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Bionomics of *Culex quinquefasciatus* within urban areas of Rio de Janeiro, Southeastern Brazil

Bionomia de *Culex quinquefasciatus* em áreas urbanas no Rio de Janeiro, RJ

ABSTRACT

OBJECTIVE: To evaluate density, parity rates, daily survival and longevity of natural populations of *Culex quinquefasciatus* in three neighborhoods with distinct socio-economic and infrastructure profiles.

METHODS: Mosquito collections of the *Culex quinquefasciatus* species were performed weekly during two four month periods, from August to November 2008 (spring) and March to June 2009 (fall), in a *favela* (slum), a suburban area and a middle class area of Rio de Janeiro, Southeastern Brazil. Collections were performed with backpack aspirators, in 20 randomly selected houses in each area per week, during 15-20 minutes per house. Ovaries were removed from captured females and classified as initial, intermediary or final stage. Furthermore, females were dissected for determination of parity based on the condition of the tracheal system. Mosquito survival rate and longevity were estimated on a per month basis for each neighborhood.

RESULTS: We collected a total of 2,062 *Culex quinquefasciatus*, but monthly vector density was not correlated with temperature and rainfall. We dissected the ovaries of 625 *Culex quinquefasciatus*, and overall, there was a higher proportion of nulliparous females during the dryer months, while gravid females were more frequent in rainy months. In the middle class neighborhood, the parity rate reached up to 93.75% with survivorship of 0.979. Lower parity and survival rates were obtained in the suburban area (as low as 36.4% parity and 0.711 daily survival). Up to 84.7% of *Culex quinquefasciatus* females could survive the eight day period needed to complete West Nile Virus incubation.

CONCLUSIONS: The survival rate of *Culex quinquefasciatus* varied significantly between the neighborhoods. This suggests that vectorial capacity and disease transmission risk may vary greatly between different urban areas, which is potentially useful information for vector control programs.

DESCRIPTORS: ***Culex*. Parity. Survival. Longevity.**

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RESUMO

OBJETIVO: Avaliar densidade, taxa de paridade, sobrevivência diária e longevidade de populações naturais de *Culex quinquefasciatus* de três localidades com diferentes perfis socioeconômicos e de infraestrutura.

MÉTODOS: Mosquitos da espécie *Culex quinquefasciatus* foram coletados semanalmente em dois períodos de quatro meses, de agosto a novembro de 2008 e de março a junho de 2009, em uma comunidade, uma área de subúrbio e um bairro de classe média no Rio de Janeiro, RJ. As coletas foram realizadas com o auxílio de aspiradores costais em 20 domicílios, aleatoriamente selecionados por semana e por área, durante 15-20 minutos por domicílio. As fêmeas capturadas tiveram os ovários removidos e classificados em estágios inicial, intermediário e final. Adicionalmente, as fêmeas foram dissecadas para determinação da paridade de acordo com as condições do sistema traqueal. A taxa de sobrevivência e a longevidade das fêmeas foram estimadas por mês, para cada localidade.

RESULTADOS: Foram coletados 2.062 *Culex quinquefasciatus*, porém a densidade mensal deste vetor não apresentou correlação com temperatura e precipitação. Dissecamos os ovários de 625 *Culex quinquefasciatus* e obtivemos maior proporção de fêmeas nulíparas durante os meses mais secos, enquanto fêmeas grávidas foram mais frequentes nos meses chuvosos. A taxa de paridade foi de até 93,75% no bairro de classe média, com sobrevivência de 0,979. Menores valores de paridade e sobrevivência foram obtidos no subúrbio (começando em 36,4% de paridade e 0,711 de sobrevivência diária). Até 84,7% das fêmeas de *Culex quinquefasciatus* poderiam sobreviver ao período de oito dias, necessário para completar o período de incubação do vírus da febre do Nilo Ocidental.

CONCLUSÕES: A taxa de sobrevivência de *Culex quinquefasciatus* variou significativamente entre os bairros, o que sugere que a capacidade vetorial e o risco de transmissão de doenças podem variar entre diferentes áreas urbanas, informação relevante para o planejamento dos programas de controle de vetores.

