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Original Article

Mosquito species occurrence in association with landscape composition in green urban areas

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(With 2 figures)

Abstract

Aedes aegypti prefers densely populated habitats, but has been shown to explore less anthropogenic environments. We investigated composition of the abundance of mosquitoes in forested areas and assessed relationships between species occurrences and different types of land use and land cover at three spatial scales (100m, 500m and 1000m). Mosquitoes were collected from October 2012 to March 2013 using oviposition traps. We collected 4,179 mosquitoes in total including at least 10 species. *Aedes albopictus* and *Limatus durhami* were eudominant species, representing 90% of all collected individuals. We found intraspecific differences in response to land use and land cover, and species response patterns were similar at all spatial scales. *Ae. albopictus* relative abundance was associated with urbanized areas, while *Li. durhami*, *Haemagogus leucocelaenus* and *Toxorhynchites* sp., abundances were associated with native forest. *Aedes aegypti* were found in five of the eight areas studied, including in an Atlantic forest fragment at a considerable distance from the forest edge (370 m). *Aedes aegypti* occurrence was not influenced by type of land use or land cover.

Keywords: Aedes aegypti, mosquito, oviposition trap, urbanization, landscape.

Ocorrência de espécies de mosquitos associada à composição de paisagem em áreas verdes urbanas

Resumo

Aedes aegypti tem como habitat preferencial áreas densamente povoadas, mas que tem se mostrado capaz de explorar ambientes menos antropizados. O objetivo desse trabalho foi investigar a abundância de mosquitos em áreas florestadas e avaliar a relação dos diferentes tipos de uso e cobertura do solo, em três escalas de paisagem (buffer de 100m, 500m e 1000m). Os mosquitos foram coletados entre outubro de 2012 a março de 2013 utilizando armadilhas de oviposição. Foram coletados 4,179 culicídeos, de pelo menos 10 espécies, destas, *Aedes albopictus* e *Limatus durhami* foram eudominantes, representando 90% do total de indivíduos coletados. As espécies de culicídeos respondem de maneira diversa aos tipos de uso e cobertura do solo, indicando que as afinidades mais evidentes se mantém nas três escalas avaliadas. A abundância relativa de *Ae. albopictus* se mostrou relacionada às áreas mais urbanizadas, enquanto a de *Li. durhami*, *Haemagogus leucocelaenu*s e *Toxorhynchites* sp., à presença da mata nativa. *Aedes aegypti* esteve presente em cinco das oito áreas estudadas, incluindo um fragmento de Mata Atlântica a uma distância considerável da borda (370m). A ocorrência de *Aedes aegypti* nas áreas analisadas não demonstrou ser influenciada pelo tipo de uso e cobertura do solo.

Palavras-chave: Aedes aegypti, mosquito, armadilha oviposição, urbanização, paisagem.

1. Introduction

Urbanization can alter the environment in ways that impact mosquito species diversity by reducing abundances or even causing local extinction of some species. A decline in population numbers of a particular species results in an empty ecological niche that may become available for invasion by other species. These new species may or may not be functionally equivalent and can potentially be harmful, possibly resulting in outbreaks of infectious diseases. Some mosquito species have high genetic and ecological plasticity and, driven by anthropogenic pressure, easily adapt to new environmental conditions. This is particularly true in urban areas neighboring green areas, which often contain an abundance of artificial containers for oviposition and ample blood sources (i.e., humans and pets), potentially leading to changes in species natural habitats (Lopes, 1997).

Among the Culicids that benefit from urban sprawl, *Aedes aegypti* is notorious due to its role as a vector of arboviruses such as Zika, dengue, and chikungunya, causing a strong impact on human health. This is an anthropophilic and synanthropic species, well known for exploring and occupying a diversity of microhabitats in intensely urbanized environments. *Aedes aegypti* habitat preferences are generally well understood, and this knowledge is fundamental for the development of effective control measures. Although this species exhibits strong preference for urban environments immature forms have been found in forested areas (Lourenço-de-Oliveira et al., 2003; Medeiros et al., 2009; Zequi et al., 2005), environments that are traditionally not covered by municipal vector control programs.

Understanding how landscape changes influence Culicidae species composition and abundance may help us to predict spatial distributions of target species under urbanization, potentially providing important insights into urban planning and prevention of disease epidemics. We investigated *Aedes aegypti* occurrence in forest areas in Porto Alegre, Brazil. We specifically evaluated potential links between types of land use and land cover (LULC) and container species composition and abundance at three spatial scales.

