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LAPY MATHAH Sabine Esther
[1.] Faculty of Sciences and Techniques, Laboratory of Biodiversity and Animal Ecology (LBDEA), University Marien NGOUABI. B.P: 69, Brazzaville
[3.] National Onchocerciasis Control Program, DELM, Ministry of Health and Population, Republic of Congo, Congo

LENGA Arsène
Faculty of Sciences and Techniques, Laboratory of Biodiversity and Animal Ecology (LBDEA), University Marien NGOUABI. B.P: 69 Brazzaville, Congo

BITSINDOU Patrick
[1.] Faculty of Sciences and Techniques, Laboratory of Biodiversity and Animal Ecology (LBDEA), University Marien NGOUABI. B.P: 69 Brazzaville, Congo
[2.] Focal point, Vector control, Ministry of Health and Population, Republic of Congo, Congo

MISSAMOU François
National Onchocerciasis Control Program, DELM, Ministry of Health and Population, Republic of Congo, Congo

Corresponding Author:
LAPY MATHAH Sabine Esther
[1.] Faculty of Sciences and Techniques, Laboratory of Biodiversity and Animal Ecology (LBDEA), University Marien NGOUABI. B.P: 69 Brazzaville, Congo
[3.] National Onchocerciasis Control Program, DELM, Ministry of Health and Population, Republic of Congo, Congo

Evaluation of some parameters involved in onchocerciasis transmission in the DJOUE fireplace in Brazzaville

LAPY MATHAH Sabine Esther, LENGA Arsène, BITSINDOU Patrick and MISSAMOU François

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Abstract
The evaluation of some entomological parameters of onchocerciasis transmission was carried out in the Djoué focus, south of Brazzaville, from July to December 2018. The entomological parameters evaluated were the density of bites of black flies, the parturition rate and the infestation rate.
Three sites were investigated, namely the enclosure of the WHO/AFRO office, the surroundings of the Djoué hydroelectric dam, and the bridge's surroundings over the Djoué. The results obtained indicate that the density of bites is on average 855 bites/man/day in the dry season against 560 bites/man/day in the rainy season. The most prolific site is the one located within the WHO/AFRO enclosure, with 1136 bites/man/day in the dry season and 721 bites/man/day in the rainy season. The parturity rate is on average 58.45% in the dry season, and 59.59% on average in the rainy season. The infestation rate was on average 1.4% in the dry season against 0.7% in the rainy season. The black flies from the WHO/AFRO site had the highest infestation rate with 1.6% in the dry season compared to 0.9% in the rainy season. The aggressiveness expressed by the black flies' capture density indicates that August and September had the highest densities. Thus, 913 bites/man/day were obtained. Regarding the parturity rate, it appeared more important in September with 67.9%. The percentage of infestation was higher in July with 31 L3 stage larvae.
These results confirm the presence of onchocerciasis in the city of Brazzaville and the designated WHO/AFRO site appears to be the most infested site. In addition, these results show that it is important to carry out regular entomological evaluations coupled with a verification of the effectiveness of the treatments initiated with Ivermectin under the Community Directive (CDTI).

Keywords: onchocerciasis, black flies, transmission, entomological parameters, Brazzaville

Introduction
Human onchocerciasis ("River blindness" or volvulosis) is a vector-borne tropical parasitosis. It is found between the 20th degree of North and South latitude and affects several million people worldwide, particularly Africa with 99% of cases [1], but also Central and South America and Yemen. It is a serious, disabling disease that causes skin lesions (onchocercal nodules, skin changes) associated with intense itching. Indeed, some patients have to scratch themselves with a knife blade or a stone in order to soothe the intensity of the pruritus. The symptoms of onchocerciasis are so severe that severe eye damage usually leads to blindness and suicides have even been recorded [2]. The number of blind and partially sighted people is currently estimated at around 5.6 million due to trachoma and 154 million more, mainly in Africa and Asia, carry the pathogen that causes it. In addition, more than 85 million people living in Africa, Latin America and the Arabian Peninsula are threatened by onchocerciasis [3]. In areas of high transmission, this disease can lead to the abandonment of fertile land by affected human communities. The socio-economic imbalances it causes are then considerable.
In Congo, the first cases of Onchocerciasis were reported by Le Boeuf in 1919 [4] in the Djoué region. A literature review on Onchocerciasis in Congo [5] revealed profound knowledge gaps in the taxonomy, distribution and ecology of vectors, as well as in the distribution, prevalence and clinical incidence of disease. Studies by Yébakima (1978-1982) have shown the existence of intense transmission in different regions of the Congo. At that time, the entomological data recorded at the observation points (the Brazzaville dam, the villages of Kinsassa and Mayama in the department of Pool) indicated a permanent presence of the insect vector Simulium damnosum sl with variations in densities in time and space.

