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Sand fly fauna (Diptera: Psychodidae), association between climatic variables and natural *Leishmania* infection in Araçatuba, Brazil

Relatório de Pós-doutorado realizado na Universidade Estadual Paulista (UNESP), Faculdade de Medicina Veterinária, Campus de Araçatuba, SP.

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Abstract

Visceral leishmaniasis (VL) is a zoonosis of major public health importance. In urban Area, *Lutzomyia longipalpis* is the primary vector of *Leishmania (L.) infantum*. This study assessed the seasonality, spatio-temporal distribution, and climatic factors associated with *L. longipalpis* abundance in Araçatuba, São Paulo State, and detected *Leishmania* spp. DNA in captured females. Monthly collections were conducted from March 2023 to February 2024 in 72 households across eight urban Area using CDC-type light traps placed indoors and in peridomestic environments. A total of 1,641 specimens (1,516 males and 125 females) were captured, with 92.4% from peridomestic Area. Area 3 had the highest density (n = 671) and was the only one with PCR-positive females (n = 3). Vector activity peaked in December 2023 (n = 335). Male abundance differed significantly among peridomestic Area, particularly between Area 3, 5, 6, and 7. In peridomestic Area, higher final temperatures increased vector abundance, while higher initial temperatures and humidity reduced it. Indoors, final temperature, humidity, and month were significant. L. longipalpis showed a defined seasonal and spatial pattern influenced by climate. The detection of PCR-positive females (area 3) showed the vector epidemiological role and the need for focused interventions to control VL.

Keywords: Phlebotominae ecology; vector-borne diseases; entomological surveillance; seasonal dynamics; canine visceral leishmaniasis

1. Introduction

Visceral leishmaniasis (VL) is a parasitic zoonosis with wide geographic distribution and is considered one of the most globally significant neglected tropical diseases. It is estimated that over 50,000 cases are reported annually, particularly in regions of Asia, Africa, and Latin America, where socioeconomic, environmental, and ecological factors contribute to the persistence and expansion of the disease [1].

In Brazil, VL is endemic in several regions and represents a serious public health concern, with increasing records of geographic expansion and urbanization of transmission, especially in recent decades [2,3].

The etiological agent of the visceral form of the disease in Brazil is *Leishmania infantum* (Kinetoplastida: Trypanosomatidae), primarily transmitted by the bite of female sand flies of the species *Lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotominae), which is the main confirmed vector in most urban transmission foci [4-6].

The spread of VL to Area previously considered disease-free has been linked to environmental changes, migratory flows, population density, and the presence of infected dogs, which are the primary urban reservoirs [3] .

In the state of São Paulo (SP), the municipality of Araçatuba marks a significant milestone in the history of visceral leishmaniasis. In 1998, the first cases of canine VL were recorded, followed in 1999 by the first reports of autochthonous human cases [7,8]. Since then, both the municipality and its surrounding Area have become endemic for the disease, with sustained transmission and continued impact on local public health [9].

This study characterized the sand fly fauna in Araçatuba, Brazil, analyzed the association with climatic variables, and evaluated natural *Leishmania* infection to support strategies for visceral leishmaniasis prevention and control in endemic and expanding Area.

2. Materials and Methods

All experimental procedures were carried out in full accordance with ethical standards and approved by the Ethics Committee on Animal Use (CEUA) of the Faculdade de Odontologia de Araçatuba and the Faculdade de Medicina Veterinária de Araçatuba (FMVA), UNESP, Campus de Araçatuba (protocol FOA nº 00870-2016).

The research was carried out in the urban area of Araçatuba (21°12′32″S, 50°25′58″W), located at an altitude of 390 meters, in the state of São Paulo, southeastern Brazil. According to data from the Brazilian Institute of Geography and Statistics (IBGE, 2024), the municipality has an estimated population of 207,775 inhabitants

(https://ftp.ibge.gov.br/Estimativas_de_Populacao/Estimativas_2024/estimativa_dou_2024.pdf) and occupies an area of 1,167.126 km² (https://www.ibge.gov.br/cidades-e-estados/sp/aracatuba.html). Araçatuba lies within the Atlantic Forest biome and is characterized by a tropical Aw climate under the Köppen–Geiger classification. The mean annual temperature is 24.1 °C, and the average precipitation reaches 1,328 mm, with the rainy season concentrated in the summer months (https://pt.climate-data.org/america-do-sul/brasil/sao-paulo/aracatuba-4222/).

