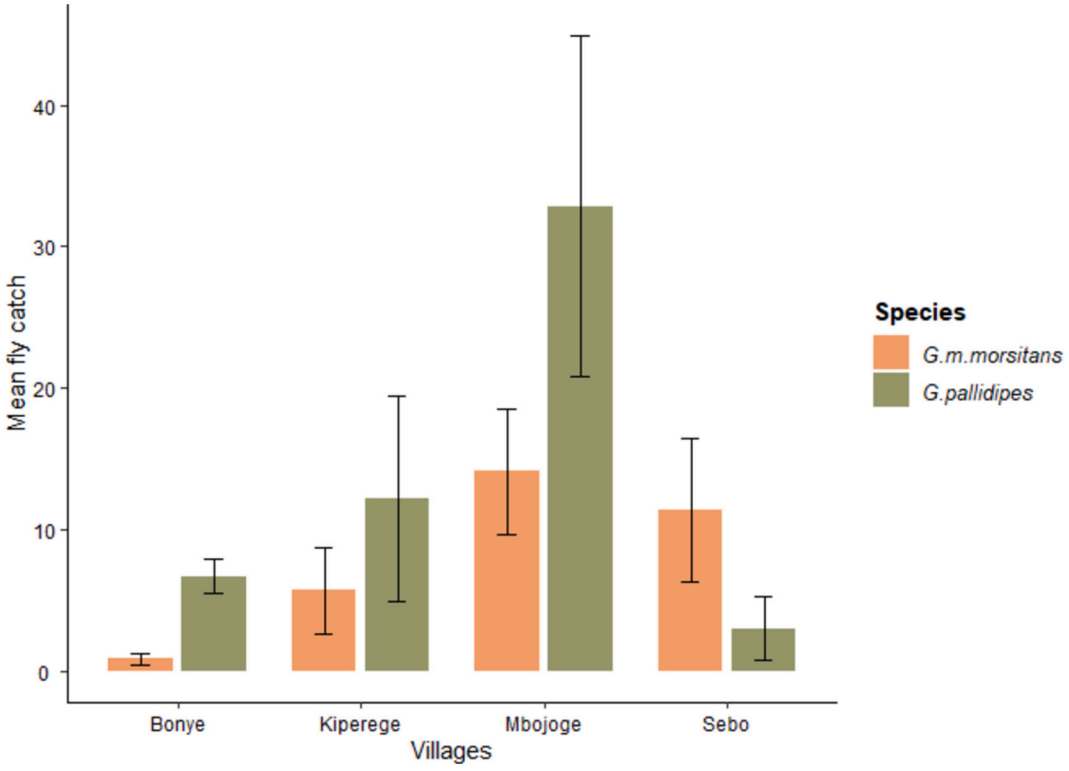

Fig. 4. A bar plot showing the distribution of tsetse fly average abundance by their species across surveyed villages.

Table 1
Tsetse fly abundance by species across season, traps, wards, villages, habitats, and months.

Characteristic	Overall, $N = 784$	*G.m.morsitans*, $N = 413$	*G.pallidipes*, $N = 371$
Seasons,			
Dry season	285 (36 %)	99 (24 %)	186 (50 %)
Wet season	499 (64 %)	314 (76 %)	185 (50 %)
Feeding status,			
Blood fed	96 (12 %)	39 (9.4 %)	57 (15 %)
Non-blood fed	688 (88 %)	374 (91 %)	314 (85 %)
Kind of traps,			
Biconical	81 (10 %)	52 (13 %)	29 (7.8 %)
NZI	422 (54 %)	201 (49 %)	221 (60 %)
Pyramidal	281 (36 %)	160 (39 %)	121 (33 %)
Wards,			
Bwakila chini	166 (21 %)	73 (18 %)	93 (25 %)
Kisaki	618 (79 %)	340 (82 %)	278 (75 %)
Villages,			
Bonye	25 (3.2 %)	5 (1.2 %)	20 (5.4 %)
Kiperege	141 (18 %)	68 (16 %)	73 (20 %)
Mbojoge	489 (62 %)	226 (55 %)	263 (71 %)
Sebo	129 (16 %)	114 (28 %)	15 (4.0 %)
Habitats,			
Bushed grassland	612 (78 %)	285 (69 %)	327 (88 %)
Farm land	43 (5.5 %)	14 (3.4 %)	29 (7.8 %)
Woodland	129 (16 %)	114 (28 %)	15 (4.0 %)
Months,			
April	104 (13 %)	57 (14 %)	47 (13 %)
February	395 (50 %)	257 (62 %)	138 (37 %)
July	18 (2.3 %)	9 (2.2 %)	9 (2.4 %)
June	267 (34 %)	90 (22 %)	177 (48 %)

Table 2
Distribution of overall fly catches, and apparent densities by sex across different traps deployed, season, wards, villages and habitat types.