The densities observed varied from 18 to 459 females / man / day. In addition, the physiological age, expressed by the parturity rate, always appeared to be high (35 to 80% of parous females) whatever the season and the point of capture; finally, parasitism by a pathogen, 0nchocerca volvulus, resulted in an infestation of 1 to 5% of parous females, with an individual transmission potential of 2.5 to 7 larvae.

The pathogen is a nematode, Onchocerca volvulus, Filarioidea (Leuckart, 1893) specific parasite of man. Vector insects are moths (Diptera - Nematoceres), small gnats whose larvae and nymphs live in running water. All species of black flies' vector belong to the genus Simulium: *S. damnosum* s.l., *S. neavei* s.l. and *S. albivirgulatum* in Africa and Yemen, and also *S. amazonicum*, *S. callidum*, *S. metallicum*, S. ochraceum in South America [6].

Regarding the main vectors, the only known species of the damnosum complex in the Congo is squamosum. The species *S. neavei* is a priori unknown there. The vectorial role of *S. albivirgulatum*, well present in the Plateaus or in Brazzaville, is still unconfirmed. Due to the biology of its vectors, the distribution of African onchocerciasis is related to areas of the strong river and river currents [7]. Recent years have been marked by an intensification of research on onchocerciasis, its diagnosis, surveillance and control operations. Many more or less effective treatments were developed, including that with ivermectin (Mectizan R), in the 1980s.

For about 15 years, through the community distributors, the National Onchocerciasis Control Program (PNLO), in collaboration with the Organization for the Prevention of Blindness (OPC), Sightsavers and the former Program African Control of Onchocerciasis (APOC), provides annual and large-scale administration of ivermectin to populations in areas with onchocerciasis [8], to eradicate this scourge. The strategy in place in the Congo, as in many other African countries, is to Treat Ivermectin with a Community Directive (TIDC). Ivermectin (Mectizan R) is a drug that kills most microfilariae and thus ensures clinical improvement in the long run (skin lesions, pruritus, mild eye damage) and decreased disease transmission [9], in order to achieve its elimination [10]. The elimination of onchocerciasis is more than ever the goal of the fight in the Republic of Congo.

In Congo, onchocerciasis is prevalent in several watersheds in the southeast of the country and affects 13 rural health districts in 5 departments (out of the 12 in the country) which are the Pool, the Bouenza, the Kouilou, the Lékoumou and the Niari.

The Republic of the Congo is distinguished by the presence of an urban transmission home in the capital Brazzaville. Strong nuisance due to high bite densities associated with the vast pre-imaginary deposits of the Congo River rapids and its tributary the Djoué in some southern neighborhoods has been shown [11].

The latest entomological studies of onchocerciasis date back to the 1980s and indicated a relatively high transmission of onchocerciasis. With the distribution of ivermectin, for about fifteen years in the riparian populations of the Djoué, the status of the main entomological indicators of the transmission of the disease in the areas of intervention of the program remains to be established. During this work, we undertook to assess the intensity of onchocerciasis transmission in the Djoué home in Brazzaville, after 15 years of implementation of the Ivermectin Treatment under the Community Directive (TIDC). The simulation nuisance felt by populations through the determination of vector densities was particularly addressed.

Material and Methods

Field of study

The study was carried out in Brazzaville, the capital of the Republic of Congo, where the average temperature is 25 ° C and annual rainfall is around 1200mm to 1500mm of water per year [12]. The annual thermal amplitude is low and fluctuates between 5 and 6 ° C. The wind speed, which is generally very low, is often less than 5m / s, except during the period of frequent tornadoes at times of the equinoxes when there are a few violent winds of short duration.

The relative humidity is important. The monthly variations are small while the daily amplitudes are high. The city of Brazzaville has a relative humidity between 88 and 94% [13]. The city covers an area of approximately 17,600 ha. It is inhabited by a population estimated at 1,838,348 million inhabitants in 2017 and whose annual growth rate is 4.8% [14].

The city of Brazzaville is bordered by the Congo River, which has an average flow of around 43,000 m3 / s. It is dominated by grassy and shrub savannah vegetation. However, there are also small forest areas in the form of forest fragments and forest galleries. These massifs occupy about 30% of the watershed. The soils of Djoué are sandy, of the depleted ferralitic type**,** with giant podzols [15]. The city is watered by numerous tributaries of the Congo River from the Batékés Plateaux, which all flow into the Congo River. These are Djoué, Djiri, Tsiémé, Mfoa, Madoukou Tsékélé, Mfilou and Kélékélé [16].