2. Material and Methods

Mosquitoes were collected from eight forested areas in Porto Alegre, Rio Grande do Sul, Brazil. The collection areas are distributed along an urbanization gradient (i.e., from municipal center to more rural areas) (Figure 1 and Table 1). Study areas were classified as urban fragments, green areas, and remnant forest fragments according to Maciel and Barbosa (2015). Green areas (4) included the Parque Farroupilha (PF; 22J, 478925.00, 6677169), the Parque Marinha do Brasil (PM; 22J, 477655.89, 6675151.10), the Parque Knijnik (PK; 22J 480451.90, 6669719.02) and the

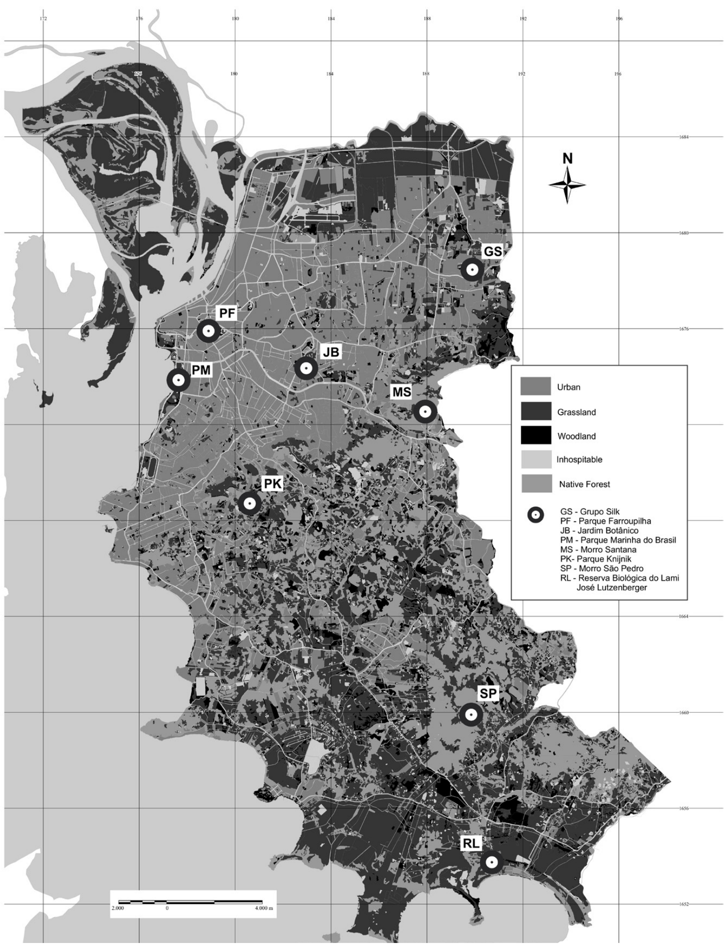

Figure 1. Map of the study area (highlighting the green areas) evaluated in Porto Alegre, RS, Brazil between October 2012 and March 2013.

Table 1. Original land use and land cover (LULC) classes as described by Hasenack (2008), and reconstructed (grouped) classes used for analyses. See Methods for more details.

Original Classes	Reconstructed Classes
Permanent Cropland Woody Vegetation Shrubland Degraded Forest Land	Woodland
Wetland Temporary Cropland Pasture Native Grasslands	Grassland
Residential Isolated Houses Rural Housing Buildings Buildings and Houses Pavilions Spontaneous Occupation	Urban
Rocky Outcrops Pavement Bare Soil Water	Inhospitable
Native Forest Land	Native Forest

Jardim Botânico (JB; 22J 482989.01, 6675651.87); these areas have a park-like structure formed by groves of scattered trees, continuous canopy, and field. Grupo Silk (GS; 22J 489947.02, 6679643.06) is a partially preserved urban, native forest fragment surrounded by densely populated area. Morro Santana (MS; 487932.01, 6673829.11) and Morro São Pedro (SP; 22J, 490116.00, 6661360.00) are Atlantic forest remnants in a permanent preservation area in granitic hills, the latter being the largest native forest fragment in Porto Alegre. The Reserva Biológica do Lami José Lutzenberger (RL; 22J, 490759.04, 6655009.08) is a municipal biological reserve of native Restinga forest fragment.

Oviposition traps were constructed from 2 L, plastic soda bottles with side openings (15 cm × 8.5 cm) cut at the label level. Trap exteriors were spray-painted with matte black paint (Chemicolor®). A 15 × 5 cm strip of hardboard (Eucatex®) was placed inside each bottle, partially immersed in 600 mL of distilled water. We installed three traps in each of the eight areas at ground level, spaced one meter away from each other in a triangular layout.

