Wing length of tsetse caught by stationary and mobile sampling methods

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Highlights

• An association exists between capture method used and body size of tsetse flies.

• Tsetse caught by fly rounds had significantly lower mean wing length than by traps.

• In 8 out of 11 months, traps had higher proportions of large females than fly rounds.

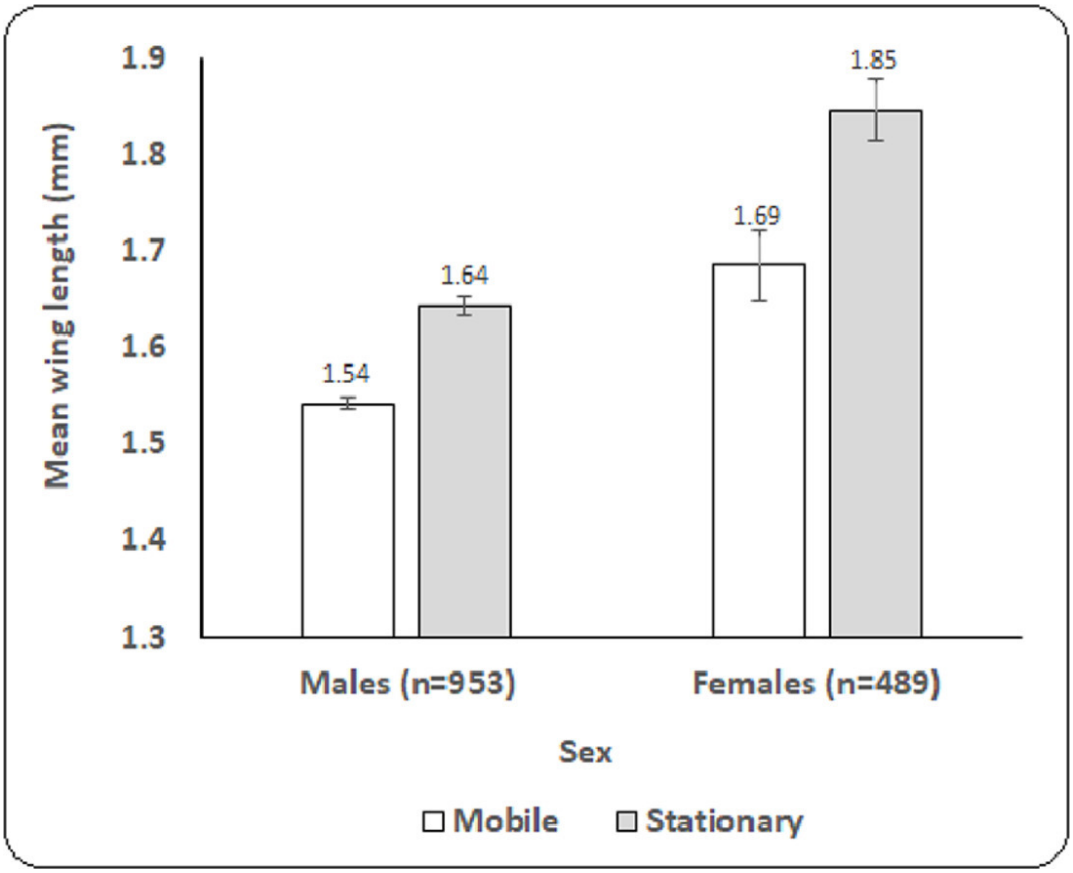
• The likelihood to capture large tsetse significantly differed between study sites.

• Possible impact on tsetse control, of size bias by traps requires further study.

Graphical abstract

Summary: The capture of tsetse flies by stationary devices is in favor of large flies and this may have implications on success of control operations.

Mean wing length of male and female flies caught by mobile and stationary tools.



Abstract

Introduction

A variety of techniques have been used to control tsetse with varying degrees of success. In a study on the population structure of *Glossina fuscipes fuscipes* that recovered after a previous vector control trial on 2 Kenyan islands, it was reported that the average fly size on the intervention islands was significantly smaller than on the none intervention islands and also compared to the size before the intervention. The conclusion was that vector control using tiny targets exerted size selection pressure on the population. The study recommended for further studies and suggested that this phenomenon could be among the reasons why targets used as a sole control method have rare reports of successful elimination of tsetse populations. Therefore, in this paper we report on a study of body size of tsetse flies caught in epsilon traps (as a stationary device) and black screen fly rounds (as a mobile trapping device).

Materials and methods

The study was carried out in eastern Zambia to test the hypothesis that the body size (measured as wing length) of *G. m. morsitans* males or females, captured by epsilon traps and fly rounds is the same.

Results

A total of 1,442 (489 females and 953 males) wing length measurements of *G. m. morsitans* were used in the analysis. It was established that tsetse flies caught by epsilon traps are on average larger than those caught by fly rounds. The likelihood of a large female or male fly being caught by traps, relative to a small one, significantly increased by 5.088 times (95% CI: 3.138-8.429) and by 2.563 times (95% CI: 1.584-4.148), respectively, $p<0.0001$, compared with being caught by fly rounds. The hypothesis was rejected.

Conclusion

This study showed that epsilon traps capture significantly larger *G. m. morsitans* than fly rounds do. Therefore, further research is recommended to verify (i) whether the predilection of traps to capture larger flies has an effect on the process of tsetse elimination when targets are used e.g. targets may take longer to reach elimination than if the predilection was not there, ii) whether different results can be obtained on ecogeographic distribution of different sizes of the species if fly rounds are used for sampling instead of epsilon traps. The results from such studies could influence the strategies used in future control operations.

Keywords: *Trypanosomiasis, Glossina morsitans, Tsetse wing length, Epsilon traps, Man-black screen fly round, Tsetse control strategy.*

