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Integrating demographic and meteorological data in urban ecology: a case study of container-breeding mosquitoes in temperate Argentina

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Cities are unique ecosystems emerging and growing worldwide due to ongoing urbanising trends. The urban–rural gradient is an excellent setting to evaluate the effect of urbanisation on the distribution of species, a matter of public health concern in the case of disease vectors. Despite this, such distributions are affected by other co-occurring variables, mainly meteorological, that may be confounded by the urbanisation gradient due to the urban heat island effect and maritime climatological conditions in the case of coastal cities. To aid in the design of ecological studies within the urban–rural transition zone, a mapping protocol was designed and applied to Buenos Aires City and its surroundings. Based on road density and district-level population counts, a detailed (1 km^2 pixel) urbanisation map was obtained which, combined with a temperature map, rendered a final urbanisation × temperature product with six classes. The resulting zonation was tested by modelling the distribution of the vector mosquitoes Aedes aegypti *and* Culex pipiens *in artificial containers. The selected model explained the occurrence of mosquitoes 59% better than chance as a function of the urbanisation × temperature categories and the natural lighting condition of the container. This novel zonation approach allows partitioning of environmental heterogeneity prior to the selection of study sites to avoid confounding gradients and provides multiple advantages, such as making comparisons across cities easier, extrapolating the results of site-scale experiments and identifying priority areas for control measures.*

Key words: *Buenos Aires, urbanisation, generalised linear mixed models, environmental gradients, mosquito vectors, arthropods*

Introduction

Cities are one of the most profoundly altered ecosystems on the planet (Collins *et al*. 2000). The process of urbanisation not only modifies the landscape by the creation of impervious surfaces and artificial structures, but also results in high densities of people, introduced plants and domestic animals. All these alter the flux of nutrients, organisms, energy and water within and between landscapes (Green and Baker 2002; McDonnell and Hahs 2008), resulting in quite similar ecological structures in town centres throughout the world (Blondel *et al*. 1984).

Along with the progressive aging of the population, the relentless trend of increasing urbanisation has affected human health by promoting a higher risk of exposure to epidemic and chronic diseases (Ezzati *et al*. 2005). In developing countries this is mainly driven by the fact that rapid population growth and low incomes have triggered large-scale immigration from rural to nearby urbanising areas, resulting in unplanned and chaotic urbanisation. In this scenario, poor water and waste management promotes different types of water and food-borne diseases and also creates suitable aquatic habitats for arthropod vectors (Ehrenberg and Ault 2005). These risks are enhanced by high degrees of social contact that increase transmission of pathogens. Therefore, the urban–rural gradient shaped by cities and their surroundings provides a good experimental setting to assess the risk posed by urbanisation on human health.

Although conceptually old, ecological niche modelling is a relatively new tool in the field of distribution ecology, which has been recently applied to the study of insects of public health importance (Peterson 2006). In particular, its use in urban mosquito research has gained worldwide interest because of the role of these insects as vectors of diseases and well-known nuisance (e.g. Ruiz *et al*. 2010; Chaves *et al*. 2011). In the continuously growing body of literature regarding this subject, the effects of climate, habitat availability and biotic interactions on the distribution and abundance of mosquito populations in urban environments have been addressed (e.g. Rey *et al*. 2006; Ruiz *et al*. 2010; Rubio *et al*. 2011; Chaves *et al*. 2013). However, some of these effects may be masked by or confounded by the urbanisation gradient and may lead to false conclusions if not treated explicitly. In particular, the urban heat island effect (i.e. the phenomenon in which a city is warmer than its adjacent rural area) is produced by modifications of the energy balance caused by furnace heating, limited surface moisture, urban structures and atmospheric pollution (Bornstein 1968). This change in temperature is confounded by the urbanisation gradient and its associated accumulation of human-generated waste (Chaves *et al*. 2009; Nguyen *et al*. 2012). Moreover, in coastal cities the temperature pattern is further complicated by the maritime location (Figuerola and Mazzeo 1998). Therefore, the objective of this study was to design a protocol for ecological studies considering thermal gradients and the level of urbanisation explicitly with respect to the urban–rural gradient. This integrative approach was applied to Buenos Aires City and its surroundings to classify the space in six urbanisation × temperature categories, and was tested using a previously published data set to model the occurrence probability of container-breeding mosquitoes.

