Spatial pattern evolution of *Aedes aegypti* breeding sites in an Argentinean city without a dengue vector control programme

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Abstract

The main objective of this study was to obtain and analyse the space-time dynamics of *Aedes aegypti* breeding sites in Clorinda City, Formosa Province, Argentina coupled with landscape analysis using the maximum entropy approach in order to generate a dengue vector niche model. In urban areas, without vector control activities, 12 entomologic (larval) samplings were performed during three years (October 2011 to October 2014). The entomologic surveillance area represented 16,511 houses. Predictive models for *Aedes* distribution were developed using vector breeding abundance data, density analysis, clustering and geoprocessing techniques coupled with Earth observation satellite data. The spatial analysis showed a vector spatial distribution pattern with clusters of high density in the central region of Clorinda with a well-defined high-risk area in the western part of the city. It also showed a differential temporal behaviour among different areas, which could have implications for risk models and control strategies at the urban scale. The niche model obtained for *Ae. aegypti*, based on only one year of field data, showed that 85.8% of the distribution of breeding sites is explained by the percentage of water supply (48.2%), urban distribution (33.2%), and the percentage of urban coverage (4.4%). The consequences for the development of control strategies are discussed with reference to the results obtained using distribution maps based on environmental variables.

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Introduction

According to the World Health Organization (WHO), dengue disease is one of the most widespread vector-borne diseases in the world (TDR/WHO, 2009). There are four different serotypes of dengue virus (DEN-1-4), all transmitted by mosquitoes of the species *Aedes aegypti*. In South America, this vector lives in urban areas (Gubler, 1997; Vezzani and Carbajo, 2008) with breeding habitats principally consisting of water-holding containers kept outside without covers. *Ae. aegypti* is characterised by its adaptations to urban environments through its capacity to breed in such containers rather than natural water collections (Vezzani and Carbajo, 2008). This adaptation includes resistance of the *Aedes* eggs to desiccation and the feeding behaviour of females, which bite at multiple occasions during each gonadotrophic cycle (Regis *et al*., 2008). These characteristics, plus a wide distribution and high densities of breeding sites, constitute fundamental factors that influence the circulation and transmission of dengue and other related viruses in the region of northern Argentina (Rotela, 2012).

The incidence of dengue has grown dramatically in recent decades, with a concomitant increasing trend in dengue outbreaks in South America during the past few years (TDR/WHO, 2009). The largest previous notified dengue outbreak in Argentina occurred in 2009, reaching subtropical regions and affecting more than 25,900 people from localities, such as Buenos Aires and Córdoba, situated near the centre of the country (Seijo *et al*., 2009; Estallo *et al*., 2014). However, the largest percentage (>90%) affected the northern provinces of Chaco, Catamarca and Salta (Argentinian Ministry of Health, 2009). During this outbreak, there were no dengue cases reported from Formosa Province (Argentinian Ministry of Health, 2009). Argentina is currently (2016) experiencing the strongest dengue outbreak in its history. Between weeks 1 and 15 of the epidemic, 56,802 cases compatible with dengue fever were confirmed by the national health system. So far, 28,008 cases are notified as indigenous and 2407 with a history of travelling to areas with local transmission and 1440 cases have been reported in Formosa Province (Argentinian Ministry of Health, 2016).

The existence of an endemic transmission cycle, introduction of cases by the movement of people across the border of countries with existing viral transmission and the absence of a vaccine (Avilés *et al*., 2003) have resulted in the development of dengue prevention mainly based on control of the vector. Effective serologic and viral surveillance programmes are often too expensive for the majority of developing countries affected by this disease, so they rely on entomologic surveillance to estimate the potential risk for virus transmission and disease (Bowman *et al*., 2016). The prevention programmes are therefore typically focused on the removal of *Ae. aegypti* breeding in order to eliminate vector larval stages (Getis *et al*., 2003), and they include elimination (source reduction) or treatment of larval habitats and insecticidal space spraying to reduce the density of adult mosquito population during periods of their occurrence (Getis *et al*., 2003). For this reason, a clear identification of vector hotspots in populated areas is an important tool for prevention programmes (Tran *et al*., 2004).

Multiple environmental factors, including biophysical and social ones, constitute a complex web that determines the spread of vector-borne diseases (Hales *et al*., 2002). Despite these complexities, various authors, Ostfeld *et al*. (2005) in particular, feel that an analysis of certain variables that have links to vectors and distribution of human cases can be a useful tool to generate space-time scenarios for dengue. In addition, spatial analysis of health events contributes to the early detection of areas involving disease transmission (Ostfeld *et al*., 2005). The Centers for Disease Control and Prevention (CDC) in the United States (USA) are clear about the fact that the detection of disease clusters allows the identification of non-random events that provides inferences about their epidemiological determinants (CDC, 1990). Surveillance tools, such as incidence maps, have been used to enhance public health operability before and during dengue outbreaks by providing visual aids for reaching a decision (Porcasi *et al*., 2012; Louis *et al*., 2014). Given that the risk generally increases with proximity to the infected host, the study of the factors that determine the spatial distribution of the host, the vector and the probability of interaction between them, are key points (Louis *et al*., 2014). In this context, spatial epidemiology, *i.e*. landscape epidemiology, can be defined as the study of the spatial variation of disease risk. This discipline, which tries to understand and explain the causes of spatial heterogeneity, is based on the idea that the spatial distribution of diseases is regulated by biological and physical variables (Ostfeld *et al*., 2005). Once the pattern is known, the future of the disease can be predicted (Pavlovsky, 1966).