1. Introduction

Tsetse flies (*Glossina spp*) are obligate blood sucking insects that transmit human and animal trypanosomiasis in sub-Saharan Africa (Vreysen et al., 2013). The causative organisms of the disease are *Trypanosoma spp* which are protozoan parasites. Trypanosomiasis is a major disease of livestock and therefore, plays a major role in constraining rural development in Africa (Swallow, 1998). In an attempt to control tsetse flies, a variety of techniques have been used in many parts of Africa (Allsopp, 1985) with varying degrees of success (Meyer et al., 2016). Despite tsetse control being considered the most desirable approach to manage African trypanosomiasis (Leak,1998) a myriad of factors is associated with limited success of tsetse control operations including mis-application of techniques, financial constraints and reinvasion of cleared areas. The changes that take place in the structure of populations as control operations progress and of populations that recover is rarely studied. The age structure is one of the commonly studied variables during tsetse control campaigns. In Zimbabwe, increasing proportions of young flies were observed in the area with insecticide treated targets compared with the untreated area (Van Sickle and Phelps, 1988), showing that stationary targets were able to control drastically the whole tsetse population. In Ghana, the percentage of non teneral flies declined from 63% before the start of aerial spraying of deltamethrin (ULV) to 33% by the end of the fourth cycle (Adam et al., 2013). However, a recent a study on the population structure of *Glossina fuscipes fuscipes* that recovered after a previous vector control trial using tiny targets on some Islands of Lake Victoria in Kenya (Tirados et al., 2015), reported that the average fly size on the intervention island were significantly smaller than the average fly size on the none intervention (Control) island and during the period before the intervention (Mbewe et al., 2018). This was observed three years after the vector control intervention that was undertaken from 2011 to 2013

and wherein *G. f. fuscipes* populations were drastically reduced by over 90% (Tirados et al., 2015). Based on the finding Mbewe et. al., (2018) concluded that vector control using tiny targets exerted size differential selection pressure and they recommended further research to understand the mechanism behind this phenomenon as it could be among the factors that explain why elimination of fly populations was rarely reported when targets were used alone to control tsetse (Meyer et al., 2016; Vreysen et al., 2013). As a possible cause of small flies recovering after the tiny target intervention, Mbewe et. al., (2018) suggested that since larger flies have a higher displacement potential than smaller flies (Vale et. al., 1984), they have a higher probability of encountering the stationary targets. Consequently, targets may have been selectively killing large flies. The suggestions of Mbewe et al., (2018) led Hargrove et al., (2019) to test the hypothesis that tsetse flies caught by epsilon traps are, on average, larger than those caught by black screen fly round. Hereto he compared the wing length of *G. pallidipes* and *G. m. morsitans* females caught from vehicle mounted electric target (VET) (Vale, 1974a) and stationary epsilon traps (Muzari and Hargrove, 1996) at Rekomitjie (Zimbabwe) in the late1980s and early 1990s. Hargrove et al., (2019) found that wing length varied only weakly with capture method in comparison with fly age or capture period and he found no reason to believe that targets would fail to eliminate tsetse populations because of the influence of body size on the mobility of tsetse flies and availability of smaller less mobile flies to targets. There is need to further explore this subject by either carrying out experiments specifically designed to address the issue and or by examining data collected from other places and on different tsetse species. In 2006 and 2007 a survey was carried out in eastern Zambia to study how the density, population structure and infection rate of *Glossina morsitans morsitans*, in an area with no control measures in place, related to the degree of habitat fragmentation. We used the data collected in this study to test the hypothesis that the

body size of *G. m. morsitans* (measured as wing length) captured by traps and fly rounds is the same.

2. Materials and methods

2.1. Study area

The study area is located between 31.788º and 31.916ºE; and between 13.916º and 14.12ºS covering parts of Katete and Mambwe districts in eastern Zambia. The area is infested by *G. m. morsitans* and *G. pallidipes* with *G. pallidipes* concentrated to the north close to the Luangwa valley (Ford and Katondo, 1973). The climatic seasons comprise of the warm and wet (November to April); cold and dry (May to August); and hot and dry (September to early November). The study locations were Chisulo, Kasamanda, Lusandwa and Zinaka with minimum and maximum distance apart of 10 to 30 kilometers, respectively. The degree of habitat fragmentation at these sites increased in the order Lusandwa, Zinaka, Chisulo and Kasamanda (Mweempwa et al., 2015)

2.2. Sampling methods

The method used to sample tsetse populations is described in detail by (Mweempwa et al., 2015) except for use of epsilon traps (Muzari and Hargrove, 1996) and measurement of body size. In brief, black-screen fly-rounds Potts (1930) were used to capture tsetse at four locations. Fly round screens (1.5 m x 1m black cotton cloth) were baited with butanone (Vale, 1980) dispensed from a 500 ml brown bottle with a mouth diameter of 22 mm attached to the top pole of the screen and octenol (Hall et al., 1984) dispensed from a 50 cm^2 (5 cm x 5 cm x 2) surface area sachet made of 250 µm thickness polythene film placed in pocket sewn on the cloth, Fig 1. Fly-rounds were carried out at an average of 8 times per month along each of the 8 transects/routes (2 per location). Transects ranged between 2.8 to 7.4 km in length and were sub-divided into 110 -120 m sectors where the fly round team (two people) stopped to capture tsetse flies that landed on the screen,

vegetation, ground and on them using hand-nets. The start and end of each sector was marked and geo-referenced. Captured tsetse flies were each placed in separate specimen tubes with a label showing the date, transect and sector number, species and sex of the fly. Fly rounds were carried out in the last half of each month from July 2006 to June 2007 at Zinaka and from November 2006 to October 2007 at Chisulo, Kasamanda and Lusandwa. Because the same transects were used for fly-rounds every month, the term “permanent fly-round (PMFR)” was used to refer to them. Epsilon traps (4 per transect, as a stationary tool) were deployed at 400 to 600m apart along fly-round transects and were baited like PMFRs. Catches by traps were collected daily during the same period PMFRs were operated.

Figure 1: *Methods used to sample tsetse. A Man operated black screen fly round. B Epsilon trap*

2.3. Measurement of wing length

Tsetse flies caught by PMFRs and traps had both of their wings removed and pasted on glass slides with transparent cello-tape and measurements were taken of the length of the “cutting edge” of the “hatchet” cell (a to b), Fig 2, of the fourth longitudinal wing vein as described by Jackson, (1946). A graduated reticule on the eye piece fitted on a binocular microscope was used to

measure the length of the “cutting edge”. Measurements read off the graticule were converted to millimeters (mm) by dividing by the magnification used.

