Spatial evaluation of larvae of Culicidae (Diptera) from different breeding sites: application of a geospatial method and implications for vector control

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ABSTRACT. Spatial evaluation of Culicidae (Diptera) larvae from different breeding sites: application of a geospatial method and implications for vector control. This study investigates the spatial distribution of urban Culicidae and informs entomological monitoring of species that use artificial containers as larval habitats. Collections of mosquito larvae were conducted in the São Paulo State municipality of Santa Bárbara d' Oeste between 2004 and 2006 during house-to-house visits. A total of 1,891 samples and nine different species were sampled. Species distribution was assessed using the kriging statistical method by extrapolating municipal administrative divisions. The sampling method followed the norms of the municipal health services of the Ministry of Health and can thus be adopted by public health authorities in disease control and delimitation of risk areas. Moreover, this type of survey and analysis can be employed for entomological surveillance of urban vectors that use artificial containers as larval habitat.

KEYWORDS. *Aedes aegypti*; dengue; mosquitoes; spatial analysis; vector control.

RESUMO. Avaliação espacial de formas larvais de Culicidae (Diptera) em diferentes criadouros: aplicação de um método geoespacial e implicações para o controle de vetores. Este estudo investiga a distribuição espacial da fauna urbana e de Culicidae e informa o monitoramento entomológico de espécies que usam recipientes artificiais como habitat larval. Coletas de larvas de mosquitos foram realizadas no município paulista de Santa Bárbara d' Oeste entre os anos de 2004 e 2006, durante visitas casa-a-casa. Um total de 1.891 amostras foi considerado, com nove espécies diferentes coletadas. A distribuição das espécies foi avaliada através do método de krigagem estatística extrapolando as divisões administrativas do município. O método de coleta adotado no presente estudo está de acordo com os métodos sugeridos aos serviços de saúde municipais pelo Ministério da Saúde e pode, portanto, ser adotado pelas autoridades públicas de saúde no controle da doença e delimitação das áreas de risco. Além disso, este tipo de levantamento e análise pode ser empregado como vigilância entomológica de espécies de mosquitos vetores que usam recipientes artificiais como habitat larval em áreas urbanas.

PALAVRAS-CHAVE. *Aedes aegypti*; análise espacial; controle de vetores; dengue; mosquitos.

Mosquitoes are among the major vectors of infectious agents in humans and animals. In the Neotropics, which have the highest endemicity for this group of insects (Ward 1982; Forattini 1996), adult females transmit parasites of malaria, filariasis and a number of viruses (*e.g.* dengue, yellow fever and encephalitis) (Rai 1991; Guimarães 1997; Foley *et al.* 2007; Fontes & Rocha 2009; Nogueira *et al.* 2009; Tauil & Fontes 2009). Although many studies have focused on the epidemiological role of mosquitoes, researchers have also emphasised their ecological importance. For example, the biomass of immature forms serves as an important food source for fish and aquatic invertebrates (Fang 2010).

During immature stages, mosquitoes inhabit a variety of larval habitats, including lakes and pools of various types, stagnant water in artificial containers and tree holes (Clements 1999; Forattini 2002). However, many habitats are species-specific, providing aquatic parameters with specific characteristics required by their respective groups (Triplehorn & Johnson 2005). In urban areas, artificial recipients represent the sites where important processes in mosquito life cycle occur, such as oviposition, larvae and pupae development and adult emergence (Clements 1999; Mwangangi *et al.* 2009).

Mosquito species generally occupy consistent habitats during immature stages. Knowledge of these sites in vector species is of obvious epidemiological relevance (Forattini 2002). Bates (1970) noted that the classification of larval habitats is essential for proper control measures, a fact reinforced by Pates & Curtis (2005), who emphasised the importance of female oviposition choice for mosquito control.

The relevance of the present study lies in the fact that several mosquitoes are adapted to anthropic environments and respond dynamically to environmental changes. Thus, further studies considering aspects of mosquito life-history in different areas are very important, particularly in regions where these insects transmit infectious agents.

Urban surveys of immature mosquitoes is of great importance because these areas possess a variety of artificial containers, which may be colonised by mosquitoes who have adapted to them (Perich *et al.* 2003), such as *Aedes* (*Stegomyia*) *aegypti*, an important vector of Dengue, Chikungunya and Yellow Fever viruses (Consoli & Oliveira 1998; Lozovei 2001; Gillott 2005).

The spatial distribution of mosquito larval habitats in urban areas is of ecological and epidemiological importance, as they can inform the application of control programs (Barbosa & Lourenço 2010) tailored to weigh the heterogeneity of given micro-areas (Honório *et al.* 2009). Few studies have attempted to study the disease risk in urban spaces using spatial pattern analysis, mainly because of the lack of knowledge by researchers related to the application of this methodology (Araújo *et al.* 2008; Cordeiro *et al.* 2011).

According to Barcellos *et al.* (2005), spatial diseases studies may aid in analyses of epidemiological data for population groups. Such research, in the context of infectious diseases, allows to capture determinants of health that are beyond the scope of the individual.