The spatial division of the municipality of Araçatuba, São Paulo, Brazil, adopted in this study, was based on the urban census sectors defined by the Brazilian Institute of Geography and Statistics (IBGE) for the 2022 demographic census, corresponding to the main district. The numbered Area (1–8) correspond to groups of contiguous census sectors, stratified according to geographical dispersion criteria and environmental characteristics. These census-based spatial units were employed for the analysis of sand fly captures in peridomestic and intradomestic environments and Leishmania PCR positivity, allowing comparisons among subregions with distinct urbanization and environmental profiles.

Two trapping cages were positioned approximately 50 cm above the ground in each household between March 2018 and July 2019. The traps were exposed for 12-hour intervals, from 7:00 p.m. to 7:00 a.m., and remained in situ for a total of 72 hours. In addition, entomological surveys were performed using seven standard CDC light traps, with their bulbs removed before deployment. These traps were placed at two distinct locations: one inside the residence, typically in the main bedroom, and another near the dog's sleeping area or at the entrance of the kennel. Thus, each trap was positioned in proximity to both human and canine hosts. Collected sand flies were counted and sexed under a stereomicroscope. Following the procedure described by Gaglio*, et al.* [10], the specimens were prepared for microscopic examination and identified to the species level using morphological criteria. As previously noted, *Lutzomyia longipalpis* females were subjected to the PCR protocol described above, following sexual differentiation and morphological confirmation.

PCR amplification of *Leishmania* DNA was performed on all conjunctival swab and popliteal lymph node samples. Genomic DNA was extracted using the PureLink® Genomic DNA Kit (Invitrogen, USA), following the manufacturer's instructions. A nested PCR assay was employed to detect *Leishmania* DNA targeting a variable region of the small subunit ribosomal RNA gene (nested SSU rRNA-PCR). Two primer pairs were utilized: the outer primers P221 (5′-GGTTCCTTTCCTGATTTACG-3′) and P332 (5′-GGCCGGTAAAGGCCGAATAG-3′), and the inner primers P223 (5′-TCCCATCGCAACCTCGGTT-3′) and P333 (5′-AAGCGGGCGCGGTGCTG-3′) [11]. Amplification of *Leishmania* kinetoplast DNA (kDNA) was

performed using the newly designed primers P221 and P332 in the first PCR reaction. Although these primers (used in the second PCR) can also amplify other kinetoplastids such as *Leptomonas* and *Crithidia*, the design of the internal primers P223 and P333 ensured specificity for the *Leishmania* genus [12]. The PCR reaction mixture was prepared by combining the outer primers of the nested PCR — forward primer P221 (10 µM; 5′-GGTTCCTTTCCTGATTTACG-3′) and reverse primer P332 (10 µM; 5′-GCCGGTAAAGGCCGAATAG-3′) — at a ratio of 10:1 between inner and outer primers. The final composition of the PCR included 10 µL of DNA template, 2 mM $MgCl_2$, 0.2 mM dNTPs, 15 pmol of each primer, 1.4 U of Taq DNA polymerase, and 1× Promega buffer. For the second PCR, 5 µL of the first PCR product (previously diluted 1:200 in ultrapure water) were used, with 2 mM $MgCl_2$, 0.2 mM dNTPs, 1.5 pmol of each primer, 0.7 U Taq polymerase, and 1× Promega buffer. The PCR protocol consisted of two amplification rounds: an initial phase of 15 cycles lasting approximately 45 minutes, followed by a second phase of 35 cycles lasting about 1.5 hours [13]. In the first round, the cycling conditions were 95 °C for 3 min, followed by 15 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 30 s, and extension at 72 °C for 30 s, ending with a final step at 95 °C for 1 min. After this step, the tubes were briefly incubated at 95 °C for 1 min, inverted several times to ensure primer dissolution, centrifuged, and returned to the thermocycler for the subsequent amplification round. The second PCR cycle began with 95 °C for 3 min, followed by 35 cycles of 94 °C, 65 °C, and 72 °C for 30 s each, and concluded with a 5 min extension at 95 °C. Double-distilled water was included as a negative control in all reactions. The amplified products were analyzed by electrophoresis on a 2.0% agarose gel containing ethidium bromide, and 10 µL of each PCR product were compared against a 100 bp DNA ladder. DNA bands were visualized under ultraviolet transillumination.

Statistical analyses were conducted using STATA/SE version 16.1 (StataCorp LLC, College Station, Texas, USA) and Microsoft® Excel® from the Microsoft 365 package. The threshold for statistical significance was established at $p < 0.05$.

To evaluate differences in *L. longipalpis* abundance among the eight urban areas studied, the data were first assessed for normality and homoscedasticity. As the dataset did not meet the assumptions for parametric testing, non-parametric methods were applied. Differences in sand fly counts among areas (peridomestic and intradomestic) were tested using the Kruskal–Wallis test, followed by Dunn's post hoc multiple comparisons with Bonferroni correction when applicable.