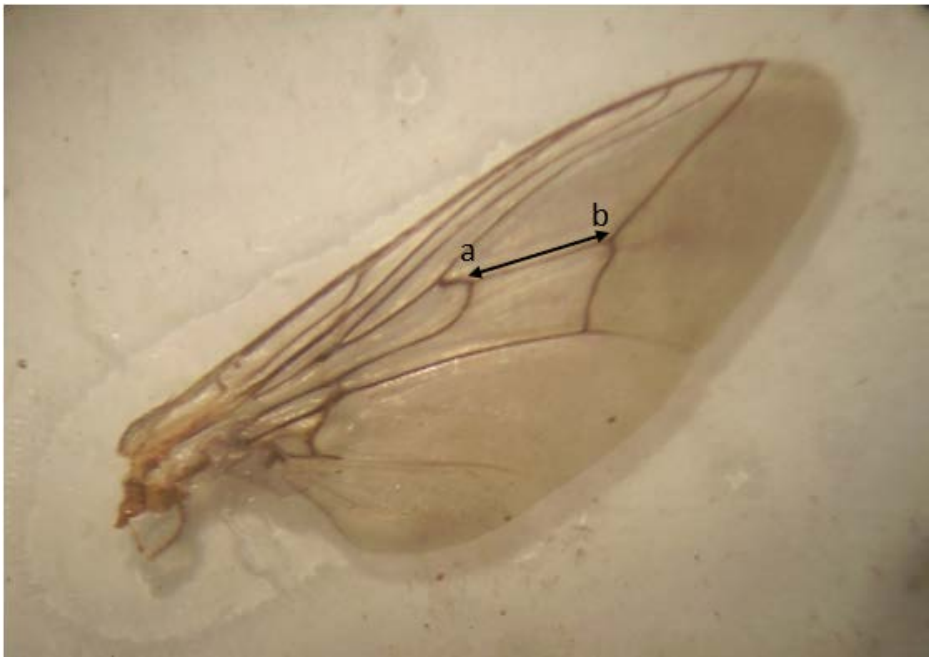


Figure 2: Distance measured on a wing

Other examinations carried out on tsetse flies included ovarian age, wing fray, infection by trypanosomes and sex ratio, covered in detail in a PhD thesis by Mweempwa, (2015).

2.4. Sample selection and data analysis

Measurements taken on the right wing of *G. m. morsitans* flies from Chisulo, Lusandwa and Zinaka study sites were used in the analysis. However, where the right-wing measurement was not available, the left-wing measurement was used instead if available. The sample size of *G. m. morsitans* caught at Kasamanda was very small (10 from PMFR and 11 from traps) and so the data were excluded from analysis. Because *G. pallidipes* was not caught at all study sites, its data were also excluded from analysis. Further, only measurements of flies caught by PMFRs and traps in same months at three sites were used in the analysis. Wing length measurements above or

below the average for each sex (measurements of two methods put together), were considered as large or small, respectively. Other data sets selected from the main database were, ovarian age and wing fray categories.

Shapiro-Wilk Normality Test was used to test for normal distribution of measurements from PMFR or trap caught male or female flies. Normal Q-Q graphs were plotted to check for normality of error terms (residuals). Logistic regression analysis on wing length data was carried out where small and large flies of the variable "Size" in the data frame were coded as "0"and "1", respectively under a new variable name "Size code". The new variable "Size code" was then used as a response variable in a series of models starting with one having the variable "Method" as the only independent variable, and successively adding other variables. The model that had the lowest AIC value was chosen for use in the analysis. The method for estimating the minimum sample size for logistic regression analysis (number of events per variable (EPV) in observational studies, (Bujang et al., 2018; Peduzzi et al., 1996) was used to check the sufficiency of data that met the selection criteria for logistic regression analysis. Because of size differences between male and female flies (Glasgow, 1970), their data were analyzed separately. R statistical software, version 3.2.2 (2015-08-14) was used in data analysis.

3. Results

A total of 3,585 *G. m. morsitans* were caught (3,191 by fly rounds and 394 by traps), (Mweempwa, 2015). Out of the 3,191, 2006 had their wing length measurements taken and out of the 2006 these, 1,223 (60.9%) met the selection criteria for this study as mentioned under data analysis sub-title. For epsilon traps, out of 394 flies, 286 had their wing length measurements taken out of which 219 (76.6%) met the selection criteria. In total 1,442 (62.9%) wing length

measurements taken on *G. m. morsitans* met the selection criteria from the two methods (953 males and 489 females), Table 1 and 2.

Table 1: Numbers of measurements at different locations

Method	Zinaka		Lusandwa		Chisulo	
	Female	Male	Female	Male	Female	Male
Trap	34	15	84	38	10	38
PMFR	171	319	177	395	13	148
Total	205	334	261	433	23	186

Table 2: Numbers of measurements in different months.

	Females		Males	
Months	PMFR	TRAP	PMFR	TRAP
November	32	2	0	0
December	37	13	167	7
January	47	14	36	11
February	46	19	88	7
March	42	25	114	19
April	41	29	76	11
May	10	7	66	9
June	17	8	38	8
July	26	4	137	8
August	30	4	106	3
September	0	0	21	3
October	33	3	13	5
Total	361	128	862	91

The distribution of numbers of wing length measurements used over ovarian age and wing fray categories for female and male flies, respectively, is shown in Fig 3. Fig 3 shows a similar distribution of measurements for both PMFR and trap caught male flies with numbers used being the main differences, since the methods used resulted in PMFRs capturing higher numbers of males than traps. For females the distributions of measurements were different and as in males PMFRs had higher numbers of captured females than traps.

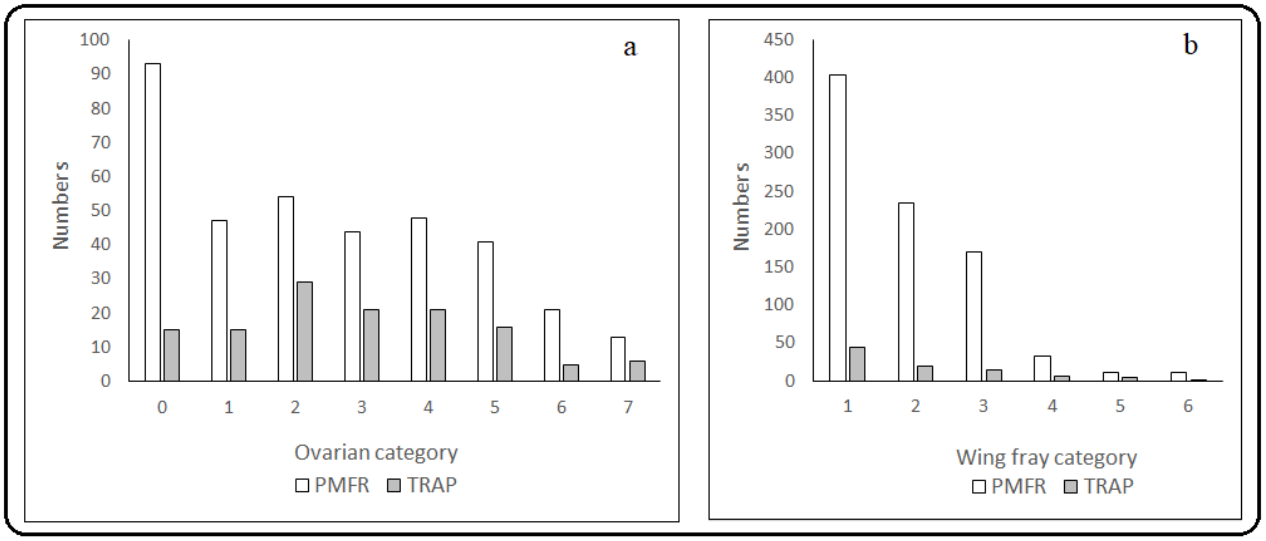


Figure 3: *Distribution of numbers of wing length measurements of G. m. morsitans over ovarian and wing fray categories. a Females and b Males.*