Earth observation satellites constitute an interesting alternative to record environmental variables that influence diseases and are therefore useful for epidemiological studies. Remote sensing (RS) techniques allow the identification of key environmental factors such as characteristics of land cover, temperature, rainfall and humidity (Rotela *et al*., 2007; Polop *et al*., 2008). Since the beginning of RS technology, studies about vector-borne diseases have focused on identifying and mapping vector habitats (Hayes *et al*., 1985) as well as assessment of environmental factors related to vector biology (Rogers and Randolph, 1991, 1993; Kitron, 1998), thereby supporting the study of epidemiology (Linthicum *et al*., 1999; Murray, 2003). RS and spatial analysis have been utilised to identify map landscape elements that collectively define vector and human population dynamics, related to the risk of disease transmission (Wood *et al*., 1992; Glass *et al*., 1992). The development of increasingly sophisticated geographic information systems (GIS) has, together with RS, provided a new set of tools for public health professionals to monitor and respond to health challenges (Morrison *et al*., 1998; Carroll *et al*., 2014). These systems help pinpoint cases and exposures, identifying spatial trends and disease clusters, correlating different sets of spatial data, and testing statistical hypotheses. Nevertheless, risk maps are still not commonly used in public health contexts (Louis *et al*., 2014). Louis *et al*. (2014) showed the great diversity of predictors and modelling approaches needed to create dengue risk maps. However, this field is only in its beginning and still evolving. In addition, different studies propose that *Ae. aegypti* prevention and control actions should be based on results obtained from spatio-temporal data, both from the field and satellite imagery (Niño, 2011; De Melo *et al*., 2012; Estallo *et al*., 2015). The increase in the quality (accuracy) of field information used for the development of predictive maps should allow public health workers to identify areas of low or high risk for adequate disease control (Rotela *et al*., 2007; Halstead, 2008; Boyer *et al*., 2014). Despite the knowledge of *Ae. aegypti* biology and the use of monitoring tools for epidemiological surveillance and vector control services, the precise detection of sites with high-density vector breeding remains poorly understood. In fact, the direction and intensity of dispersion of vector breeding sites in urban areas over time is still unknown. The Argentinian government is currently working on an integrated dengue risk stratification system (Porcasi *et al*., 2012). Although its national scale module is well established, local scales need a deeper understanding. Several national academic teams are studying spatial vector dynamics in different cities and the kind of output that needs to be established to allow the use of predictive models in an operative context. Furthermore, a number of ideas have been proposed for the use of vector control strategies based on predictive maps generated from local environmental variables and field data. The purpose of this study is to analyse the temporal dynamics of the spatial distribution of *Ae. aegypti* breeding sites in a city that has not instituted any vector control actions. Clorinda City, Formosa Province, Argentina is such a place, from where three years of dengue vector data are available.

Materials and Methods

Study area

The City of Clorinda (25°17'S, 57°43'W) is located in the Province of Formosa, Argentina, 4 km from the Paraguayan border on the right bank of Pilcomayo River, 10 km before its confluence with Paraguay River (Figure 1). This city lies in the humid Chaco region and presents

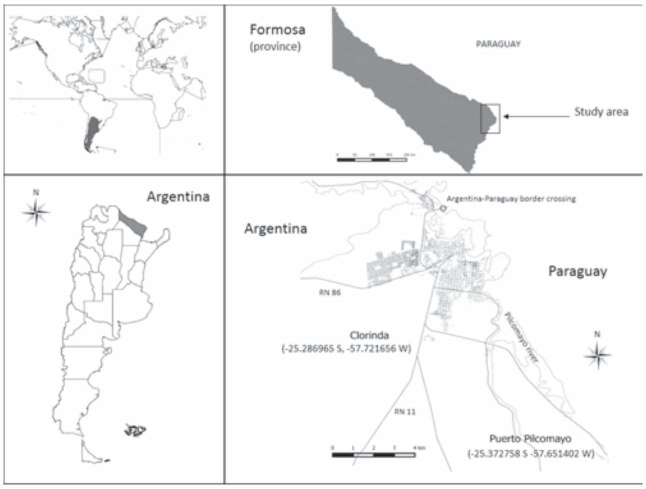

Figure 1. Geographic area of *Aedes aegypti* larval sampling in Clorinda and Puerto Pilcomayo (Formosa Province, Argentina), 2012-2014.

a subtropical climate without a dry season. Annual median temperature is about 23°C and the mean annual precipitation 1300 mm, due to a rainy season stretching from October to May (Masuh *et al*., 2003; Gurtler *et al*., 2009). The urban area is comprised by 980 blocks of buildings and the entomologic surveillance area of this study included 16,511 houses belonging to these blocks. Each house was georeferenced by the use of a handheld GPS equipment (Garmín™ eTrex Legend® HCx; Garmin, Olathe, KS, USA). Clorinda City is characterised by its ethnic cultural diversity, due to the presence of several indigenous groups and migrant populations from the bordering countries, above all from Paraguay. This feature produces an important effect on the cultural, social and economic profile of these communities (Masuh *et al*., 2003).

Entomologic data

From October 2011 to October 2014, the presence and abundance of *Ae. aegypti* breeding sites in Clorinda City were registered and in each time 20% of the total number of houses was selected by random distribution sampling for monitoring. During this period, a total of 12 entomological surveys were performed (Table 1) and the results grouped by year in order to conduct space-time analysis. In all households from a selected block that had agreed to participate, the water containers were grouped into different categories (tires, tanks, drums, barrels vases, *etc*.) and the total number of containers recorded, including information on which had water and which had larvae. All larvae were collected and moved to the Entomologic Laboratory of Fundación Mundo Sano in Clorinda City for taxonomic determination using a specific morphological key (Rossi and Almirón, 2004). Fundación Mundo Sano is a scientific, non-governmental foundation in Argentina engaged in control and prevention of communicable diseases. Houses found to have at least one container with one or more *Ae. aegypti* larvae or pupae were considered a positive site. For each year, the entomological indices at the housing unit level were calculated using stegomyia indices: house index (HI)=the number of positive homes/number of houses inspected) ×100; and Breteau index (BI)=the total number of breeding sites/number of houses inspected) ×100 (Ibañez-Bernal and Gomez-Dantes, 1995).

The free Quantum GIS Desktop, version 2.6.1. Brighton (QGIS) (http://www.qgis.org/) software was used to build the point vector layers (.shp) locating samples points, recording sites of mosquito presence, collecting abundance data for each survey and grouping them per year. Based on those layers, the QGIS *heatmap* tool (estimating the density of positive sites) was used to create hotspot maps for each year. Moreover, cluster analysis methodology was applied to identify spatial clusters (Kulldorf and Hjalmars, 1999). Sites with presence of larval stages were indicated as positive cases (1) and those without were indicated as negative (0). The analysis consisted of a spatial scan through the superposition of exploratory circles over sites with a record of larval presence. Each circle was seen as a possible cluster and, taking into consideration the number of events inside and outside an expected number of events, the probability was calculated. The circle that presented the maximum probability and excess in the number of events observed *vs* expected, was defined as the most probable cluster (Kulldorf and Hjalmars, 1999). Based on the results of this exploratory analysis and the knowledge of the roads and demographic stratus of the city, we defined five particular zones. The temporal development, based on the mean numbers of positive breeding sites for each zone for each of the three sampling years, was analysed in order to evaluate whether the zones in the urban geography presented different responses to the environmental annual oscillation.

