

## Short Communication

# Nocturnal Activity Rhythm of *Nyssomyia intermedia* (Diptera: Psychodidae: Phlebotominae) in Campus Fiocruz Mata Atlântica, an American Cutaneous Leishmaniasis Transmission Area, Rio de Janeiro, Brazil

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### Abstract

**Introduction:** *Nyssomyia intermedia* is an important vector of American cutaneous leishmaniasis. **Methods:** Sand flies were captured in a Shannon trap and after landing on human collectors and retrieved monthly. Nocturnal activity was estimated using a log-linear model. **Results:** Peak sand fly collection in the Shannon trap occurred in summer and winter, and peak fly collection on humans occurred in spring and summer. **Conclusions:** *Ny. intermedia* was captured in both the hottest and coldest months, indicating its adaptability to different seasons and the possibility of disease transmission at any time of the year in Campus Fiocruz Mata Atlântica, Brazil.

**Keywords:** *Nyssomyia intermedia*. Nocturnal activity rhythm. Campus Fiocruz Mata Atlântica.

Sand flies exhibit fluctuations in various behavioral activities, such as sugar-feeding, host-seeking, blood-feeding, mating, and oviposition. Most sand flies are crepuscular or nocturnal in their blood-feeding habits, but species-specific differences are observed in peak activity periods, which can play a role in the vectorial capacity of different species<sup>1</sup>.

Alterations in the epidemiological profile of leishmaniasis have been reported in several regions of Brazil and are so ubiquitous that there is no doubt regarding the ability of *Nyssomyia intermedia* to invade environments, leading to striking vegetation destruction and participation in the leishmaniasis transmission cycle.

In the city of Rio de Janeiro, outbreaks of American cutaneous leishmaniasis (ACL) have followed the progression of urbanization, resulting in a discontinuous area of occurrence. Today, ACL transmission occurs primarily in the areas surrounding Pedra Branca Massif<sup>2</sup>.

This knowledge would make it possible to determine when there is an increased risk of contracting the disease and would contribute to a better understanding of the local ACL epidemiological profile in the Caminho da Cachoeira Community at Campus Fiocruz Mata Atlântica (CFMA).

Field activities were undertaken by staff of the Laboratório Interdisciplinar de Vigilância Entomológica em Diptera e Hemiptera (LIVEDIH) of the Instituto Oswaldo Cruz and the State Institute of the Environment (INEA/RJ/PR n°010/06). To reduce health risks, all individuals involved were educated on fieldwork biosafety and were appropriately equipped. The capture site was a private farm, therefore it was not necessary to obtain a field work license from government agencies. All sand fly captures were authorized by Mrs. Maria Jovelina Ferreira de Freitas, owner of the study site in the community of Caminho da Cachoeira Community in CFMA.

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The western region of CFMA is an environmental conservation area predominantly consisting of secondary Atlantic Forest vegetation, which is a dense shady forest, that is located at 22°56' 26"S, 43°28'50"W. The sand fly capture monitoring station at our study site was established on the border of the Caminho da Cachoeira Community, adjacent to the peridomiciliary area of the last house in the community, which contained four kennels and a henhouse, at a distance of approximately 9 m (Figure 1).

During the 24-month study period (January 2012 to December 2013), sand flies were captured for 12 consecutive hours (06.00 pm to 06.00 am) once each month, except in June and November 2012 and January and March 2013, when heavy rains rendered collection impracticable. In total, our study included 240 hours of capture time.