Numbers of flies were plotted over wing length, Fig 4. The distribution for both female and male data showed similar distributions for both PMFR and trap caught flies. For females PMFRs had higher numbers than traps to the left of 1.9 mm wing length and for traps it was the reverse. For male flies a similar picture is seen around a wing length of 1.85 mm.

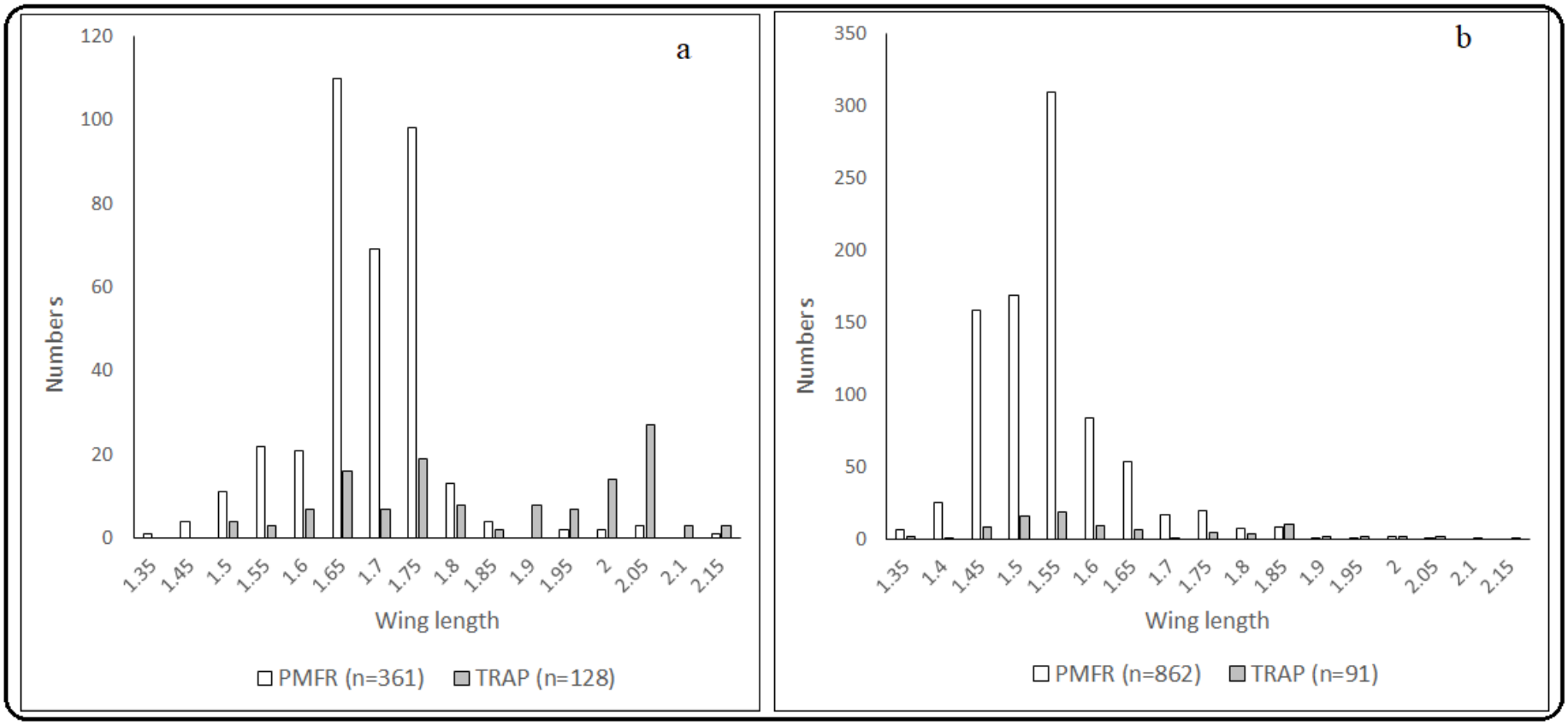


Figure 4: *Distribution of numbers of wing length measurements of G. m. morsitans over wing length. a Females and b Males.*

The Shapiro-Wilk Normality Test on wing length of male or female flies from PMFRs or traps showed that whether caught by PMFRs or traps, wing length in *G. m. morsitans* was not normally distributed ($w = 0.89313$ and 0.92021 for wing length of female flies caught by PMFRs and traps, respectively, $p<0.0001$. In males $w = 0.87383$ and 0.9043 for wing length of flies caught by PMFRs and traps, respectively, $p<0.0001$). Normal Q-Q plots showed similar results, but being not normally distributed was more prominent for male than female data.

The wing length in female flies caught by PMFR ranged from 1.35 mm to 2.15 mm with a median at 1.70 mm, Fig 5. The range in flies caught by traps was from 1.50 mm to 2.15 mm with a median at 1.82 mm. In males, wing length ranged from 1.35 mm to 2.05 mm with a median at 1.55 mm in PMFR caught flies and from 1.35 mm to 2.15 mm also with a median at 1.55 mm in trap caught flies. The distribution of wing length of *G. m. morsitans* females caught in traps was shifted to the right or high in comparison with those caught by PMFR, Fig 5. In male flies, the two distributions had similar medians.

The wing length means of male and female flies caught by PMFRs were significantly lower than the means of corresponding sexes caught by traps as observed by lack of overlap of 95% confidence intervals, Fig 6. In PMFR caught males and females the means were 1.54 mm and 1.69 mm with 0.003 and 0.005 standard errors, respectively, while in trap caught flies, respective means were 1.64 mm and 1.85 mm with 0.019 and 0.016 standard errors, respectively. The difference in mean wing length between fly-round and trap caught females was 0.16 mm which was 9.26% of the overall mean of 1.73 mm (mean of fly round and trap caught females together). In males the difference was 0.1 mm which was 6.45% of the overall mean of 1.55 mm.