DESCRITORES: ***Culex*, Paridade. Sobrevivência. Longevidade.**

INTRODUCTION

Culex quinquefasciatus Say is a cosmopolitan mosquito with worldwide distribution, more frequent in tropical and subtropical areas in association with human dwellings. The adult females lay eggs preferentially in relatively large, permanent habitats with high concentrations of decomposing organic matter, such as sewage effluents and septic tanks. However, immature stages of this species can be found in artificial containers often filled with polluted or organic-rich water, but rarely co-exist in the same container with the dengue vector *Aedes aegypti*.

Besides causing nocturnal discomfort and allergic responses, *Cx. quinquefasciatus* has important medical relevance. This species is established as the main vector of both *Wuchereria bancrofti* Cobbold in cities of the Northern/Northeastern Brazil, including Salvador, Maceió, Recife and Belém,[24] and *Dirofilaria immitis* Leidy in Brazilian coastal cities such as Florianópolis, Rio de Janeiro, Recife and São Luiz.[1] Moreover, *Cx. quinquefasciatus* is a competent vector of St. Louis encephalitis (SLE)[18] and West Nile virus (WNV).[13] Public health authorities from all over the world realized the need to monitor birds, horses and mosquito populations for the prediction of WNV circulation in the end of the 90s, after an outbreak of WNV in New York City was followed by rapid dissemination throughout the country.

WNV plagues the Americas from the North of South America to Canada and its activity is observed in Mexico, countries of Central America, Venezuela, Colombia and Argentina.[14] A recent report has observed the presence of WNV antibodies in horses of the Brazilian *Pantanal* biome, but all the tested mosquitoes and caimans were negative.[22] The vectorial competence of native *Cx. quinquefasciatus* populations or other species to WNV still remains unknown. Although the surveillance of WNV in mosquitoes, caimans and migratory birds has detected no WNV genome or antibodies up to now, the presence of WNV in horses of the *Pantanal* suggests this virus has already invaded Brazil. However, no human cases were registered in the country up to August 2011 (Nota Técnica 28/2011).

Little is known about bionomic aspects of *Cx. quinquefasciatus* in Brazil, especially those parameters governing vectorial capacity. The daily survival probability of adult females is crucial for understanding disease transmission, since small increases in longevity may exponentially augment the vectorial capacity.[11] Vectors must survive longer than the pathogen extrinsic incubation period to be able to infect the host by bite. Girard et al[12] have shown that a lifespan of at least eight days is required for WNV transmission. Daily survival rates may be calculated by either a mark-release-recapture experiment or by dissecting the ovaries of wild-caught individuals and assessing their parity rate and gonotrophic cycle length.[7]

This study aimed to assess *Cx. quinquefasciatus* density, survival rates, longevity and age structure in three neighborhoods with distinct socioeconomic statuses.

METHODS

A field study was performed simultaneously in three urban neighborhoods of Rio de Janeiro, Southeastern Brazil: Tubiacanga (22°47'08" S; 43°13'36" W), Amorim (22°52'30" S; 43°14'53' W) and Vila Valqueire (22°53'17" S; 43°22'20" W). Tubiacanga is an isolated, low middle-class suburban area surrounded in about 70% of its extension by the Guanabara Bay, which has an area of approximately 380 km² and is bordered by eight counties with 7.6 million inhabitants. Tubiacanga has 867 standard houses and 2,902 inhabitants, with human density of 337.4 inhab/hectare, distributed over 14 square blocks. Amorim is a typical overcrowded poverty-stricken Brazilian *favela* (slum), with narrow alleys and irregular blocks, located in an isolated area surrounded by large highways and an amply vegetated and non-residential area. Amorim lacks regular piped water distribution and garbage collection, and has poor basic sanitation. In Amorim, there are 2,992 people living in 897 premises within ten square blocks, with a population density of 901.2 inhab/ha. Valqueire is a middle-class urban area surrounded by a secondary forest, with paved streets, regular municipal water supply and garbage collection. At a distance of 500 m, an urban polluted stream about 5 m wide flows through part of the study area. Valqueire has 2,962 inhabitants in 895 houses spread over 15 square blocks, with human density of 145.5 inhab/ha. The neighborhoods of Tubiacanga, Amorim and Valqueire were referred to as suburbia, slum and middle-class areas, respectively.

