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Preliminary study of the spatial distribution of *Glossina fuscipes fuscipes* (Diptera: Glossinidae) in the Bipindi Forests and surrounding areas (South Cameroon)

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Abstract
An entomological study was conducted in the Southern region of Cameroon, one of the most infested areas of African human trypanosomiasis, to determine the spatial distribution of the primary biological vector of trypanosome species, *Glossina fuscipes fuscipes*. In September 2022, twelve Vavoua traps were set up early in the morning before 8 a.m. in three types of habitats (anthropized areas, streams, and paths) in Bipindi, Lambi, and Memel, localities in the Ocean Division, and traps inspected after 10 to 11 hours of exposure. A total of 141 hematophagous flies (116 specimens, 82.27%, belonging to the Glossinidae family and 25 individuals, 17.73%, to the Tabanidae family) were collected. The dominant species, *Glossina fuscipes fuscipes*, was present in all environments with an apparent trap density of 0.805. This species was captured more in the Lambi locality (65.52%) and in the habitat representing the trails (50.86%), as well as in the Lambi trails (43.10%). In the study site, females of *Glossina fuscipes fuscipes* were more abundant than males, with a sex ratio of (F/M) = 1.83. These results confirm that the abundance and dominance of Glossina fuscipes fuscipes species vary significantly with inspected localities and habitats in Bipindi forests and surrounding areas.

Keywords: Abundance, glossina, trail, sex ratio, hematophagous vectors

Introduction
The locality of Bipindi is a historical hotbed of Human African Trypanosomiasis. Until the 1990s, only the Fontem, Mamfé, and Campos outbreaks were considered active HAT outbreaks (Grebaut *et al*., 2001). The first suspected cases of resurgence occurred in 1994 in Lolodorf and Kribi. Between 1998 and 1999, several trypanosomiasis cases were detected in the villages of Lambi and Bijouka, with a prevalence reaching 3.5% (Grebaut *et al*., 2001). Despite efforts to neutralize the pathogen, new HAT cases are still diagnosed. Thus, screening campaigns conducted by OCEAC researchers repeatedly revealed positive HAT cases. On June 21, 2021, the HAT team organized a screening campaign in the district of Bipindi (CMA registry of Bipindi; Lollo, personal communication); it included three villages (Bijouka, Bohgouama, Ebiminbang). This screening campaign highlighted five positive cases (despite the population's reluctance to voluntary testing), including one case in Bidjouka, three in Ebimimbang, and two in Bohgouana. These new cases sufficiently demonstrate the phenomenon of hat resurgence, challenging the health authorities. Most of the studies carried out in the locality of Bipindi have aimed to eradicate the pathogen of trypanosomiasis; very few have something to do with vectors (Biology, Ecology, Ethology, Genetics, etc.), which play an essential role in human and animal health (Mavoungou *et al*., 2008) [12], given their involvement in the vector transmission of infectious agents. Indeed, tabanids are vectors of Loa loa filariasis transmitted by Chrysops silacea Austen 1907 and Chrysops dimidiata Austen 1906 (Wanji *et al*., 2002; Fain, 1978) [8, 16]. Numerous species of the genus Glossina, male and female, ensure the transmission of HAT to humans (Cren-Travail & Rotureau, 2016; Zinga Koumba *et al*., 2015; Bosson-Vanga *et al*., 2012; Mbida Mbida *et al*., 2009; Courtin *et al*., 2005; Aksoy, 2003; Laveissière *et al*., 2000) [2, 3, 5, 6, 12, 15, 21]. The impact of the invasion of these blood-sucking flies has repercussions on the economies of several countries (Abdoulmoumini *et al*., 2015; Mamoudou, 2008) [1, 13]. Their control, therefore, appears to be a privileged means in the fight against vector-borne diseases. However, few vector control operations have been successful to date. The cause of these failures is insufficient knowledge of the vector and its environment, resistance to an increasing number of insecticides, or an inadequate control strategy. Knowledge of fluctuations in abundance and distribution research is fundamental in improving strategies and methods for controlling these blood-sucking insects. Thus, health officials should focus more on forest areas with disease outbreaks when controlling trypanosomiasis vectors. For example, Mounioko *et al*. (2018) [16] reported that Glossina fuscipes fuscipes species is the primary vector of HAT in forest areas. The conditions in this area are favorable for the development of the vector. Also, logging and hunting are the primary activities in Bipindi (Melombo, Ndoua, Madounga, etc.), where several headquarters of companies (BOISCAM, CUF, FEEMAM, CIFOA, LFM, and several illegal exploitations). These activities promote a permanent flow of populations between the forest (Trypanosome reservoir and natural habitat of tsetse flies, mechanical vectors of HAT) and the villages, with the possible consequence of an increase in the probability of host-vector encounters and pathogen exchanges between humans, fauna