Land cover

Images from the satellite SPOT 5 (http://www.geoimage.com.au/satellite/spot-5) were used to characterise environmental coverage types in Clorinda City. This commercial imaging satellite system provides a 10-m multispectral resolution image in its SPOT 5 product J with four spectral bands: green (0.50-0.59 μm), red (0.61-0.68 μm), near infrared (0.78-0.89 μm), and middle infrared (1.58-1.75 μm). All images used were supplied by National Commission on Space Activities (CONAE) in Argentina (http://www.conae.gov.ar/index.php/). The SPOT image from April 29, 2013 (approximately in the middle of sampling period) was used to generate a land cover classification and

Table 1. Temporal sequence of entomologic sampling surveys for *Aedes aegypti* breeding sites in Clorinda City, 2012-2014.

Sampling number	Start date (day/month/year)	End date (day/month/year)
17	17/10/2011	16/02/2012
18	16/02/2012	04/06/2012
19	05/06/2012	31/08/2012
20	23/08/2012	07/11/2012
21	07/11/2012	04/03/2013
22	04/03/2013	27/05/2013
23	23/05/2013	13/08/2013
24	13/08/2013	07/10/2013
25	07/10/2013	28/11/2013
26	05/12/2013	06/05/2014
27	07/05/2014	05/08/2014
28	06/08/2014	27/10/2014

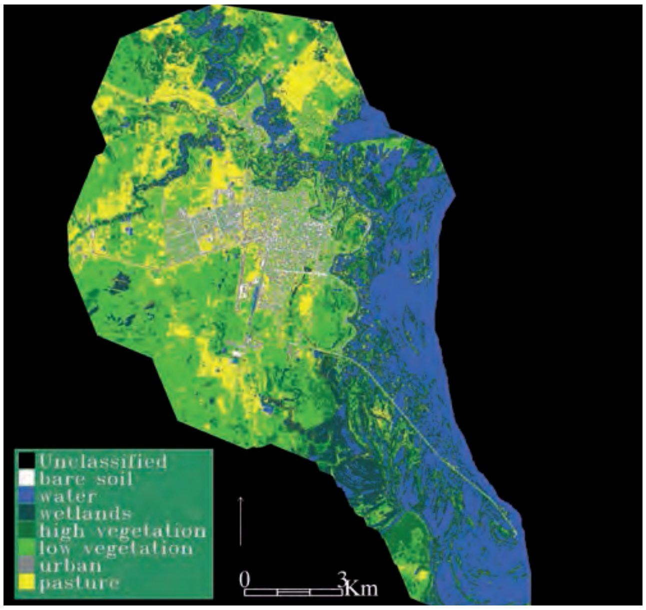

Figure 2. SPOT 5 satellite image of the Clorinda Region with seven different classes of landscape variables. Unsupervised classification (K-means).

macro-environmental products of the study area (Figure 2). Unsupervised classification (K-means) was performed to classify the study area image following the methodology indicated by Rotela *et al*. (2010). Seven land cover classes were defined: *bare soil*; *superficial water or water*; *wetlands*; *low vegetation (grass)*; *high vegetation (trees)*; *urban buildings*; and *pasture/crops*. Through the use of Google Earth, a set of 40 control points (ground truth) was created for each environmental cover to corroborate the accuracy of the classification. The confusion matrix used with validation control points showed an overall accuracy of 77.6% and a Kappa coefficient of 0.74. Some classes (water, pasture/crops, low and high vegetation, wetlands) reached values above 80% accuracy, while other (bare soil and urban buildings) presented 50 and 76% accuracy, respectively. QGIS, version 2.6.1 and ENVI, version 4.8 (Exelis Visual Information Solutions, Boulder, CO, USA) software were used to create classification images and vectors to assess accuracy level of classifications.

Based on the land cover classes created, two different types of macro-environmental variables were generated from each class and expressed as i) *Buffer Image* (Figure 3) here called *Bare soil distribution*, *Low vegetation distribution, etc*.; and ii) *percentage* of each land cover class (Figure 4) by applying the methodology described by Rotela *et al*. (2010). The window size for the generation of the percentage map was 31×31 pixels, attributing the average value of the window pixels to the central pixel. To generate the new land cover classes (distribution and percentage), we considered a 300-m diameter buffer zone, based on available evidence of the *Ae. aegypti* flying range (Trpis and Hausermann, 1986; Ordoñez *et al*., 1997) that was accepted as the average habitat of the species. All these analyses were performed with the ENVI 4.8 geospatial software. In addition, a hotspot buffer layer shapefile was created as a distance map to critical points (cemetery and garbage dump). Finally, the normalised burn ratio thermal (NBRT) layer was included as proxy for the annual land surface temperature according to Espinosa *et al*. (2016). This index includes near infrared, short wave infrared (SWIR) and thermal infrared Landsat bands (http://landsat.gsfc.nasa.gov/). These composites are made from Level L1T orthorectified scenes, using the computed top-of-atmosphere reflectance (Holden *et al.,* 2005) and can be obtained from several Earth data repositories, for example in Google Earth Engine.

Subsequently, information provided by the Argentinean National Institute of Statistics and Censuses - INDEC, processed with Redatam +Sp (http://www.indec.gov.ar) was used to generate a layer that included demographic information related to the availability of drinking water (public network) (Figure 4). This layer reflects the lack of this public service as an indicator of the use of outdoor water storage containers that could function as *aegypti* breeding sites. The INDEC data were transformed, by the QGIS software, into a vector layer including percentage values of this service.