The climate in Rio de Janeiro is characterized by a dry winter (May-August) and a wet summer season (November-March). The sites are approximately 15 km apart from each other in a straight line, which suggests differences in air temperature and precipitation influencing mosquito biology in each area, i.e., development time, survival and competition due to breeding sites availability. All three areas are subject to intense rainfall in the summer (November-March), but the suburbia and slum areas are dryer than the middle-class neighborhood between May-September (Figure 1). Meteorological data were recorded at the nearest meteorological station from

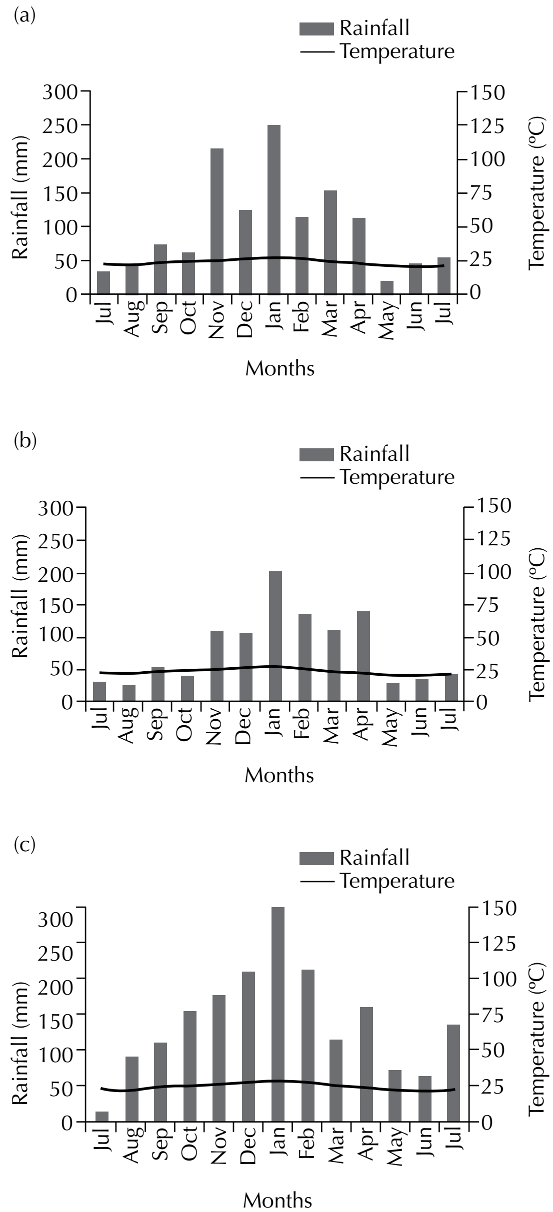

Figure 1. Rainfall (mm) and mean temperature. Slum (A), suburbia (B) and middle class (C) areas of Rio de Janeiro, Southeastern Brazil, July 2008 to July 2009.

each study area to estimate the role of meteorological variations in vector population density and survival.

Mosquito collections were performed in two four-month periods, from August to November 2008 (spring) and from March to June 2009 (fall), preceding and succeeding the winter (the season in which *Cx. quinquefasciatus* has its peak of density).[20,21] We monitored adult *Cx. quinquefasciatus* density on a weekly basis by collecting mosquitoes with backpack aspirators in 20 randomly selected houses per area. The backpack aspirator is frequently pointed out as the most effective collection method for adult mosquitoes, since it captures males and females of all physiological statuses, affording adult density estimates. Aspiration was carried out inside and in the peridomestic environments during 15-20 minutes per house. Collected mosquitoes were brought to the laboratory for identification and sequential assays.