The city of Brazzaville and its surroundings were originally covered with forest and savannah. The latter was composed of Poaceae and Annonaceae (Annona arenaria Thonn). We could also note species that are typical of clay soils such as Hypparhenia diplandra (Hack) Stapf and Bridelia ferruginea (Benth) and those of sandy soils (Loudetia demeusei De Wild and Hymenocardia acida Tul.). The forest presented various aspects: copses or groves on plateaus, zones of mesophilic forest occupying about 5% of the surface and gallery forests along the rivers [17].

The protocol for baseline studies relating to the long-term impact of APOC operations provides for the capture point to be located near the roost closest to the watercourses selected for medical surveys [18]. According to hydrometric records, the Djoué experiences its maximum low water level in September and its flood period from December to March. In order to allow a comparison with the results of previous studies, the capture sites chosen are the same as those used by Yébakima (1978-1982). These sites are around the Djoué Bridge, a few meters upstream from it.

Fig 1: Djoué Bridge site

Fig 2: Djoué dam

Fig 3: WHO / AFRO city, about 2 km from the Djoué bridge

Materials

The biological material consisted of captured black flies. Data collection sheets, hourly collection bags, hemolysis tubes and a cooler were used in the field. In the laboratory, a Visiosonic 2000 brand microscope, a Carl Zeiss brand binocular magnifier, a dissection kit, 80% alcohol, ether, chloroform, physiological fluid, and slides and coverslips were used to observation, storage or dissection of samples.

Methods

It was a descriptive and cross-sectional study. The National Onchocerciasis Control Program (PNLO) distributes ivermectin on an annual basis. The study population consisted of all black flies captured at the sites. All the black flies were captured during the biting process. The study was spread over the year 2018 over 5 months, from July to September for the dry season and the months of November and December for the rainy season from 7 a.m. to 12 p.m. in the morning and from 12 p.m. to 5 p.m. in the evening. The catch rate was 3 consecutive days per month and per site. For practical reasons, the fourth week of each month has been chosen for the catches.

Entomological indices

Several variables were determined, namely: (i) The density of the vector, which is expressed by the average number of blackflies captured in relation to the total number of investigators, according to the number of sessions, (ii) the Parturity rate expressed by the proportion of parous female blackflies (having already laid eggs) among the captured and dissected blackflies, (iii) the Infection rate which indicates the proportion of dissected parous female blackflies harboring evolutionary larvae (stage L1, L2, L3) of *Onchocerca volvulus* on the number of parous flies dissected and (iv) The infectivity rate (Individual transmission potential) which provides information on the proportion of dissected parous black flies containing L3 infecting larvae of Onchocerca volvulus in the salivary glands on the number of parous flies dissected.

The collection of blackflies was carried out by a team of six investigators. At each site selected, the capture sessions were organized as follows:

Given the diurnal activity of the blackflies, the captures were made from 7 a.m. to 5 p.m. by three pairs of captors. For each site, two captors took turns every hour. Each captor was provided with a reserve of hemolysis tubes, carded cotton, hourly collection bags, a watch and an hourly recording sheet of catch results; blackflies were captured using the classic method [19]. Given the low location of the bites of the females of *S. damnosum* s.l, the captor's mission was to capture, using a hemolysis tube, all the females that came to rest on his bare and exposed legs.

As onchocerciasis is a cumulative disease, the risk to human bait is minimal. However, at the end of the project, the investigators each received a dose of ivermectin and were included on the list of those who benefit annually.

The captured blackflies being intended for the study of their infestation by O. volvulus, it appeared essential that the females be caught before they bite in order to avoid the loss of the infecting larvae possibly located at the level of the proboscis.

Blackflies were collected directly using a hemolysis tube, with only one female per tube.

The tubes containing the blackflies were grouped in time slots, wrapped with a damp towel and placed in a cooler. The conditioned blackflies were transported to the laboratory.

All the blackflies brought back to the laboratory have been identified; thus, part of the sample (40%) of blackflies was dissected and the other part (60%) intended for studies by "pool screening" was kept in alcohol diluted at 80%, then sent to Ouagadougou in Burkina Faso for analysis. Regarding the dissections, each female was killed with chloroform and dissected in a drop of physiological water under a binocular magnifying glass. The blackfly dissection was performed in the abdomen to allow examination of abdominal organs and tissues (Malpighi tubes, ovaries, fat) for estimation of physiological age. The dissection then consisted of separating the three parts of the insect's body (head, thorax and abdomen), observing them and possibly tearing the proboscis at the level of the head in order to look for L3 larvae of *O. volvulus* and other parasites. The estimation of physiological age in *S. damnosum* s.l. was limited to a simple distinction between parous and nulliparous females. In current practice, the characteristics of abdominal fat (abundant in nulliparous females, reduced or absent in parous females) and Malpighi tubes (opaque in nulliparous females, clear in parous females) allow an experienced observer to separate parous females from nulliparous females [20-21].