Modelling vector-presence probability

In order to assess the contribution of each of the selected variables in the prediction of suitable *Ae. aegypti* breeding sites in the City of Clorinda, the maximum entropy (MaxEnt) program, version 3.3.3a software (http://www.cs.princeton.edu/~schapire/maxent/) was run to generate vector presence probability maps based on the macro-environmental layers created (Peterson, 2001, 2003; Elith *et al*., 2006; Rotela *et al*., 2007). It was run with 1000 repetitions using 75% of vector presence points for model training and 25% for validation. In general, the MaxEnt algorithm detects non-random relationships between two datasets: i) georeferenced records of species presence, and ii) a set of raster land cover types representing the environmental and demographic variables considered relevant to determine *Ae. aegypti* distribution (Phillips *et al*., 2006). The environmental dataset used consisted of 23 raster format variables of 10-m spatial resolution (Appendix 1).

The ecological modelling calculates the *probability of presence* of

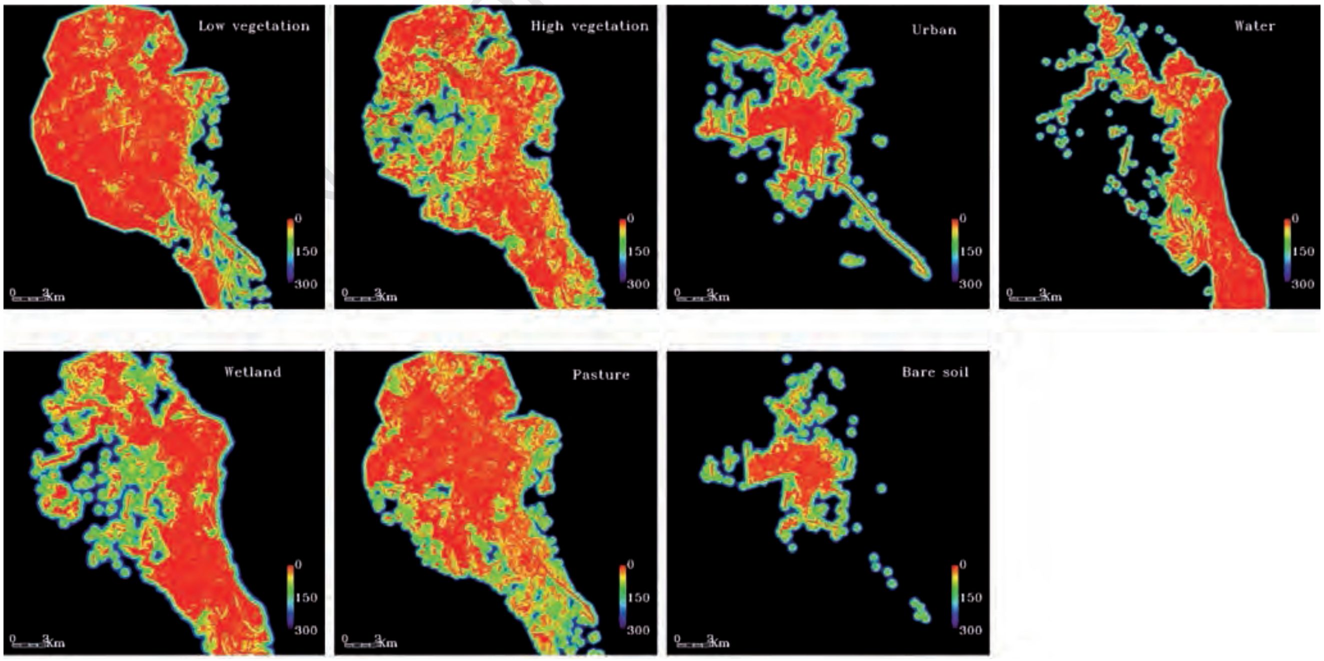

Figure 3. Distance maps centred on Clorinda City. The various landscape variables are as indicated in the legend (300 m buffer).

vector breeding sites based on environmental and demographic variables and the recorded presence of vector breeding sites for training and testing. All *Ae. aegypti* breeding sites from the 2014 survey in Clorinda were used. In order to do that, a product compiling all variable values was generated. Thus, each pixel of the study area represents different landscape values (from the set of variables shown in Appendix 1) and an associated value indicating the probability of the presence of a breeding site. For this analysis, two basic premises were taken into account, one related to the presence of sites where the *Aedes* species successfully grows, and another referred to the selected environmental variables that adequately represent the ecological requirements of this species. Each presence site was indicated by a pair of geographic coordinates (WGS84 Datum) and represented places where *Ae. aegypti* breeding sites were found during the sampling period.

Results

From 2012 to 2014, 12 samplings recording *Ae. aegypti* breeding sites in about 2500 houses during the entire sampling period were performed and recorded including the two stegomyia indices (HI and BI) obtained for each year (Table 2). The number and distribution of *Ae. aegypti* breeding sites found in Clorinda City for each sampling year are presented in Table 2 and Figure 5.

Figure 6 shows the result of hotspot analysis per sampling year based on entomological data. A broad distribution with evidence of a small high-density zone north of Clorinda City and a high-density region in the centre can be seen. The southwest to northeast cluster line remained similar during the three years.

The infestation pattern observed each year led us to define the five regions presented in Figure 7A. The annual density of breeding sites for each year and each zone, extracted from Figure 6 data, are presented in Figure 7B. Centre-west and southeast regions present more similar abundance levels each year to the total city pattern (A2013>A2012>A2014), while the centre-east and northern regions show different inter-annual variability (A2012>A2013>A2014) (Figure 7B). Although a general temporal pattern can be recognised in the previous analysis, we can see that some city zones have a dynamic of their own, a remarkable finding considering the small size of Clorinda City that does not have any systematic control activities.

MaxEnt software results show that the spatial pattern of our vector data is principally related to the set of indicators presented in Table 3, which underlines the importance of the percentage of the contribution and permutation of the model's principal variables. The predictive map obtained by the model was assessed with the measurement of the area

Table 2. Entomologic samplings performed in Clorinda City showing the House and Breteau indices obtained.

Year	Samplings (n)	Houses visited (n)	Positive houses (n)	Breeding sites (n)	HI (%)	BI (%)
2012	4	7468	1027	1135	13.75	15.19
2013	5	10,224	881	1392	8.61	13.61
2014	3	7030	612	981	8.7	13.95

HI, house index; BI, Breteau index.