and the environment (Epstein *et al*., 1993) [7]. Therefore, within this context, an entomological study based on the spatial distribution of Glossina fuscipes fuscipes was conducted near the Bipindi forestry exploitation in southern Cameroon.

Materials and methods

Study area

In September 2022, a prospective study was conducted in three localities in the district of Bipindi (Bipindi, Lambi, and Memel). The district of Bipindi (3°4'23.5902 N, 10°24'15.71652 E) is located about 75 km from the Atlantic Ocean, in the Southern Region of Cameroon in the Ocean division (Figure 1). The site has an equatorial climate characterized by four seasons. The climate is equatorial; the landscape is marked by the predominance of dense evergreen forest with, around the houses, an interweaving of several facies (fields, fallows, cocoa fields, forest islands, streams, and lowlands) constituting the village terroir, irrigated by a particularly dense hydrographic network, and fed by numerous streams from the Ngovayaang massif, supplying was to the villages (Grebaut *et al*., 2001).

Methodology

Sampling

Trap Layout

Tsetse and blood-sucking flies were captured using Vavoua traps (see Laveissière & Grebaut 1990). This monoconical trap has a mesh cone with a string at the top for support and is visually attractive. Thirty-three Vavoua traps were set (Figure 1) at a rate of 12 per locality (Bipindi, Lambie, and Memel) in anthropogenic environments, river banks, and paths. The anthropogenic environments are sites where people regularly gather for recreation or relaxation after activities. The activities around the banks of the rivers include bathing, laundry, washing dishes, or other activities such as digging sand. Trails are paths regularly used by the inhabitants of the study area to get to the water, the field, or the forest. In each chosen habitat, a system of three Vavoua traps at least 500 m apart was installed in order to take into account the diversity of the habitats of each trapping site. The traps were surveyed daily at 12 p.m., and the blood-sucking insects captured were transported to the laboratory for further identification.

