

Abundance of *Lutzomyia longipalpis* and *Nyssomyia whitmani*, the *Leishmania* spp. vectors in northeastern of Argentina: Are spatial and temporal changing patterns consistence?

María Soledad Santini^{a,b,c,*}, Regino Cavia^{b,d}, María Gabriela Quintana^{b,c,e,f},
Mariana Manteca Acosta^{b,c,g}, Oscar Daniel Salomón^{b,c,e}

^a Instituto Nacional de Parasitología "Dr. Mario Fatała Chaben" (INP)-ANLIS, Malbrán-Ministerio de Salud de la Nación, Buenos Aires, Argentina

^b Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

^c Leishmaniasis Investigation Network of Argentina (ReDILA)

^d Dto. de Ecología, Genética y Evolución, FCEN, UBA e Instituto de Ecología, Genética y Evolución de Buenos Aires (UBA-CONICET), Buenos Aires, Argentina

^e Instituto Nacional de Medicina Tropical (INMeT)- ANLIS, Malbrán- Ministerio de Salud de la Nación, Puerto Iguazú, Misiones, Argentina

^f Instituto Superior de Entomología (INSUE), FCNeIML, UNT, San Miguel de Tucumán, Tucumán Argentina

^g Centro Nacional de Diagnóstico e Investigación en Endemo-epidemias (CeNDIE)-ANLIS, Malbrán-Ministerio de Salud de la Nación, Buenos Aires, Argentina

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ABSTRACT

Lutzomyia longipalpis and *Nyssomyia whitmani* were incriminated as vectors of *Leishmania* sp. Spatial heterogeneity together with seasonal changes in abundance constitute important elements for the understanding of the dynamics of vector populations, and there are fundamental for the development of adequate prevention and control strategies. The aim of this work was to compare the spatial and seasonal abundance of *Lu. longipalpis* and *Ny. whitmani* at a city spatial scale between two periods separated by three years. To study the spatial distribution, we compared the abundance distribution of these species at two warm times, 2011 with 2014. Respect to inter-annual seasonal abundance changes, we compared the four seasons of the year between two periods (2011–2012 vs 2014–2016). The spatial distribution for both species were found to be distributed mainly in the same areas of the city in both periods. We change for: Respect to the seasonal pattern of abundance, we observed that seasonal patterns showed changes between periods. Our study defines the 'where' and 'when' implement the actions to mitigate leishmaniasis cases.

1. Introduction

Leishmaniasis are zoonotic diseases caused by protozoan parasites from the genus *Leishmania* (Kinetoplastida: Trypanosomatidae). They are transmitted to the vertebrate host by phlebotomine sand flies (Psychodidae: Phlebotominae). In America, *Lutzomyia longipalpis* (Lutz and Neiva, 1912) is the main vector of *Leishmania infantum* Nicole, 1908, the aetiological agent of Visceral Leishmaniasis (VL) (Lainson & Rangel, 2005). However, respect to Tegumentary Leishmaniasis (TL), in Puerto Iguazú in the Northeast of Argentina *Nyssomyia whitmani* (Pinto, 1926) was incriminated as the main vector of *Leishmania braziliensis* Vianna, 1911, (Salomón et al., 2009a).

The distribution and epidemiology of parasitic diseases in urban and periurban areas of endemic countries have been changing as

development and urbanisation progresses (Mott et al., 1990; Rodrigues et al., 2019; Santos et al., 2021). While *Lu. longipalpis* in the last few decades, has adapted to be an urban or peri-urban species (Souza et al., 2002; Rangel & Lainson, 2009; Fernández et al., 2012; Pinheiro et al., 2013; Salomón et al., 2009b; María Soledad Santini et al., 2013; Dorval et al., 2016), *Ny. whitmani* is a species of forest and rural areas (Souza et al., 2002; Rangel & Lainson, 2009; Fernández et al., 2012; Pinheiro et al., 2013; Salomón et al., 2009a; Santini et al., 2013; Dorval et al., 2016), but also shows a trend to colonize periurban areas (Rangel & Lainson, 2009; Santini et al., 2013; Neves et al., 2021; Santini et al., 2018).