Statistical analysis of the data

The data from this study were captured with the Excel 2010 software and processed by the Chi-square statistical test with two degrees of freedom. The statistical test allowed a multivariate analysis to explain the dependence of the quantitative variables on the qualitative variables at the significance level of 5% using the Xlstat

Results:

The results obtained on the blackfly density, the parturity rate and the infectivity rate of the captured and dissected blackflies in the dry season are given in Table 1 below.

Table 1: Numbers of flies captured, dissected and preserved according to the capture sites (Brazzaville, dry season 2018)

Characteristics	Capture Sites				
	Bridge DJOUE	DJOUE DAM	WHO/AFRO City	Summary 2018	P
Number of day of capture	9	9	9	27	
Blackflies caught	4299	8561	10223	23083	
Blackflies Dissected	1721 (40%)	3426 (40%)	4089 (40%)	9236 (40%)	0,05
Black flies preseved	2578 (60%)	5135 (60%)	6134 (60%)	13847 (60%)	
Average/day	478	951	1136	855	
Blackfly Dissected	1721	3426	4089	9236	
Black flies Nulliparous	739 (42,94%)	1477 (43,11%)	1622 (39,67%)	3838 (41,55%)	0,01
Black flies Parous	982 (57,06%)	1949 (56,89%)	2467 (60,33%)	5398 (58,45%)	
Black flies Parous	982	1949	2467	5398	
Blackfly Infected	14 (1,43%)	22 (1,13%)	38 (1,54%)	74 (1,37%)	0,3
Black flies Uninfected	968 (98,57%)	1927 (98,87%)	2429 (98,46%)	5324 (98,63%)	
Blackfly Infected	982	1949	2467	5398	
Non-infectious black flies	975 (99,29%)	1943 (99,69%)	2453 (99,43%)	5371 (99,50%)	0,3
Infectious black flies	7 (0,71%)	6 (0,31%)	14 (0,57%)	27 (0,50%)	
Total number of larvea	17	27	55	99	
Number of L3 leading	11	11	28	50	

The density of blackfly bites according to the capture sites in the dry season.

The results obtained in the three sites indicate that the average is 855 bites / man / day, in the dry season. The peak of bites is obtained between 7 a.m. and 9 a.m. at all sites. The most prolific site is that of the OMS / AFRO city (1136 bites / man / day) followed by that of the Djoué dam (951 bites / man / day) then the Djoué bridge (478 bites / man / day). Statistical tests show that there is no significant difference between capture sites and blackfly densities P> 0.05.

Parturity rate of blackflies according to catch sites in the dry season

The observed parturity rates vary increasingly from one site to another (see Table I). Thus, it was 56.89% at the Djoué dam; 57.06% at the Djoué bridge and 60.33% at the OMS / AFRO city. Statistical tests show that there is a significant difference between the values observed depending on the capture sites (P <0.05).

The infection rate of females by *O. volvulus* according to the capture sites in the dry season

The average infestation rate obtained varies increasingly from one site to another (see Table 1): thus, it was 1.54% at the WHO / AFRO city; 1.43% at the Djoué bridge and 1.13% at the Djoué dam. Statistical tests show that there is no significant difference between the infection rates of the different sites P> 0.05.

Infectivity rate of females by *O. volvulus* according to the capture sites in the dry season

The average infection rates observed vary increasingly from one site to another (see Table I above). It was 0.78% at the WHO / AFRO city; 0.55% at the Djoué dam and zero at the Djoué bridge. Chi-square statistical tests show that there is no significant difference between the infestation rates of the different sites P> 0.05.

Table 2 below presents the different entomological indicators of onchocerciasis transmission in the rainy season.

Table 2: -Numbers of flies captured, dissected and preserved according to the capture sites (Brazzaville, rainy season 2018)

Characteristics	Capture sites				
	Bridge DJOUE	DJOUE DAM	WHO/AFRO City	Summary 2018	P
Number of day of capture	6	6	6	18	
Blackflies caught	1940	3805	4327	10072	
Blackfly Dissected	776 (40%)	1522 (40%)	1731 (40%)	4029 (40%)	0,9
Blackflies Preserved	1164 (60%)	2283 (60%)	2596 (60%)	6043 (60%)	
Average / day	323	634	721	560	
Blackfly Dissected	776	1522	1731	4029	
Black flies Nulliparous	309 (39,82%)	617 (40,54%)	702 (40,55%)	1628 (40,41%)	0,5
Black flies Parous	467 (60,18%)	905 (59,46%)	1029 (59,45%)	2401 (59,59%)	
Black flies Parous	467	905	1029	2401	
Blackfly Infected	465 (99,57%)	898 (99,23%)	1020 (99,13%)	2383 (99,25%)	0,9
Black flies Uninfected	2 (0,43%)	7 (0,77%)	9 (0,87%)	18 (0,75%)	
Blackfly Infected	467	905	1029	2401	
Non-infectious black flies	467 (100%)	900 (99,45%)	1021 (99,22%)	2388 (99,46%)	0,2
Infectious black flies	0 (0%)	5 (0,55%)	8 (0,78%)	13 (0,54%)	
Total number of larvea	2	13	11	26	
Number of L3 leading	0	7	9	16	

The density of blackfly bites according to the capture sites in the rainy season.