Containers were sampled quarterly from October 2012 to March 2013. Oviposition traps were left in the field throughout the experimental period. During collections the content of the traps was filtered, larvae and pupae were removed, and the water was returned to the trap. The water volume was replenished to the original 600 mL if necessary. Fourth-instar larvae were sacrificed immediately after collection and stored for later identification. First to third instar larvae were raised in the laboratory in 135 mL plastic containers with distilled water. Rearing containers were covered with voile fabric, and larvae were fed fish food (Tatramin®) until reaching the forth instar. Pupae were kept in distilled water until they emerged. Adult and fourth-instar larval identification was carried out using taxonomic keys by Lane (1953) and Forattini (2002). Genera were abbreviated according to Reinert (2009).

Landscape analyses were performed using LULC maps from the Environmental Assessment of Porto Alegre (Hasenack, 2008), which is based on QuickBird® imaging. Briefly, for each sample area we calculated the LULC class percentages at three different spatial scales, defined by circular buffers around each triangular trap arrangement with radii measuring 100 m, 500 m, and 1000 m. Culicids typically do not disperse further than 1000 m from the breeding site, which informed our choices for experimental buffer radius. The twenty original LULC classes were grouped into five classes considered relevant for this study (see Table 1). The class "water" was reclassified as "inhospitable", as the sampled Culicidae did not use these bodies of "water" *sensu* Hasenack (2008) for oviposition. GIS analyses were performed using ArcView 3.2 software (ESRI).

We followed the classification proposed by Cardoso et al. (2011) to determine the degree of constancy of species: 'constant' species were present in more than 50% of the samples, 'accessory' species were present in 25 to 50% of the samples, and 'incidental' species appeared in less than 25% of the samples. Dominance (Dom.) was defined as the relative abundance of each species in the sample, where: eudominant ≥ 10%, dominant 5 ≤ 10%, subdominant 2 ≤ 5%, recessive 1 ≤ 2% and rare <1% (Cardoso et al., 2011).

We used redundancy analysis (RDA) to explore the association between the composition and abundance of container-inhabiting Culicidae species and landscape structure at three different spatial scales. Abundance data were Hellinger-transformed (Peres-Neto et al., 2006; Legendre and Legendre, 2012). Analyses were performed using R software (R Core Team, 2012).

3. Results

We collected a total of 4,179 mosquitoes belonging to six genera, including at least ten species. *Aedes albopictus* (Skuse, 1894) and *Limatus durhami* (Theobald, 1901) had the highest relative abundances and occurred in all surveyed areas (Table 2). *Ae. albopictus* was the most dominant species, and was constant in all areas. *Limatus durhami* was incidental at PM, accessory at PK and PF, and constant in the remaining areas. *Haemagogus leucocelaenus* (Dyar and Shannon, 1924) was constant at SP, and *Toxorhynchites* sp. was constant at PK and SP. *Culex quinquefasciatus* and *Sabethes albiprivus* (Theobald, 1903) were considered incidental; only one individual was collected for the latter.

Ae. aegypti was classified as subdominant and was found in five of the eight areas, including three green areas (PM, PF e JB), the GS urban fragment, and the permanent forest fragment (MS). The highest *Ae. aegypti* abundances

Table 2. Numbers of collected specimens, dominance (Dom.), species constancy (**A-C**), and relative abundance (%) of Culicids captured with oviposition traps in eight green areas in Porto Alegre, RS, Brazil; (**A**) = Incidental; (**B**) = Acessory; (**C**) = Constant.

	GS	PF	PM	JB	MS	SP	RL	PK	Total	Dom.	%	Acr.
Aedes albopictus	643 (C)	470 (C)	649 (C)	223(C)	44 (C)	96 (C)	259 (C)	105 (C)	2,489	EUD	59.56	Ae.alb.
Limatus durhami	73 (C)	4 (A)	25 (B)	398 (C)	254 (C)	106 (C)	401 (C)	8 (A)	1,269	EUD	30.37	Lm.dur.
Aedes aegypti	8 (B)	35 (C)	28 (A)	5 (A)	17 (A)				93	SUB	2.23	Ae.aeg.
Aedes sp.						2 (A)			2	RAR	0.05	Ae.sp.
Aedes fluviatilis			20 (B)	6 (A)		2 (A)			28	RAR	0.67	Ae.flu.
Culex dolosus				10 (A)	26 (A)			69 (A)	105	SUB	2.51	Cx.dol.
Culex quinquefasciatus			34 (A)						34	RAR	0.81	Cx.qui.
*Haemagogus leucoceleanu*s				3 (A)	15 (B)	84 (C)			102	SUB	2.44	Hg.leu.
Toxorhynchites sp.	13 (B)	1 (A)	2 (A)	3 (A)	7 (B)	12 (C)	5 (B)	13 (C)	56	REC	1.34	Tox.sp.
Sabethes albiprivus					1 (A)				1	RAR	0.02	Sa.alb.
									4,179		100	