Both sand fly species show spatial and temporal variations in their abundances (Brandão-Filho et al., 2011; Donalisio et al., 2012; Fernández et al., 2012, 2013) and different studies show the distribution

* Corresponding author at: Av. Paseo Colón 568, 1°, CP 1063, CABA, Argentina.
E-mail address: msantini@anlis.gob.ar (M.S. Santini).

of these species at the village/city spatial scale (de Souza et al., 2014; Oliveira et al., 2008; Santini et al., 2018). Moreover, several works show different patterns of abundance associated to seasonal environmental fluctuations. Abiotic factors as temperature, relative humidity, rainfall including wind have been studied as possible factors correlated with phlebotomine sand fly seasonal dynamics (de Andrade et al., 2014; de Oliveira et al., 2013; Oliveira et al., 2008; Santini et al., 2018). However, studies simultaneously examining the city abundance distributions of both species, vectors of TL and VL, during two periods to compare abundance inter-annual spatial and seasonal abundance changes are scarce.

The inter-annual consistency of the spatial distribution was studied at city scale for *Lu. longipalpis*, with regard to the distribution of clusters of high abundance, and surrounding areas of lower or null abundance (Berrozpe et al., 2017, 2019; Fernández et al., 2013; Gómez-Bravo et al., 2017; Salomón et al., 2009).

The persistence of these patterns, in addition to the interest from the ecology about population dynamics and landscape fragmentation, served as the basis for proposing a surveillance-control strategy based on the 'hot spot' hypothesis (Salomon, 2020). Spatial consistency over years could contribute to define 'where' to make focal interventions, and seasonal consistency could define 'when', without the need for monitoring the abundance of this vectors throughout the year. Finding patterns of abundance consistent in time and space would avoid the need for constant monitoring at a whole city scales for preventive purposes, while would allow to concentrate management efforts at the required times and places.

In the focus of Puerto Iguazú, Misiones province, Argentina, we reported factors associated with the spatial distribution of abundance of both species by cross sectional surveys (Quintana et al., 2020), the annual seasonal pattern with *Lu. longipalpis* peaking in early autumn, while *Ny whitmani* was more abundant in spring and summer (Santini et al., 2018).

Spatial heterogeneity together with seasonal changes in abundance constitute important elements for the understanding of the dynamics of vector populations (Liebhold, Rossi, & Kemp, 1993), and there are fundamental for the development of adequate prevention and control strategies (Felicangeli et al., 2006). Therefore, the aim of this work was to compare the spatial and seasonal abundance of *Lu. longipalpis* and *Ny. whitmani* at a city spatial scale in Puerto Iguazú between two periods separated by three-four years, to evaluate if the patterns remain. This approach will allow at local scale to develop differential health messages, recommendations, and interventions, and to the proper allocation of resources according to VL or TL risk. Also serve at the regional scale level to consolidate the surveillance and control strategy for each of these zoonoses, based on the spatiotemporal persistence of phlebotomine vector populations (Thomaz-Soccol et al., 2018). Furthermore, these approaches contribute to understand the dynamics when two vectors at different stages of urbanisation occur in the same city, *Lu. longipalpis* of recent but established colonization and with steady or declining LV transmission, and *Ny. whitmani* encroaching the urban the most urbanised area with TL sporadic foci in periurban.

2. Materials and methods

2.1. Study area

This study was carried out in the city of Puerto Iguazú, Misiones, Argentina (25° 36' S, 54° 35' W). Since this city is one of the most important touristic areas of Argentina due to its proximity to Iguazú waterfalls.

This area belongs to the Paranaense forest ecoregion, a subtropical humid forest from the Amazonian domain Oyarzabal et al., (2018). The weather is subtropical without dry stations and hot summers. The coldest months are June and July, with a mean minimum temperature of 11.6 °C and 10.8 °C respectively, while the warmest month is January

with a mean maximum temperature of 31.8 °C. Rainfalls are abundant all around the year with average annual values of 1919 mm (Data from National Meteorological Service).