Materials and methods

Area description

Buenos Aires City is the second megalopolis in South America and the capital of Argentina, located at the shore of the de la Plata River estuary (Figure 1). The climate is temperate humid–subhumid, with annual precipitation in the range 600–1200 mm W–E and annual mean temperatures 14–17°C S–N (Magrin *et al*. 1997). The study area includes 47 districts (Figure 1), covering a true urbanisation gradient from Buenos Aires City (14 308 inhabitants/km^2) to several small towns of low population density (<10 inhabitants/km^2) (INDEC 2010a) located up to 140 km from the city centre. The original grassland has been modified by agriculture, farming and human settlements that dissected the natural matrix along transportation corridors in all directions except the east (Morello *et al*. 2000).

The most abundant container-breeding mosquitoes in Buenos Aires are *Aedes aegypti* and the *Culex pipiens* complex (Rubio *et al*. 2011; Vezzani *et al*. 2011). Both are cosmopolitan species and recognised worldwide as vectors of diseases to humans and domestic animals (Forattini 2002). In Argentina, *Ae. aegypti* has been responsible for more than 30 000 cases of dengue fever since its resurgence in 1998 (Vezzani and Carbajo 2008; Zambrini 2011), whereas *Culex pipiens* has been implicated as the vector of Saint Louis encephalitis virus (Diaz *et al*. 2006). Both mosquito species were found to be infected by the canine haemoparasite *Dirofilaria immitis* (Vezzani *et al*. 2011).

Urbanisation map

The aim of the urbanisation map was to obtain a fine-scale resolution following a procedure capable of producing good detail, but based on easily accessible and available input data. This was achieved by combining road maps (OpenStreetMap 2013) with demographic data from the last national census (INDEC 2010a). A 1 × 1 km cell grid was generated in ArcGis 9.3 (ESRI 2008) and the road layer was clipped with the grid in order to measure road length per cell. Each pixel was assigned a given population number by weighting the total population of the district by the fraction of road length of the district located within the pixel. Afterwards, pixels with one and two road segments (corresponding to routes and highways) were excluded with a mask, because in districts with very high population numbers they were assigned an unrealistically elevated population density. For further analyses three urbanisation categories were defined: low (<1000 inhabitants/km^2), middle (1000–5000) and high (>5000).

The urbanisation map obtained was compared with a non-areal functional classification (INDEC 2010b) in which each previously delimited censual radius was categorised as urban if all premises in it were located within a locality of ≥2000 inhabitants; as rural if within a locality of <2000 inhabitants or dispersed in open fields; and as mixed if containing both urban and rural premises. The proportion of pixels of each of the three urbanisation categories defined above (urban, rural and mixed) were calculated.