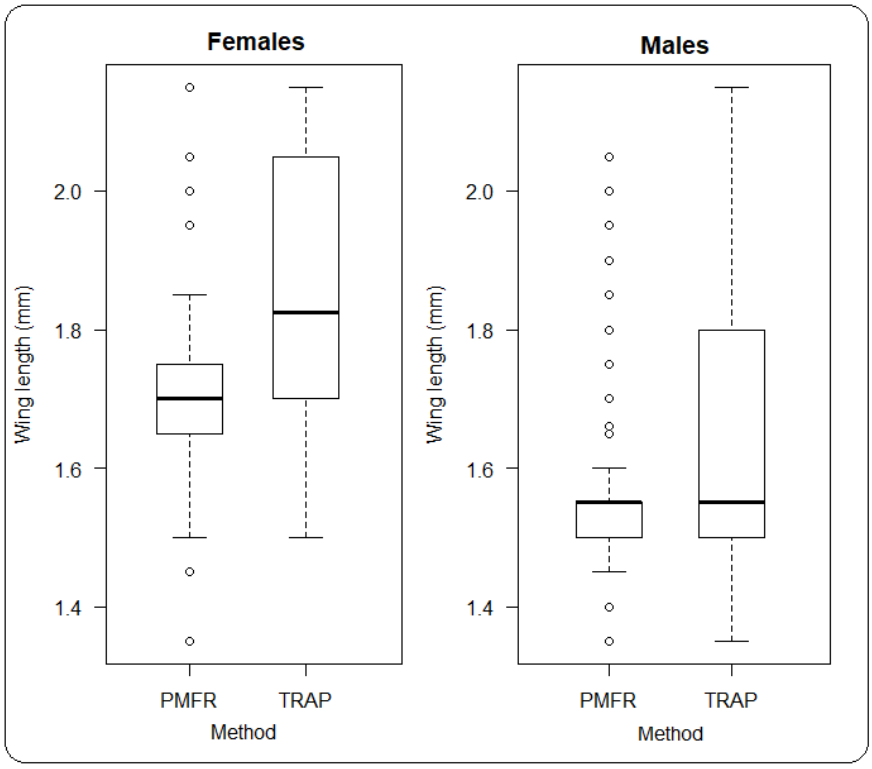


Figure 5: Spread of wing length about the median for female and male flies.

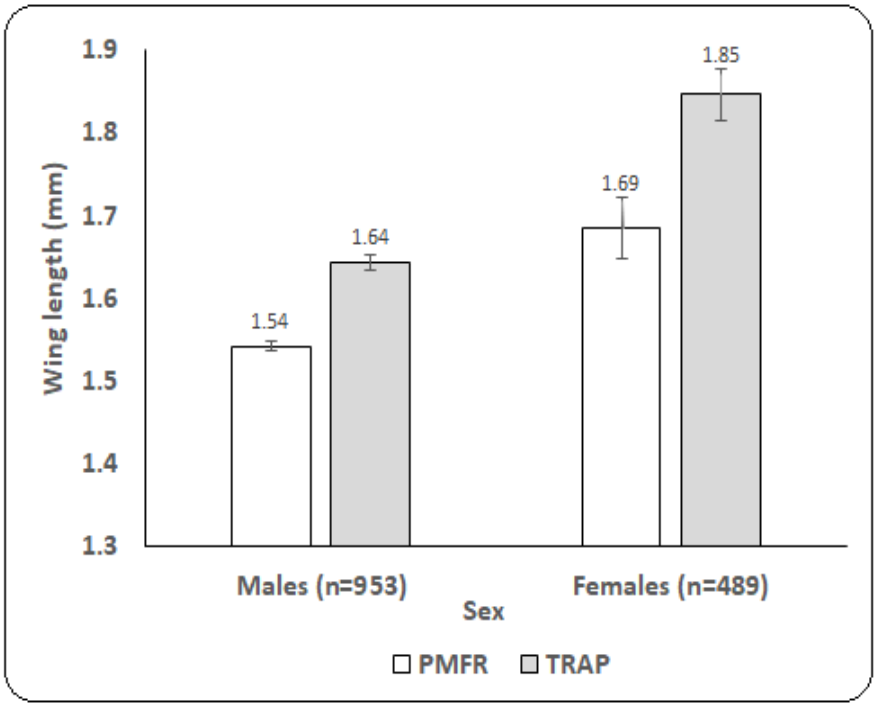


Figure 6: *Mean wing length of male and female flies caught by PMFRs and traps.*

Four multiple logistic regression models were run on female data and another four on male data. In both sets of models, the four independent variable model had the lowest AIC value. The range of AIC values was 610.97 – 627.22 for models on female data and 1018.4 – 1056.7 for models on male data. A sample size of 300 was calculated to be the minimum required to run a logistic regression analysis with four independent variables (Bujang et al., 2018 and Peduzzi et al., 1996) and thus a multiple regression analysis was carried out on both male and female data using: Method; Ovarian category or Wing fray; Month of capture and Study site as independent variables and size code 0 (for small) and 1 (for large), as a response variable. Regression analysis results showed an association between sampling method and whether the fly caught was large or small. They showed that female flies caught by traps were 5.088 times (95% CI: 3.138-8.429; $p < 0.0001$) more likely to be large ones (relative to small ones) than those caught by PMFR while males were 2.563 times (95% CI; 1.584-4.148; $p < 0.0001$) more likely to be large ones than those caught by PMFR, Table 3, thereby rejecting the hypothesis. In other words, as one moves from PMFR to trap catches, the likelihood that a caught female or male fly was large, relative to being small, significantly increased by 5.088 and 2.563 times, respectively.