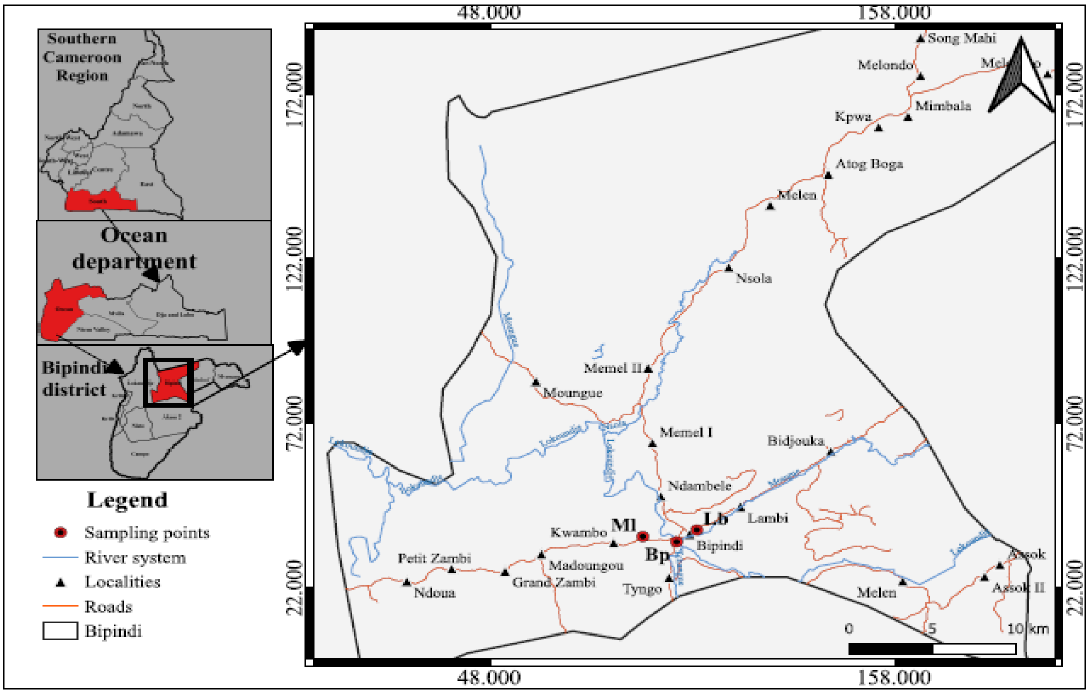

Fig 1: Different study areas showing the position of Vavoua traps.

Vector capture and identification

During the daily surveys carried out at noon during the work period by prospected biotope, captured tsetse and other blood-sucking flies were put into the vials with labels bearing the date, locality, and biotope of collection and transported to the Special Mission for the Eradication of Glossins, Ministry of Livestock, Fisheries and Animal Industries (MINEPIA), Yaoundé. The captured insects were put in pill boxes containing 70 % alcohol for further identification and counting in the laboratory. For this purpose, tsetse flies and other biting flies were sorted and separated from other insects, then counted and identified using a binocular magnifying glass and relevant u identification keys published by Brunhes *et al*. (1998) [4] and Pollock, 1992. [15] The shape of the lower forcipules allowed effective identification of *Glossina fuscipes fuscipes* species.

Statistical analysis

Abundance of mechanical vectors

The data were analyzed directly by considering the absolute abundances observed « n_i » or converted into binary presence/absence (+/-) data. The relative abundance of a species "i" noted $p_i = \frac{n_i}{n}$ is the proportion of occurrence of that species "i" relative to the total number of individuals of all species captured "n". p_i being a pure number, we express it as a percentage, i.e. $p_i = \frac{n_i}{n} \times 100$. As part of the study of the composition of the fly community, we determined species richness and dominance. The absolute abundance data were arranged in descending order to construct the diagrams. To assess the richness of the fly community studied, seven statistical indices were determined: (1) the number of taxa caught (or observed wealth) "S_{obs}"; (2) the total number of specimens captured (or sample size) "$n = \sum n_i$"; and (3) the Berger-Parker dominance index "$D = \frac{n_{max}}{n}$". The abundance of the tsetse species studied was reflected by its bulk density per trap and per day (DAP) calculated according to the following formula

$$DAP = \frac{Number\ of\ tsetse\ fly\ captured}{Number\ of\ traps * number\ of\ day\ of\ captures}.$$

Sex-ratio

The sex ratio is the ratio of the number of males to the number of females in a population. It is calculated according to the formula

$$\frac{Number\ of\ males}{Number\ of\ females}.$$

Diversity index

Diversity represents the richness associated with the distribution of individuals by species in the community. Diversity was assessed using the Shannon-Weaver Diversity Index "H'";

$$\hat{H}' = -\sum_{i=1}^{S} p_i Log_2(p_i).$$

Regularity index

The regularity of the taxa from the communities represents the regularity of the distribution of individuals among the different taxa of the community. We assessed species regularity using Pielou's Regularity Index "

