# Spatiotemporal dynamics of *Lutzomyia longipalpis* and macro-habitat characterization using satellite images in a leishmaniasis-endemic city in Argentina

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Abstract. The spatiotemporal population dynamics of Lutzomyia longipalpis (Lutz & Neiva, 1912) (Diptera: Psychodidae) were evaluated in a city in Argentina in which visceral leishmaniasis is endemic. Over 14 sampling sessions, 5244 specimens of five species of Phlebotominae (Diptera: Psychodidae) were captured, of which 2458 (46.87%) specimens were L. longipalpis. Generalized linear models were constructed to evaluate the associations between L. longipalpis abundance and explanatory variables derived from satellite images. The spatial variable 'stratum' and the temporal variable 'season' were also included in the models. Three variables were found to have significant associations: the normalized difference vegetation index; land surface temperature, and low urban coverage. The last two of these were associated with L. longipalpis abundance only during summer and winter, respectively. This variation between seasons supports the development of models that include temporal variables because models of distributions of the abundance of a species may show different critical variables according to the climatic period of the year. Abundance decreased gradually towards the downtown area, which suggests that L. longipalpis responds to a meta-population structure, in which rural-periurban source populations that persist over time may colonize adjacent areas. This information allows for a spatiotemporal stratification of risk, which provides public health authorities with a valuable tool to help optimize prevention measures against visceral leishmaniasis.

**Key words.** *Leishmania infantum*, Phlebotominae, generalized linear models, land surface temperature, normalized difference vegetation index, urban coverage, vegetation coverage, visceral leishmaniasis.

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# Introduction

Outbreaks of infectious diseases have both spatial and temporal dimensions, and differences in the distribution of vectors may arise as a result of spatial heterogeneities, patterns of vector-host contact, and variations in population susceptibility and environmental factors that are dynamic over time (Werneck, 2008). In Latin America, visceral leishmaniasis (VL) is a zoonotic infectious disease caused by Leishmania infantum (Nicolle, 1908) (syn. Leishmania chagasi), a trypanosomatide parasite (Kinetoplastida: Trypanosomatidae), the main vector of which in the Americas is the phlebotomine sandfly Lutzomyia longipalpis. Although it has been known historically as a rural endemic disease, VL has reached endemic and epidemic proportions in many large Latin American cities since the latter part of the last century and has come to represent a serious public health problem (Desjeux, 2004; Salomón et al., 2015). Currently, VL is considered an emergent zoonotic urban disease because the domestic dog serves as the parasite's main reservoir and because its incidence and geographical distribution have recently increased (Gould et al., 2013).

In Argentina, between 1925 and 2001, only 16 cases of human VL were recorded in rural areas (Salomón et al., 2001). In 2008 the presence of L. longipalpis was recorded for the first time in the city of Corrientes (Salomón et al., 2009). Five years later, a transversal study in the same location showed this vector of the aetiologic agent of VL to be the dominant phlebotomine species, and its distribution to be associated with macro-habitat variables such as vegetation estimated from satellite images analysis (Berrozpe et al., 2017). Therefore, following the transversal study in Corrientes city, the objective of the present study was to assess L. longipalpis population dynamics in space and time in this VL-endemic city in Argentina in order to find associations with seasonal and environmental variables at a macro-habitat scale derived from the digital processing of satellite images, and to obtain spatiotemporal maps of L. longipalpis occurrence and abundance probabilities.

#### Materials and methods

#### Study area

The study was conducted in the city of Corrientes, in northeastern Argentina ( $27^{\circ}28'08''$  S,  $58^{\circ}49'50''$  W). The city is located on the east shore of the Paraná River, in the humid Chaco ecoregion (Cabrera, 1976). The urban area is approximately  $60 \text{ km}^2$  in size (Fig. 1) and has a population of 328 868 inhabitants. Like most cities in the region, Corrientes has a complex and heterogeneous urban landscape, which includes a downtown area with a higher population density and well-developed infrastructure, and periurban areas with houses located in a green matrix.

Corrientes has a subtropical climate without dry seasons. Temperatures vary from 8 °C to 21 °C in winter and from 20 °C to 32 °C in summer. Precipitation is abundant throughout the year with annual values of 1400–1900 mm [National Meteorological Service, 1981–2010 (http://www.smn.gov.ar).