This study sought to survey the Culicidae of larvae in different types of urban breeding sites in the municipality of Santa Bárbara d'Oeste (São Paulo, Brazil). Samples were collected during house-to-house visits between February 2004 and February 2006 with the assistance of municipality health workers. Probability maps of the principal Culicidae species in different types of larval habitats were created using these sampling data. We sought to improve understanding of spatial distribution of Culicidae both in Santa Bárbara d' Oeste and other areas with risk of dengue transmission (Brasil 2002). Applications of geospatial methods for vector control are also discussed.

MATERIAL AND METHODS

Study Area. The municipality of Santa Bárbara d' Oeste (22°45'15"W, 49°22'46"S) has a total area of 241 km² and a population of 180,148 and is part of the Campinas metropolitan area in São Paulo State (Brasil 2010; Fig. 1). The region' s landscape is characterised by irregular relief, with type CWA climate (Köppens classification; tropical humid with dry winters) and a mean annual rainfall of 1,252.26 mm (Santa Bárbara d' Oeste 2010).

Field Protocols. The Santa Bárbara d' Oeste (subsequently SBO) Municipal Secretary of Health, in accordance with the requirements of the Federal Ministry of Health' s National Dengue Control Plan (Brasil 2002), has divided the city into four areas, which are further subdivided into 21 sections distributed in a logical grouping of blocks. The office also maintains a 30-person staff of municipal vector control workers and three area supervisors. The Culicidae inventory in SBO was undertaken between February 2004 and February 2006 by health workers at the municipality's Center for Zoonosis Control. The protocol was followed on all workday, between 8:00 and 16:00, during collection months.

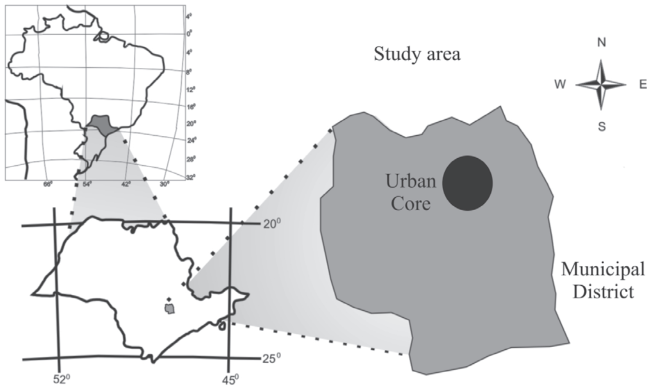


Fig. 1. Study Area.

Sector employees entered homes when authorised by residents and performed an active search for breeding and larval sites in intra- and peri-domiciliary areas. The visits were designed to find sites with proper conditions for the development of immature Culicidae and subsequent mechanical or chemical (larvicide) inactivation or elimination of breeding sites. These professionals also informed residents about dengue prevention and control methods.

House-to-house campaigns were performed in each of SBO' s urban areas, except in strategic sites (e.g. tyre repair shops, junkyards and cemeteries) and special buildings (*e.g.* schools, hospitals and colleges), which have their own working arrangements and require different protocols.

Breeding sites were identified following the prevailing classifications (SUCEN 1997; SUCEN 2001): Type-1: tire; -2: plant pot and drip tray; -3: tin, jar, bottle; -4: bucket, washtub, barrel; -5: water tank; -6: bottle; -7: other removable container and -8: other non-removable container.

Staff collected the largest number of larvae possible using a disposable Pasteur pipette into plastic collection tubes with 70% ethanol. Tubes were properly covered and the cap sealed with adhesive tape. Bottles were labelled with collection date, sample number, larval habitat and residence address and block.

Mosquito Identification. Species identification of larvae was undertaken through a dichotomous key with the assistance of a microscope (Lane 1953; Consoli & Oliveira 1994; Forattini 2002). Following identification, the material was discarded except for infrequent species, which were kept for later revision to the diagnosis.

Analysis Georeferencing. The data collected were used to populate a geo-spatial databank with georeferenced breeding sites within SBO city blocks using a Universal Transverse Mercator (UTM) projection. This allowed for the geometric placement of larval habitats within a base map of the city, for which a database adjustment enabled georeferencing using AUTO CAD (Autodesk 2000). These data were exported to SURFER (Golden Software 1997) and compiled into a probability distribution map using the presence/absence of the four most common species: *Aedes aegypti*, *Aedes fluviatilis*, *Aedes albopictus* and *Culex quinquefasciatus*. Thus, four larval spatial distribution maps were created for SBO.

Subsequently, indicative kriging was utilized as described by Elliott *et al.* (2000), Simão (2001) and Lawson (2006). This exploratory analysis, derived from Daniel G. Krig, estimates a spatial covariance matrix. Here presence/absence data were utilized in determining a non-biased inference procedure and the error associated to the estimated value (Camargo 1998). For any given location, this yields an average value and estimates of proportions for certain values that are above or below a cut-off (Simão 2001). The indicative kriging is based on models developed for variography (study of variogram, which in turns is a basic supporting tool for the kriging techniques, which can represent the quantitative change of a regionalized phenomenon in space), with indicator-transformed variables (Camargo 1998).

The final product of these analyses consisted of four maps showing the frequency probability distribution of the culicid larval habitats studied.