$$E_1 = \frac{H'}{H'_{max}}$$

Results

Abundance and composition of flies caught

Overall, 141 blood-sucking flies belonging to the families Glossinidae and Tabanidae were captured, including 89 biting flies (63.12%) in Lambi, 45 (31.93%) in the village of Memel, and 7 (4.96%) in Bipindi. The highest catch was observed in Lambi and the lowest in Bipindi (Figure 2). About 46.81% of Dipterans were recorded in the trails, 52 (31.88%) in streams, and 23 (16.31%) in anthropogenic areas. The highest sampling was observed in streams, while the lowest was in anthropogenic regions (Figure 3). The Kruskal-Walli's test shows no significant difference between the three locations ($p = 0.0822$) or the three habitat types ($p = 0.5775$). Of the vectors captured, 116 individuals, or a frequency of 82.27%, were tsetse flies, and 25 specimens, or 17.73%, were tabanids.

During the trapping sessions, 4 species of Tabanidae and one species of Glossinidae were identified. Species of the Tabanidae family belong to the genera *Tabanus* and *Chrysops*. The genus *Tabanus* had had three species, while the genus Chrysops had only one species: Chrysops sp. For the Glossinidae, only *Glossina fuscipes fuscipes* species were identified.

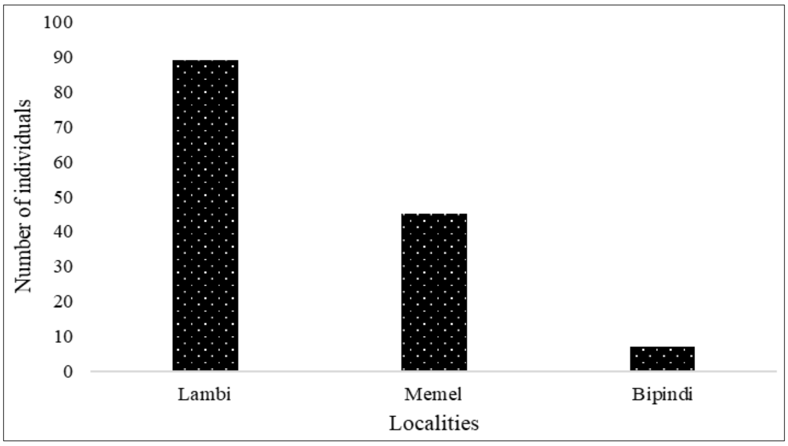

Fig 2: Distribution of blood-sucking fly individuals captured per locality, in September 2022 in the Ocean Division

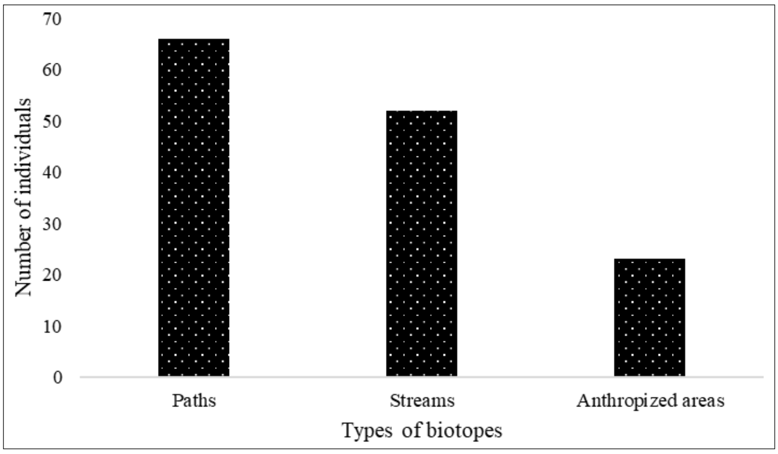

Fig 3: Distribution of bloodsucking fly individuals captured per habitat type in September 2022 in the Ocean Division