#### Entomological sampling

The entomological sampling design was based on a previous stratification of the study area. Prior to entomological collections, the city was divided into three environmental strata (urban centre/periurban/rural-periurban) based on the mean values of the normalized difference vegetation index (NDVI) estimated from four representative satellite images obtained in each season between 2013 and 2014. The NDVI was calculated using the following equation:

$$NDVI = (NIR - R) / (NIR + R)$$

where NIR and R are the reflectance values in the near-infrared and red bands, respectively, recorded by the operational land image (OLI) sensor of the Landsat Data Continuity Mission 8 (LDCM 8), with a spatial resolution of 30 m.

Using four LDCM 8 satellite images of Corrientes city (obtained on 23 December 2013, 9 February 2014, 14 April 2014 and 19 July 2014), a mean NDVI was calculated for every pixel (each selected image corresponded to a season). The total values obtained were then divided into three parts with the same amounts of data, each corresponding to a specific environmental stratum.

In each stratum, six sampling sites were selected to give a total of 18 collection sites. Two sites in each stratum corresponded to high-abundance sampling sites defined by Berrozpe *et al.* (2017), and the other four were determined by ensuring that sites were separated by at least 200 m to avoid the overlap of 100-m radius buffer areas for each site. Sites with dense vegetation, organic material on the soil and blood sources such as dogs and farm animals were selected to raise the probability of sandfly captures (Salomón *et al.*, 2015) (Fig. 1).

Two trapping sessions per season were performed (one was performed in autumn for operational reasons) from September 2014 (spring) to August 2016 (winter), giving a total of 14 sessions. At each sampling site, a REDILA-BL trap (Fernández *et al.*, 2015) was installed near the residence at a height of 1.5 m and operated for three consecutive nights without rainfall, from approximately 17.30 hours to 08.30 hours the following day.

The captured phlebotomine sandflies were separated from the rest of the entomological material at the laboratory using magnifying glasses. They were clarified with a lactic acid-phenol solution, observed by optical microscope at 40× magnification and classified according to morphological keys (Galati, 2003). Females of *Evandromyia cortelezzii* (Brètes, 1923) and *Evan-dromyia sallesi* (Galvao & Coutinho, 1941) were determined as females of the *Evandromyia cortelezzii-sallesi* species complex.

#### Environmental data

The possible associations of *L. longipalpis* abundance over time with environmental variables were analysed. Macro-habitat scale variables were obtained from the digital processing of 14 LDCM 8 satellite images with less than 15% cloud coverage, which were acquired 20–35 days prior to each entomological sampling session. Images were radiometrically calibrated and geographically corrected (U.S. Geological Survey, 2013). For

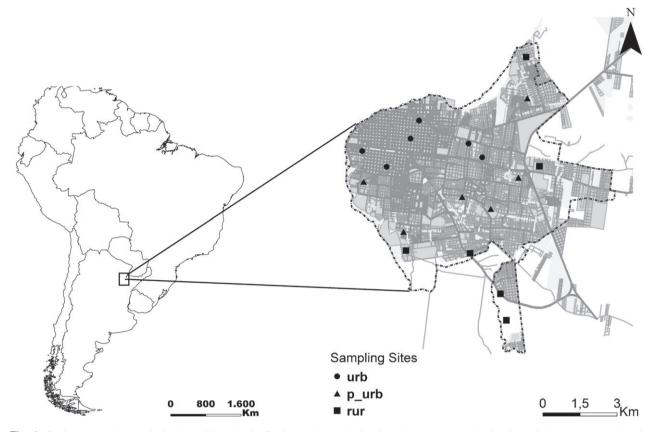


Fig. 1. Study area and entomological sampling sites in Corrientes city. urb, sites in urban centre; p\_urb, sites in periurban areas; rur, sites in rural-periurban areas.

each entomological sampling site, the average NDVI, normalized difference water index (NDWI) and land surface temperature (LST) were obtained in a buffer area with a radius of 100 m. In addition, the percentages of land use/surface coverage of the buffer areas were obtained.