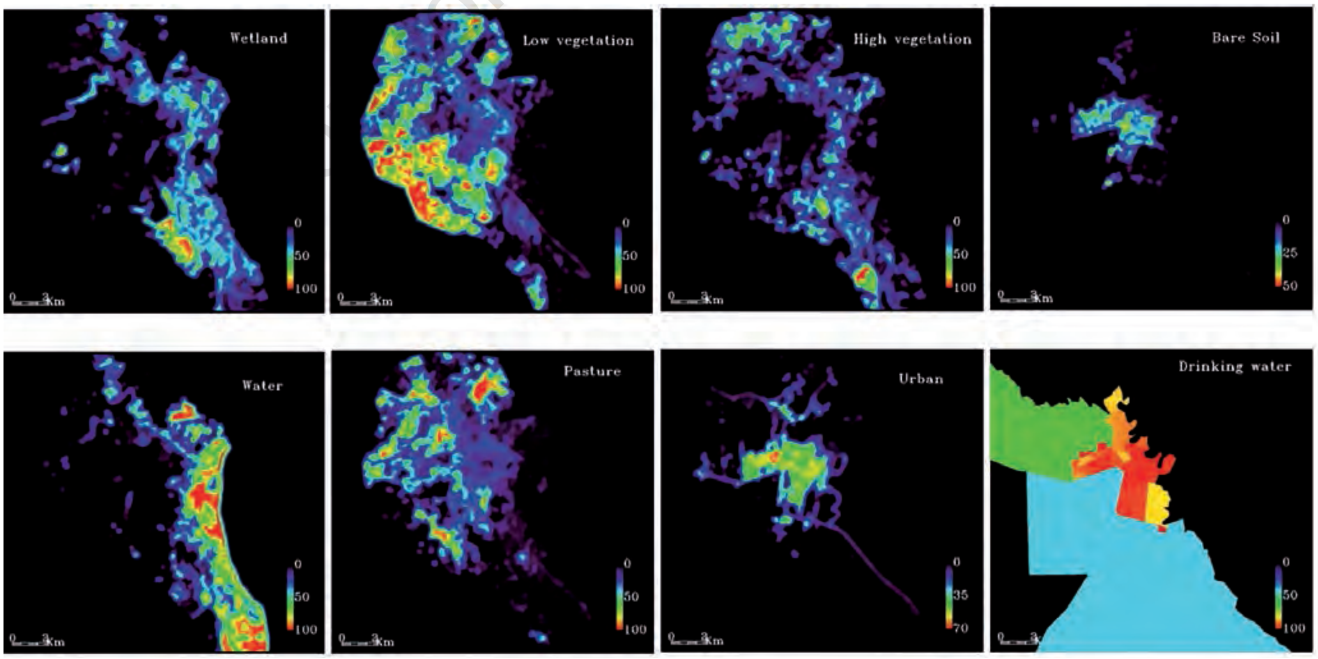

Figure 4. Variable class percentage maps at Clorinda City. The various landscape variables are as indicated in the legend (300 m buffer).

under the curve using receiver operating characteristic analysis, which indicates the global accuracy of the test (Deleo, 1993). In this case, the analysis scored 0.965 with a standard deviation of 0.003, which means that the predictive ability of our model generated for the 2014 dataset must be classified as very good according to the scheme mentioned by Parolo *et al*. (2008). The model predicted that the best environmental variables explaining 85.8% of the distribution of the breeding sites were water containers (48.2%), urban building distribution (33.2%) and urban building percentage (4.4%) (Table 3). The probability map for *Ae. aegypti* breeding sites generated by this ecological niche model is presented in Figure 8.

Discussion

This study presents relevant results on how *Ae. aegypti* breeding sites are distributed during three years of spatial pattern dynamics in a small town in the North of Argentina. The focus is based on the different results that can be found between typical analysis based on only one year of sampling data and the temporal evolution of patterns when no control intervention is performed. Relevant results about *Ae. aegypti* breeding site distribution in the study area are presented taking into account dynamic spatial patterns that estimate the evolution of vector populations in an environment free of vector control. Maps showing high-density of *Ae. aegypti* breeding sites indicate a greater proportion and persistence (in time) of four principal cluster areas. The increase in density of positive breeding sites from 2012 to 2014 was observed in association with the increase in the stegomyia indices (both HI and BI). This behaviour is in agreement with observations obtained from other areas under no vector control (Rotela, 2012) in Puerto Iguazú City, but differs from the results obtained by Espinosa *et al*. (2016) in the City of Tartagal, where there was a significant reduction in breeding site density related to vector control activities.

Clorinda's high-density clusters are characterised by a deficit in the supply of potable water, especially during the summer months (Garelli *et al*., 2011) promoting the accumulation of a diverse array of containers for water storage near the houses. Generally, most of these containers are uncovered, thereby constituting excellent breeding sites for *Ae. aegypti*. A previous study conducted in Clorinda City concluded that this practice is due to a cultural pattern adopted to face the lack of access to water that constitutes a complex set of factors influencing the abundance of breeding sites and mosquito population dynamics (Garelli *et al*., 2011). In addition, the lack of a relationship between precipitation