Table 3: Logistic regression analysis results

Variable	Females				Males			
		95% CI		p-value		95% CI		p-value
	Odds ratio	Lower	Upper		Odds ratio	Lower	Upper	
Trap	5.088	3.138	8.429	0.0001***	2.563	1.584	4.148	0.0001***
Ovarian or wing fray category								
0	Reference							
1	0.960	0.460	1.983	0.913	Reference			
2	1.156	0.598	2.232	0.665	1.099	0.743	1.616	0.633
3	1.385	0.682	2.819	0.367	1.285	0.831	1.974	0.255
4	0.887	0.434	1.796	0.739	2.353	1.123	4.841	0.021*
5	2.369	1.132	5.022	0.023*	1.925	0.612	5.592	0.238
6	2.843	1.091	7.506	0.032*	1.315	0.323	4.461	0.676
7	1.186	0.378	3.546	0.763				
Month								
April	Reference							
November	0.398	0.119	1.216	0.117	-	-	-	-
December	0.767	0.334	1.749	0.529	0.757	0.389	1.488	0.415
January	0.502	0.225	1.101	0.088	1.259	0.529	2.922	0.595
February	1.012	0.475	2.155	0.976	1.668	0.865	3.266	0.13
March	1.636	0.772	3.505	0.201	1.39	0.73	2.687	0.321
May	0.444	0.097	1.737	0.261	2.587	1.221	5.567	0.014*
June	1.210	0.395	3.688	0.737	2.215	0.921	5.33	0.075
July	0.949	0.318	2.783	0.924	1.322	0.679	2.606	0.415
August	2.025	0.824	5.076	0.126763	0.798	0.323	1.931	0.62
September	-	-	-	-	0.344	0.052	1.343	0.177
October	0.672	0.233	1.894	0.456	0.488	0.121	1.64	0.271
Study site								
Chisulo	Reference							
Lusandwa	6.113	1.862	23.345	0.004**	0.511	0.323	0.809	0.004**
Zinaka	4.321	1.323	16.290	0.021*	0.359	0.22	0.583	0.0001***

**P<0.05, **P<0.01, ***P<0.001. Small and large female flies (less or greater than 1.73 mm wing length) coded as "0" and "1", respectively. The respective wing length for male flies is 1.55 mm.*

Further, such increase in likelihood of a large female being caught relative to a small one , ranged from 1.156 – 2.843 times over ovarian categories 2, 3, 5, 6 and 7 and this was significant for category 5 and 6 relative to category 0, the reference category, $p < 0.04$. However, the likelihood reduced insignificantly by 4.0% and 11.3% for ovarian categories 1 and 4, respectively, $p > 0.700$.

In male flies, the likelihood increased over the whole range of wing fray categories and was significant for wing fray category 4 relative to wing fray category 1, the reference category, $p = 0.021$. As regards month of capture, no significant increase or reduction in the likelihood was observed relative to the likelihood in April, the reference month in females. For males, a significant increase by 2.587 times was observed in the month of May, $p = 0.014$. For females significant increases in likelihood were observed at Lusandwa and Zinaka, 6.113 and 4.321 times, respectively, relative to the likelihood at the most fragmented site Chisulo, $p < 0.005$ while the reverse was true for males. Monthly means of wing length of all flies caught by PMFR and traps (herein referred to as Overall joint means) and means of wing length above and below the overall joint means (herein referred to as upper or lower joint means, respectively) were calculated as in Hargrove et al., (2019). The means in the upper half were 5.2 - 19.5% greater than those of the lower half in females, Fig 7a and were 7.0 - 18.9% greater than those in the lower half in males, Fig 7b. The largest deviation from the overall joint means occurred in the rainy season for both sexes, with a peak in March for females and in January for males. The absolute means in the lower half remained relatively constant in deviation with the overall averages during the whole study period.

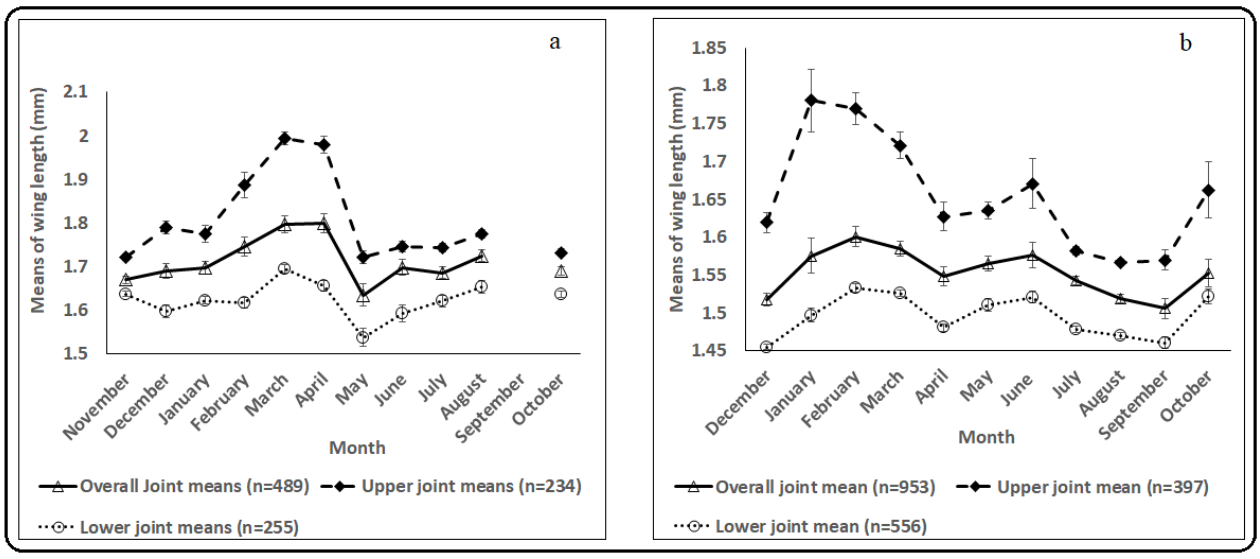


Figure 7*: Means of wing length in the upper and lower half. a upper and lower joint means for female flies. b upper and lower joint means for male flies*

Proportions of male or female flies with wing length in the upper half for each method were separately calculated. In March and April the proportions of trap-caught females with wing length in the upper half were significantly greater than those caught by PMFRs and the proportions of those caught in traps were always greater except in May, July and October (thus they were more numerous in 8 of the 11 months) Fig 8a. For males caught in traps, proportions of those with wing length in the upper half were significantly greater in January and June than for those caught by PMFRs, and proportions of those caught in traps were always greater except in October (thus they were more numerous in 10 of the 11 months) Fig 8b.