The NDWI reflects the water content in vegetation with values ranging from -1.00 to +1.00. It was calculated using the following equation:

# NDWI = (NIR - SWIR) / (NIR + SWIR)

where NIR is reflectance in the near-infrared band, and SWIR is reflectance in the short-wave infrared band recorded by the OLI sensor of the LDCM 8, with a spatial resolution of 30 m.

The LST is defined as the long-wave thermal energy emitted from the earth's surface and is obtained through the application of split-window algorithms on bands 10 and 11 of the thermal infrared sensor of the LDCM 8 (Jiménez-Muñoz *et al.*, 2014), with a spatial resolution of 100 m.

The percentages of land use/surface coverage of each entomological site buffer area were obtained through supervised classifications, from which four macro-habitat environmental variables were defined: high urban coverage; low urban coverage; low vegetation coverage, and high vegetation coverage. Supervised classifications were validated to establish the accuracy of the products obtained following the criteria recommended by Chuvieco (2010). Visual analyses were performed on false urban colour RGB 7-6-4 compositions using bands 7, 6 and 4 from the OLI sensor of the LDCM 8. The accuracy of the supervised classification is quantified according to the percentages of pixels correctly classified within each class and to overall accuracy and kappa coefficients (Manandhar *et al.*, 2009).

Climatic variables, as accumulated millimetres of rainfall and minimum and maximum average temperatures (°C) 30 days prior to sampling, were obtained from the National Meteorological Service (Station Corrientes AERO: 27°27′ S, 58°50′ W). The stratum of each sampling site and the season of each sampling session were also taken into account in the analysis (Table 1).

The 'abundance' of *L. longipalpis* was defined as the cumulative abundance recorded during the three consecutive sampling nights for each sampling session, whereas the 'occurrence' of *L. longipalpis* was defined as the percentage of sites at which at least one individual was trapped during any of the three sampling nights in each sampling session.

# Data analysis

Generalized linear models (GLMs) were constructed to evaluate the associations between *L. longipalpis* abundance and the various explanatory variables. Exploratory analyses were

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Table 1.	Variables considered	l to evaluate Lutzomyi	<i>i longipalpis</i> abundai	nce in Corrientes c	ity, 2014–2016.

Variable	Abbreviation	Description
Precipitation	precip	Accumulated millimetres of rainfall 30 days prior to sampling
Maximum temperature	tmax	Average maximum temperature (°C) 30 days prior to sampling
Minimum temperature	tmin	Average minimum temperature (°C) 30 days prior to sampling
Stratum	stratum	Type of stratum: urban centre/periurban/rural farms
Season	season	Season of the entomological sampling session
Normalized difference vegetation index	NDVI	Average*NDVI around the sampling site
Land surface temperature	LST	Average*LST around the sampling site
Normalized difference water index	NDWI	Average*NDWI around the sampling site
High urban coverage	cov1	Proportion <sup>†</sup> of high urban development soil coverage around the sampling site
Low urban coverage	cov2	Proportion <sup>†</sup> of low urban development soil coverage around the sampling site
Low vegetation coverage	cov3	Proportion <sup>†</sup> of low density or dispersed vegetation coverage around the sampling site
High vegetation coverage	cov4	Proportion <sup>†</sup> of high and dense vegetation coverage around the sampling site

\*Average value within a 100-m radius (n = 130 pixels) around the sampling site.

†Proportion of coverage within a 100-m radius around the sampling site.

**Table 2.** Abundances of Phlebotominae, its relative abundance and the percentage of positive samples by stratum, collected in Corrientes city during 2014–2016.

Stratum	Species	Male	Female	Male : female ratio	Total	Relative abundance	Positive samples (%)
Urban centre	Lutzomyia longipalpis	320	131	2.44	451	98.47	53.57
	Nyssomyia neivai	4	3	1.33	7	1.53	5.95
	Total	324	134	2.42	458	100	
Periurban	Lutzomyia longipalpis	449	174	2.58	623	64.03	58.33
	Nyssomyia neivai	216	78	2.76	294	30.21	28.57
	Migonemyia migonei	31	18	1.72	49	5.04	10.71
	Micropigomyia quinquefer	0	1	_		0.10	1.19
	Evandromyia cortelezzii–sallesi	0	6	_	6	0.62	7.14
	Total	696	277	2.51	973	100	
Rural-periurban	Lutzomyia longipalpis	1063	321	3.31	1384	36.82	69.09
	Nyssomyia neivai	1250	719	1.74	1969	52.38	67.85
	Migonemyia migonei	267	138	1.93	405	10.78	44.05
	Micropigomyia quinquefer	0	3	_	3	0.005	2.38
	Evandromyia cortelezzii–sallesi	0	52	_	52	0.015	17.85
	Total	2580	1233	2.09	3813	100	