Geostatistical Modeling. The procedures of Niño (2008, 2011) and Pfeiffer *et al.* (2008) were used to test the autocorrelation among *Ae. aegypti* larval habitats. This utilized a stochastic approach and an omnidirectional semivariogram as a set of patterns of continuous points: i.e., a graphical representation showing no directional tendency in the data and, in this case, the spatial distribution is called isotropic (Pannatier 1996; Camargo 1998).

Statistical Analysis. A chi-square test was used to verify the homogeneity of *Ae. aegypti* in the breeding sites and to test for an association between occurrence in breeding sites and other attributes (chi-square for two independent samples). This test followed the recommendations of Siegel & Castellan (1988) and was performed using SAS 6.0 (Sas/Stat 1989), adopting a significance level of 5%.

RESULTS AND DISCUSSION

Composition and co-occurrences. A total of 1,891 larvae were collected and identified at the Centre for Zoonosis Control. Of this, 1,641 (86.78%) were collected from larval habitats containing a single species. Conversely, 250 samples (13.22%) were found in cohabitation with two or more species in the same breeding site (Table I). The distribution of artificial recipients was as follows: Type-1: 7.93%, Type-2: 24.96%, Type-3: 17.82%, Type-4: 15.60%, Type-5: 5.44%, Type-6: 0.42%, Type-7: 17.50% and Type-8: 10.31%. At least six species and three undefined taxa were collected. In order of frequency, these were: *Aedes* (*Stegomyia*) *aegypti* (Linnaeus), *Aedes* (*Stegomyia*) *albopictus* (Skuse), *Culex* (*Culex*) *quinquefasciatus* (Say), *Aedes* (*Ochlerotatus*) *fluviatilis* (Lutz), *Culex* Coronator Complex, *Limatus* sp., *Anopheles* (*Nyssorhynchus*) *strodei* (Root), *Aedes* (*Ochlerotatus*) *scapularis* (Rondani) and *Psorophora* sp. (see Fig. 2A). Occurrence of species found in isolation, cohabitation and total values in different types of containers are shown in Table I.

Aedes aegypti predominated in cohabitation with second species. The highest cohabitation frequencies were: *Ae. aegypti* – *Ae. albopictus* (33.2%), *Ae. aegypti* – *Cx. quinquefasciatus* (25.6%) and *Ae. aegypti* – *Ae. fluviatilis* (19.2%). Cohabitation was not as common as was observed by Hribar *et al.* (2001) in Florida, where *Ae. aegypti* and *Cx. quinquefasciatus* were the most common species found together in multiple infestations. Although research in São Sebastião (São Paulo State) found *Ae. albopictus* was replaced by *Ae. aegypti* in urban areas and in household recipients (Passos *et al.* 2003), other studies in Florida (Juliano 1998) and Brazil (Braks *et al.* 2004) showed *Ae. albopictus* to be more competitive than *Ae. aegypti* in recipients with high larval density and limited resources.

In field conditions, competition from *Ae. albopictus* to *Ae. aegypti* is stronger but does not seem to impact interspe-

Table I. Distribution of larvae of Culicidae species found in different breeding sites in conditions: isolation, cohabitation and total (%). Municipality of Santa Barbara d' Oeste, SP (2004–2006). Breeding sites: Type 1: tire; Type 2: plant pot and plant pot drip tray; Type 3: tin, jar, bottle; Type 4: bucket, washtub, barrel; Type 5: water tank; Type 6: bottle; Type 7: other removable container and Type 8: other non–removable container.