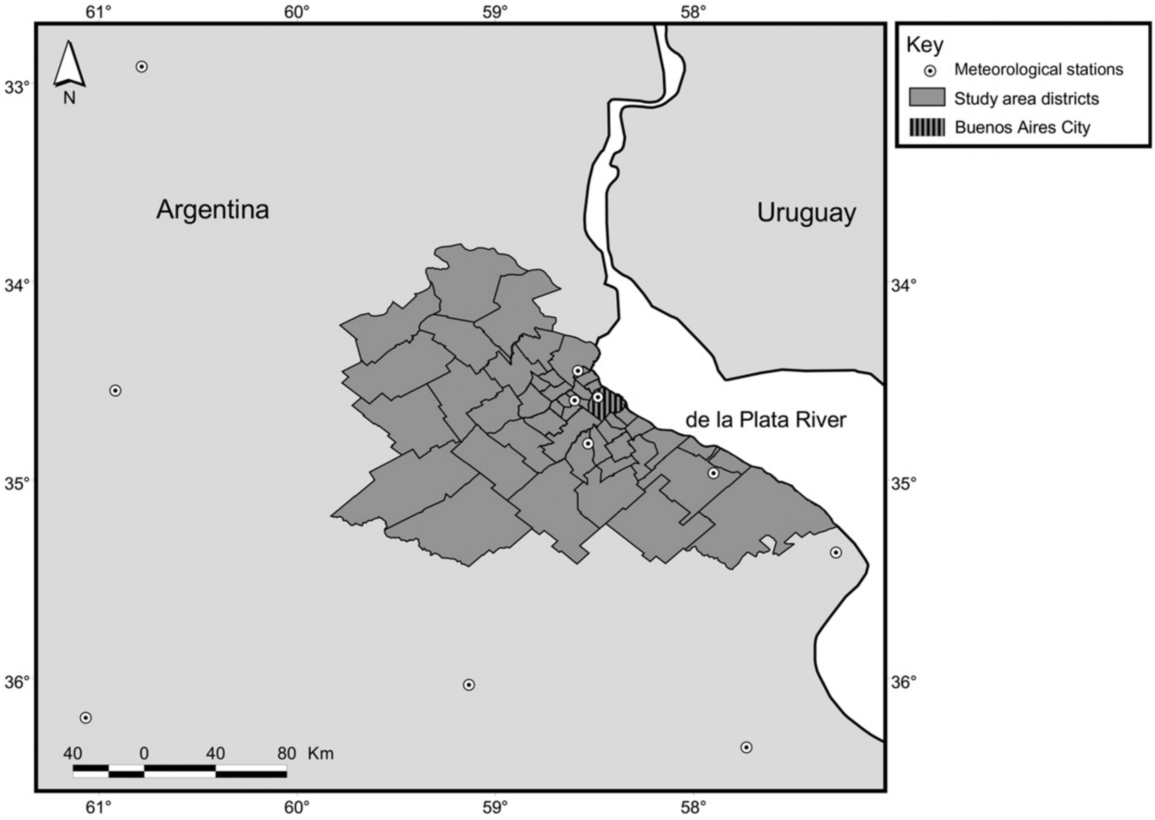

Figure 1 **Study area and location of the 11 meteorological stations from which temperature data were obtained**

Temperature map

The selection of the appropriate meteorological variable/s must be based on knowledge regarding the bionomy of the taxon under study. For the current biological model, i.e. mosquitoes, the mean annual temperature was selected because in temperate regions it plays a key role in oviposition and larvae development (Clements 1999). In the study area, thermal heterogeneity might be particularly important for container-breeding mosquitoes given that Buenos Aires is close to the southern distribution limit of *Ae. aegypti* (Carbajo *et al.* 2006) and within the transition zone between the two species of the *Cx. pipiens* complex (Forattini 2002).

Temperature information was provided by the National Meteorological Service, which has 11 stations within or <200 km distant from the study area (Figure 1). To obtain a surface, mean monthly values for the period 2001–2010 were interpolated using a spline type tension (weight=0.1, number of points=12). This method was preferred over weighted inverse distance because it works better when the number of points is relatively small and it is best to represent smoothly varying surfaces (Childs 2004). Pixels were classified in two categories (cold ≤16.5°C, warm >16.5°C) using this threshold of annual mean temperature for the pixels in which 278 randomly selected piles of used vehicle tyres were inspected for mosquitoes. This decision had the methodological basis of splitting the variability of the study area in half, under the assumption that the inspected tyre piles were a random representation of all available piles.

Final product

The three urbanisation categories (low, middle and high) were combined with the two temperature categories (cold and warm) by superimposing both maps in ArcGis, rendering six categories in the final product.

Mosquito modelling

Rubio *et al.* (2011) conducted a survey of immature mosquitoes from November 2009 to May 2010, during which 2038 water-filled tyres grouped in 279 piles were inspected. A total of 9337 *Ae. aegypti* and *Cx. pipiens* larvae were collected and identified from 650 infested tyres; see Rubio *et al.* (2011) for detailed sampling design. The final database excluded five tyres surveyed and one complete pile due to lack of georeferencing. The six urbanisation × temperature categories defined above were well-represented in the database, with >10 piles within each category.

The occurrence of mosquitoes was analysed with generalised linear mixed models, an extension of generalised linear models that include random terms in addition to the usual fixed effects to account for any potential lack of spatiotemporal independence among tyres due to repeated sampling within the same pixel (Chaves 2010).

Mosquito occurrence per tyre was modelled assuming a binomial distribution of errors and applying the logistic function as a link. In total, 15 models were built and the model that yielded the lowest Akaike Information Criterion, as a measure of goodness-of-fit, was selected (Zuur *et al*. 2009). Each model included a measure of temperature (either continuous, i.e. the exact temperature value for that pixel, or categorical with two levels, cold and warm), a measure of urbanisation (continuous, i.e. the exact value of population density per km^2 for that pixel, or categorical with three levels: low, intermediate and high) and the lighting conditions for each tyre (categorical with two levels, open to sunlight and shaded) as fixed factors. Lighting was considered due to its high relevance for both mosquito species in previous studies within the region (Vezzani and Albicócco 2009; Rubio *et al*. 2011). The interaction term between the temperature and the urbanisation variables, either in their categorical or continuous forms, could not be evaluated given the correlation between both gradients. The urbanisation × temperature variable (categorical with six levels) solved this problem and provided a measure of both temperature and urbanisation. Each of these fixed factor combinations (temperature + urbanisation + lighting condition) were examined along with one of three random terms, namely the sampling occasion (seven groups, one per month), the pixel code (190 groups) and the pixel code per month (260 groups). Once the best model was selected, the levels in a factor that were not significantly different (if any) were merged together (Nicholls 1989).