Diversity of blood-sucking flies by locality and habitat type

The highest catch was recorded in Lambi (63.12%) and the lowest in Bipindi (4.96%). However, the Shannon diversity index was higher at Memel (H' = 0.7893) compared to Bipindi (H' = 0.5983) and Lambi (H' = 0.4158). Similarly, the Piélou equitability index shows that blood-sucking flies were more homogeneously distributed in Bipindi (E = 0.8631) and Lambi (E = 0.5999), while in Memel (E = 0.4904), the distribution of flies was heterogeneous. In Bipindi, five (5) species of blood-sucking flies were recorded against two (2) species in each of the localities Lambi and Memel. The Berger-Parker index indicates the dominance of *Glossina fuscipes fuscipes* at all three locations (Table I).

The highest catch of Diptera was in rivers (46.81%), and the lowest was in anthropogenic environments (16.31%). However, the Shannon diversity index was higher in anthropogenic areas (H' = 1.273) compared to trails (H' = 0.3382) and Rivers (H' = 0.2712). Similarly, the Piélou equitability index shows that blood-sucking flies were more evenly distributed in anthropogenic areas (E = 0.7907), while the distribution in trails (E = 0.4879) and rivers (E = 0.3912) was heterogeneous. The anthropogenic environments had five (5) blood-sucking fly species, while trails and rivers had two (2) species. The Berger-Parker index indicated a dominance of *Glossina fuscipes fuscipes* in trails and rivers (Table I).

Table 1: Diversity indices of blood-sucking flies caught by locality and habitat types in the Ocean Division, in September 2022

	Locality			**Habitat types**		
Indices	Memel	Lambi	Bipindi	Anthropized areas	streams	paths
Species richness	5	2	2	5	2	2
Shannon index	0,7893	0,4158	0,5983	1,273	0,2712	0,3382
Pielou index	0,4304	0,5999	0,8631	0,7907	0,3912	0,4879
Berger-Parker index	0,7778	0,8539	0,7143	0,3913	0,9231	0,8939

Abundance and sex ratio of *Glossina fuscipes fuscipes*

A total of 116 specimens of *Glossina fuscipes fuscipes* were recorded, with a bulk density of 0.805 /trap/day. Of these, 76 (65.52%) were captured in Lambi, 35 (30.17%) in Memel, and 5 (4.31%) in Bipindi. The highest catches were from Lambi and the lowest from Bipindi (Figure 4). Fifty-nine individuals (50.86%) were sampled in the trails, 48 (41.38%) in the rivers, and 9 (7.76%) in anthropogenic environments. The maximum catches were from the trails, and the minimum was from anthropogenic environments (Figure 5). Kruskal-Walli's test showed no significant difference between the three locations (p = 0.1112) or the three habitat types (p = 0.3373).

The bulk density per trap at Lambi, Meme, and Bipindi was 0.527, 0.243, and 0.035 Glossina fuscipes fuscipes/trap/day, respectively. The bulk density in trail, river, and anthropogenic environments was 0.41, 0.3, and 0.063 *Glossina fuscipes fuscipes*/trap/day, respectively. In the anthropogenic environment, the bulk density was 0.05 and 0.007 *Glossina fuscipes fuscipes*/trap/day in Memel and Limba, respectively. In trials, the bulk density was 0.0625 and 0.3472 *Glossina fuscipes fuscipes*/trap/day in Memel and Lambi, respectively. In river banks, the bulk density was 0.125, 0.17361, and 0.03472 *Glossina fuscipes fuscipes*/trap/day in Memel, Lambi, and Bipindi, respectively.