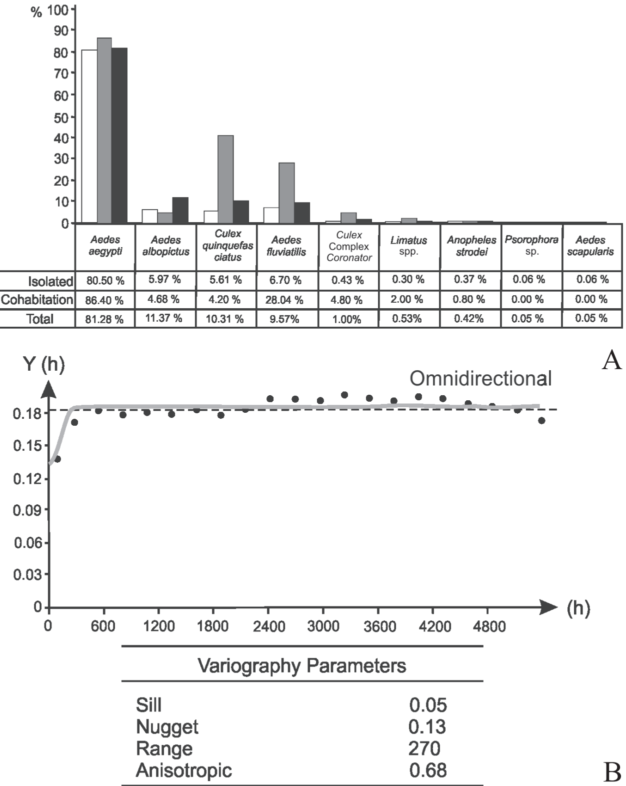
Species	Types of breeding sites							
	1	2	3	4	5	6	7	8
Aedes aegypti								
Isolated	7.95	28.84	17.71	14.46	5.37	0.30	18.02	7.34
Cohabitation	8.33	16.67	24.07	21.30	4.63	–	21.30	3.70
Total	8.00	27.13	18.61	15.42	5.27	0.26	18.48	6.83
Aedes albopictus								
Isolated	11.22	29.59	20.41	10.20	3.06	4.08	15.31	6.12
Cohabitation	8.55	23.93	24.79	17.09	1.71	–	23.08	0.85
Total	9.77	26.51	22.79	13.95	2.33	1.86	19.53	3.26
Culex quinquefasciatus								
Isolated	10.75	17.20	22.58	10.75	4.30	–	7.53	26.88
Cohabitation	10.68	12.62	20.39	23.30	4.85	–	18.45	9.71
Total	10.71	14.80	21.43	17.35	4.59	–	13.27	17.86
Aedes fluviatilis								
Isolated	3.64	3.64	2.73	23.64	11.82	–	17.27	37.27
Cohabitation	7.04	5.63	15.49	30.99	5.63	–	16.90	18.31
Total	4.97	4.42	7.73	26.52	9.39	–	17.13	29.83
Culex coronator complex								
Isolated	–	–	14.29	14.29	–	–	14.29	57.14
Cohabitation	–	–	25.00	25.00	8.33	–	25.00	16.67
Total	–	–	21.05	21.05	5.26	–	21.05	31.58
Limatus sp.								
Isolated	–	40.00	40.00	20.00	–	–	–	–
Cohabitation	–	20.00	–	40.00	20.00	–	20.00	–
Total	–	30.00	20.00	30.00	10.00	–	10.00	–
Anopheles strodei								
Isolated	–	–	–	–	–	–	16.66	83.33
Cohabitation	–	–	–	–	50.00	–	0.00	50.00
Total	–	–	–	–	12.50	–	12.50	75.00
Psorophora sp.								
Isolated	–	–	100.00	–	–	–	–	–
Cohabitation	–	–	–	–	–	–	–	–
Total	–	–	100.00	–	–	–	–	–
Aedes scapularis								
Isolated	–	–	–	100.00	–	–	–	–
Cohabitation	–	–	–	–	–	–	–	–
Total	–	–	–	100.00	–	–	–	–

cific competition associated with coexistence or exclusion (Juliano *et al.* 2004). However, further studies may clarify the factors responsible for facilitating coexistence of two or more mosquito species for each type of urban recipients.

Aedes aegypti was found alone in containers in 1,321 samples (69.85%) and was found cohabiting with other species 216 times (11.42%). The major *Ae. aegypti* breeding sites were of Type-2, Type-3, Type-4 and Type-7. Pinheiro & Tadei (2002) found that dishes and plant pots were the most frequent recipients in Manaus households, Amazonas State, Brazil. These containers maintain the annual cycle of *Ae. aegypti* because they are not dependent on seasonal rainfall. Similar results were found in a survey of immature stages found in containers during vector control operations in the Campo Grande neighbourhood of Rio de Janeiro (Silva *et al.* 2006). Here, the most important larval habitats were drains (26.3%), plant pots (25.5%), bottles/tins/plastic containers (16.2%) and bucket/washtub/barrel/clay containers (10.6%). The respective percentage of each *Ae. aegypti* larval habitats was very similar to that found for *Ae. albopictus* (see below): Type-2, -3, -4 and -7 were responsible for 79.64% of the total (Table I). These are known to yield high positivity for this species. Focks & Chadee (1997) found that barrels, buckets and small containers accounted for about 90% of the total habitats.

Aedes aegypti habitats varied in all sectors and in all areas of SBO, a result that demonstrates the importance of micro-scale geographic sampling in the development of control strategies to reduce infestation in susceptible regions. Statistical analysis indicated a relationship between habitats and areas and/or sectors: habitats occurrence was not independent of sectors within areas ($\chi^2 = 252.553$; $P < 0.0001$) or of areas without considering sectors ($\chi^2 = 61.71$; $P < 0.0001$). Thus, the occurrence of a given breeding site is related to the area and/or the sector in which it was located. In the autocorrelation test of *Ae. aegypti* habitats, an exponential isotropic semi-variogram was obtained, *i.e.*, the same parameters were found for different directions of sampling. This semi-variogram demonstrated a spatial correlation between habitats at a distance of up to 270 m. Beyond this distance, there was no significant relationship (Fig. 2B). It is noteworthy that this value is similar to that recommended for the control of breeding sites when cases of dengue are confirmed (SUCEN 2001), which corroborates the importance of spatial distribution data for vector control and the delineation of transmission risk areas.

Pereira (2001) analysed the productivity of intra- and peridomiciliary breeding sites in Santos (São Paulo State) and showed that peridomiciliary habitats played important roles in *Ae. aegypti* infestation. In SBO, peridomiciliary larval habitats were those containing the most *Ae. aegypti* larvae, highlighting the importance of municipal awareness campaigns. Teixeira *et al.* (2002) emphasized not only the importance of education strategies but also continuity in measures used to control this vector species. Although the knowledge of the measures necessary to reduce residential infestation is usually satisfactory for the population, it is not accompanied by effective control measures (Chiaravalloti-Neto 1997).