Variables		Overall fly catch	Catch(M ± SE)	Overall FTD	Male FTD	Female FTD
Traps	NZI	422	5.41 ± 0.63	4.98	1.26	3.72
	Pyramidal	281	4.07 ± 0.62	4.01	1.17	2.84
	Biconical	81	1.80 ± 0.36	1.87	0.37	1.50
Season	Wet season	499	4.06 ± 0.45	4.27	1.16	3.11
	Dry season	285	4.13 ± 0.60	2.72	0.62	2.11
Wards	Bwakila chini	166	2.41 ± 0.50	19.72	4.03	15.69
	Kisaki	618	5.02 ± 0.41	63.41	17.61	45.80
Villages	Mbojoge	489	5.43 ± 0.58	42.85	11.50	31.35
	Sebo	129	3.91 ± 0.75	20.56	6.11	14.44
	Bonye	25	1.13 ± 0.23	4.17	0.50	3.67
	Kiperege	141	3.38 ± 0.83	15.56	3.53	12.03
Vegetation	Bushed grassland	612	4.98 ± 0.50	55.41	14.20	41.21
	Farmland	43	1.19 ± 0.20	7.17	1.33	5.83
	Woodland	129	3.91 ± 0.75	20.56	6.11	14.44
Grand total		784		83.13	21.64	61.49

abundant than *G.pallidipes* (Table 1), likely due to the presence of their preferred hosts and suitable habitats for them to survive. *G.m. morsitans* prefer feeding on warthogs (Gaithuma et al., 2020), and *G.pallidipes* prefer feeding on warthog, giraffe and elephant (Muturi et al., 2011; Nyingilili and Malele, 2016). But, on the absence of their preferred hosts, both species blood-feed on cattle, goats and human (Clausen et al., 1998). Several wild animals' marks (foot prints and faeces) such as for elephants, Impala, lions and baboons were observed during the survey. This indicates the presence of the wild hosts visiting the interface. In addition, cattle and goats frequently crossed the trapping sites heading to or from the grazing areas. Thus, serving as the blood meal source for identified species. There could be more *Glossina* species in the area, but since this study focused on the area outside of the protected area (interface) only two species were collected. Therefore, future surveys should sample flies in and out of the protected area to get more tsetse flies species available in the area.

Most tsetse flies were collected from Kisaki ward than Bwakila chini. The villages within each wards recorded significant different fly abundance. The highest fly catch occurred in Mbojoge villages (the village within kisaki ward). This can be attributed to its location or the distance of the protected area to the surveyed villages and hosts availability in these sites (Table 4 and Fig. 6). This finding is consistent with the results from other studies done elsewhere (Luziga et al., 2017; Salekwa et al., 2014; Ngonyoka et al., 2017; Gashururu et al., 2021). All these studies report the decrease in tsetse flies abundance with an increase in the distance from the PA boundary. The trapping sites in Kisaki ward were closer to the boundary compered to Bwakila chini. Also, large herds of livestock