2.2. Entomological sampling

Each sample household was selected using the 'critical site' criterion (Quintana et al., 2020). One REDILA-BL light trap (Fernández et al., 2015) was set in each sampled site. Traps were placed 1.5 m above the ground in the henhouse or when it was absent, within the outside hens or dogs resting places. Traps were active during the night, without rain or strong wind, approximately from 5:00 p.m. to 9:00 a.m., and were placed for two consecutive nights in the first sampling (2011–2012) and three consecutive nights in the second one (2014–2015), to standardize the capture effort of different simultaneous projects.

All phlebotomine sand fly were dried and preserved prior to processing. The specimens were cleared with lacto-phenol. The identification by species and sex were performed according to Galati (2018) under a microscope 40X (Zeiss®, 400x).

All of the sampled households were geopositioned with a hand-held Global Positioning System Garmin (eTrex® HC series model) and located within a map of Puerto Iguazú using QGIS (QGIS Development Team, 2016).

2.3. Sampling design

To study the spatial distribution, we compared the abundance distribution of *Lu. longipalpis* and *Ny. whitmani*, within the city of Puerto Iguazú at two warm times, 2011 (December 14th – 17th) with 2014 (November 26th – December 02nd). This period was selected because in this latitude it is during this period that the new reproductive generations emerge (Manteca Acosta et al., 2021; Salomón et al., 2004). In both studied periods the city of Puerto Iguazú was divided in quadrants of 400 by 400 m, 51 quadrants in 2011 and 54 quadrants in 2014. The quadrant areas were the same in both sampling periods. Although the sampled households were selected using the same criterion of 'critical site', were not always the same ones due to changes in the micro-environment that modified its quality as critical sites.

Respect to inter-annual seasonal abundance changes, we compared the four seasons of the year (spring, summer, autumn, and winter) between two periods: 2011–2012 (first period) vs 2014–2016 (second period). In this study we compared the same season, although there are not the same precise days due to logistical issues. We contrasted the results to Santini et al., 2018 (from 24 to 27 September 2011, 14 to 17 December 2011, from 19 to 22 March 2012, and 25 to 28 June 2012), with the new data presented in this article of November 26th to December 02nd (Spring to 2014), March 3th to 5th (Fall to 2015); August 10th to 12nd (winter to 2015); October 19th to 21st (Spring to 2015) and February 1st to 4th (Summer to 2016), by logistic reason we incorporated this second moments of sampling in the summer season. Respect to date poll of March 3th to 5th 2015, due to it was not possible to carry out the survey post march 21 too due to logistical reasons, this period was analysed as Fall given the proximity of the date to the beginning of the autumn equinox. In order to be able to carry out this comparison, the cross-sectional study 2015–2016, was divided into three categories according to the abundance of sand flies collected. The categories were null abundance: zero phlebotomine sand flies per trap night; intermediate abundance: 1 – 15 individuals per trap night; and highest abundance: more than 15 individuals per trap night. From these three categories, 9 houses were randomly selected, 3 from each category, to be sampled during the days described above.

2.3.1. Spatial distribution analysis

The abundance of each species in each household was estimated by the trap success, expressed as the number of individuals caught/number of nights the traps were set (Seber, 1973), since the trapping effort was

different in both periods. Before comparing the abundance distribution within the city between the two periods (spring 2011 vs spring 2014), the spatial dependence of the abundance of both species and periods within the whole city was analysed. For this goal the spatial autocorrelation was studied by semivariograms of the abundance of *Lu. longipalpis* and *Ny. whitmani* (Legendre and Legendre 2013). To estimate the range, the nugget and the sill, we fitted the semivariogram function using a spherical model. To account for anisotropy, four directions were considered: 0°, 45°, 90° and 135°. Then, if corresponded, vector abundances were interpolated using an ordinary kriging procedure, according to the parameters estimated from the semivariogram functions of each species and period, to predict their abundance distribution within the whole city. Afterward, we overlapped the interpolated abundance maps of each phlebotomine sand flies species for both studied periods to analyse the changes on their spatial distribution. For this goal, the interpolated abundance was categorised into three levels: low abundance (less than 0.5 individuals per trap night), intermediate abundance (from 0.5 to 5 individuals per trap night) and high abundance (more than 5 individuals per trap night). We computed the percentages of areas characterised by no changes in the phlebotomine species abundance. Semivariograms analysis and kriging interpolation were conducted using *sp* package (Pebesma & Bivand 2005) for R 3.0.0 (R Development Core Team 2013), while maps were handled using QGIS program (QGIS Development Team, 2016).