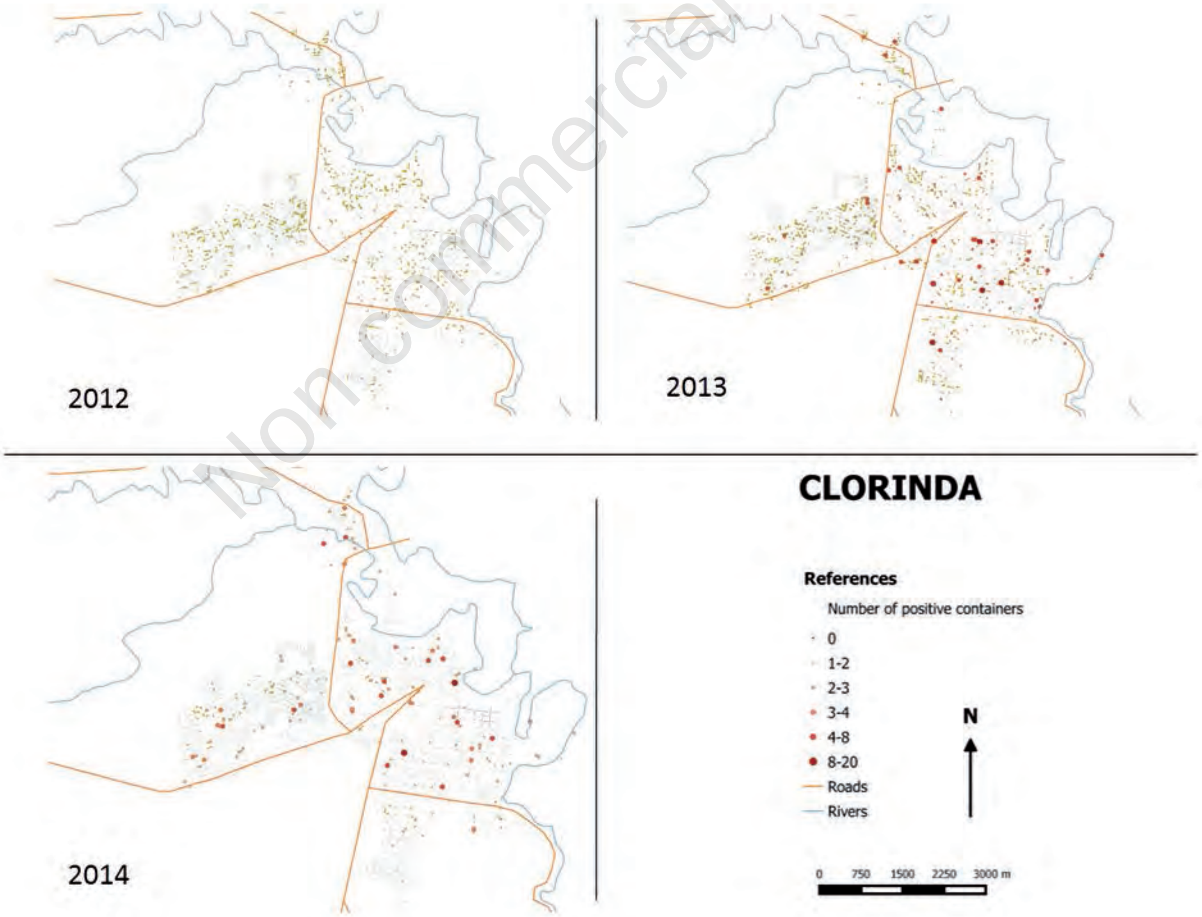

Figure 5. Distribution of total number of surveyed houses in Clorinda City expressing the number of positive containers found for *Aedes aegypti* larvae in each sampling year.

and number of breeding sites found in Puerto Iguazú City (Rotela, 2012) highlights the importance of the use of water storage containers and the increase of mosquito breeding sites independently of this weather variable. Therefore, a chain is created resulting in an increase in vector density leading to an increase in vector-human contact that finally produces a higher viral transmission rates among the human population during epidemic events (Siqueira-Junior *et al*., 2008; Eisen and Lozano-Fuentes, 2009). This converts the detection of vector density changes into an important factor in the epidemiology of the disease (Niño, 2011).

Table 3. Relative contribution of the environmental variables in the maximum entropy model.

Variable	Percent contribution°	Permutation importance$^{\#}$
Water supply (INDEC, 2010)	48.2	18.2
Urban building distribution	33.2	37.7
Urban building percentage	4.4	2.9
Hotspot distribution	4.1	3.8
NBRT§ temperature distribution	2.6	3.3
Bare soil distribution	2.5	0
Low vegetation percentage	1.2	4
Pasture/crops percentage	0.5	2.4
NBRT§ temperature	0.5	5.4
Pasture/crop distribution	0.5	1.2

INDEC, Argentinean National Institute of Statistics and Censuses; NBRT, normalised burn ratio thermal. °The increase in regularised gain was added to the contribution of the corresponding variable for each iteration of the training algorithm; #for each environmental variable, the values of that variable on training presence and on background data were randomly permuted; §normalised burn ratio thermal.