	Aedes aegypti	*Aedes albopictus*	*Culex quinquefasciatus*	*Aedes fluviatilis*	*Culex* Complex *Coronator*	*Limatus* spp.	*Anopheles strodei*	*Psorophora* sp.	*Aedes scapularis*
Isolated	80.50 %	5.97 %	5.61 %	6.70 %	0.43 %	0.30 %	0.37 %	0.06 %	0.06 %
Cohabitation	86.40 %	4.68 %	4.20 %	28.04 %	4.80 %	2.00 %	0.80 %	0.00 %	0.00 %
Total	81.28 %	11.37 %	10.31 %	9.57%	1.00%	0.53%	0.42%	0.05 %	0.05 %

A

Variography Parameters	
Sill	0.05
Nugget	0.13
Range	270
Anisotropic	0.68

B

Fig. 2. A. Percentage of occurrence of species found in the survey (1,641 isolated, 250 Cohabitation and 1,891Total); B. Exponential isotropic semivariogram adjusted.

Effective participation of local human population in the control of *Ae. aegypti* is of fundamental importance for success of any program. Chiaravalloti-Neto *et al.* (2003) evaluated the participation of local population in the control of *Ae. aegypti* in Catanduva, São Paulo state. The authors found that the relationship between the population and the public sector was not collaborative and that micro-geographic idiosyncrasies were not considered in dengue-control programs. Throughout Brazil, intra- and peridomiciliary habitats of *Ae. aegypti* represent major challenges for vector control, as these recipients require integrated control strategies by both government and local population. The principal factors responsible for *Ae. aegypti* infestation are plant pots and drip trays, incorrectly stored recyclable items, and the neglect of health practices in homes. Hence the importance of inter-agency coordination in SBO for the control of mosquito infestation.

Of additional concern is that the elimination of the major breeding sites may not necessarily control the species, as *Ae. aegypti* is ecologically flexible and can colonise sites originally considered secondary (Forattini 2002). According to Focks *et al.* (2000), the removal of the larval habitats in an area to control immature stages can lead to female dispersal to other regions. The primary *Ae. aegypti* breeding sites (Types-2, -3, -4 and -7) were found in all four areas of SBO. Distribution analysis of SBO' s sectors showed idiosyncratic variations that should be considered in the design of control efforts and specific health education for the populations of certain sectors in the city. Statistical analyses indicated that the incidence of *Ae. aegypti* is not statistically equal in the breeding sites; nor is it when considering the distribution of the breeding sites among the four areas.

Although socioeconomic characteristics of the different populations within SBO neighbourhoods were not analysed, the distribution map of dengue cases in the city and the map of the probability of finding *Ae. aegypti* (Fig. 3A) highlight the existence of areas with higher risk of disease transmission. These include the more populous and urbanized neighbourhoods, which are usually the first to present positive cases of dengue fever during outbreaks. This may be associated with the substantial flux of persons commuting to other cities for work.

Container Types-5 and -7 (water tanks and other removable, respectively) were abundant in SBO areas 1 and 2 of the spatial distribution maps of breeding sites. This may be a consequence of an area of unsanctioned housing (shantytown), where residents do not have a proper water supply network. Nearby, the Nova Conquista neighbourhood is also characterised by social and environmental risk factors because a portion of the population derives its income from recycling and often improperly handle/store containers. Thus, efforts to vector control by removing breeding sites in this and similar neighbourhoods are problematic because the recipient are, obviously, linked to residents' livelihoods, which makes recurring the transmission of dengue in this area in a single epidemic period.

The epidemiological importance of *Ae. albopictus* is partly a consequence of its capacity to occupy different environments and its potential to bridge the introduction infectious agents from wild habitats into the urban environment (Gomes *et al.*