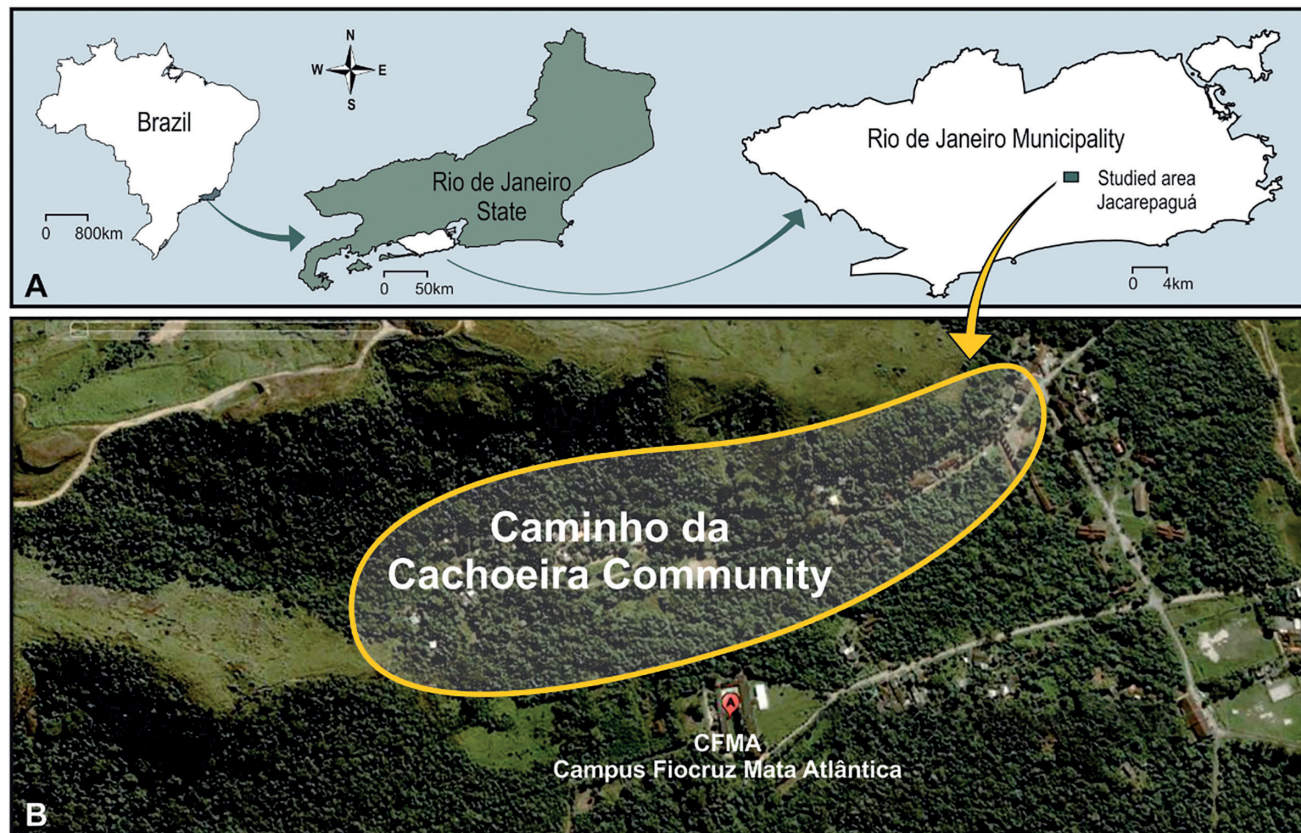
Sand flies were attracted to the Shannon trap by a light source (LED lamp) and to two staff members who acted as human collectors. Every hour, the collected specimens were separated into individual Falcon tubes to evaluate frequency and detect potential vector species. At the same time, attraction to humans was evaluated hourly by collecting the specimens that landed on the human collectors into separate Falcon tubes. Temperature and relative humidity were also recorded hourly. The captured insects were transported to LIVEDIH, where they were clarified as follows: (1) KOH 10% was poured into a

well containing the phlebotominae, submerging them for 3 h to soften the chitin. (2) Then, the phlebotominae were transferred, with a stylet, to another well containing acetic acid P.A., and incubated for approximately 20 min to neutralize the KOH. (3) Next, the phlebotominae were washed by transferring them to a well containing type II water and incubating for 20 min. (4) Finally, the insects were transferred to a lactophenol solution and incubated for 24 h to finalize the diaphanization process for taxonomic identification using the nomenclature of Galati<sup>3</sup>.

The outcome was the number of sand flies collected per hour. The expected number of sand flies per hour,  $\lambda_t$ , represents *Ny. intermedia* nocturnal activity where  $t = 0$  is 06.00 pm;  $t = 1$  represents is the time period from 06.00–07.00 pm; and  $t = 2$  is 07.00–08.00 pm, and so on. This rate was determined to be a log-concave function of time, as follows:

$$\lambda_t = \chi e^{-\phi(t-\mu)^2}$$

where  $i$  represents the time (in hours) after 6.00 pm, which was used to estimate the exact time when peak activity occurred;  $\phi$  represents the variability in flight throughout the night, with smaller values indicating more sand flies around the peak time; and  $\chi$  represents the expected number of sand flies at the peak hour. A log-linear model was fitted for different seasons, and then the parameters ( $i$ ,  $\phi$ , and  $\chi$ ) were estimated directly from the model coefficients<sup>4</sup>.