2.3.2. Seasonal pattern of abundance change

To analyse if the seasonal patterns of abundance of *Lu. longipalpis* and *Ny. whitmani* were different in both periods studied Generalized Linear Mixed Models were used (GLMM, Zuur et al., 2009), with Negative Binomial error structure and the log-link function since over dispersion were observed with Poisson error structure. The response variable was the trap success of *Lu. longipalpis* or *Ny. whitmani*. Since the number of sand flies caught depended on trap-effort and it was not constant in each trap period, we used the logarithm of this value as the Negative Binomial offset according to Zuur et al. (2009). The site was included as a random effect since in each period the sites were sampled repeatedly in each season. The season and the period studied (period 1: 2011–2012; period 2: 2015–2016) were tested as explanatory variables and evaluated as fixed factors. Since, for the second period, the city was stratified to study the seasonal changes of abundance of sand flies and a subset of three sites with low, intermediate, and high abundance were randomly selected, a fixed factor of stratum was tested in the model to account this sampling design protocol. For this, all sites were assigned to stratum according to the observed abundance on the corresponding period. Interaction terms between significant variables were added if they contributed to a better fit of the model. Candidate models were built based on the significance of change of deviance of the factors included and compared using the Akaike Information Criterion (AIC) for model selection, reporting only models with $\Delta AIC < 2$ in relation to the best-fit model with the lowest AIC (Burnham & Anderson, 2002). For each species, the simplest models are reported. Statistical analyses were performed with *glmmADMB* package (Skaug et al., 2011) for R 3.0.0 (R Core Team, 2013). When it was necessary, *a priori* multiple comparisons were performed using the *multcomp* (Hothorn et al., 2008) and *car* (Fox and Weisberg, 2011) packages from the R software (R Core Team, 2013).

3. Results

A total of 4004 *Lu. longipalpis* and 1549 *Ny. whitmani*, were captured with 621 trap-nights effort in the entire study. A total of 3727 *Lu. longipalpis* and 1295 *Ny. whitmani* corresponds to the first period (347 trap-nights), while 277 *Lu. longipalpis* and 254 *Ny. whitmani* corresponds to the second period (274 trap-nights).

In the two spring periods analysed looking for inter-annual spatial distribution changes, both species showed a spatial isotropic autocorrelation of their abundances. *Lutzomyia longipalpis* abundance was

autocorrelated up to 600 m in 2011 (sill = 17; nugget = 0) and changes up to 1000 m in 2014 (sill = 600; nugget = 0), while *Ny. whitmani* abundance autocorrelation does not change between periods as it was up to 450 m in 2011 (sill = 40, nugget = 0) and in 2014 (sill = 500; nugget = 0).

Regarding the spatial distribution of the interpolated abundance, *Lu. longipalpis* was found to be distributed mainly in the same areas of the city three years after the first sampling. According to our categorization of abundance, in 76.4% of the city area, the abundance of *Lu. longipalpis* does not change from 2011 to 2014 (Fig. 1A). In both periods, this species showed the higher abundances in the northwest of Puerto Iguazú, close to the main rivers and that matches with the most urbanized environments of this city. On the other hand, most of the area with *Lu. longipalpis* trap success was where the abundance was lower than 0.5 individuals per trap night in both periods (45.03%). Consistently with the recent urbanisation process, *Lu. longipalpis* abundance gradually decreases towards the periphery of the city, with areas with low abundance of *Lu. longipalpis* in both periods, or with intermediate abundance in one period and the lower abundances in other period (Fig. 1A).