The average number of bites was 560 bites / man / day in the rainy season. The site of the WHO / AFRO city remains the most prolific. The statistical test shows that there is no significant difference between simulid densities and the different sites P> 0.05.

Parturity rate of blackflies according to the capture sites in the rainy season

The parturity rate remained relatively constant at the three sites with respectively 60.18% for the Djoué bridge site, 59.46% for the Djoué dam and 59.45% for the WHO / AFRO. Statistical tests show that there is no significant difference between the purity rates and the different sites (P> 0.05).

The infection rate of females by *O. volvulus* according to the capture sites in the rainy season

As in the dry season, the highest infection rate was observed in the WHO / AFRO city with 0.87%, while it was 0.77% at the Djoué dam and 0.43 % at the Djoué bridge. Statistical tests show that there is no significant difference between and infection rates at and different sites P> 0.05.

Female infectivity rate by *O. volvulus* according to the capture sites in the rainy season

The rate of infectivity by *O. volvulus* resulted in a percentage of 0.78% for the site of the WHO / AFRO city which thus appeared to be the most important, the other two sites have shown rates of 0, 55% and 0% respectively for the Djoué dam and Djoué bridge site, therefore much lower than during the dry season.

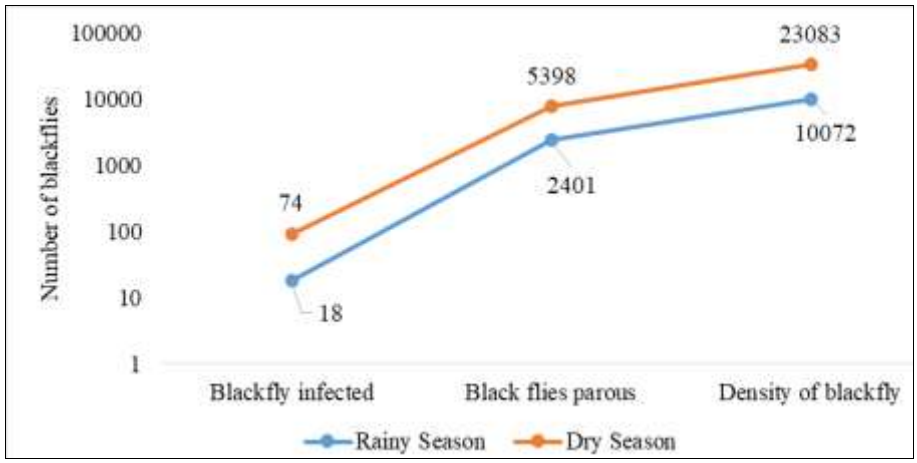

Fig 4: Distribution of black flie's indices by capture season in Brazzaville in 2018.

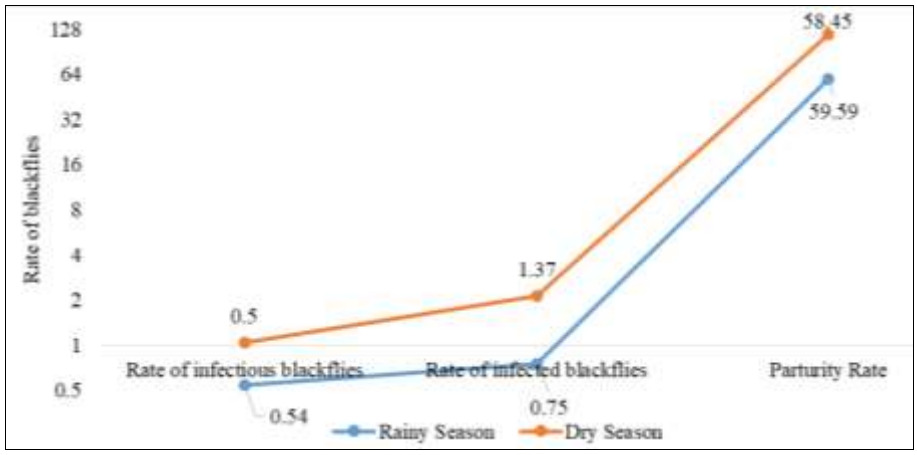

Fig 5: Distribution of blackflie's parturity, infestation and infection rates by capture season in Brazzaville in 2018.