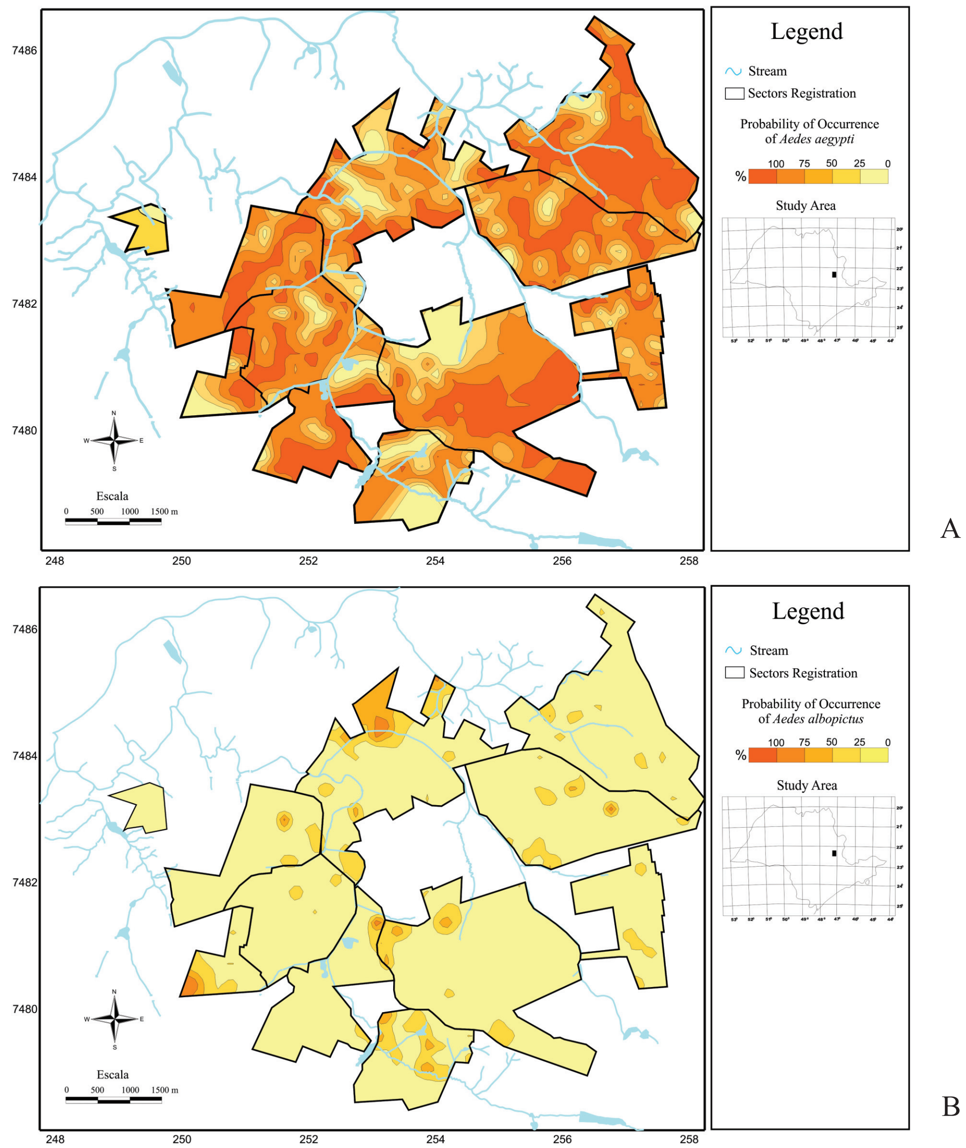


Fig. 3. A. Map of probability of occurrence of *Aedes aegypti* in sectors registration from Santa Barbara d' Oeste City-SP 2004 to 2006; B. Map of probability of occurrence of *Aedes albopictus* in sectors registration from Santa Barbara d' Oeste City-SP 2004 to 2006.

1992). In the SBO culicid inventory, larvae of this species were found in all types of larval habitats (Table I) and it was the species with the highest frequency in single-species recipients. The most important larval habitats for *Ae. albopictus* were Types-2, -3 and -7 (Fig. 3B). This pattern changed only in SBO area 4, where breeding site Type-4 had a more important role, potentially a result of the region' s proximity to the rural area. In these less-urban areas, animals (particularly horses) are abundant and it is not uncommon to find buckets and barrels used as troughs. In the district of Pedrinhas, Ilha Comprida municipality (São Paulo State), Forattini *et al.* (2001) estimated the potential emergence of permanent large-scale *Ae. albopictus* breeding sites and suggested that organic matter in these sites may influence the emergence of adult mosquitoes. Sites containing more than ten litres of water and decomposing organic matter is essentially what the troughs described above provide.

In light of its potential as an arbovirus vector, health authorities should monitor the distribution of *Ae. albopictus* and analyse possible behavioural and physiological changes imposed by the crossover effect of the control exerted on *Ae. aegypti* (Santos 2003).

Culex quinquefasciatus was most frequently collected in sites of Type-2, -3, -4 and -8, which corroborates the findings of Consoli *et al.* (1984) and confirms the eclectic habits of a species that utilises both undisturbed ponds and artificial breeding sites that vary from clean and extremely polluted water. The spatial distribution of this species in SBO is shown in Fig. 4A.

Urbinatti *et al.* (2001) evaluated the prevalence of *Cx. quinquefasciatus* and confirmed it was the only species that occurred in aquatic environments with high concentrations of organic matter. In the present study, they were usually found