Temporal variation in sand fly abundance (monthly comparisons) and the influence of microclimatic variables (initial and final temperature, initial and final humidity) were assessed using Generalized Linear Models (GLMs). Given the distributional characteristics of the data and the presence of overdispersion, models were fitted using a square-root link function, appropriate for count datasets with heterogeneous variance.

For each GLM, Likelihood Ratio Tests (LRTs) were used to determine the statistical significance of individual predictors. In the peridomestic models, the explanatory variables tested included, initial temperature, final temperature, initial humidity, final humidity, month.

Similarly, intradomestic models evaluated the same climatic variables and monthly variation. When significant monthly effects were detected, pairwise post hoc contrasts with Bonferroni adjustment were performed to identify specific differences between months.

Spatial distribution analyses, kernel density estimation, and buffer zone evaluations were conducted separately (described in the GIS subsection), and were not part of the inferential statistical models.

3. Results

In Araçatuba, a total of 1,226 *L. longipalpis* were collected, including 1,148 from peridomestic areas (87.4% males; 1,003/145 males/females) and 78 from intradomestic (80.8% males; 63/15 males/females) (Table 1). Peridomestic abundance was markedly higher in Area 3 (n=671; 622 males and 49 females), followed by Area 6 (n=201) and 2 (n=92), while Area 8 had the fewest specimens (n=2). Intradomestic, the highest total was recorded in area 4 (n=37), with much lower values elsewhere (Table 1). Over the study period, peridomestic captures peaked in December 2023 (n=335), and the indoor maximum occurred in August 2023 (n=19), with a small rise in females in February 2024 (n=4). Microclimatic conditions at the capture sites showed little variation among areas: initial temperature ranged from 26.9 to 28.0 °C, final temperature from 21.2 to 21.9 °C, initial humidity from 46.0% to 51.9%, and final humidity from 56.7% to 59.5%.

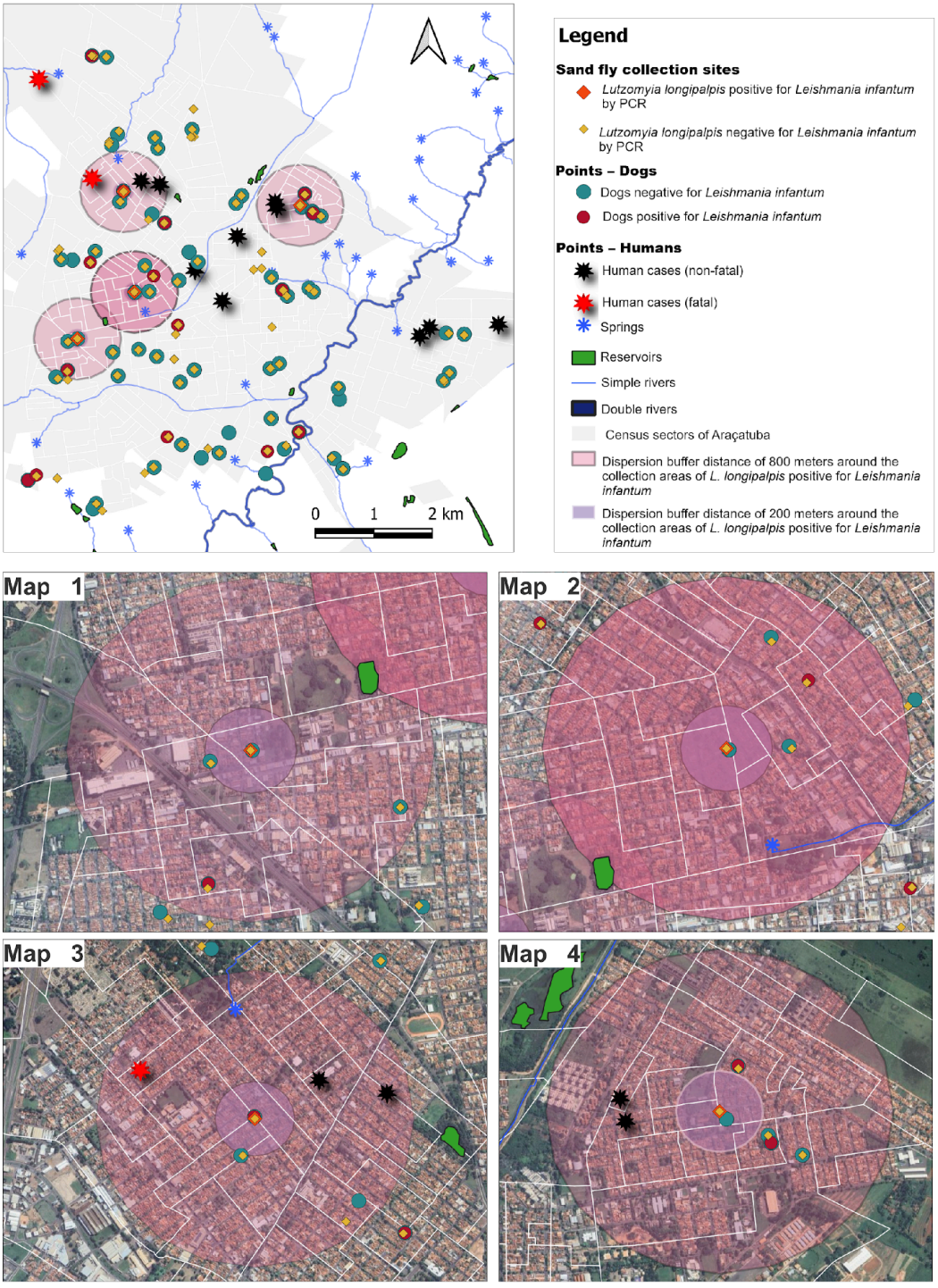
Table 1. Absolute frequency of *Lutzomyia longipalpis* by capture location, in the peridomestic and intradomestic areas, in the urban area of Araçatuba, SP, Brazil, from March 2023 to February 2024.