were in PF and PM with 35 and 28 specimens, respectively. *Ae. aegypti* was constant in PF, accessory in GS and PM, and incidental in JB and MS.

The RDA for the 100 m scale showed that the LULC variables explained 76% of the total variation in species abundances (Figure 2). The first and second axes accounted for 55% and 13% of the variation, respectively. The RDA for the 500 m scale showed that 79% of the total variation in species abundances was explained by landscape variables, with 50% being explained by the first axis and 21% by the second (Figure 2). Finally, analysis at the 1000 m scale showed that LULC explained 73% of the total variation in species abundances, 45% by axis 1 and 21% by axis 2 (Figure 2).

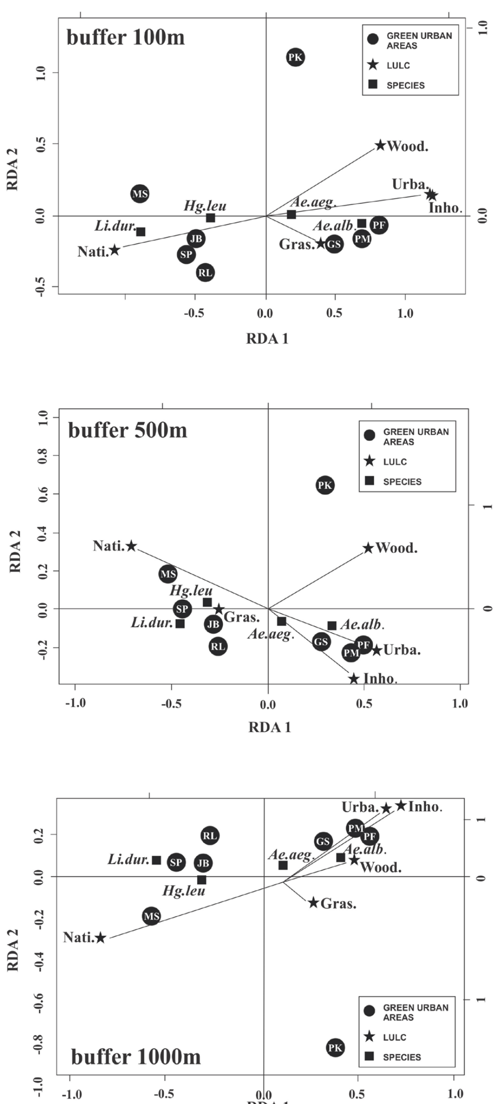

Figure 2. Ordination diagram from redundancy analysis (RDA) of Culicid abundance and LULC classes at three spatial scales (100m, 500m and 1000m) around each oviposition trap site.

The first axis explained the majority of the variation in species abundances, and represents the landscape structure gradient. This gradient ranges from more preserved environments composed of complex natural structures and high vegetation cover, to urban settings with less vegetation cover, less shade, and higher rates of human movement. The pattern of associations between species and habitats holds fairly constant at all three spatial scales. GS, PM and PF appear at the right side of the RDA diagram and were associated with the variables URB, INO and NAT. MS, SP, JB and RL were situated on the left side (along the first axis), and were associated with NAT, as expected. In terms of the species' responses to environmental variables, *Li. durhami* (correlation with axis 1: -0.61; -0.58; -0.53) and *Hg. leucocelaenus* (correlation with axis 1: -0.27; -0.33; -0.36) appear to be associated with more forested environments. In contrast, *Ae. albopictus* appears near URB (correlation with axis 1: -0.47; -0.42; -0.39) at all three spatial scales. *Culex dolosus* is strongly correlated with the second axis, in association with MS and PK.

This study documents *Sa. albiprivus* and *Li. durhami* for the first time in Porto Alegre. *Sa. albiprivus* was previously found in three cities in Rio Grande do Sul: Derrubadas, Garruchos and Santo Antônio (Cardoso et al., 2005), while *Li. durhami* has been recorded in Nova Bassano, Nova Petrópolis (Cardoso et al., 2005) and Maquiné (Cardoso et al., 2011).