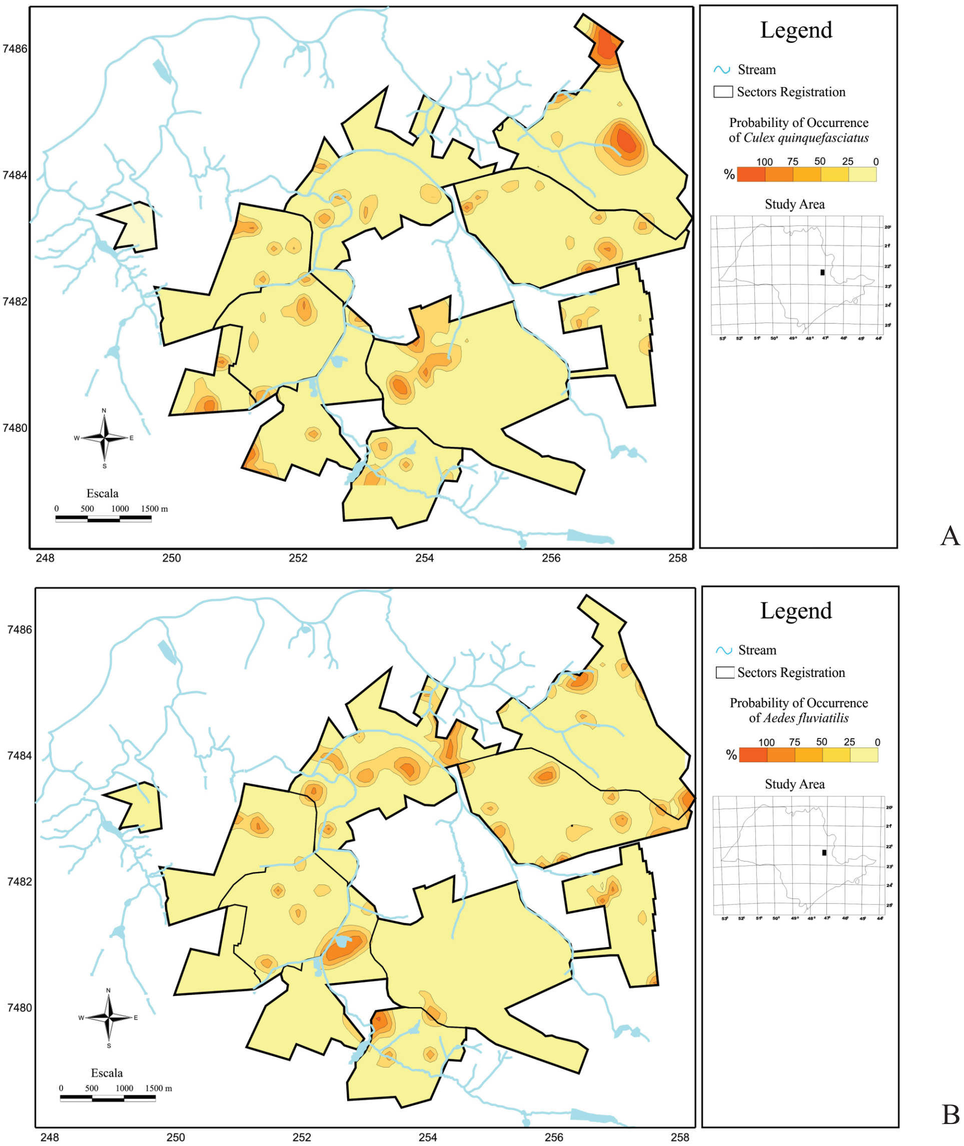


Fig. 4. A. Map of probability of occurrence of *Culex quinquefasciatus* in sectors registration from Santa Barbara d' Oeste City-SP 2004 to 2006; B. Map of probability of occurrence of *Aedes fluviatilis* in sectors registration from Santa Barbara d' Oeste City-SP 2004 to 2006.

in collections from turbid and eutrophic water: e.g. grease traps, septic tanks and storm-water containment boxes (Type-8: other non-removable). Other containers, such as ditches, canals, sewers and drains, which were not considered herein, may have allowed the development of additional *Cx. quinquefasciatus* immatures.

The ecological range of *Ae. fluviatilis* (it uses both artificial and natural breeding sites) may explain this species' frequency as the fourth most common mosquito in our survey, accounting for 9.57% of the sample total (5.82% in isolation and 3.75% in cohabitation). *Aedes fluviatilis* adaptive potential makes it an important species in monitoring efforts, particularly as it is a potential vector of avian malaria and has been experimentally infected with Yellow Fever virus and *Dirofilaria immitis* (Consoli & Oliveira 1998). The spatial distribution of *Ae. fluviatilis* in SBO is shown in Fig. 4B. The species was more frequently found in larval habitat of the Types-4, -7 and -8, suggesting that it may have a preference for containers with more solar irradiation and higher volume.

Less frequent species were also grouped into a distribution map (Fig. 5). Positive sites were generally closer to areas with some arboreal vegetation for *Limatus* sp., *Culex* Coronator Complex, *An. strodei*, *Psorophora* sp. and *Ae. scapularis*.

Human disturbance of natural habitats can alter species behaviour and the consequent synanthropy can enable the establishment of disease vectors (Forattini *et al.* 1995). In SBO, this threat is highlighted by forests within the municipality and a dam close to rural forest fragments a short distance from the urban perimeter. In light of these issues, there is a need for further studies of species in these areas and the adoption of relevant vector-control initiatives (Zequi *et al.* 2005). It is thus important to conduct arboviruses studies, such as with mosquito species in urban parks.

Nineteen representatives of the *Culex* Coronator Complex were collected in SBO, of which seven (36.84%) were found in isolated conditions and twelve (63.15%) cohabited the same breeding site with another species. Most samples were found in container Type-8 (other non-removable). Others were collected in breeding sites Type-3, -4 and -7. Among the *Culex* Coronator Complex samples, higher larvae frequencies were observed in the vicinity of areas with forest coverage. This observation was also true for the Anophelinae, underscoring the importance of such scenarios in the occurrence of SBO vector species.

We found ten specimens of Sabethini, all of the genus *Limatus*. Of those found alone in their recipients, two were in habitat Type-2, two in Type-3 and one in Type 4. Of those found cohabiting with other species, three were in breeding site Types-2, -4 and -5 (with *Cx. quinquefasciatus*), one in breeding site Type-7 (with *Ae. aegypti*) and one in breeding site Type-4 with (*Ae. albopictus*). The genus *Limatus* can be found in natural habitats (Guimarães *et al.* 2000) and also in artificial containers, with Types-2, -3 and -4 being most common in the SBO. Further studies that include capture of adults in forest environments may contribute to understanding of this genus distribution in SBO.