**FIGURE 1:** (A) Map of Brazil, Rio de Janeiro State, and Rio de Janeiro Municipality, highlighting the study area in the Jacarepaguá neighborhood. (B) Google Maps image showing the capture station at Caminho da Cachoeira Community, Campus Fiocruz Mata Atlântica, Jacarepaguá, Rio de Janeiro, Brazil.

A total of 14,497 sand flies were captured over the two-year sampling period (56.3% in 2012 and 43.7% in 2013), which belonged to the genera *Brumptomyia*, *Expapillata*, *Lutzomyia*, *Micropygomyia*, *Migonemyia*, *Nyssomyia*, *Pintomyia*, and *Psychodopygus*. The most common species was *Ny. intermedia* (93.8%), followed by *Mg. migonei* (3.4%), *Pi. fischeri* (1.3%), and *Ps. h. hirsutus* (0.8%); these four ACL vector species represented 99.3% of the collected specimens. *Ny. intermedia* was the most frequently captured species in the Shannon trap in 2012 (95%) and 2013 (93.6%) and from humans in 2012 (90%) and 2013 (89.3%).

The nocturnal activity of *Ny. intermedia* was estimated using two years of seasonal observation data collected from the Shannon trapping. The highest sand fly abundance was observed in winter and summer, with different peak activity times (10.49 pm and 00.56 am, respectively). In the transitional seasons, late spring and autumn, activity peaked at 00.55 am and 11.38 pm, respectively (Figure 2). Nocturnal activity based on the landing of *Ny. intermedia* on humans was estimated using the same method. Similar to the Shannon trap results, the highest sand fly abundance was observed in spring and summer; again with different peak activity times of 00.32 am and 00.55 am, respectively. In winter and autumn, activity peaked at 00.00 am and 10.55 pm, respectively. These results revealed the tendency for sand flies to be attracted to humans and feed on blood earlier in the night (Figure 3).

The vectorial capacity of *Ny. intermedia* was discovered by Aragão in Rio de Janeiro<sup>5</sup>, and the species has been found to be naturally infected by flagellates in the states of Paraná<sup>6</sup>, São Paulo<sup>7</sup>, Espírito Santo<sup>8</sup>, and Rio de Janeiro<sup>9</sup>. It has also been found to be naturally infected by *Leishmania (Viannia) braziliensis* in Pau da Fome, Cachamorra, Cabuçú, Lameirão Pequeno, and CFMA (previously Juliano Moreira Colony) using a polymerase chain reaction (PCR) multiplex non-isotopic hybridization assay<sup>10</sup>. These findings support the hypothesis that this sand fly species is the main vector of ACL in the state of Rio de Janeiro.

The occurrence of ACL outbreaks in the city of Rio de Janeiro has followed the progressive urbanization, which has created discontinuous areas of infection. The activity of *Ny. intermedia* in colder months may be related to epidemiological risk factors, since low temperatures may cause the sand flies to seek more favorable conditions, bringing them into contact with humans and enabling the transmission of *L. (V.) braziliensis* within domiciles. *Ny. intermedia* is recognized for its anthropophilic behavior and tendency to invade homes to obtain blood and acquire shelter. Campbell-Lendrum et al. highlighted the endophagic and endophilic characters of *Ny. intermedia* and its positive phototaxis, which increase its opportunities to encounter a human host. Its tendency to search for domiciliary shelter may explain, in part, the decline in the outside abundance of *Ny. intermedia* after midnight, as by that