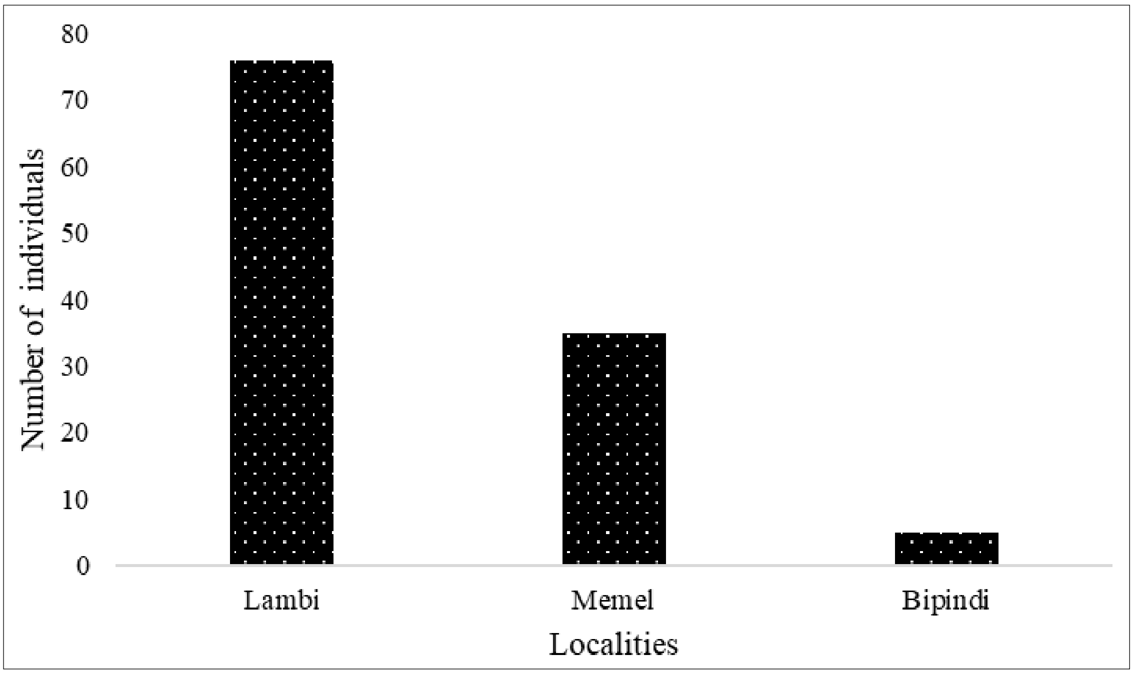

Fig 4: Distribution of *Glossina fuscipes fuscipes* individuals captured per locality in September 2022 in the Ocean Division.