To assess the accuracy of the selected model, the agreement Kappa index (K), which indicates the classification improvement of the final model over chance (Fielding and Bell 1997) and overcomes unequal number of presences and absences (Titus and Mosher 1984), was calculated. Given that the predicted values are a probability, K was calculated for each 0.01 cut-off point between the whole range of possible values (0–1) and the point that provided the best value of K was reported as the optimal. The residuals were plotted to check for normality and semivariograms were built to discard any remnant spatial dependency. Modelling was performed in R 2.11.1 (R Development Core Team 2012) with lme4 (Bates *et al*. 2011) and Design (Harrell 2009) packages, and semivariograms were built in S-plus 8.0.

Results

The vast majority (87.5%) of the study area was classified within the low urbanisation category, whereas 7.7% and 4.8% corresponded to the middle and high categories, respectively (urbanisation map, Figure 2A). The study area yielded highly variable values of road density, ranging between 0 and 259 road segments and 0 and 34.6 km length per pixel. This heterogeneity resulted in a highly detailed urbanisation map, with several districts including the three categories within their boundaries. Only Buenos Aires City and a few neighbouring districts were classified as highly urbanised for their entire surface. Of all pixels classified herein as low urbanised, 75.6% matched the functional rural category, whereas both the middle and highly urbanised pixels corresponded to the functional urban category (90.2 and 100%, respectively) (Table 1).

The observed temperature pattern in the study area (Figure 2B) is a variation of the flat E–W isotherm expected by latitudinal effects due to the combined action of the moderating influence of the river and the heating influence of Buenos Aires City. In fact, when temperature values from this point were excluded from the input data, the interpolation rendered a fair N–S temperature gradient (data not shown). It is noteworthy that the temperature range between both extremes of the gradient reached 3.75°C.

The urbanisation × temperature map (Figure 2C) shows that the urban sprawl occurred on both temperature categories, extending mainly E–W for the cold category and NW–SE for the warm category. As expected, most of the highly urbanised area was located in warmer areas adjacent to the river, whereas the middle urbanisation category was evenly distributed between cold and warm and the low urbanisation category occurred more frequently in the cold category.

The best model for mosquito occurrence included the urbanisation × temperature categories combined in three groups and the lighting condition as fixed factors, and the pixel code for each month as a random term. The selected model predicted the input data 59% better than chance (K = 0.59, cut-off point = 0.36), and the random term had a standard deviation of 1.64. Model residuals had a normal distribution and no spatial autocorrelation.

The probability of finding mosquitoes was higher at the less urbanised end of the gradient for both temperature categories along with the urban extreme at low temperatures, intermediate in middle urbanisation category and minimum in the high urbanised–warm category (Figure 3). For all groups a shaded condition favoured mosquito occurrence over sunlit tyres. It is worth noting