Area	Peridomestic		Intradomestic		Positive PCR - *Leishmania*	
	Total	CI 95%*	Total	CI 95%*	Total	CI 95%*
Female						
1	7.0	1.9 - 12.1	3.00	-0.4 - 6.4	1.0**	1.0 - 3.0
2	10.0	2.7 - 17.3	1.00	-1.0 - 3.0	1.0**	1.0 - 3.0
3	49.0	20.7 - 77.3	1.00	-1.0 - 3.0	3.0**	0.4 - 6.4
4	15.0	0.8 - 29.2	3.00	-0.4 - 6.4	0.0	NC
5	11.0	0.1 - 21.9	1.00	-1.0 - 3.0	0.0	NC
6	48.0	11.5 - 84.5	3.00	-0.4 - 6.4	0.0	NC
7	4.0	0.1 - 7.9	3.00	-0.4 - 6.4	0.0	NC
8	1.0	-1.0 - 3.0	0.00	NC	0.0	NC
Total	145.0		15.0		5.0	
Male						
1	40.0	6.1 - 73.9	3.0	-0.4 - 6.4		
2	82.0	27.0 - 137.0	3.0	-1.4 - 7.4		
3	622.0	229.5 - 1014.5	11.0	0.9 - 21.1		
4	64.0	16.7 - 111.3	34.0	1.1 - 66.9		NC
5	26.0	-2.7 - 54.7	5.0	0.6 - 9.4		
6	153.0	75.0 - 231.0	5.0	-1.5 - 11.5		
7	15.0	5.3 - 24.7	2.0	-0.8 - 4.8		
8	1.0	-1.0 - 3.0	0.0	NC		
Total	1003.0		63.0			
Total						
1	47.0	12.6 - 81.4	6.0	1.2 - 10.8		
2	92.0	33.8 - 150.2	4.0	-0.8 - 8.8		
3	671.0	255.2 - 1086.8	12.0	1.7 - 22.3		
4	79.0	24.9 - 133.1	37.0	3.9 - 70.1		NC
5	37.0	-0.4 - 74.4	6.0	1.2 - 10.8		
6	201.0	99.2 - 302.8	8.0	0.7 - 15.3		
7	19.0	7.9 - 30.1	5.0	0.6 - 9.4		
8	2.0	-1.9 - 5.9	0.0	NC		
Total	1148.0		78.0			

*: Confidence interval

Kernel density maps showed multiple hotspots of *L. longipalpis* abundance in central–eastern urban sectors (Figure 1).