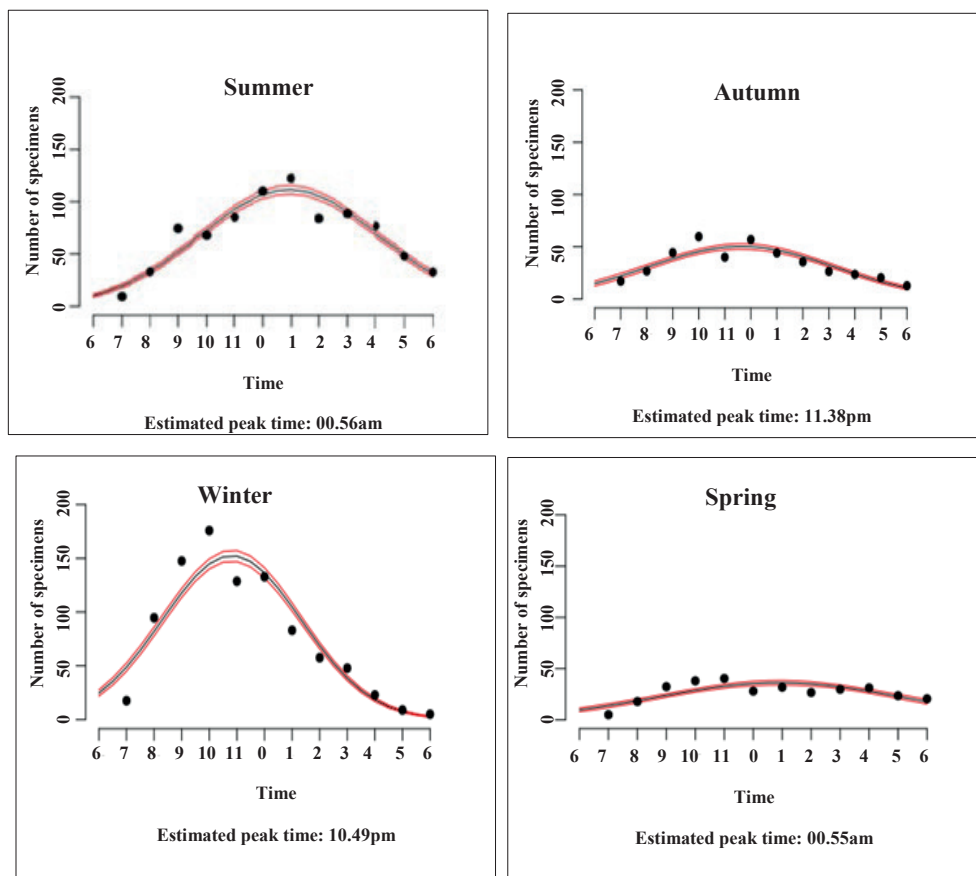
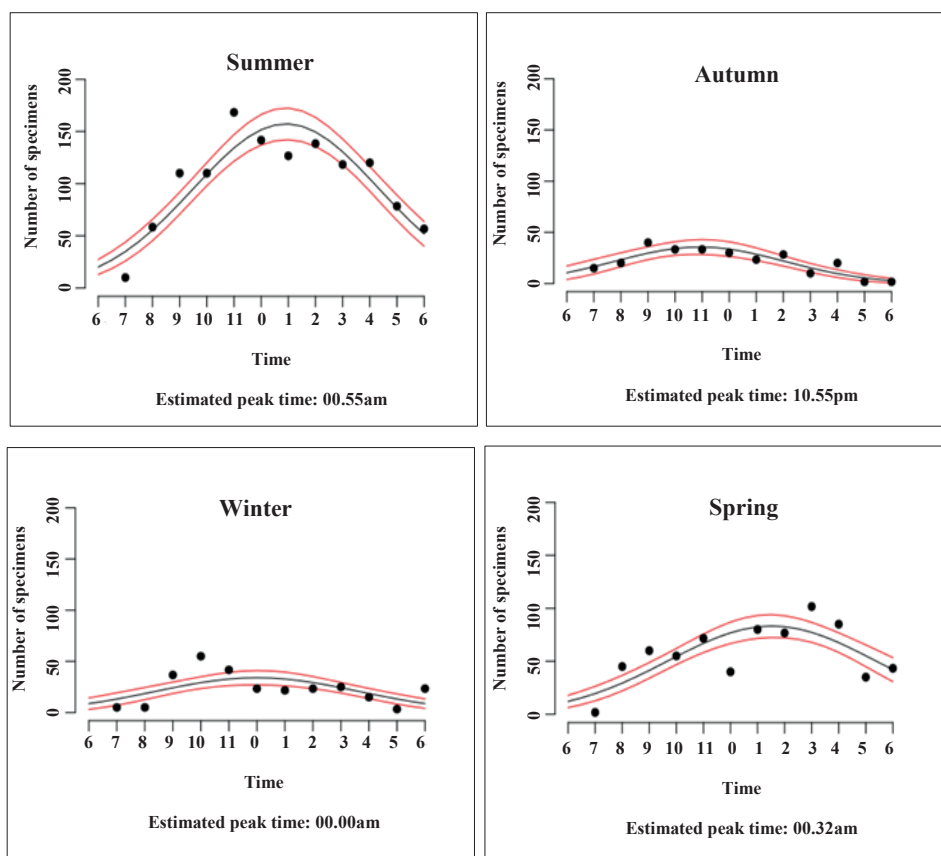


FIGURE 2: Nocturnal activity of the sand fly *Nyssomyia intermedia* captured in Shannon traps in the transitional area during the period from January 2012 to December 2013, with estimated peaks for each season.