Anopheles strodei larvae were found in eight habitats, six of these in isolated conditions and two in cohabitation with other species. Its distribution relative to larval habitats was as follows: six specimens in the Type-8 (other non-removable), one in the Type-7 (other removable) and one specimen in the Type-5 (water tank). In most cases, *An. strodei* was found in habitats containing large-volume of water such as pools and small springs (Type-8). The occurrence of *An. strodei*, a secondary vector of malaria, stresses the importance of monitoring rural and the habitats of the Anophelinae

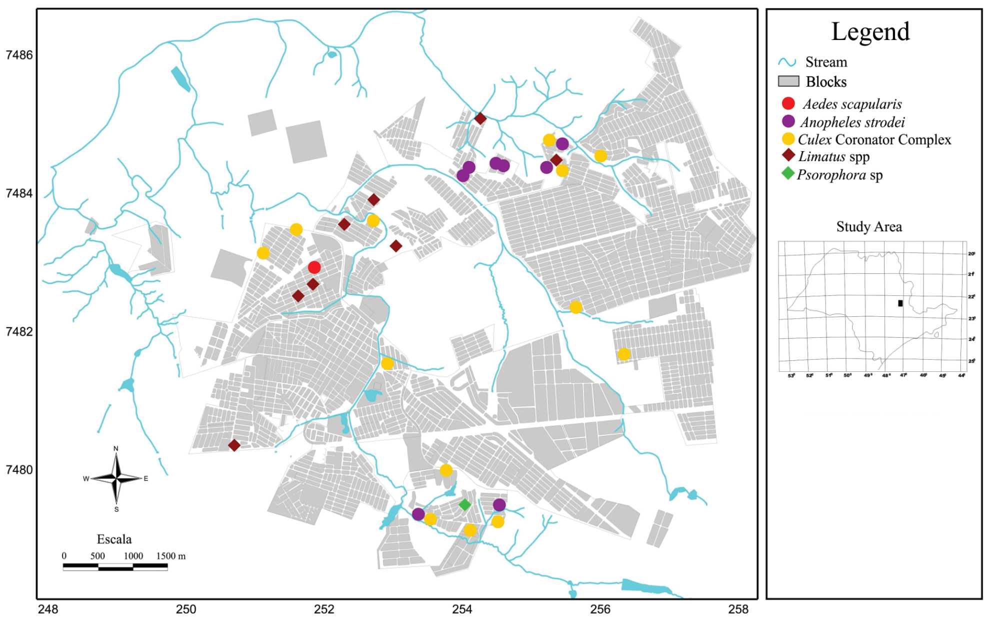


Fig. 5. Map of spatial distribution of rare Culicidae from Santa Bárbara d' Oeste City-SP 2004 to 2006.

larvae. Our findings corroborate Forattini (2002), who posited that this species returns to forested areas to rest after hematophagy in urban areas (Forattini 2002).

Psorophora sp. was found only once, suggesting that artificial breeding sites have little influence on the species' population dynamics. The genus' use of temporary breeding sites, such as marshes, swamps, animal tracks and tyres, may explain *Psorophora* relative scarcity in urban environments (Forattini 2002). In SBO, a single specimen was collected from the habitat Type-3 (tin, jar, bottle), suggesting that artificial containers cannot sustain populations of *Psorophora* sp.

Aedes scapularis, like *Psorophora* sp., was also found only once, but in breeding site Type-4 (washtub, bucket, barrel). Despite its low frequency, previous work with this species suggests a higher potential for domiciliation, with breeding site preferences for puddles, excavations and other ground-level aquatic environments (Forattini 2002). Presence of *Ae. scapularis* in an urban setting is of importance to epidemiological surveillance agencies, as this species can transmit various pathogens: it has been found naturally infected with several viruses (Melao, Ilhéus, Venezuelan equine encephalitis) and with *Dirofilaria immitis* (Forattini 2002).

The maps presented in this paper represent the spatial distribution of the most common mosquito species in the municipality of Santa Bárbara d' Oeste. Additional mapping is needed to understand the population dynamics of these species, particularly *Ae. aegypti*, and to verify if the areas of highest probability remain so throughout the year and whether there is a relationship between these areas and large-scale non-removable breeding sites, which may account for the infestation of smaller residential larval habitats.

Our probability-of-occurrence maps should serve as baselines for future dengue epidemiology studies in the municipality. We conclude that SBO displays serious risk factors for the manifestation of severe cases of the disease. This worry is founded on the continuous transmission of dengue since 1995, with the circulation of serotypes DEN-1, DEN-2 and DEN-3. Two deaths from Dengue Haemorrhagic Fever have already been documented (in 2010). In light of the maps provided herein, there may be a greater likelihood of dengue transmission in areas where *Ae. aegypti* is most likely to occur.

New geographic information systems (GIS) technologies increase the potential to aggregate different forms of data and constructing indicators at different spatial levels (Bailey& Gatrell 1996). It is herein suggested that public authorities adopt GIS technologies to identify areas at risk for disease transmission and increase educational activities for targeting audiences and empower the responsible authorities for planning and management at the municipal, state and federal levels.

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