performed to establish the distribution of *L. longipalpis* abundances in order to determine the link function to use in the GLMs. Pearson's tests were performed to identify correlated continuous independent variables to avoid collinearity problems within the models (Fig. S1). Finally, negative binomial GLMs were settled using the MASS statistical package for R software (Venables & Ripley, 2002; R Core Team, 2016).

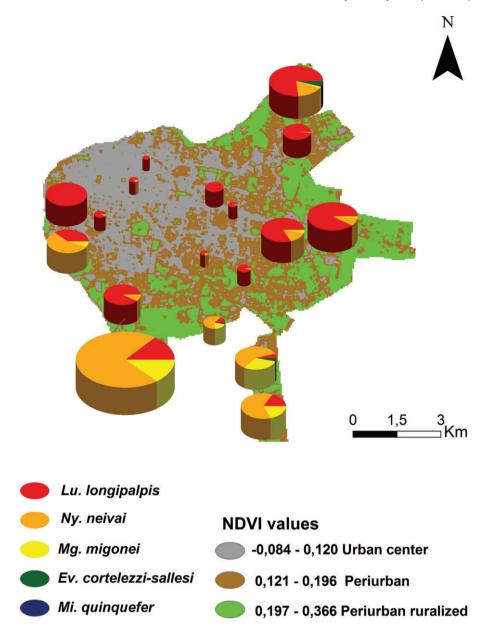
A model simplification was performed by a step-by-step elimination of terms using the Akaike information criterion adjusted for low sample sizes (AICc) (Johnson & Omland, 2004), using the *MuMIn* package for R (Barton, 2016). Terms that did not decrease the AICc by at least two units were not retained in the model. Therefore, the resulting models were selected based on their AICc values, considering the model with the lowest AICc as the best model of each set (Burnham & Anderson, 2002). Estimates, standard errors and confidence intervals (CIs) (with 95% confidence) of the selected models were calculated using 1000 bootstrap replications. Finally, Tukey's contrast tests were performed to evaluate the differences between levels of the categorical variables contained in the final model. The significant variables associated with the spatial distribution of *L. longipalpis* were used to build up a cartographic output through the overlap of each variable taken into account in the final model.

Occurrences of the other Phlebotominae species registered in this study in the urban centre and periurban strata were low or null, and therefore no models were run for these species.

#### Results

# Entomological sampling

A total of 5244 individuals of five phlebotomine species were captured. These represented *L. longipalpis* (n = 2458), *Nyssomyia neivai* (Pinto, 1926) (n = 2270), *Migonemyia migonei* (França, 1920) (n = 454), *E. cortelezzii–sallesi* (n = 58) and *Micropigomyia quinquefer* (Dyar, 1929) (n = 4). *Lutzomyia longipalpis* was the most abundant species and was heterogeneously distributed among the three city strata, presenting a gradual reduction in abundance and occurrence towards the urban centre (Table 2). This species also had the highest rates



**Fig. 2.** Species composition, spatial distributions and accumulated abundances of Phlebotominae captured in Corrientes city during 2014–2016, showing the stratification of the city according to normalized difference vegetation index (NDVI) values per stratum. [Colour figure can be viewed at wileyonlinelibrary.com].

of occurrence in the three strata. The rural stratum presented the highest abundance. However, the most abundant species in this stratum was *N. neivai* rather than *L. longipalpis*. With reference to the urban centre and periurban strata, *L. longipalpis* represented 98.47% and 64.03%, respectively, of captured sandflies. *Nyssomyia neivai*, *Mig. migonei*, *E. cortelezzii–sallesi* and *Mic. quinquefer* were virtually confined to the city margins (Fig. 2). Sampling sites selected by the extensive transversal work of Berrozpe *et al.* (2017) accounted for 58.54% of the *L. longipalpis* collected and 69.03% of the *N. neivai* collected.