The interpolated abundance of *Ny. whitmani* showed that it was also distributed mainly in the same areas of the city, in both periods (Fig. 1b). In the 67.2% of the city area the abundance of *Ny. whitmani* do not change in the compared periods. In both moments *Ny. whitmani* was observed distributed in 'hot spots' within the city of Puerto Iguazú, close to patches of forest. For this species, most of the area with *Ny. whitmani* trap success was also where the abundance was lower than 0.5 individuals per trap night in both periods (45.98%).

3.1. Seasonal pattern of abundance change

We observed that for both species the models with greater support were those that included the interaction between the season and the period studied (*Lu. longipalpis*: $LRT_3 = 24.58$, $p < 0.001$, *Ny. whitmani*: $LRT_3 = 13.63$, $p = 0.003$) and the stratum (*Lu. longipalpis*: $LRT_2 = 30.88$, $p < 0.001$, *Ny. whitmani*: $LRT_2 = 25.72$, $p < 0.001$) indicating that seasonal patterns showed changes between periods. During 2011–2012, the minimum abundances of *Lu. longipalpis* was observed in winter, while in the spring and summer intermediate values were observed, but in 2014–2016, the minimum abundances were observed in winter and in spring. In summer, intermediate abundances were observed. For both periods *Lu. longipalpis* showed the highest abundance in autumn (Fig. 2a). While for *Ny. whitmani* we could observe during the first period of study that the most abundant seasons were spring and summer, and the minimum abundances were in autumn and winter. For the second period the abundances were similar between all seasons, although we can observe a trend to be greater in the first summer and autumn (Fig. 2b).

4. Discussion

The present study compares in Puerto Iguazú, Argentina, the inter-annual seasonal and spatial distribution of abundance of *Lu. longipalpis* and *Ny. whitmani*, the most important species for VL and TL transmission respectively, and also the most abundant ones. The results showed that these two species occupy almost the same areas within the city despite the interval of three years between captures. By contrast, patterns of seasonal population abundance were evident different in the two periods of study and for both vector species.

To understand the spatial distribution of phlebotomine sand flies in a given place, in order to compare spatial patterns and trends between different locations, extrapolate generalizations, and propose intervention measures, it is necessary to describe the historical context of sand fly colonization, the leishmaniasis incidence, and the size and urban quality of the city, mainly with actively spreading species as *Lu. longipalpis* in changing environments or changing epidemiological scenarios