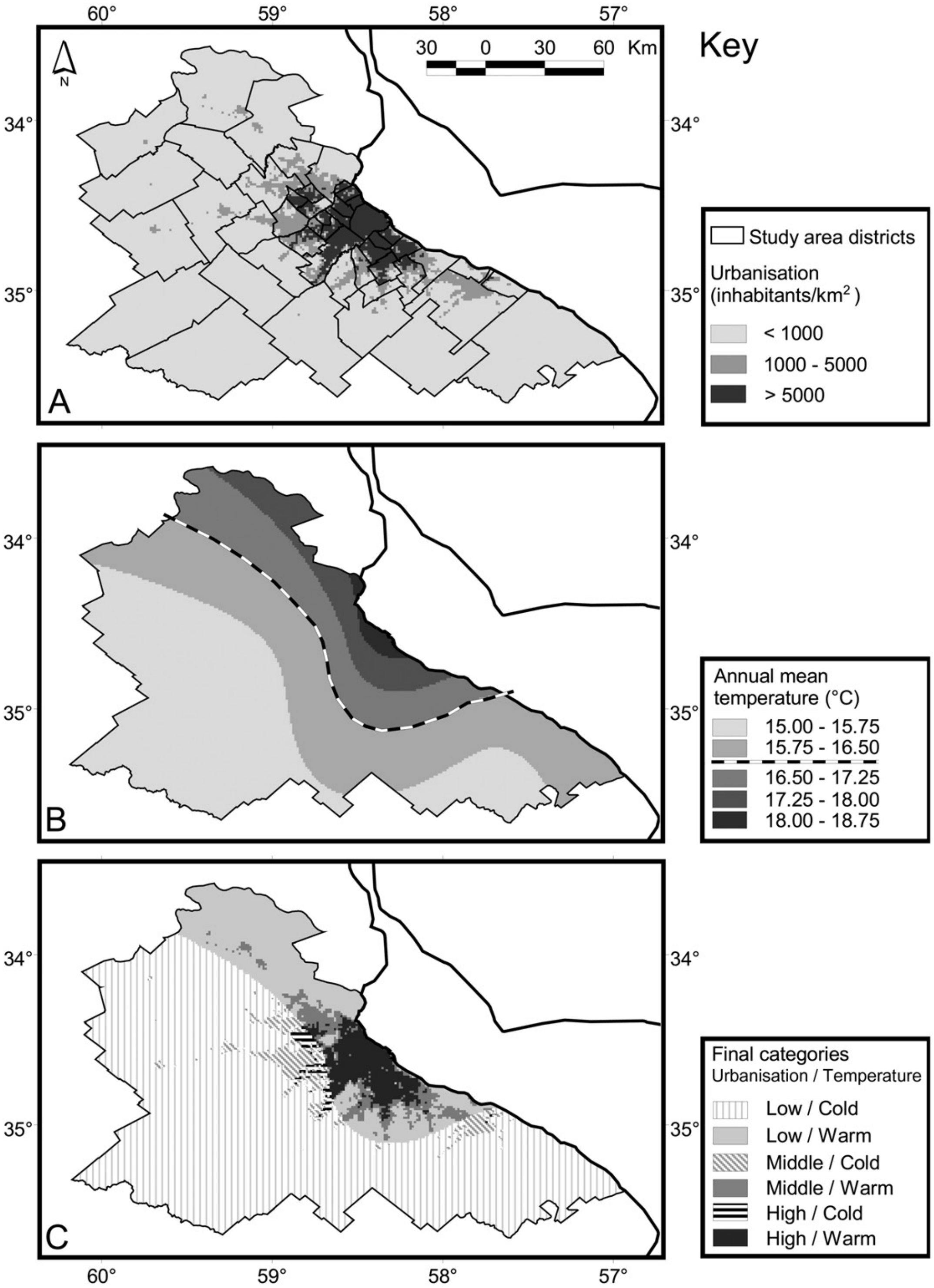

Figure 2 (A) Map of urbanisation density (inhabitants/km²) arranged in three categories (low, middle, high). (B) Map of temperature gradient (°C) and delimitation between cold and warm categories (dashed line). (C) Final map of six categories of urbanisation × temperature

Table 1 Percentage of coincidence (number of pixels) in each of the urbanisation classes, present classification and functional classification

		Present classification in inhabitants/km^2		
		Low (<1000)	Middle (1000–5000)	High (>5000)
Functional classification	Rural	75.6 (15 303)	1.4 (25)	0.0 (0)
	Mixed	18.1 (3659)	8.4 (149)	0.0 (0)
	Urban	6.3 (1269)	90.2 (1602)	100.0 (1109)