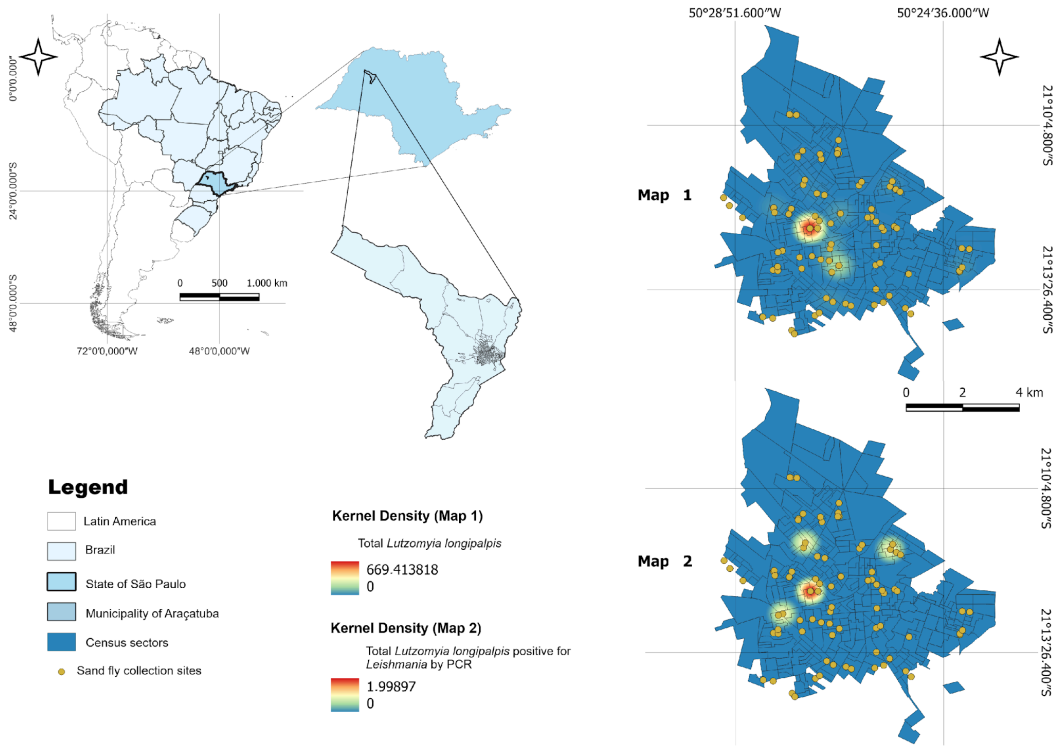
Figure 1. Map showing buffer zones (200-800 m) around *Lutzomyia longipalpis* collection sites by *Leishmania infantum* presence (PCR).



A second kernel surface based on PCR-positive *L. longipalpis* indicated smaller and more focal intensity peaks (0 – 1.999). Buffer analyses centered on positive-vector sites (200 m and 800 m) revealed co-location of canine infections and human VL cases, including fatal cases, within or

adjacent to these rings, especially in four clusters displayed in the high-resolution panels (Figure 2). Hydrological features, including springs, small streams, and drainage channels, intersected several of these clusters.

Figure 2. Spatial distribution and density of *Lutzomyia longipalpis* and specimens positive for *Leishmania* by PCR in the municipality of Araçatuba, São Paulo, Brazil.



Nonparametric comparisons revealed heterogeneity among peridomestic sites for male counts ($p < 0.01$), but not for females ($p = 0.141$). Dunn–Bonferroni post hoc analysis identified significant differences between Area 3 vs. 5, 6 vs. 5, 3 vs. 7, and 6 vs. 7 (all $p < 0.01$). Intradomestic, male counts differed between area 4 and Area 2 and 7 ($p < 0.05$), while female indoor counts did not differ among Area (Table 3).

Table 3. Multiple comparisons between different areas in the distribution of phlebotomine sandflies, in peridomestic and intradomestic areas, in the urban area of Araçatuba, SP, Brazil, during the period from March 2023 to February 2024.

Area	Peridomestic			Area	Intradomestic		
	p[1]	Z-statistic	p Ajusted[2]		p[1]	Z-statistic	p Ajusted[2]
			♀[3]				
	0.1414				0.8228		
			♂[4]				
Area 3 vs. Area 5	0.0001	4.0105	0.0008	Area 4 vs. Area 2	0.0285	-3.0773	0.0292
Area 6 vs. Area 5		-4.0095	0.0009	Area 4 vs. Area 7		3.1305	0.0244
Area 3 vs. Area 7		3.5778	0.0049				
Area 6 vs. Area 7		3.5721	0.0050				
			Total				
Area 3 vs. Area 7	0.0031	3.1041	0.0267		0.1341		
Area 6 vs. Area 7		3.0999	0.0271				

[1]: Kruskal-Wallis; [2]: Dunn's test with Bonferroni adjustment; [3]: Female; [4]: Male

Peridomestic activity showed a clear summer peak, with the highest monthly total in December 2023 (n=335) and the lowest in September 2023 (n=8). From intradomestic, captures peaked in August 2023 (n=19) and dropped to zero in September 2023. Overall, peridomestic captures ranged from 40 to 335 and indoor captures from 0 to 19 throughout the study period (March 2023–February 2024). Detailed monthly totals by sex are presented in Table 4.