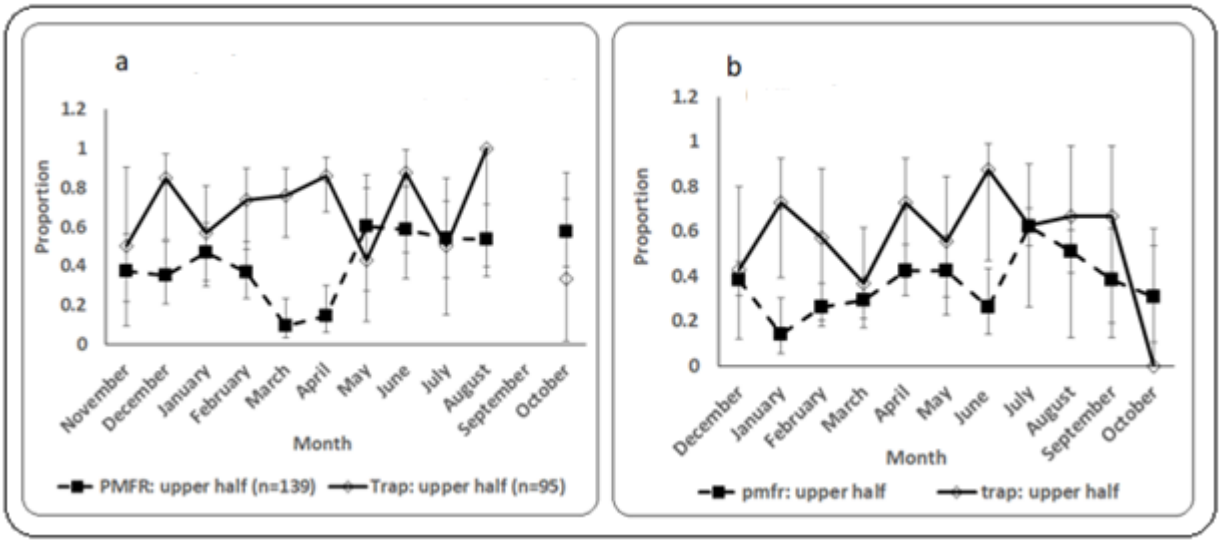


Figure 8*: Proportion of flies with wing length in the upper half of the mean wing length of flies caught by each method separately. a females. b males.*

4. Discussion

This study has added body size among tsetse variables on which sampling by available methods is biased towards certain sections of populations. Analysis of data on *G. m. morsitans* collected by epsilon traps and man operated black screen fly rounds in eastern Zambia has shown significantly different likelihoods of capturing individuals of different sizes and therefore, significant differences in wing length means between samples of the two methods. The analysis has shown reason not to accept the hypothesis that the body size (measured as wing length) of *G. m. morsitans* captured by epsilon traps and fly rounds is the same, Fig 6, Table 3, Fig 7a and b. Probably, this is because, larger tsetse flies are the most mobile (Vale et al., 1984). Since traps are stationary, an active, host seeking tsetse fly is more likely to find them. As tsetse flies of larger species or sex are the most mobile (Vale et al., 1984) and most available to stationary odour baits (Vale et al., 1974b), they are the most likely to find stationary traps. Hence the observed variability of wing length between trap and fly round caught flies. Further, since this study has

shown that traps capture larger *G. m. morsitans* of the same sex, it suggests that larger flies of the same sex and species are also the most mobile.

The significant increase in likelihood of a large *G. m. morsitans* female being caught relative to a small one in Lusandwa and Zinaka compared with that in Chisulo, Table 3, and the significant reduction of the same in male flies showed that with pooled PMFR and trap data, Chisulo had a lower and higher proportion of large female and male flies, respectively, compared with proportions at the other two sites (lowest and highest at Chisulo and Lusandwa, respectively for females and lowest and highest at Zinaka and Chisulo for males), Fig 9a. In females, the proportion of large flies at Chisulo was significantly different with the proportion at the least fragmented study site Lusandwa ($\chi^2 = 7.3788$, df = 1, p-value = 0.007), whereas in males the difference was significant with proportions at both other sites ($\chi^2 = 48.32$, df = 2, $p < 0.0001$). However, graphs from separated PMFR and trap data showed that, in relation to fragmentation levels at study sites, the proportions of large females from PMFRs had a similar pattern (though insignificant between sites) as with the pooled data, but proportions for males were the reverse to those of females (lowest and highest at Lusandwa and Chisulo, respectively) and were significant between the proportion at Chisulo and the other two sites, Fig 9b. For traps, proportions of large females were lowest and highest at Zinaka and Lusandwa respectively and were significant between the proportion at Lusandwa and those at the other two sites, Figure 9c. The same pattern occurred in males. It is evident from the three graphs that the proportion of large females tended to increase with reducing fragmentation while that of large males increased and reduced when sampling was carried out with traps and fly rounds, respectively. This is suggestive that large female *G. m. morsitans* tend to shun fragmented areas while in males the ecogeographic

distribution of large and small ones seems to be dependent on the method used to sample them.

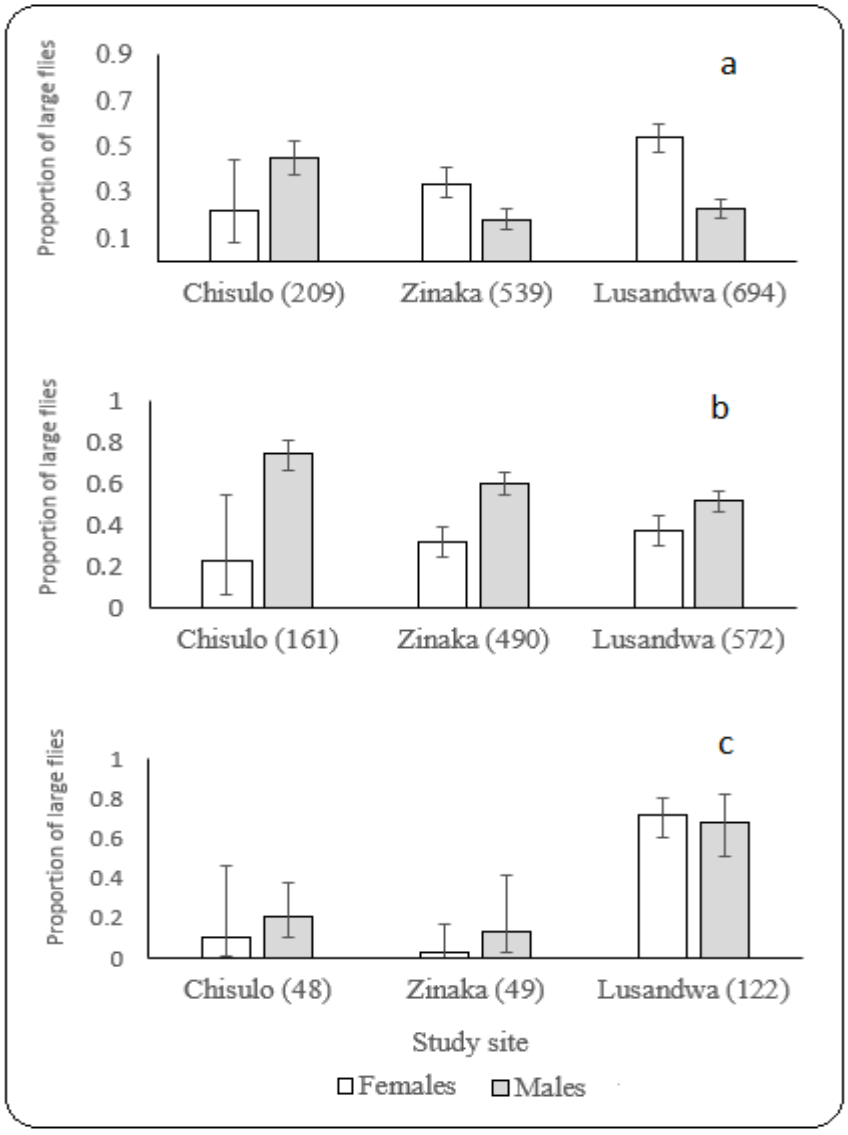


***Figure 9**: Proportions of large flies at study sites. a pooled methods data. b PMFR data. c trap data. Number in brackets is sample size.*