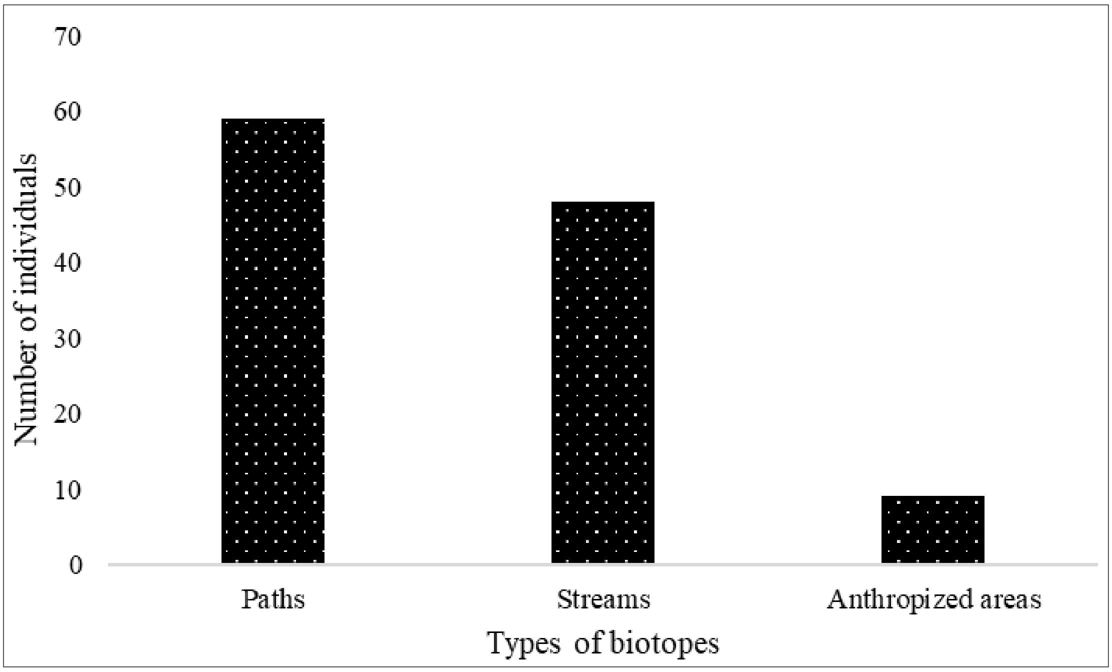

Fig 5: Distribution of individuals of *Glossina fuscipes* captured per habitat type in September 2022 in the Ocean Division.

Depending on the anthropogenic environments, the population of Glossina fuscipes fuscipes was eight individuals (6.90%) in Memel and one (0.86%) in Lambi. None of the individuals were present in Bipindi. For trails, 59 individuals were present in specimens, 50 in Lambi (43.10%) and 9 in Memel (7.76%). Finally, in the rivers, 48 individuals were captured, including 25 in Lambi (21.55%), 18 in Memel (15.52%), 5 in Bipindi (4.31%), and no specimens in Bipindi (Figure 6).

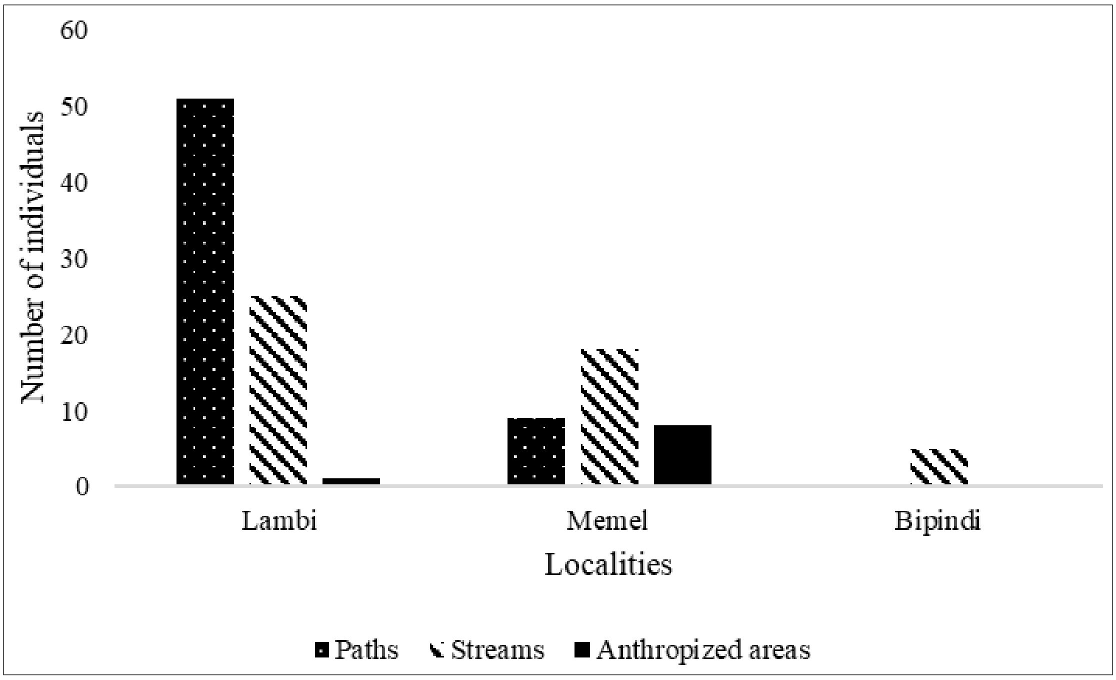

Fig 6: Distribution of Glossina fuscipes captured in September 2022 in the Ocean Division