Table 4. Number of *Lutzomyia longipalpis* specimens, by sex (male and female) and trap location (peridomestic and intradomestic) over several months, in the urban area of Araçatuba, SP, Brazil, from March 2023 to February 2024.

Period	Trap location													
	Peridomestic							Intradomestic						
	♂[1]	♀[2]	T[3]	Med[4]	SD[5]	Min[6]	Max[7]	♂[1]	♀[2]	T[3]	Med[4]	SD[5]	Min[6]	Max[7]
march, 2023	104	8	112	0.6	3.8	0	41	7	0	7	0.0	0.3	0	3
april, 2023	77	13	90	0.4	2.0	0	23	5	2	7	0.0	0.2	0	2
may, 2023	71	6	77	0.4	3.4	0	36	0	1	1	0.0	0.1	0	1
june, 2023	31	9	40	0.2	1.2	0	13	9	3	12	0.1	0.4	0	4
july, 2023	65	6	71	0.4	3.9	0	49	7	2	9	0.1	0.3	0	2
august, 2023	33	12	45	0.2	1.0	0	10	19	0	19	0.1	1.1	0	15
september, 2023	5	3	8	0.1	0.8	0	6	0	0	0	0.0	0.0	0	0
october, 2023	52	10	62	0.3	2.3	0	19	1	0	1	0.0	0.1	0	1
november, 2023	53	8	61	0.4	2.5	0	23	2	0	2	0.0	0.1	0	1
december, 2023	292	43	335	1.7	12.9	0	164	1	2	3	0.0	0.1	0	1
january, 2024	107	12	119	0.6	3.9	0	42	7	1	8	0.0	0.4	0	6
february, 2024	113	15	128	0.6	5.0	0	60	5	4	9	0.0	0.2	0	1

[1]: Male; [2]: Female; [3]: Total; [4]: Media; [5]: Standard Deviation; [6]: Minimum; [7]: Maximum

Post hoc GLM comparisons revealed significant temporal variation in sand fly abundance across months. In peridomestic environments, December 2023 showed markedly higher counts than most other months (all $p < 0.001$), including April, May, July, August, and November, confirming a strong seasonal peak at the end of the year. In contrast, June and October 2023 presented significantly lower counts compared with April 2023 ($p < 0.05$). In intradomestic aereas, significant differences were observed mainly between August 2023 and other months, particularly December 2023, March 2023, May 2023, and November 2023 ($p < 0.05$). These results, summarized in Table 6, indicate clear seasonal fluctuations in both peridomestic and intradomestic sand fly activity, with maximum abundance concentrated in the warmer, more humid summer months.

In intradomestic GLMs, likelihood ratio tests indicated that final temperature ($\chi^2 = 3.93$, df = 1, $p = 0.047$) and final humidity ($\chi^2 = 3.02$, df = 1, $p = 0.082$) were the only variables influencing sand fly counts, with positive and negative effects, respectively. Month effects were also significant ($\chi^2 = 34.38$, df = 11, $p < 0.001$), confirming temporal variation in indoor captures. Model coefficients revealed a positive association between final temperature (Estimate = +0.01287, SE = 0.0058, z = 2.22, $p = 0.027$) and phlebotomine abundance. In contrast, final humidity showed a slight negative relationship (Estimate = −0.00377, SE = 0.00184, z = −2.05, $p = 0.041$) (Table 5).

Table 5. Variations in sandfly counts, analyzed using a Generalized Linear Model (Square Root Link Function), in peridomestic and intradomestic areas of Araçatuba, SP, Brazil, from March 2023 to February 2024.