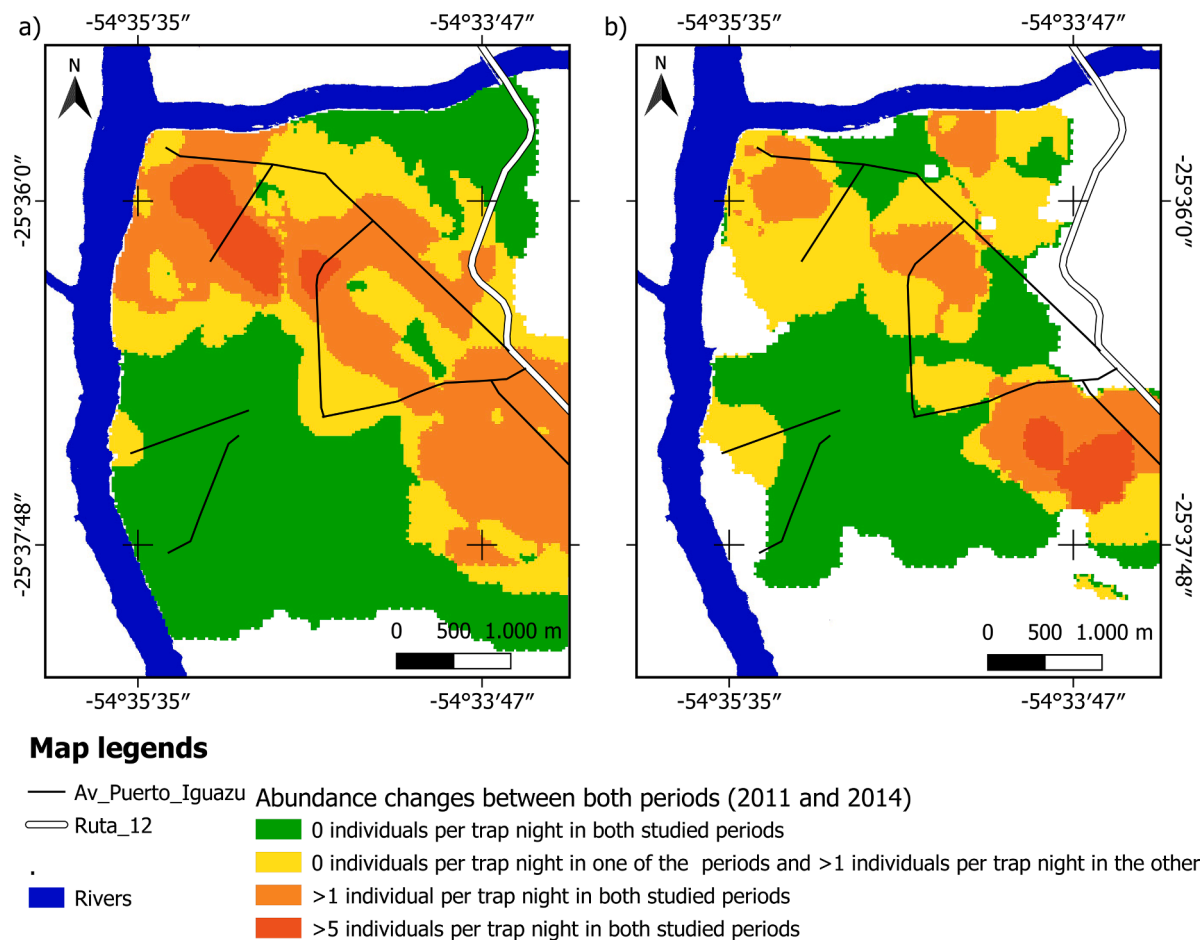


Fig. 1. Spatial distribution: Spatial distribution changes map of a) *Lu. longipalpis* and b) *Ny. whitmani* estimated on the base of the overlapped interpolated trap success maps of each species in both studied periods (2011 and 2014) in the city of Puerto Iguazu, Argentina. In green is represented the area of the city where the species presented null abundances in both studied periods, in orange the area where the species showed abundances higher than one individual per trap night in both studied periods, in red the area where the species showed abundances higher than five individuals per trap night in both studied periods and in yellow the areas where the abundances changes: null abundance in one of the periods and abundances higher than one individual per trap night in the other period.

(Salomon, 2020). Puerto Iguazu, surrounded by rivers with riparian forest and national reserves, is a growing but relatively small city, without a highly urbanised downtown and green patches even in its centre, as many other localities in the region. In Puerto Iguazú, *Ny. whitmani* was found in 2005 after an increase of TL cases associated with forest and recent settled population in new deforested areas (Salomon et al., 2009); however, the presence of this species in the sylvan habitats of Iguazú department area were known from the former scattered collections during the period 1940–1950 (Castro, 1959). On the other hand, *Lu. longipalpis* was reported in Puerto Iguazú urban area for the first time in the year 2010 (Salomon et al., 2011), the two first human cases of VL in 2014, and in the same year even *Ny. whitmani* in the rural areas, 6 km afar of the more urbanised neighbourhoods was found infected with *L. infantum* (Moya et al., 2015). The pattern of human TL incidence in Puerto Iguazú is still sporadic with limited outbreaks associated with forest activities or forest contiguity, while no more humans VL cases were notified since 2014, and urban visceral canine prevalence rates decreased from 26.18% in 2014 to 17.50% in 2018 (Lamattina et al., 2019; Salomón et al., 2016).