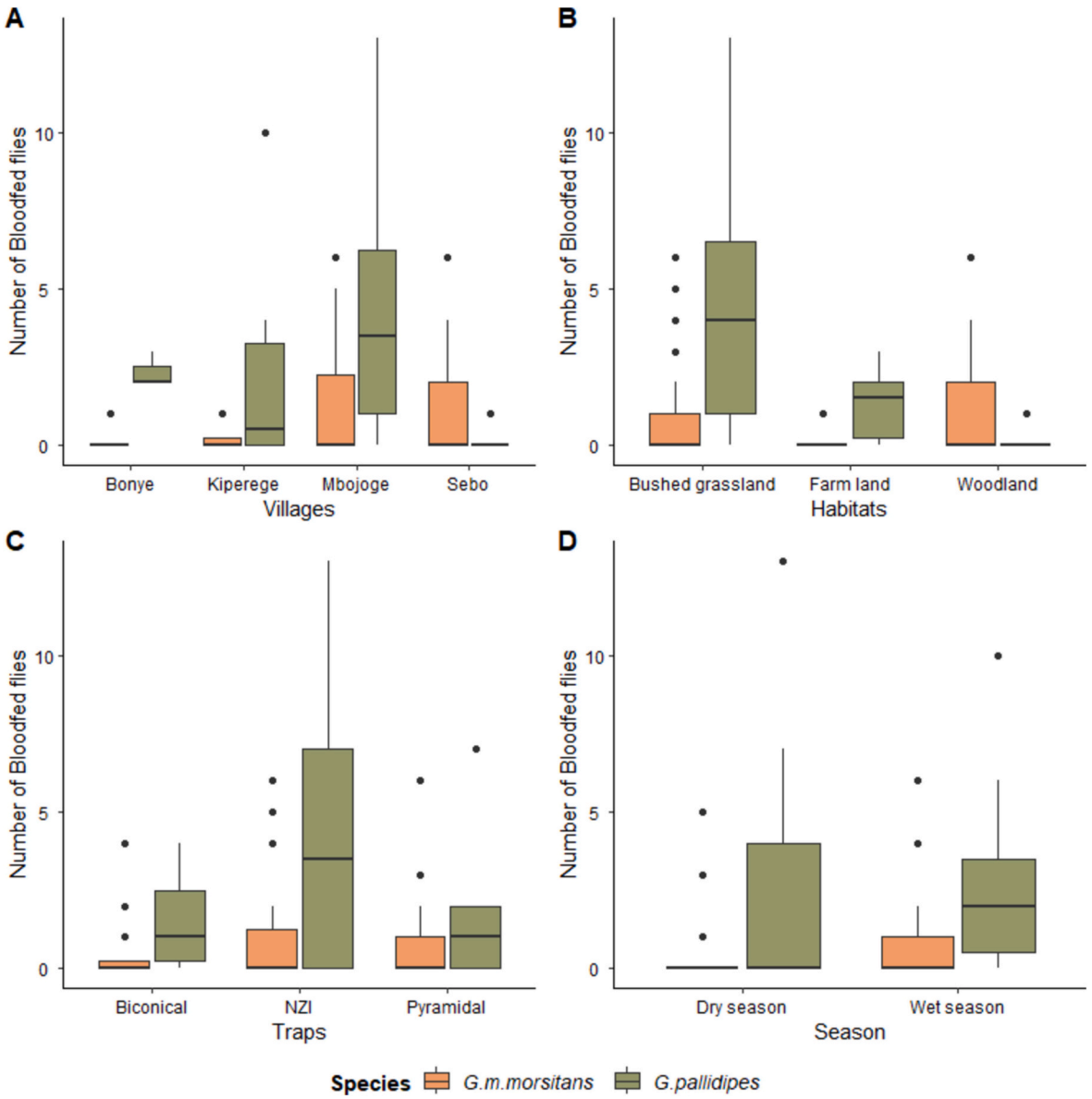

Fig. 5. The distribution of blood fed tsetse flies across: A: Villages, B: Habitats, C: Traps and D: Seasons.

Table 3
The distribution of the number of tsetse flies by species across sex and feeding status.

Species	Sex		Feeding Status	
	Female	Male	Blood fed	Non-blood fed
G.m.morsitans	276 (67 %)	137 (33 %)	39 (9.4 %)	374 (91 %)
G.pallidipes	308 (83 %)	63 (17 %)	57 (15 %)	314 (85 %)
Total	584 (74 %)	200 (26 %)	96 (12 %)	688 (88 %)

crossing and grazing in the interface could have influenced tsetse flies abundance. Ngonyoka et al. (2017) reports that, available hosts at the interface play a great role in sustaining existing tsetse flies population. Apart from people that were seen working in their farms, several wildlife and livestock were also observed during trapping period. Among them, impala, cattle and goats were dominant especially in Kisaki ward. Such interaction could pose a significant risk to both human and livestock. Therefore, future studies can assess the trypanosome infection status in both tsetse flies and livestock so as to quantify the potential trypanosome risk available in the surveyed human-wildlife-livestock interface.

Table 4
The number of trapped tsetse flies across different locations and their distance from the protected area boundary.

Villages	Village location		Distance from PA (m)	Number of trapped tsetse flies
	Latitude	Longitude		
Mbojoge	−7.6276	37.5528	4342.678	489 (62.37 %)
Sebo	−7.4931	37.6664	773.714	129 (16.45 %)
Bonye	−7.3954	37.8635	3965.576	25 (3.19 %)
Kiperege	−7.38	37.7749	9231.970	141 (17.98 %)

Most flies were caught during wet season compared to dry season. This can be attributed to availability of favourable weather condition during wet season and tsetse flies' behaviour. Tsetse flies survival and reproduction mainly depend on average temperature and relative humidity (Nnko et al., 2021). On the other hand, during the dry season, tsetse flies aggregate in dense vegetation and disperse during the rainy season (Robinson, 2003; Leak et al., 2008). This behaviour affects tsetse fly catches (Nnko et al., 2017). Therefore, it is more likely even in the surveyed habitats to record high fly abundance when trapping during wet season than dry season. Moreover, hosts (especially wild animals) travel short distance searching for good pasture during wet season. Hence, the blood-feeding flies including tsetse flies, which depend on these hosts, were highly collected during wet season compared to dry season.