Generally, females were more abundant than males, with a sex ratio of (F/M) = 1.83. This value varied according to the habitat. Indeed, in Lambi, the sex ratio (F/M) was 2.45. However, at Bipindi and Memel, males were more abundant than females, with a sex ratio of 1.5 and 1.06, respectively. Similarly, in the trials, the sex ratio (F/M) was 1.81, while in the stream, it was 2.43. However, in anthropogenic environments, the males were more abundant than females, with a sex ratio of 0.5.

Discussion

Tsetse flies were largely the bloodsucking flies captured (using Vavoua traps) predominating, corroborating the results of several authors. Similarly, of the 5 species of blood-sucking flies collected, the species *Glossina fuscipes fuscipes* was dominant in the three localities and two habitats (trails and rivers) according to the Berger-Parker index. Several authors have also reported the majority abundance of Glossina fuscipes fuscipes in South Sudan

(Yatta *et al*., 2016) [20], in Northern Uganda (Opiro *et al*., 2022) [17], in Gabon (Zinga Koumba *et al*., 2015) [21]. This abundance and dominance of the tsetse species captured may be attributed to the effectiveness of Vavoua traps in capturing tsetse flies rather than other HTA vectors (Zinga Koumba *et al*., 2015) [21] and because it is a species of Nemorrhina found mainly in vegetation near river systems (Eteme Enama, 2019) [8].

Although there was no significant difference in the number of captured insects in the three localities and the three habitats, a maximum capture of the tsetse species, *Glossina fuscipes fuscipes*, was in trails (biotope) in Lambi (locality), thus highlighting a risk of HAT transmission in these areas. The study also reveals that the apparent density of this species is also high in Lambi and the trails; the same is true for its abundance in the paths of Lambi. Biting flies and *Glossina fuscipes fuscipes* species in these environments may have favored ecological conditions for their development. This could be explained by the fact that this species is the major vector of trypanosomosis in humans, reptiles, cattle and guibs, most of which are found in forest areas (Itard, 1986) [11].

The locality of Lambi, a hotbed of trypanosomiasis (Grebaut *et al*., 2001) [10], has a savannah climate with a dry winter (Aw) according to the Köppen-Geiger classification, with significant rainfall during the year. This factor probably influenced the density and dispersal of tabanids and *Glossina fuscipes fuscipes*. The trails used by forest operators are environments frequently visited by humans who go to the forest to carry out their daily activities. Individuals of the *Glossina fuscipes fuscipes* species are always on plants lying in wait-along trails and rivers waiting for humans. According to studies conducted by Laveissière *et al*. (2000) [12], trails are preferred sites for tsetse flies.

In this study, more female Glossina fuscipes fuscipes were captured than males, probably because females have longer lifespans. However, in the villages of Bipindi and Memel (localities) and the anthropized area (biotope), males were more abundant than females. The sex ratio of tsetse flies depends on the location of the capture points (Laveissière *et al*., 2000) [12]. Males probably prefer shiny and open environments such as the anthropized area and the Bipindi district.

Conclusion

This preliminary study focused on the spatial distribution of *Glossina fuscipes fuscipes* near Bipindi forest in southern Cameroon. This study determined an apparent density per trap of 0.805. The distribution of this species varies from one locality to another and from one biotope to another. The maximum number of captures of this species was made in the Lambi locality and at the paths in the three localities, as well as at the paths in the Lambi locality. This species, which is the predominant vector for humans and animals, represents a risk to human health, so knowledge of its distribution is important for vector control. A more in-depth study should be carried out to determine the spatial dynamics and even the temporal distribution of tsetse flies in South Cameroon, in order to prevent the resurgence of this vector-borne disease.

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