Table 3: Monthly distribution of the entomological indicators of blackflies in each capture site in Brazzaville in 2018.

Characteristics	Capture Month						P
	July	August	September	November	December	Totals	
Black flies capture	6879	8218	7986	6373	3699	33155	0,9
Black fly Dissected	2753 (40%)	3289 (40%)	3194 (40%)	2549 (40%)	1480 (40%)	13265 (40%)	
Black fly Preserved	4126 (60%)	4929 (60%)	4792 (60%)	3824 (60%)	2219 (60%)	19890 (60%)	
Average of blackflies caught per day	764	913	887	708	411	738	
Blackfly Dissected	2753	3289	3194	2549	1480	13265	0,001
Blackflies Nulliparous	1725 (62,66%)	1088 (33,08%)	1025 (32,09%)	905 (35,50%)	723 (48,85%)	5466 (41,21%)	
Blackflies Parous	1028 (37,34%)	2201(66,92%)	2169 (67,91%)	1644 (64,50%)	757 (51,15%)	7799 (58,79%)	
Blackflies Parous	1028	2201	2169	1644	757	7799	0,001
Blackfly infected	48 (4,67%)	5 (0,23%)	21 (0,97%)	15 (0,91%)	3 (0,40%)	92 (1,18%)	
Blackflies uninfected	980 (95,33%)	2196 (99,77%)	2148 (99,03%)	1629 (99,09%)	754 (99,60%)	7707 (98,82%)	
Blackfly infected	48	5	21	15	3	92	0,01
Black flies Non infectieuse	29 (60,42%)	1 (20,00%)	17 (80,95%)	3 (20,00)	2 (66,67%)	52 (56,52%)	
Black flies Infections	19 (39,58%)	4 (80,00%)	4 (19,05%)	12 (80,00%)	1 (33,33%)	40 (43,48%)	
Total number of larvas	57	12	30	23	3	125	
Number of L3 leading	31	7	12	15	1	66	

Comparisons of proportions were carried out with the Chi-square test with four degrees of freedom

Density of blackfllie's bites according to the months of capture

From the table above, 33,155 black flies were captured in 5 months and over 45 days at three different sites, 13,265 (40%) were dissected, all belonging to the *Simulium damnosum* complex. The average bite was 738 bites / man / day in 2018.The aggressiveness expressed by the capture density of blackflies indicates that the months of August and September, are those where the greatest numbers of bites are observed. Thus, during this period, on average, 913 bites / man / day were obtained against an average of 411 bites / man / day in December. The general trend is a slight increase in density from July to September (764 - 913 to 887), followed by a very marked decrease from November to December (708 to 411).

Parturity rate of blackflies according to the months of capture (average physiological age of biting females): Of 13265 dissected black flies, 7799 or 58.79% were parous, against 5466, or 41.21% which were nulliparous. Comparison of the rates obtained by Pearson's chi-square test reveals a significant difference between months for P = 0.001 <0.05. We observe an increase in the rate of parous females from July to August and September with respectively, 37%, 67% and 68%, followed by a strongly accelerated decrease from November to December with 64 and 51%.

Infection rate of females by *O. volvulus* according to the months of capture

Among the 7799 parous blackflies, 92, or 1.18% were infected against 7707 or 98.82% of uninfected blackflies. The difference obtained by the Fischer chi-square test is significant between months for P = 0.001 <0.05. These rates are pa particularly high in July (4.67%). They fell very low in August with 0.23%. In September and November, the rates remain very low (0.97% and 0.91%), before falling in December (0.40%).

Infectivity rate of females by O. volvulus (In number of L3s in the head) ("Individual transmission potential")

Among the 92 infected black flies, 40 or 43.48% were infectious, against 52 or 56.52% of non-infectious black flies. The difference obtained by the Fischer chi-square test is significant between the months for P = 0.01 <0.05. The average load for all dissected females is 1.85 L3 / head per infectious female. It is 1.82 for the month of July with 31 L3 larvae, when a large majority of infectious females are observed and 1.70 for the months of August, September, November and December. The average body-wide L3 load is 2.9 per infectious female. It emerges from this study that the number of infected females is 2.3 times higher than that of infectious females. The number of evolving larvae is 1.9 times greater than that of infective larvae (L3) in the cephalic part.