NZI traps outperformed all other types of traps used in this study. It attracted the highest proportion of both recognized tsetse fly species across all trapping sites, followed by Pyramidal and Biconical traps (Table 1). Malele et al. (2016) also reported on the varying trapping success of different traps in different ecosystems. Because NZI traps are very successful at trapping savannah species of tsetse flies, the study recommend NZI and Pyramidal traps for tsetse flies control at the assessed human-wildlife-livestock interface where savannah species predominate.

There was significant variation in tsetse fly abundance across surveyed habitats. Most flies were collected from bushed grassland habitat followed by woodland and very few in a farmland (Table 2). This finding align with other studies conducted elsewhere (Reid et al., 2000; Salekwa et al., 2014; Ngongolo et al., 2019) which recorded higher fly abundance in animal grazing zones including bushed grassland. The two species identified; *G.m.morsitans,* and, *G.pallidipes,* inhabits mainly open woodland, grass land and bush-land (Leak et al., 2008). Therefore, recording the highest fly abundance in bushed grassland is likely due to the availability of blood meal sources. Tsetse flies in bushed grassland and woodland habitats, can feed on wild animals and livestock, which often visits the area for grazing. In a farmland habitat, there is habitat distraction and strict protection for livestock to graze, hence only human being and perhaps less preferred wildlife (examples; reptiles and birds) visit the areas.

Only 96 tsetse flies (12.24 % of total catch; 80 Female, 16 Male) were blood fed. Among them, 57 flies (48 Female, 9 Male) were *G. pallidipes* and 39 flies (32 Female: 7 male) were *G.m.morsitans*. Observed small proportion of blood fed flies could be attributed the tsetse flies' behaviors. Most engorged tsetse flies often remain in shades and resting sites and the hungry roam around reaching for blood meal sources. This finding is supported with the study done by Farikou et al. (2010), which reported only 4.7 % of the total fly catch with blood in their guts. Therefore, targeting the blood fed population using traps could end up catching larger proportion of the hungry flies. Furthermore, the highest abundance of blood fed flies occurred during wet season in a bushed-grassland habitat and were trapped using NZI trap (Fig. 5). This finding cement on the frequent visitation of the blood meal hosts in bushed grassland habitats and the minimum dispersal of both flies and their potential blood meal hosts during wet season. Also, the traps are designed to exploit host-seeking behaviour, especially when lured with olfactory baits. Therefore, it is not surprising that most of the tsetse caught were unfed.

5. Conclusion

The two Glossina species identified; *G.pallidipes* and *G.m.morsitans* were the only two potential vectors found. The results suggest that they may be implicated in the transmission of AAT and HAT in areas of close proximity to wildlife. The small abundance of trapped tsetse flies in the trapping points that were far from the protected areas relative to those which were close to the protected area indicates the possibility of controlling trypanosome infection by minimizing the interaction between livestock and tsetse flies, particularly those tsetse flies which also feed on wild animals. The study finally recommends NZI and Pyramidal traps in addition to existing tsetse flies control strategies in controlling tsetse flies in the areas where human, livestock and wildlife interact. This underscores the need for blood meal analysis to assess transmission risks and guide existing tsetse control programs.

Ethical consideration

Research permits were obtained from Tanzania Wildlife Research Institute and the Tanzania Commission for Science and Technology (permits number: 2022–735-NA-2022-082) and from the Sokoine University of Agriculture Research and Publication Committee (reference number SUA/DRRTC/R/186/18).