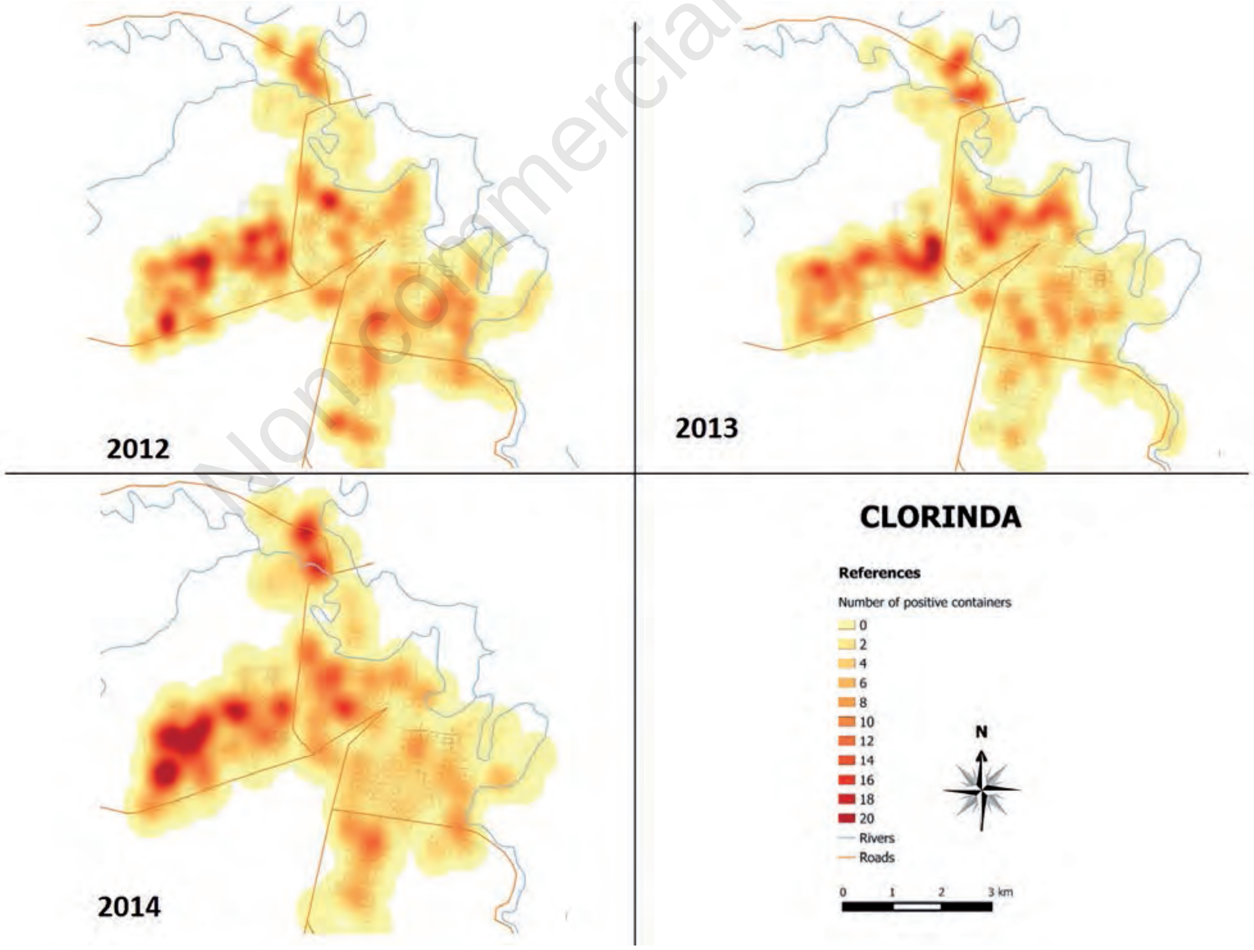

Figure 6. Annual density in the summer-autumn period of *Aedes aegypti* breeding sites in Clorinda City.

The presence of high-density breeding sites, which remained stable during all the study period, was found to be strongly related to suitable areas predicted by the ecological niche model based on environmental and socioeconomic conditions. Indeed, the spatial dataset of vectors and environmental variables used to generate the probability map allowed predicting over 95% of the positive breeding sites. The variables found to be most contributing to the breeding site distribution are consistent with results reported by Porcasi *et al*. (2012) and Rotela *et al*. (2012) for other cities in northern Argentina. However, the findings are in contrast to findings by Vezzani *et al*. (2005) for microhabitat suitability in the southern latitudes, *e.g*. Buenos Aires, a fact that reinforces the results obtained here taking into account that one of the key aspects to reduce the abundance of the vector involves the identification of *Ae. aegypti* breeding sites (Aviles *et al*., 2003).

Considering the complexity of variables that affect the distribution of anthropophilic vectors such as *Ae. aegypti*, spatial analysis tools and GIS applied in the current study allowed the generation of a map showing positive breeding sites as well as the determination of the more significant variables supporting this development. The predictive power with respect to the presence of *Ae. aegypti* breeding sites produced by the model generated for Clorinda City is in accordance with Parolo *et al*. (2008). The vector presence probability maps, with values exceeding 85%, clearly showed that the increased risk was associated with three principal clustered sectors in the city, strongly related with hotspots (one located in the North, one in the West and one in the East of the city). The convergence of Heatmap clusters and the three sectors identified as the most likely areas with vector presence (*Ae. aegypti* breeding sites) in contrast to the sectors with lower probabilities (0-30%) shows the suitability of our model in environments where vector control has not been implemented.

In Clorinda City, and in accordance with Ostfeld *et al*. (2005), WHO (2014) and Troyo *et al*. (2009), RS information has been used to determine the type of land cover that favours the presence of breeding sites and survival of *Ae. aegypti*. The performance obtained by the MaxEnt model could allow the establishment of the proposed methodology, to generate continuous breeding site maps, like a valid one when used with contemporary field data. The great quantity of field data collected and analysed in Clorinda City allowed us to evaluate the model's predictive capacity, confirming the necessity to use large time series of local data (entomological and environmental) for the construction of predictive models, which allows the application of more efficient control actions (Louis *et al*., 2014). In addition, our results support previous predictive models developed by Porcasi *et al*. (2012), Rotela *et al*. (2010), and Rotela (2012) offering tools to improve health system responses with respect to vector and virus transmission control, which have relevance not only for dengue but also other vector-dependent viral diseases.

Conclusions

As usual, the results do not completely agree with the results expected by the study team. Our hypothesis was that in a small city without systematic control activities, the breeding site pattern NB(x,y,t) should have a quasi-uniform time evolution for the whole city. This means that NB(x,y,t)=f(x,y)*g(t), *i.e*. the relative spatial pattern, should basically be the same modulated only by the temporal function (the city as a whole is expected to respond similarly to the annual climatic variability). In this case, the spatial map obtained for a specific year, could be used for others years, because the relative values are temporally invariant. Inversely, however, it is clear that our results (Figure 7B) show a differential temporal behaviour for each intra-city zone. For example, from 2012 to 2013, in the southern and southeastern areas, there is an increase in the mean number of breeding sites, meanwhile in the North and Centre-East, the risk decreased from 2012 to 2013. This means that our hypothesis is clearly rejected. Thus, more studies are needed to understand this intra-city dynamic and its regulatory micro- and macro-environmental variables.