Lutzomyia longipalpis and *Ny. whitmani*, showed spatial segregation in both sampling periods, *Lu. Longipalpis* colonizing more urbanized landscapes and *Ny. whitmani* periurban and rural landscapes, as it was already reported (Pinheiro et al., 2013; Quintana et al., 2020). Although the city area and their characteristics are changing (Fernández et al., 2020), these changes seems not intensive or extensive enough yet to produce a substantial change in the distribution of both vectors probably

regarding the lack of significant modification of the main environmental determinants of abundance of each species at the analysed spatial scale (Fernández et al., 2010, 2012; Quintana et al., 2020). In this sense, the autocorrelation up to 450 m for *Ny. whitmani* was the same for the two sampling periods. On the other hand, for *Lu. longipalpis* was 600 m in the first period of study and up to 1000 m in second period, in the same range to those reported for the city of Posadas, also in Misiones province, Argentina, where from the year of the first case of human VL was up to 590 m, and 688 m two years later (Fernández et al., 2013). The differences between cities, in the second sampling, could be due to the different physiognomy of both locations, being Posadas larger and more urbanised than Puerto Iguazú, but both showed a trend to increase the distance of autocorrelation with the time elapsed from the former cases, in Posadas around 100 m after two years, and in Iguazú 400 m after four-five years. Although more time series data from other cities are needed to confirm that it is a trend and not a stochastic variation, the fact suggests that after the first broad spatial colonization, the areas with higher abundance of *Lu. longipalpis*, as a proxy of *Leishmania infantum* transmission risk, clustered in the most environmentally suitable places, less frequent or more dispersed and distant from each other. This phenomenon may also different abundance of catches in each period, as discussed in the following paragraph, which restricts the catch to sites where adverse conditions are more attenuated or source ‘hot spots’. The clusters of higher abundance could be discrete ‘islands in bigger cities as Posadas (Fernández et al., 2013) and localities as Clorinda with a clear downtown (Gómez-Bravo et al., 2017), or coalescent broader areas in

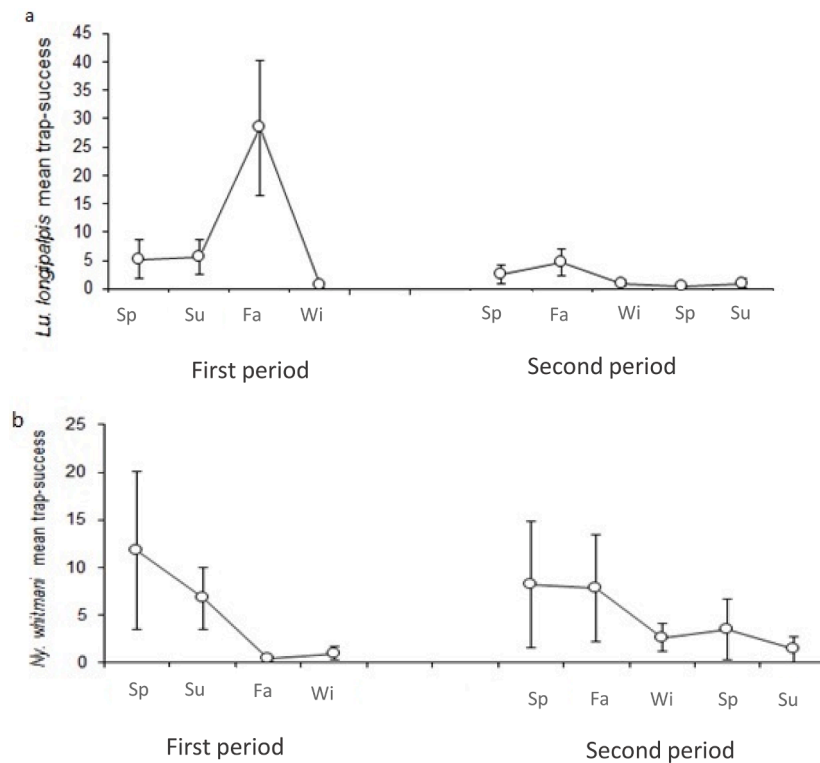


Fig. 2. Seasonal changes: Inter-annual seasonal changes of the mean trap-success (\pm standard error) observed for a) *Lu. longipalpis* and b) *Ny. whitmani*, between both studied periods, 2011–2012 (first period) vs 2014–2016 (second period) in the city of Puerto Iguazu, Argentina. References: Sp (Spring), Su (Summer), Fa (Fall), Wi (Winter).

small vegetated cities as Puerto Iguazú.