CRediT authorship contribution statement

Filbert E. Mdee: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Venance T. Msoffe:** Writing – original draft, Methodology, Investigation, Data curation. **Eliakunda M. Mafie:** Writing – review & editing, Writing – original draft, Supervision, Data curation,

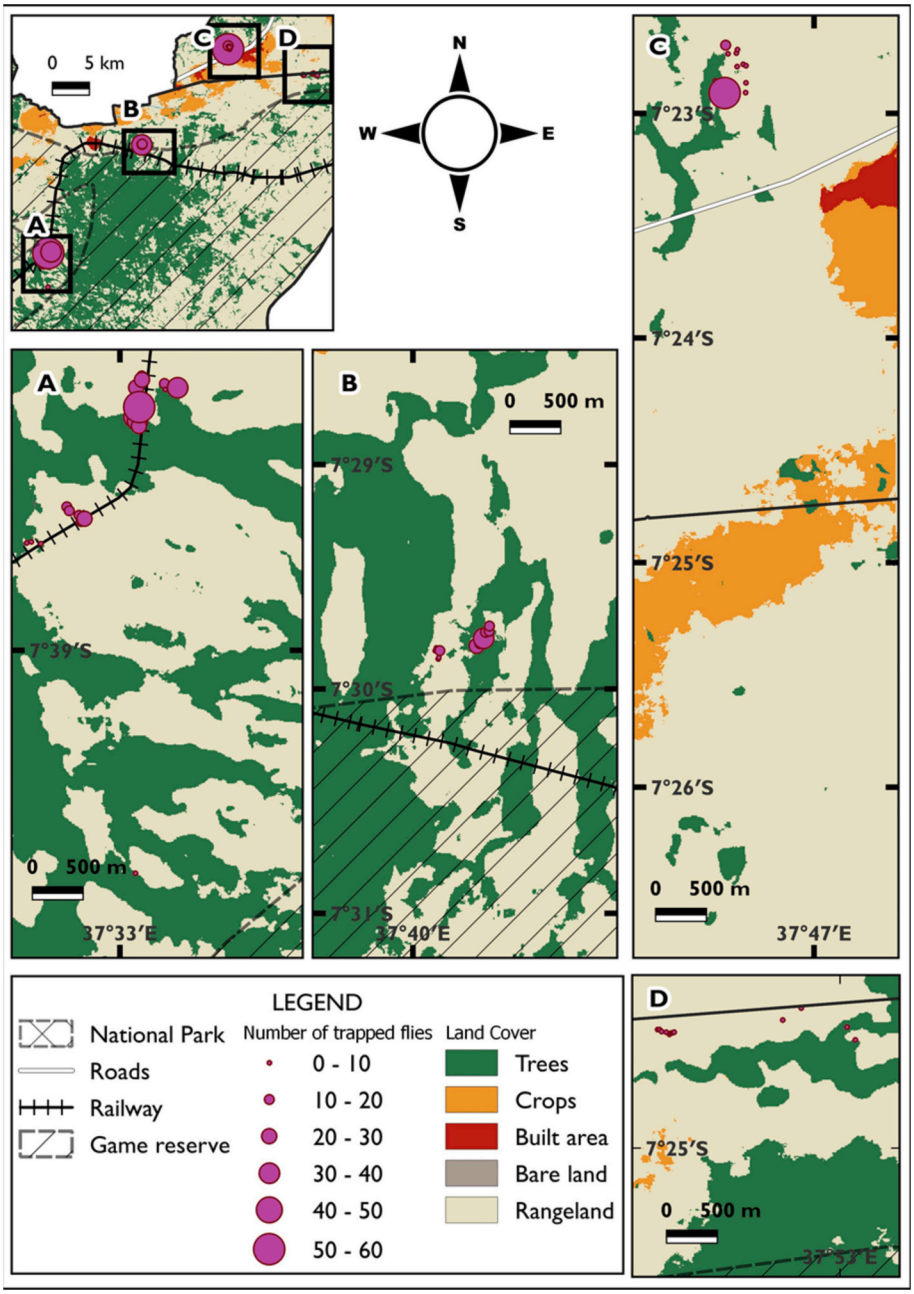

Fig. 6. A map showing the number of tsetse flies trapped in different habitats, at various distance from the protected area boundary. This map was developed using freely available shape files (at https://www.diva-gis.org/datadown and https://data.humdata.org/dataset/cod-ab-tza) and land cover data for 2021 to 2022 freely available in the ArcGIS Living Atlas of the World (https://www.esri.com/arcgis-blog/products/arcgis-living-atlas/imagery/global-land-cover-updates-2/).

Conceptualization. **Ladslaus L. Mnyone:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgement

The authors sincerely appreciate the financial support from The Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development (ACE II IRPM-BTD) under WORLD BANK for sponsoring this study. Special thanks should go to Mr. Ngahama, and all village chairpersons in the surveyed villages for their support during field trapping of the tsetse flies. They also appreciate the advice and support from staffs of the Institute of Pest Management (IPM) and Tanzania Veterinary Laboratory Agency (TVLA) Tanga. Thank you all.

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