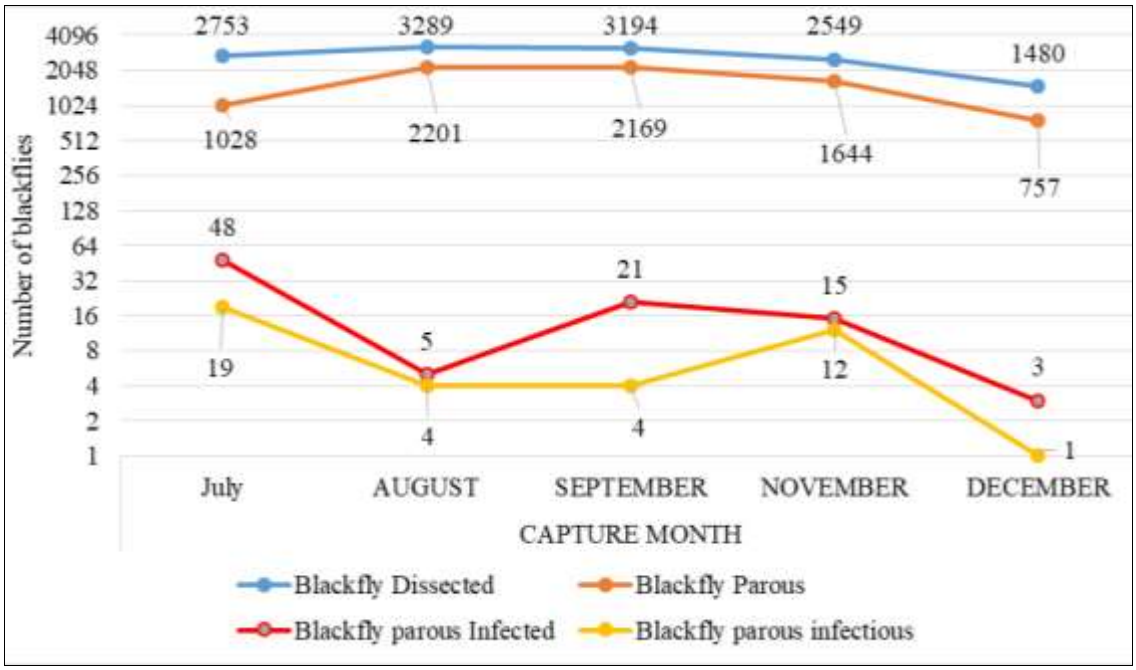

Fig 6: Monthly distribution of simulidian indices during captures in Brazzaville in 2018.

Discussion

This study has updated research on entomological indicators of Onchocerciasis transmission in the Djoué area in Brazzaville. Thus, a new assessment of the endemic onchocerciasis can be made, 15 years after the launch of the mass treatment program with ivermectin. Morphologically determined blackflies belong to the Simulium damnosum complex. On this subject, indicate that cytotaxonomic studies have shown that the cytotype involved in Brazzaville is *Simulium squamosum* [11].

Quantities of pitting: To bring the data of the entomological survey closer to hydrological fluctuations, the study period therefore covered the periods of main low water (July to September) and of rising water levels more than the flood itself (November to December).

Regarding rainfall, the survey period which spanned from July to December is well representative of the end of the great dry season (July-September) and the peak of the great rainy season (November-December). The latter in principle is more favorable to the movements of blackflies, the humidity is very high in all seasons and conducive to flight [11].

In our study, the catches of blackflies were mainly carried out during the low water period, known as the long dry season (July-September) and during the flood period (November -

December). The deposits observed in the Pont du Djoué site are mainly low-water deposits, made up of slabs and rocky boulders forming the bed of the river and which emerge along the course and the banks of the river with grassy vegetation. However, between November and December, an abundant rise in the flow of water that can partially or even completely cover the deposits is observed. Annual rainfall totals in Djoué are around 1300 mm in the dry season and 2000mm in the rainy season (from south to north of the Djoué watershed). This pluviometric abundance is justified by the atmospheric conditions, which are the dominance of low intertropical pressures, the unsubsidized maritime trade winds, the intensity of thermal convection and the relatively high relief [22].

The Djoué River is known to be particularly favorable to the development of black flies [11]. It should be remembered that along this stream, the simulid density (Imagos) is maximum (more than 855 bites / man / day obtained during this study and 170 bites / man / day obtained by [23] when a maximum of supports is available for the laying and the development of the pre-imaginal stages (Talani *et al*., 1997). On the other hand, during flood periods, when the number of egg-laying supports for females is considerably reduced, due to the partial or total immersion of the supports, a very significant drop in the density of the pre-imaginal stages can be noted [18]. 18 bites / man / day obtained in 2006 by Soungalo *et al*., Compared to 560 bites / man / day observed during this study during the so-called high water period. In short, it emerges that the dry season can be considered conducive to the proliferation of blackflies (855 bites / man / day in the dry season against 560 bites / man / day in the rainy season).

In fact, at the confluence of the Djoué with the Congo River, the dry season is synonymous with a period of low water. The rocks are flush with the water level and present numerous supports for female egg-laying and the development of blackfly larvae.