**FIGURE 3:** Nocturnal activity of the sand fly *Nyssomyia intermedia* captured on human collectors in the transitional area during the period from January 2012 to December 2013, with the estimated peaks for each season.

time, they would have entered homes, making it one of the most important factors in the transmission of ACL<sup>11</sup>.

In our study, more specimens were captured in winter and summer, with peaks at 10.49 pm and 00.56 am, respectively. Additionally, *Ny. intermedia* attraction to humans peaked at 00.55 am in summer and at 00.32 am in spring. It is noteworthy that *Ny. intermedia* was captured during every sampling period. In a study conducted in Vale do Ribeira in the state of São Paulo, Gomes and Galati observed *Ny. intermedia s. l.* activity rhythms throughout the night in residual and extra forest environments. In a poultry coop, activity peaked between 08.00 pm and 10.00 pm, whereas in the human attraction study, activity peaked at approximately 08.00 pm. Maximum activity in the Shannon trap occurred between 10.00 pm and 11.00 pm<sup>12</sup>. In a Shannon trap study conducted in the municipality of Itupeva, in the southeast region of the state of São Paulo, Mayo et al. found that *Ny. intermedia s. l.* was at its highest density in the coldest and driest period of the year (April–September), with the highest activity between the second and fifth hour after twilight, reaching a peak in the third hour, at approximately 08.00 pm<sup>13</sup>. In a study conducted on the coast of São Paulo state, Brito et al. reported the presence of *Ny. intermedia s. l.* throughout the night, based on a six-hour manual collection and 12-hour Shannon trap capture, although the highest frequencies occurred in the first five hours after twilight, with a decrease in the third hour. Based on hand collections from Shannon traps throughout the year,

the highest peaks were observed in May–August<sup>14</sup>. In a study conducted in a peridomestic area of Iporanga municipality, São Paulo state, 47.4% of the *Ny. intermedia s. l.* specimens and 50.5% of the *Ny. neivai* specimens were captured between 06.00 pm and 07.00 am, and 70% of the specimens of both species were captured between 06.00 pm and 00.00 am. The authors reported that the activity of *Ny. intermedia* females peaked between 07.00 pm to 08.00 pm; and the highest hourly average for *Ny. intermedia* females on the black trap occurred in winter, while the activity peak for males was between 07.00 pm to 08.00 pm in the summer<sup>15</sup>. All studies performed in the state of São Paulo showed similar *Ny. intermedia s. l.* rhythms, varying between 07.00 pm and 10.00 pm. In the city of Petrópolis, in the state of Rio de Janeiro, Souza et al. reported peak activities (varying from 07.00 pm to 10.00 pm) for species that were similar to those in the studies conducted in São Paulo state. The authors stated that the seasonal activity peaks in Petrópolis occurred between 09.00 pm and 10.00 pm in winter and spring, respectively, and between 08.00 pm and 09.00 pm in summer and autumn, respectively. Therefore, our results, which showed *Ny. intermedia* activity peaking between 10.00 pm and 01.30 am, differed somewhat from the results of previous studies<sup>1</sup>.

A study by Souza et al. conducted in Caminho da Cachoeira Community demonstrated the sylvatic cycle of ACL and its connection with disease transmission in CFMA communities, which showed a clear predominance of *Ny. intermedia*<sup>1</sup>.

Our studies indicate that, for the people of Caminho da Cachoeira Community, the time period with the highest risk of being bitten by *Ny. intermedia*, the main transmitter of ACL, is between 10 pm and 1 am. This coincides with the time when residents are returning from their professional, educational, and religious activities, putting them at greater risk of infection. Therefore, we emphasize the need to adopt policies involving health education, environmental management, and an understanding of the basic concepts of the disease, to ensure the success of an integrated program of entomological surveillance and ACL control in this community.

*Ny. intermedia* has been reported as the main vector of ACL in the state of Rio de Janeiro. Reports of its presence in both domiciliary environments and preserved forests indicate its wide-ranging habit and leave no doubt regarding its ability to invade environments influenced by anthropic activity, where it becomes established and participates in a peridomestic transmission cycle. Therefore, it is relevant that in our study *Ny. intermedia* was captured in both the hottest and coldest months, indicating the adaptability of this vector to different seasons and that disease transmission can occur at any time of the year in CFMA, Rio de Janeiro city, Brazil.

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### Conflict of interest

The authors declare that there is no conflict of interest.

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