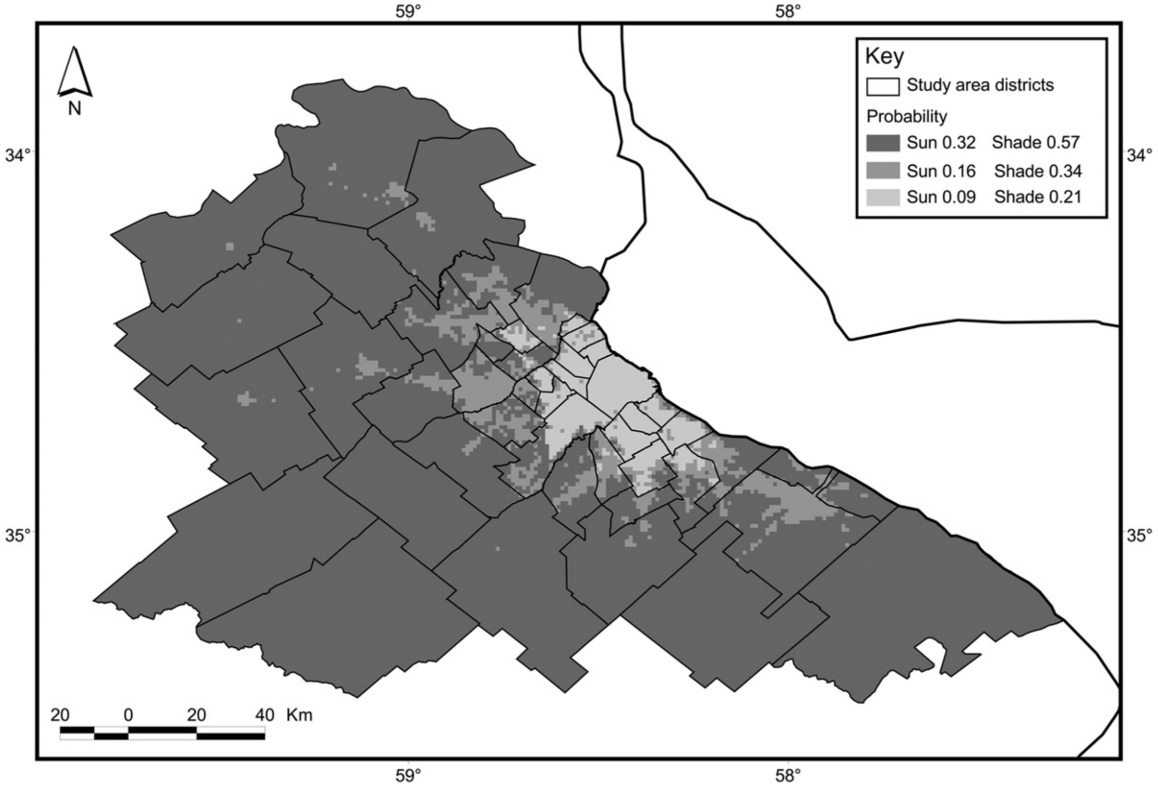

Figure 3 Probability of occurrence of container-breeding mosquitoes for each urbanisation × temperature group in sunlit and shaded conditions, according to the fixed part of the selected model

that the probabilities presented in Figure 3 are the result of the fixed factors alone, and lack the random term effects to simplify their display. Although the model yielded a seasonal pattern (Figure 4), it was not the objective of this study to describe it.

Discussion

As most ecological gradients involve several co-occurring variables, to investigate population patterns and their potential causal factors a comprehensive approach acknowledging the complex nature of biological systems is required (McDonnell and Hahs 2008). Our study generated a zonation of the environmental heterogeneity explicitly in the context of the urbanisation gradient that could be applied in the design of ecological surveys. To our knowledge, this is a novel approach that could help to unravel the effect of each factor on the distribution of different target organisms. This methodology could be applied to urban landscapes around the world, making comparisons of the effect of human activities on a certain biological model across cities with different spatial configurations easier. Partitioning the landscape can also be useful to extrapolate the results of site-scale experiments

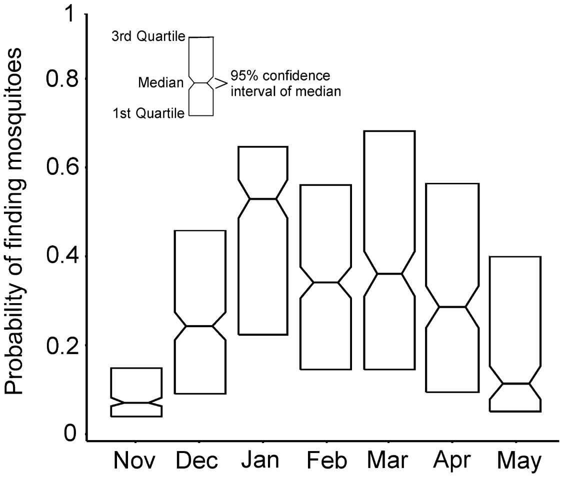

Figure 4 Probability of occurrence of container-breeding mosquitoes as a function of the sampling month predicted by the selected model

and to identify priority areas for control measures, as proposed by Hobbs and McIntyre (2005) for broader-scale biogeographical regions.