The paucity of good, shady breeding grounds in fragmented areas was thought to be one of the factors that determine the ecogeographic distribution of female flies but since it affects both small and large females yet their ecogeographic distributions were not similar, suggests that the paucity of good breeding grounds is not the main factor that determines their ecogeographic distribution. Probably it is the variation in temperature and saturation deficit between fragmented and non-

fragmented areas. For males, segregation against small male flies, especially in the hot dry season (Phelps and Clark, 1974) was thought to be among the reasons for a high proportion of large male flies at the most fragmented site, but this was contradicted by results obtained by carrying out same calculations using rainy season data only (season when segregation of small males is at its minimum, if present). It appears a plausible explanation is that while traps become less available to less mobile small males in non-fragmented areas (thereby raising the proportion of large ones in traps there, relative to the proportion in fragmented areas), fly rounds become more available to them, thereby lowering the proportion of large ones in fly rounds there, relative to the proportion in fragmented areas. The reverse can be said for fragmented areas. The change in proportion of different sizes within short distances seems to be consistent with what Vale and Cumming (1976) reported that the mean size of tsetse can differ in localities just a few kilometers apart.

The lack of similarity in distributions of numbers of ovarian age categories between PMFR and trap caught females, Fig 3a, could be attributed to age sampling bias (Hargrove, 1991 and Warnes, 1997) towards young females inherent in the PMFR. It appears that if PMFRs were not biased in favor of young female flies, especially of ovarian category 0, the distribution pattern of numbers of ovarian categories for the two methods were going to be approximately the same, just as the patterns of numbers of wing fray are the same in males, Fig 3b. The bias of traps in favor of old female flies seems to have the highest impact in the middle ovarian categories 2-5. Had it not been for the biases of the two methods, the distribution patterns would generally look correlated to the declining numbers in the population as the age of female flies increases. The distribution of wing length occurrences for females and males, Fig 4a and b, respectively, clearly demonstrates the bias of traps in favor of large flies in both sexes relative to the fly round method. This is in support of significantly higher mean wing length for trap caught flies as shown in Fig 6 and the significantly

higher likelihood of a large fly being caught in a trap than a small one relative to fly rounds as shown in Table 3.

The absolute means of wing length in the upper half, Fig 7, deviated farthest from the overall averages in the rainy season in both females and males. The rising parts of Fig 7a and b in the rainy season show a steeper rise for males than for females, suggesting that the response rate in body size adjustment to prevailing and changing conditions (which in this case is an increasing body size as the rainy season progresses (De Deken et al., 1997; Van den Bossche 1999) was higher in males than in females, hence an earlier attainment of a peak mean wing length in males (January) than in females (March). Female flies have longer lifespan than males (FAO, 1982) and so the late attainment of the peak mean wing length could also be due to longer lifespan.

The gap over most months between graphs of Fig 8, both being graphs of proportions of wing length in the upper half of each method separately, demonstrates the superiority of traps in catching larger flies than fly rounds.

It is worth noting that the study had shortcomings but had to be done anyway because the data used were the only available at the time. Firstly, the study used data collected from a study not specifically designed to test the hypothesis. A study specifically designed to test the hypothesis would for example compare a stationary screen (electrocuted or with glue) to a mobile screen instead of an epsilon trap (which is completely different from a screen in not only architecture but also in mode of action). Secondly, in the logistic regression analysis the study used wing length means obtained by combined data from mobile screens and epsilon traps. In the opinion of authors, the best wing length means to use, would have been the respective true populations means for the two sexes. Since the true population mean may not be easily attainable, a stationary trap e.g. the epsilon trap may be incorporated as a reference method in a study design where the size of

flies caught by a mobile screen method are compared with the size of those caught by a stationary screen method in all seasons. If hot dry season data only is required, then the refuge trap (Vale, 1971) may be used as a reference method as it gives a more representative mean wing length of the population. The use of monthly wing length means from flies caught by one method and calculating the proportion of those with wing length in the upper half of the mean for flies captures by that method, Fig 8, may have produced more deviated results from the true population mean, given that sampling methods have known inherent sampling biases (Hargrove, 1991 and Warnes, 1997). Further rigorous studies are recommended.

Carrying out studies on whether the predilection of traps to capture larger flies has an effect on the process of tsetse elimination when targets are used would provide a basis to make a decision on whether the suggestion by Mbewe et al., (2018) that the rare reports of successful elimination of tsetse populations when targets are used alone (Meyer et al., 2016 and Vreysen et al.,2013) may be due to selective killing of tsetse based on size is correct or not. In the absence of such a study there should be no reason to worry about use of targets to eliminate populations of tsetse as examples of such elimination exist (Vale et al., 1986; Vale et al., 1988; Willemse, 1991 and Hargrove, 2003).

5. Conclusion

This study has shown that epsilon traps are biased towards capturing larger *G. m. morsitans* tsetse flies compared with fly rounds. Therefore, further research is recommended to verify (i) whether the predilection of traps to capture larger flies has an effect on the process of tsetse elimination when targets are used e.g. targets may take longer to reach elimination than if the predilection was not there, (ii) whether different results can be obtained on ecogeographic distribution of different sizes of the species if fly rounds are used for sampling instead of epsilon traps. The results from such studies could influence the strategies used in future control operations.

Consent for publication

Not applicable.

Availability of data and materials

All datasets used and/or analysed during the current study are available from the corresponding authors upon reasonable request.

Competing interests

The authors declare that they have no competing interest.

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Authors' contribution

CM conceived the study, collected samples, analysed the data and wrote the draft manuscript. NJM, RDD critically revised the manuscript. All authors read and approved the final version of the manuscript.

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