Aedes albopictus was dominant in green urban areas. This species is known to be well adapted to transition zones between urban and non-urban/forest habitats. According to Forattini (2002), such habits result from being able to utilize breeding sites and blood sources from both environments. The high incidence of *Ae. albopictus* in our study was thus expected (Lourenço de Oliveira et al., 2004; Medeiros et al., 2009).

4. Discussion

Our analyses indicated an association of *Li. durhami* and *Hg. leucocelaenus* with non-urban/forest habitats, with *Li. durhami* occurrence being strongly associated with native forest. This contradicts Lopes (1997), who compared the two species and characterized *Li. durhami* as "strongly prone to domiciliation" based on its ability to use artificial containers for breeding. Following this logic, *Hg. leucocelaenus* can be considered better adapted to non-urban habitats, preferring to oviposit in tree holes and bamboo nodes (Forattini, 2002). Our choice of artificial container favored oviposition by *Li. durhami*, possibly influencing our results and leading to categorization of the species as "wild" compared to *Hg. leucocelaenus*. Capturing *Sa. albiprivus* with oviposition traps is unusual, as this species typically does not utilize artificial containers; it prefers to oviposit in phytotelmata such as tree hollows, bamboo internodes, bromeliads, and leaf axils (Forattini, 2002).

Toxorhynchites sp. was found in all sample areas, including those with higher urban influence. They frequently co-occurred with *Ae. aegypti*, *Ae. albopictus*, and *Hg. leucocelaenus*. Species belonging to this genus exhibit predatory behavior in larval stages (Forattini, 2002; Albeny et al., 2010), and have been evaluated for their potential role as biological control agents (Collins and Blackwell, 2000). However, because we did not record *Toxorhynchites* larval instars during the collections, we were not able to assess any negative effects on mosquito vector populations.

While *Ae. aegypt'*s proclivity to urban environments is well understood, our analyses did not reveal specific associations of the species with anthropogenic environments. Furthermore, in the current study it was less prone to urban habitats than *Ae. albopictus*, known for its affinity to peri-urban habitats. This discrepancy implies that factors related to LULC cannot fully explain *Ae. aegypti* occurrence in green areas of the city, and that perhaps other factors are equally or more influential.

The average maximum flight distance for female mosquitoes is around 100 m (Forattini, 2002). At the PF, JB, PM, and GS sites where the oviposition traps were installed within 100 m of populated areas, species distributions may be explained by the presence of nearby sources of human blood. At the MS site, however, traps were placed more than 370 m away from human-occupied areas, and human movement and activity within the study site was uncommon. According to Reiter et al. (1995), *Ae. aegypti* dispersal is driven by search for oviposition sites. The methodology employed by the city of Porto Alegre's vector control administration, which includes breeding site suppression, could be causing *Ae. aegypti* to disperse to neighboring green areas in search of oviposition sites. Forattini (2002), Lourenço de Oliveira et al. (2004) and Reiter et al. (1995) also highlighted the inefficiency of such vector control strategies, as it leads to species dispersion rather than elimination.

The availability of artificial breeding sites such as trash and containers used in religious rituals, especially around forests, can also contribute to the presence of *Ae. aegypti* in forest patches and fragments (e.g. the MS area). According to Lourenço de Oliveira et al. (2004), forest remnants can serve as refuge for this species, harboring the necessary conditions for re-colonization of other areas in the absence of proper population control by authorities. The explicit preference for human blood as a food source and artificial containers as breeding sites can also explain habitat expansion of *Ae. aegypti* to forested areas adjacent to urban centers. The occurrence of *Ae. aegypti* at the MS site is thus likely not indicative of late adaptation to natural or native areas, but rather another aspect of its well-known opportunistic behavior in terms of seeking habitat for reproduction and shelter.

In conclusion, we observed *Ae. aegypti* breeding in areas not considered characteristic for the species, far from the reach of city vector control operations. We also found that mosquito species respond differently to types of land use and land cover, and that they show affinities to LULC classes that are similar at the three spatial scales evaluated. *Ae. albopictus* was dominant in the study. Occurrence of *Hg. leucocelaenus*, an important regional vector of jungle yellow fever (Cardoso et al., 2010), was associated with native forest habitat, but was also found in areas with frequent human traffic such as the Botanical Garden (JB). Knowledge from this study can contribute to urban planning and development of future strategies to limit the transmission of arboviruses in Porto Alegre.

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