During this study, a total of 33,155 black flies were captured within 45 days at all capture sites and 13,265 of these were dissected. The quantities of daily bites to which an individual is theoretically exposed are significant, with a variation which goes from the simple to the double between December and July. They range from 416 bites / man / day in the rainy season to 970 bites / man / day in the dry season. These densities are significantly higher than those reported by Yébakima *et al*. in 1982, at the Djoué sites (from 18 for the rainy season to 170 females / man / day for the dry season). The emergence at low water of rocky thresholds which create important rapids, favorable to the productive sites of blackflies, while at high water these thresholds are covered on too great heights of water, or are subjected to currents too violent for allow the attachment of *S. damnosum* sl larvae.

The relative abundance of black flies at different sites can also be influenced by human use of these sites. Indeed, the blood meal confers on the female her reproductive capacity, its intensity being conditioned by the blood meal.

Blackfly bites during low water in our study area are much lower than those recorded further downstream, along the Congo River (13,000 bites / man / day ur in Inga, nearly 300 km from Brazzaville). By comparison with other African households, they are likely to cause some discomfort among corporations linked to the outdoors and called upon to frequent the river (fishermen, boatmen, market gardeners, sand shooters, etc.) or even among resident residents in their daily outdoor activities.

Parturity rate (average physiological age of biting females):

The values obtained for physiological age are between 35 and 80% as observed by Yébakima in 1982. They are on average 57 and 60% for this study. The synchronism of the three monitoring values of the parturity rate from September to December gave respectively 67.91%, 64.50% and 51.15%. Young blackflies (nulliparous) were captured at their production roost (43% at the Djoué Bridge and dam, respectively 5m and 10m from the production roosts). The presence of riparian human populations therefore gives them the opportunity to have their first blood meal near the river, while older females are rather observed far from roosts (61% at WHO / AFRO) about 800m from production lodges, compared to 56% at the other two sites). It is also likely that the abundance of the population around the Djoué River, which is currently higher compared to 1982, is an important factor in simulid productivity. In fact, in 1982, Brazzaville had 504,000 inhabitants with an increase rate of 6.33%, [24, 14] however, according to the last population census, the 1 Makélékélé and 8 Madibou districts which border the Djoué river, have 254,404 and 184,226 inhabitants respectively [25] which is almost the total population of Brazzaville in 1982.

The rate of infectivity of females by *O. volvulus*

Although with the exception of July, the rates of infectivity (number of parous females carrying L3 in the head), are low, transmission has been continuous throughout the duration of the study, despite regular annual ivermectin distributions, at at least one site. Each month, the rates are of the same order of magnitude at the three sites. The percentage of blackflies infected with 0 volvulus results in an infection of between 0.5 and 1% of parous females, thus expressing an individual transmission potential of 1 to 3 larvae. Yébakima *et al*. in 1982 in the same sites had observed 1 to 5% of infectious pares females with a transmission potential of 2.5 to 7. This difference can be justified by the real and regular intake of ivermectin by the neighboring populations for about fifteen years. Although treatment with ivermectin has dramatically reduced the potential for transmission (from 7 to 3 L3 larvae), the disease continues to be rampant. Thus, under these conditions, during their meals, the absorption of microfilariae by blackflies is then low or zero. Indeed, as shown by our results compared to those of Yébakima, it appears that regular intake of ivermectin drastically reduces the microfilarial burden in populations subject to treatment. Although the increase in the population leads to an increase in simulid density, the actual intake of ivermectin considerably decreases this density. This decrease can also be justified by accelerated urbanization or the transformation of the study area (construction of hotels and tourist sites near the lodge); by climatic conditions (abundance of rains, etc.); by the different frequentation of the places of the lodgings by the local populations (washing cars, extraction of construction materials,...), as well as by the evolution of the habits of the surrounding people more alert on the subject.

Conclusion

At the end of this study, it emerges that the density of blackfly bites per man per day has remained very high for more than three decades, particularly in the selected sites. However, studies performed during the same time periods indicate permanence of the disease, although a reduction in continued transmission has been observed. In addition, it appears necessary to strengthen Ivermectin Treatment under

Community Directive (CDTI) in the neighborhoods bordering Djoué because it has been shown that the regular intake of ivermectin by the population drastically reduces the potential of transmission of Onchocerca volvulus sl larvae In addition, it appears important to apply it to people living or working in the WHO / AFRO City, which is a highly heterogeneous environment where there are offices, restaurants, dwellings with dense wooded vegetation, and where more than 400 people work. An entomological and epidemiological assessment of the disease over three years, for example in the areas concerned, would be judicious, while ensuring that the population is aware of the need for the effective intake of ivermectin. In addition, the aim will be to strengthen public awareness of the harmful effects of onchocerciasis, with a clear and scientific explanation of the origin of this disease, which some local populations consider to be linked to witchcraft.

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