While the spatial comparison 2011 vs. 2014 was carried out in the spring season of the year, during the 2014 the weather conditions were not favourable for the capture of adults, this could explain the low abundances in that year. However, through this study, we were able to observe that the ‘hot spots’ are maintained, regardless of the weather conditions. The spatial distribution of these high-abundance areas remains almost the same in the city in the two periods studied, these places could be used to locate the ‘where’ for focused activities of surveillance or control. Therefore, it is probable that an intervention on ‘hot spots’ could have impact on the abundance of the sand fly population of the whole city and so the risk of leishmaniasis transmission, if they act as persistent source populations in a metapopulation dynamics, as they remain even in the cold seasons as it was observed in the results. However, while *Lu. longipalpis* ‘hot spots’ in urbanized blocks with intermingled green patches make this strategy feasible, the *Ny. whitmani* clusters of high abundance in highly forested or ruralized areas make it unworkable. Therefore, for *Ny. whitmani* would be more advisable to propose environmental integrated measures as backyard and domestic animal breeding dwelling improvement, and physical barriers by increasing the distance from wooded fringes (Salomon, 2019).

In order to determine if a ‘when’ for focused interventions is also possible the seasonal pattern of abundance between the two sampling periods was also analysed. Both species were more abundant during warm-template periods of the year. *Lu. longipalpis* was most abundant in early autumn in both periods studied, confirming its seasonality (Holman et al., 2013). For other phlebotomine sand flies vectors with similar regular pattern and autumn peaks, the insect control during autumn was recommended because in this season there are the higher proportion of infected sand flies after long individual survival and consecutive cohorts, and each individual has the highest reproductive potential before winter (Salomón et al., 2004).

Nyssomyia whitmani, on the other hand showed a more irregular pattern between years, so it could require monitoring to define a season

with the highest abundance, with temporal longer series. However, other studies for this species, even in the same location of this study, show that the temporal monitoring of newly emerged individuals explain the seasonal variation patterns of adults, and the warmest months are those of greatest emergencies (Manteca Acosta et al., 2021; Pinheiro et al., 2013), although this pattern could be more extended in time where the climatic conditions are more stable, as in domestic animal dwellings, places where the species could be present all year round (María Soledad Fernández et al., 2013; Manteca Acosta et al., 2021).

The approach of those study, comparing successive periods, contributes to identify the period and sites of leishmaniasis potential risk transmission. Our study defines the ‘where’ and ‘when’ implement the actions to mitigate LT and LV cases. These should be focused on these potential source sites, and in seasons with minimum abundances and maximum reproductive potential of individuals, in order to improve intervention effectiveness and thus decrease the probability of occurrence of cases in the intervened city.

Some risk factors can be decreased by applying control strategies tailored to each eco epidemiological entity, ensuring continuity, adjusting these strategies to any new environmental change and improving them through specific research (Desjeux, 2001). The results of this study are of epidemiological relevance, since within an integrated control strategy, they reinforce a real perspective of changing the vector control paradigm to focus exclusively on the areas and times of greatest impact. The sites considered as ‘hot spots’ showed the presence of sand flies even at times of minimum abundance, the most inhospitable moment for these insects (Santini et al., 2018).

5. Conclusions

In conclusion, applying prevention and control strategies in a targeted manner, only in a few sites for a determined period of time, would increase the cost-effectiveness of the intervention, increasing the impact on the abundance and population dispersion of these vectors. This

strategy could be applied in many other foci of VL and TL co-occurrence, while the approach contributes to the required background knowledge for rational interventions by leishmaniasis risk distribution models, and characterization of determinants of critical sites.

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Ethics approval and consent to participate

The procedures were approved by the ethical committee (CEIC, Dr Carlos Barclay Office for Human Research Protection, IRB Registration 00,001,678-USA; Res. No. 1108/26/2014), and all the field sampling in peridomestic environments included informed consent. The householders were informed about the results with the guarantee of confidentiality, together with prevention recommendations based on environmental management.

Declaration of Competing Interest

It should be clarified that the authors of this article, declares that there are no conflicts of interest regarding the publication of this article.

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