*Lutzomyia longipalpis* was collected in all sampling sessions; however, higher abundances of the vector occurred during spring

and summer and the lowest abundances were recorded in winter. The variations in abundances of the remaining phlebotomine species were similar. The seasonal variations in the occurrence and abundance of *L. longipalpis* along the sampling years are shown in Fig. 3. The occurrence of this species was associated with NDVI average annual values ranging from 0.11 to 0.21. With respect to LST, abundance was related to average annual LST values in the buffer areas of entomological collection sites of between 17.16 °C and 18.97 °C, and to summer values of between 19.19 °C and 20.39 °C.

In terms of macro-habitat characterization, the sites at which the vector was most abundant were associated with high

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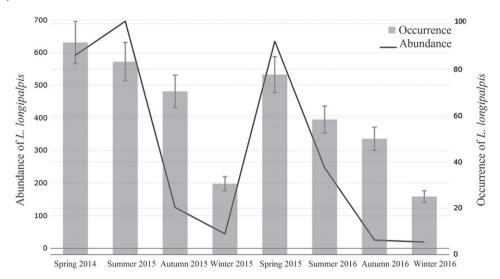


Fig. 3. Seasonal variations in occurrences and abundances of *Lutzomyia longipalpis* in Corrientes city, 2014–2016. Occurrence was defined as the percentage of sites at which at least one individual was trapped during any of the three sampling nights in each sampling session.

environmental heterogeneity defined as equivalent proportions of land coverage types in buffer areas and with NDVI values ranging from 0.16 to 0.26 in the most favourable period (mid-spring/summer). Extreme NDVI and LST values, recorded in the present study in Corrientes city, corresponded to the lowest *L. longipalpis* abundances.

### Statistical analysis

Pearson's correlations between all numerical independent variables were evaluated. Variables showing a high correlation with a *P*-value of < 0.01 were not taken into account in model construction. Consequently, model simplification was carried out starting from a model that contained six variables and their double interactions: NDVI; LST; low urban coverage (cov2); low vegetation coverage (cov3); season, and stratum.

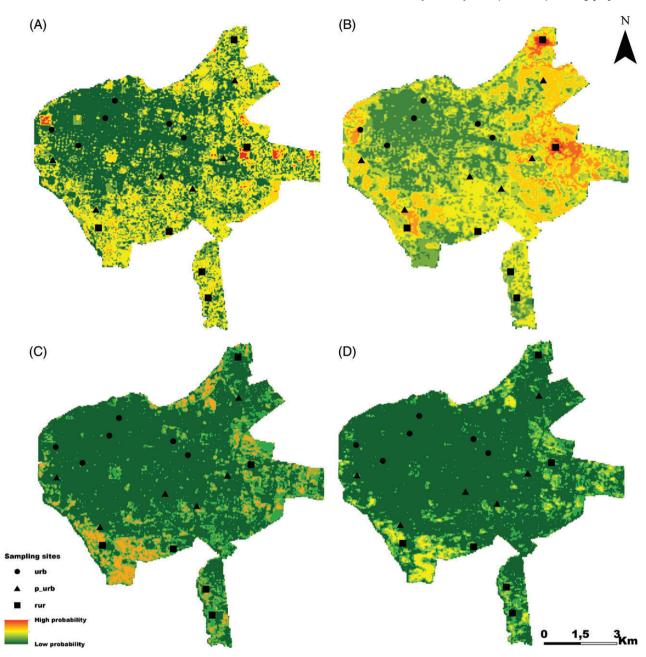
The result of removing variables according to the AICc was a model with three significant terms: NDVI + season\*LST + season\*cov3. Adding one term to this model would represent an AICc increment of at least 3.612 units, and its AICc was 86.685 units lower than that of the null model. A Tukey's contrast test was then performed to evaluate differences in *L. longipalpis* abundance between seasons, and showed a significant difference between abundance in summer and abundances in the other seasons (P < 0.01). Figure 4 shows the models resulting from the superimposition of the variables associated with *L. longipalpis* abundance. The final model standard errors and CI limits calculated by a bootstrap of 1000 iterations are shown in Table 3.