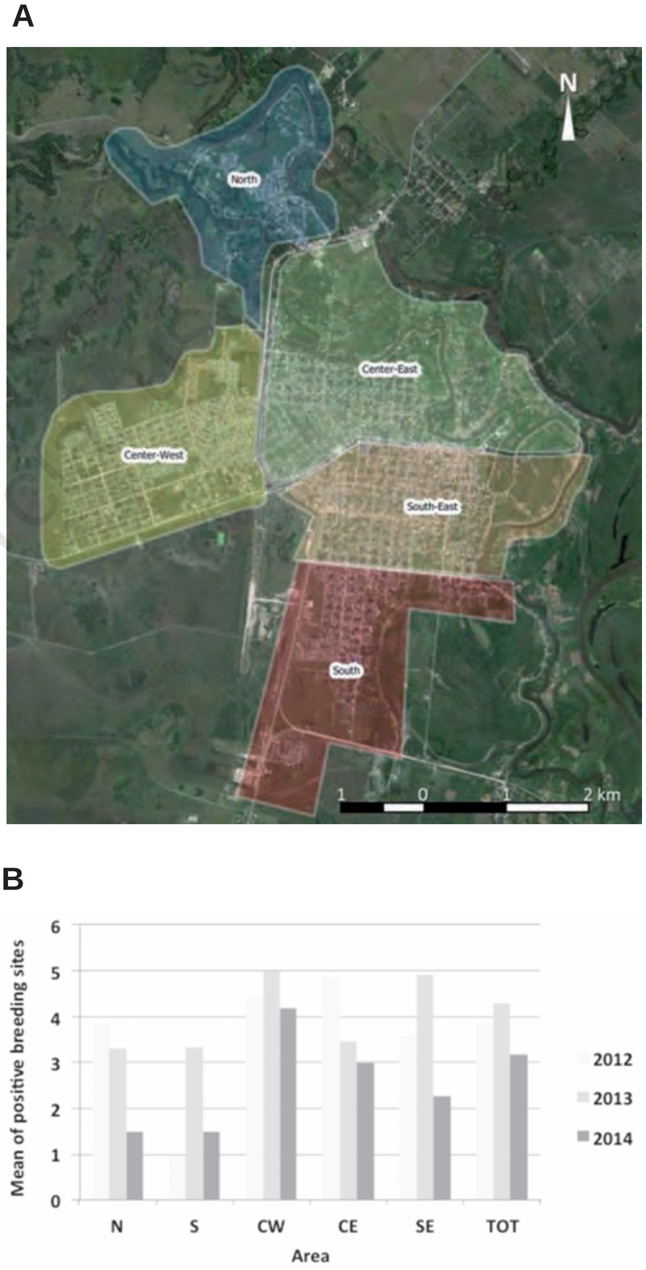

Figure 7. A) Regions in Clorinda City defined for particular temporal analysis; B) mean of positive breeding sites per house for areas showed in A) for the three sampling years. N, North; S, South; CW, Centre-West; CE, Centre-East; SE, Southeast; TOT, total annual.

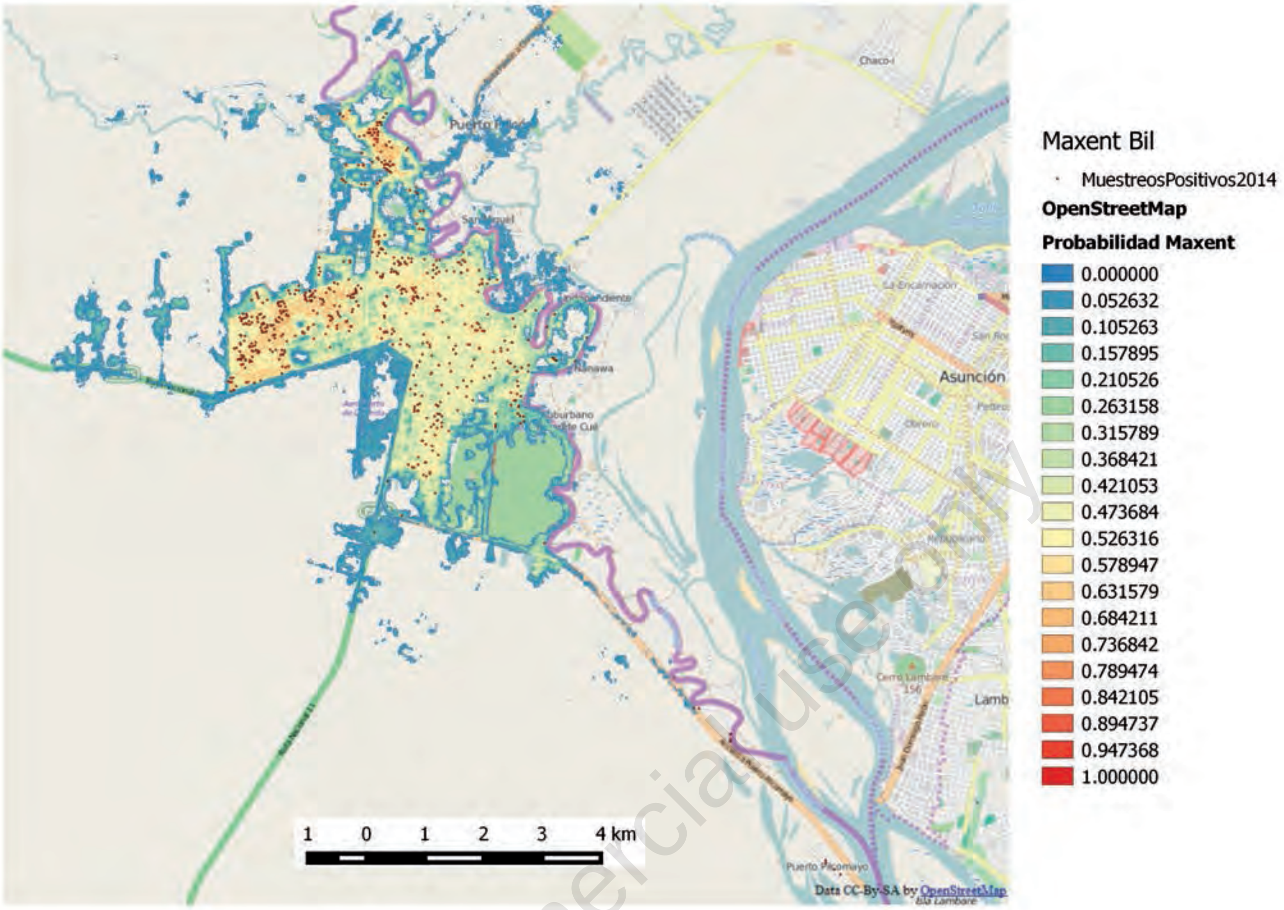

Figure 8. Probability map for *Aedes aegypti* breeding sites generated by the maximum entropy model (MaxEnt). Blue areas represent a null risk for developing breeding sites, while red areas are the most suitable habitats for vector breeding sites.

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