Urban–rural transitions have generally been presented as transects (i.e. linear gradients) cutting through a specific section of the landscape (e.g. Luck and Wu 2002; Morse *et al.* 2003). Transects are useful for representing changes as a cross-section because they allow the examination of changes across the profile directly. However, the pattern obtained is highly dependent on the placement of the transect and details of the unintersected areas are lost. As it has been largely recognised that urbanisation creates complex non-linear gradients, the use of multidimensional methods such as the landscape grid presented herein provides a more accurate representation of urban–rural transitions. However, because it is more difficult to display the absolute values for a continuous variable across the landscape, data tend to be displayed as categorised variables (Hahs and McDonnell 2006).

Road density has been previously used as a proxy for urbanisation (e.g. Green and Baker 2002; Dickerson 2010; Khanderwal and Goyal 2010), although this and the percentage of impervious surface ignore the vertical dimension and may represent a limitation in some instances. In this regard, the urbanisation map produced provides a more accurate and realistic basis for ecological studies than the currently available data from national agencies. Whereas the functional rural and mixed categories were unambiguously associated with low urbanised areas, the urban category was almost evenly distributed among low, middle and highly urbanised pixels. This is because some districts are large and highly heterogeneous, therefore the threshold of 2000 inhabitants and the limits of a locality may be misleading. Comparing both classifications, ours could be redefined as 'rural' (<1000 inhabitants/km^2) and 'urban' (≥1000).

The regionalisation of the landscape in terms of climatic conditions has been undertaken before, generally at broader spatial scales; e.g. Bettolli *et al.* (2010) at the country level. The approach developed herein identified subtle (1–2°C) temperature differences that may be confounded by urbanisation in a given climatic region. In Buenos Aires, the urban heat effect is relatively mild given that winds bring the moderating influence of the de la Plata River, the terrain is flat and the street grid encourages effective urban ventilation (Leveratto *et al.* 2000). However, in other cities lacking these moderating factors, the difference between urban and rural temperature may be further exacerbated and selecting sites with similar meteorological conditions to evaluate the effect of urbanisation on a certain organism may be crucial. The delimitation of temperature categories should be carefully examined considering the characteristics of both the area (which determine the temperature range) and the biological model under study (which may, for instance, present key physiological features at certain temperature thresholds). Surprisingly, an exhaustive bibliographical search provided little evidence/data on this matter. We hypothesise this is mainly due to the fact that in developed countries, in which most research is performed, there are no large unplanned urban areas; urbanisation consists of urban nuclei interspersed with rural areas. In contrast in parts of South America and Southeast Asia very steep urbanisation gradients are the norm.

The proposed approach outlined in this study is applicable to any biological model where the underlying assumption is that it is affected by urbanisation and meteorological conditions. The approach could be extended to other environmental gradients as long as they co-occur with the urbanisation gradient and are relevant to the biological model of interest, e.g. altitude. Mosquito populations are perfect for this type of approach because urbanisation and temperature are clearly recognised factors controlling their distribution (e.g. Clements 1999; Cox *et al.* 2007). However, in Buenos Aires and other temperate cities, the confusion between such gradients hampers the simultaneous analysis of both variables and their interaction due to collinearity issues. The protocol presented in this study solved this problem by defining urbanisation × temperature categories, which were appropriate explanatory factors for the probability of finding larval life stages of *Ae. aegypti* and *Cx. pipiens*. The results reaffirmed the importance of considering the interaction between both factors, given the marked difference in the probability of harbouring mosquitoes between cold and warm temperatures within the same urbanisation category (high). Our study design was based solely on used tyres and excluded other types of artificial containers typically found in rural and urban areas.

Temporally the population density of Buenos Aires City has not changed significantly in the past 20 years (INDEC 2010a). The suburbs continue to grow and the urban fringe continues spreading over formerly rural areas. Therefore, the zonation developed in this study should be updated periodically, based on new population data (which are typically gathered at the national level on a decadal basis) and the corresponding road density layer. Public and widely available population and environmental data, if correctly processed, may aid in the selection of sampling sites to facilitate the study of the effects of environmental gradients on specific organisms and especially those that pose a threat to human health, such as mosquitoes.

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