# Discussion

In the present study, 14 sampling sessions were carried out in 18 peridomestic units and spatial and temporal variables were analysed to describe *L. longipalpis* abundances in the city of Corrientes.

Occurrences of L. longipalpis at a city scale were high, with 60.31% of sites sampled with captures. In the rural-periurban stratum, both the lowest abundance of L. longipalpis in relation to other phlebotomine species (36.82%) and the highest proportion of positive samples (69.04%) were recorded. This stratum presented the greatest absolute abundances, which may be related to high environmental heterogeneity, the presence of farmyard animals, deficiencies in public sanitation infrastructure and greater vegetation coverage, all of which are favourable factors for vector breeding (Lainson & Rangel, 2005; Queiroz et al., 2012), not only for L. longipalpis, but also for other species that are better adapted to this ruralized environment, such as N. neivai (Quintana et al., 2012). By contrast, L. longipalpis is dominant in areas of greater urban infrastructure development. Prevalences and occurrences in these strata are similar to those described in other cities (Fernández et al., 2013; Gómez-Bravo et al., 2017; Santini et al., 2018). The high rate of occurrence, added to the presence of VL-infected canines, which act as Le. infantum reservoirs, increases the risk for transmission to the human population.

The gradual decrease in abundance towards the downtown area suggests that the distribution of L. longipalpis populations may be consistent with a metapopulation distribution, in which the rural-periurban stratum serves to provide source sites at which populations persist over time and are capable of colonizing adjacent areas. Pulliam's source-sink metapopulation model was also suggested for N. neivai in Argentina (Quintana et al., 2012). The demonstration of this dynamic and the geographic location of source populations have relevance for vector control strategies because anti-vector interventions can be focused on these 'hot spots'. As almost 70% of the L. longipalpis specimens collected were captured in the rural-periurban stratum, these hot spots could be assimilated to the core transmitters of the 80-20 theory, which states that these sites may serve to contribute vectors to sites with lower abundances (Woolhouse et al., 1997). Thus it could be assumed that control of these hot spots will reduce the probability of vector-human contact because the



**Fig. 4.** Probability of *Lutzomyia longipalpis* occurrence during (A) spring, (B) summer, (C) autumn and (D) winter in Corrientes city, 2015–2016, based on satellite images from which variables significantly associated with the spatial distribution of *L. longipalpis* [normalized difference vegetation index (NDVI), land surface temperature (LST) and low vegetation coverage (Cov3)] were derived and used to build up a cartographic output through the overlap of each variable according to its relative weight in the final model. urb, urban centre sampling sites; p\_urb, periurban stratum sampling sites; rur, rural–periurban stratum sampling sites. [Colour figure can be viewed at wileyonlinelibrary.com].

20% of sites with vectors may be responsible for 80% of vector abundance and dispersion in the city. These assertions must be empirically corroborated.

The species diversity of the phlebotomine community also decreased from the rural-periurban stratum towards the demographic centre, where only two species were captured (Table 2). The adaptation of *L. longipalpis* to rapidly changing environmental conditions may explain the dominance of *L. longipalpis*  over other phlebotomine species in the urban centre, and may play an important role in VL urbanization recorded in recent years (Salomón *et al.*, 2015). In the urban centre, lower *L. longipalpis* abundances may reflect reductions in space for the growth and sustainability of large populations and fewer food sources.

In relation to statistical models, variables derived from the digital processing of satellite images, such as the NDVI, LST and

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<b>Table 3.</b> Variables of the negative binomia	l generalized linear model explaining the	the spatiotemporal distribution of <i>Lutzomvia lo</i>	<i>ngipalpis</i> abundance.

	Estimator	SE	CI lower limit	CI upper limit
Intercept	-21.37048	12.36862	-49.55	1.61
Spring	27.81094	19.p563	-11.86	66.61
Summer <sup>†</sup>	461.23687	186.72423	112.4	935.4
Winter	7.18829	22.34686	-36.638	52.298
NDVI†	13.10252	3.70103	3.21	18.85
LST	0.07377	0.04399	-0.0074	0.1778
cov3	-0.0054	0.01443	-0.0332	0.0202
Spring*LST	-0.09305	0.06649	-0.2226	0.0463
Summer*LST†	-1.58351	0.65339	-3.222	-0.391
Winter*LST	-0.02713	0.0773	-0.186	0.1281
Spring*cov3	-0.02012	0.02226	-0.0711	0.0172
Summer*cov3	-0.2171	0.29854	-0.5255	0.1686
Winter*cov3 <sup>†</sup>	-0.17657	0.06169	-0.3157	-0.0797

†Significant variables.