Variables/Area	Likelihood ratio tests			Generalized Linear Model (GLM)			
	X^2	df	p	Estimate	SE	z	p
Peridomestic							
Initial temperature	10.82	1	0.001	-0.0228	0.0070	-3.232	0.001
Final temperature	49.37	1	<0,001	0.0430	0.0058	7.460	< 0,001
Initial humidity	13.37	1	<0,001	-0.0080	0.0020	-3.999	< 0,001
Final humidity	1.15	1	0.284	0.0021	0.0018	1.127	0.260
Months	236.84	11	<0,001				
august, 2023 - april, 2023				-0.1231	0.0529	-2.326	0.020
december, 2023 - april, 2023				0.5476	0.0610	8.982	< 0,001
february, 2024 - april, 2023				0.0554	0.0573	0.968	0.333
january, 2024 - april, 2023				-0.0483	0.0606	-0.797	0.425
july, 2023 - april, 2023				0.0966	0.0683	1.414	0.157
june, 2023 - april, 2023				-0.2104	0.0523	-4.021	< 0,001
may, 2023 - april, 2023				-0.0411	0.0517	-0.794	0.427
march, 2023 - april, 2023				-0.0486	0.0582	-0.835	0.404
november, 2023 - april, 2023				-0.0243	0.0581	-0.419	0.675
october, 2023 - april, 2023				-0.1476	0.0527	-2.802	0.005
september, 2023 - april, 2023				-0.1549	0.0868	-1.785	0.074
Intradomestic							
Initial temperature	0.113	1	0.737	-0.0033	0.00709	-0.465	0.642
Final temperature	3.932	1	0.047	0.01287	0.0058	2.217	0.027
Initial humidity	0.528	1	0.467	9.39e-4	0.00215	0.436	0.663
Final humidity	3.017	1	0.082	-0.00377	0.00184	-2.051	0.04
Months	34.377	11	< 0,001				
august, 2023 - april, 2023				0.16995	0.05535	3.070	0.002
december, 2023 - april, 2023				-0.05938	0.06135	-0.968	0.333
february, 2024 - april, 2023				0.02396	0.05832	0.411	0.681
january, 2024 - april, 2023				-0.01723	0.06102	-0.282	0.778
july, 2023 - april, 2023				0.05931	0.0707	0.839	0.402
june, 2023 - april, 2023				0.0551	0.05333	1.033	0.301
may, 2023 - april, 2023				-0.08227	0.05413	-1.520	0.128
march, 2023 - april, 2023				-0.06828	0.05849	-1.167	0.243
november, 2023 - april, 2023				-0.06306	0.06026	-1.046	0.295
october, 2023 - april, 2023				-0.08462	0.05558	-1.522	0.128
september, 2023 - april, 2023				-0.02827	0.09073	-0.312	0.755

Consistently, post hoc contrasts demonstrated that August 2023 had significantly higher indoor counts than March, May, October, and November 2023, as well as December 2023 ($p < 0.05$ for all) (Table 6). These results suggest that intradomestic sand fly activity increased with warmer and drier conditions, reaching its highest levels in late winter (August).

Table 6. Post hoc comparisons (GLM) for the different months, in the peridomestic area, using Bonferroni correction, in the urban area of Araçatuba, SP, Brazil, from March 2023 to February 2024.

Compared Variable (months)	Estimated Difference	Standard Error	Z-value	Bonferroni adjusted p-value
Peridomestic				
december 2023 vs april 2023	-0.5476	0.061	-89,815	< 0,001
june 2023 vs april 2023	0.2104	0.0523	40,213	0.044
august 2023 vs december 2023	-0.6707	0.0636	-105,538	< 0,001
august, 2023 vs july, 2023	-0.21973	0.0621	-353,851	0.027
august, 2023 vs february, 2024	0.49217	0.0572	861,178	< 0,001
december, 2023 vs january, 2024	0.59589	0.0528	1,129,477	< 0,001
december, 2023 vs july, 2023	0.45099	0.0855	527,402	< 0,001
december, 2023 vs june, 2023	0.75803	0.0681	1,112,457	< 0,001
december, 2023 vs may, 2023	0.58865	0.0672	875,582	< 0,001
december, 2023 vs march, 2023	0.59615	0.0616	967,412	< 0,001
december, 2023 vs november, 2023	0.57194	0.0628	910,794	< 0,001
december, 2023 vs october, 2023	0.69515	0.0611	1,137,613	< 0,001
december, 2023 vs september, 2023	0.70248	0.1028	683,251	< 0,001
february, 2024 vs june, 2023	0.26586	0.0656	405,486	0.003
february, 2024 vs october, 2023	0.20298	0.0546	372,059	0.013
february, 2024 vs june, 2023	0.30703	0.0625	491,018	< 0,001
Intradomestic				
august, 2023 vs december, 2023	0.22933	0.0652	3.515	0.029
august, 2023 vs may, 2023	0.25223	0.0527	4.785	< 0,001
august, 2023 vs march, 2023	0.23823	0.0697	3.420	0.041
august, 2023 vs november, 2023	0.23302	0.0647	3.599	0.021
august, 2023 vs october, 2023	0.25458	0.0598	4.260	0.001

4. Discussion

The analysis of seasonality, spatiotemporal distribution, and climatic factors associated with the abundance of *L. longipalpis* in Araçatuba reveals an epidemiological pattern consistent with findings reported in other endemic regions of Brazil, while also highlighting local specificities that may influence surveillance and control strategies. The peak of vector activity recorded in the peridomestic area in December 2023 confirms the strong influence of seasonality on the species' population dynamics, a finding widely documented in endemic areas, such as in Dracena (SP), where eight years of monitoring identified peridomestic predominance and a population increase at the beginning of the rainy season [14,15].

In Campo Grande (MS), vector activity remained constant throughout the year but exhibited marked peaks immediately after periods of heavy rainfall [16], a phenomenon also observed in Pernambuco [17], reinforcing that seasonality responds to microclimatic variations that directly affect the survival, reproduction, and dispersal of the species [18].

In the present study, statistical modeling (GLM) revealed a positive association be-tween higher final temperatures and the abundance of sand flies in the peridomicile area, while higher initial temperatures and humidity exerted a significant negative effect. These findings align with the observations of [19], who identified a positive correlation between the abundance of *L. longipalpis* and increases in temperature and accumulated precipitation, as well as variations in humidity, suggesting that the daily oscillation of environmental parameters may modulate the vector's nocturnal activity and capture rates. In urban settings, studies have shown that population dynamics may also be influenced by structural and socioeconomic factors, including irregular solid waste disposal, forest fragments, and water drainage, which directly affect the availability of shelters and food sources [20]. Although climatic factors explain a substantial portion of temporal variation, anthropogenic aspects must also be incorporated into the interpretation of spatial patterns. In contrast to the present findings, Garcia*, et al.* [21] did not identify a correlation between phlebotomine abundance and climatic factors such as temperature and precipitation.

The heterogeneous distribution of sand flies among the study areas, with a marked concentration in Area 3—the only site with PCR-positive females for *Leishmania* spp.—reinforces the presence of microhabitats favorable to the maintenance of the vector cycle. Studies conducted in Argentina by Santini*, et al.* [22] demonstrated that macrohabitat variables, such as vegetation cover and proximity to water bodies, are associated with the presence of the vector, while abundance is more closely related to microenvironmental conditions, including shading, the presence of animals, and the accumulation of organic matter. In Araçatuba, the spatial overlap between capture points of infected females, human and canine cases, and hydrological features

suggests the coexistence of environmental factors and domestic reservoirs that favor transmission.

The predominance of captures in the peridomicile area observed in this study is consistent with the literature and has relevant epidemiological implications. Chicken coops, although not competent reservoirs for *Leishmania*, have been described as structures that increase the attraction and maintenance of sand fly populations in domestic areas, indirectly contributing to the risk of transmission [23-25].

The detection of infected females at only one focal point underscores the importance of targeted surveillance strategies that prioritize areas with higher vector density and confirmed risk, thereby optimizing control resources. Moreover, the correlation between areas of greater vector abundance and the presence of infected dogs reinforces the role of the canine reservoir in maintaining urban transmission [26-28].

The combination of temporal and spatial analyses leads to the recommendation of integrated, seasonal, and geographically focused actions. The population increase during the rainy season and the detection of infected vectors in a delimited hotspot justify the in-tensification of vector control measures—such as residual insecticide application, envi-ronmental management, and reservoir control—during and in the months preceding the seasonal peak. Similar strategies have proven effective in reducing vector density in Bra-zilian urban settings when applied with spatial and temporal precision [29]. Continuous surveillance, coupled with the incorporation of predictive climatic variables, can enhance the early detection of high-risk periods and guide preventive interventions.

In summary, the results from Araçatuba reaffirm that the ecology of *L. longipalpis* is strongly modulated by seasonal and microenvironmental factors, yet also dependent on anthropogenic determinants that shape the vector's spatial distribution. The convergence with findings from other endemic regions strengthens the external validity of the observed patterns and supports the recommendation of targeted interventions synchronized with seasonal dynamics, integrating entomological surveillance, reservoir control, and environmental improvements as pillars of sustainable management of urban visceral leishmaniasis.

5. Conclusions

The results obtained in Araçatuba confirm that the dynamics of *Lutzomyia longipalpis* are strongly modulated by seasonal, climatic, and microenvironmental factors, showing population peaks during the rainy season and a heterogeneous spatial distribution with well-defined hotspots of vector risk. The detection of *Leishmania* spp.–infected females in a specific area reinforces the importance of targeted surveillance and control approaches that consider not only seasonality but

also the environmental and urban contexts that favor the maintenance of the transmission cycle. The convergence of these findings with recent evidence from different endemic regions of Brazil supports the adoption of integrated strategies that combine continuous entomological surveillance, environmental management, reservoir control, and seasonal interventions scheduled for periods of highest risk, as fundamental pillars for effectively reducing visceral leishmaniasis transmission in urban environments.

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