Standard errors (SEs) and confidence intervals (CIs) were calculated by 1000-replication bootstrapping.

cov3, low vegetation coverage; LST, land surface temperature; NDVI, normalized difference vegetation index.

the percentage of scattered and low-density vegetation coverage (cov3) in the buffer area around the sampling sites, showed statistically significant associations with *L. longipalpis* abundance. The variables LST and low-density vegetation coverage showed significant levels of association with *L. longipalpis* abundance during only the summer and winter, respectively. This shows that the effects of critical variables associated with vector abundance may vary between seasons, supporting the development of models that contain temporal variables. Furthermore, this means that models that are constructed to search for associations between vector abundances and critical explanatory variables should discriminate between climatic periods.

The increases in L. longipalpis abundance and distribution during mid-spring and summer and its persistence until the beginning of autumn indicate that this period represents the time of greatest risk for human-vector and reservoir-vector contact. Similar temporal dynamics of L. longipalpis populations have been recorded in other regions (de Melo Ximenes et al., 2006; da Paixão Sevá et al., 2017; Gómez-Bravo et al., 2017). In a longitudinal study carried out in Mato Grosso do Sul, L. longipalpis was associated with NDVI (Oliveira et al., 2012). However, de Andrade et al. (2014) did not find associations between L. longipalpis abundance and NDVI in the same study area. This may be because the variables considered in their study were assessed individually rather than through multivariate analysis, and because only one image was used (de Andrade et al., 2014). These data contrast with those presented in this study and with the spatiotemporal work performed by Bavia et al. (2005), in which a temporal series of images was used. These results further support the need to take into account seasonal patterns when assessing associations between vector abundance and environmental variables.

The interannual differences in *L. longipalpis* abundance found in this study may reflect the vector control activities carried out by the Corrientes Zoonoses Department during the dengue outbreak in the summer of 2015–2016 (G.A.F. 2016, Corrientes Zoonoses Department, personal communication). Spraying with pyrethroids according to the environment, local population

dynamics, methods and product formulations has been reported as effective at least in the short term for L. longipalpis control (Lainson & Rangel, 2005; Barata et al., 2011; Salomón et al., 2015). Similar control spraying against Aedes aegypti (Diptera: Culicidae) may have affected L. longipalpis populations. The present authors were unable to obtain details of the actual schedule of spraying activities against Ae. aegypti in order to investigate associations between these interventions and L. longipalpis space-time abundance distribution. However, spraying was most intense during summer in the downtown area, although it was performed throughout the city, and hence may produce some bias implied by decreasing abundance mainly in the urban centre. By contrast, dispersion from the urban centre to the periurban area as a result of the insecticide is unlikely (Santini et al., 2010). However, other factors such as regional-scale climate factors, which were not taken into account in this study, may also explain the interannual variation in vector abundance.

In conclusion, assessing vector abundance and associated variables in time is necessary to estimate the level of risk for vector-human contact and to design vector control strategies. Thus, annual abundance curves will allow for evaluation of the best time at which to apply vector population control measures in accordance with the reproductive potential of the species. The availability of satellite images, together with the ease with which coverage indices and proportions from digital processing can be obtained, and their integration into geographic information systems, make these variables an optimal resource when analysing the eco-epidemiology of VL and other vector-borne diseases (Bavia et al., 2005; Ferro et al., 2015; Roy et al., 2016). Nevertheless, seasonal oscillations in explanatory variables should always be taken into account when analysing possible associations between vector abundance and environmental factors. The resulting data and maps allow the risk stratification of a defined area and time period according to the probability of occurrence of the associated vector. This information is useful for vector dispersion control and provides public health authorities with a valuable tool with which to optimize the stratification of risk

within a city, and to enhance VL prevention and control measures.

# **Supporting Information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Pearson's correlations for the explanatory variables.

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The authors declare no conflicts of interest.

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