All females captured with backpack aspirators had their ovaries removed and preserved in saline solution. The skeins of the ovarian tracheal system were evaluated under a microscope. The irreversible uncoiling of skeins indicates parity.[8]

Ovaries of dissected females were classified according to Christophers,[5] with stages 1, 1-2, and 2 grouped as initial stages of development, stages 3 and 4 grouped as intermediate and stage 5 classified as final (gravid females). Parity of natural populations of *Cx. quinquefasciatus* for each season and area was calculated as the number of parous divided by the total number of dissected females. Survival rate was estimated for each neighborhood according to the Davidson[7] method: $S = \sqrt[g]{\text{parity rate}}$, where *S* was the survival rate and *g* was the duration of the gonotrophic cycle, which was assumed to be three days.[9,15] Mosquito longevity was calculated as $1/-\log_e S$, and the proportion of females that survived eight days was defined as S.[6] Eight was the probable duration of the extrinsic incubation period for the WNV virus.[12] We performed parity, survival and longevity analysis on a monthly basis due to the limitations of weekly female collections.

We analyzed the correlation between meteorological variables (minimum, average and maximum temperatures and rainfall) and *Cx. quinquefasciatus* density by a Spearman correlation test. Data on mosquito density per week were correlated with meteorological variables in the same week as well as in the two weeks prior to field collection.

Parity rates between August-November/2008 and March-June/2009 were compared using a chi-square test to evaluate whether the rates differed regarding the study area and season.

RESULTS

A total of 2,062 *Culex quinquefasciatus* was collected in the three neighborhoods with a sex ratio remarkably biased to males (Table 1): 851 mosquitoes in the middle-class site (295 females, with a sex ratio of 1:2.88 female:male), 972 in the suburbia (282 females, sex ratio of 1:3.44) and 239 in the slum (103 females, sex ratio of 1:2.32). The percentage of female *Cx. quinquefasciatus* per area ranged from 29% (in the suburbia district) to 43% (in the slum).

More *Cx. quinquefasciatus* were collected from August to November/2008 (spring) than during March-June/2009 (fall) (55.9, 80.2 and 65.3% of the collections pertaining to the slum, middle-class and suburbia districts, respectively). The collection of male and female *Cx. quinquefasciatus* in the three neighborhoods was generally not correlated with minimum, average and maximum temperatures. The exception was that the maximum temperature observed in the week of collection was negatively correlated with the number of *Cx. quinquefasciatus* females in the three areas (Middle-class: $R^2 = 0.366$; Suburbia: $R^2 = 0.61$; Slum: $R^2 = 0.60$). No correlation was established for mosquito density and rainfall (data not shown).

Table 1. Number of *Culex quinquefasciatus* males and females collected in each neighborhood per month. Rio de Janeiro, Southeastern Brazil, August 2008 to June 2009.

Month/year	Middle-class			Suburbia			Slum		
	Males	Females	Total	Males	Females	Total	Males	Females	Total
Aug/08	93	34	127	157	55	212	17	11	28
Sept/08	81	64	145	135	73	208	15	16	31
Oct/08	50	36	86	171	61	232	26	15	41
Nov/08	70	48	118	93	34	127	32	24	56
Mar/09	42	16	58	29	16	45	10	4	14
Apr/09	122	27	149	50	23	73	16	6	22
May/09	54	42	96	33	13	46	11	5	16
Jun/09	44	28	72	22	7	29	9	22	31
Total	556	295 (34.6%)	851	690	282 (29.0%)	972	136	103 (43.0%)	239

We dissected ovaries of 88.1%, 96.1% and 91.3% of *Cx. quinquefasciatus* females collected in the middle-class, suburbia and slum areas, respectively. *Cx. quinquefasciatus* age structure presented significant variation among the three neighborhoods (Figure 2). Parity rate varied from 64.0% (June/09) to 93.75% (November/08) in the middle-class area, but no significant variation was observed between the two collection periods (χ^2 = 1.67, p > 0.05). A higher proportion of nulliparous females was observed during the dryer months, but more gravid (stage V) females were collected in rainy months in the middle-class and suburbia neighborhoods.

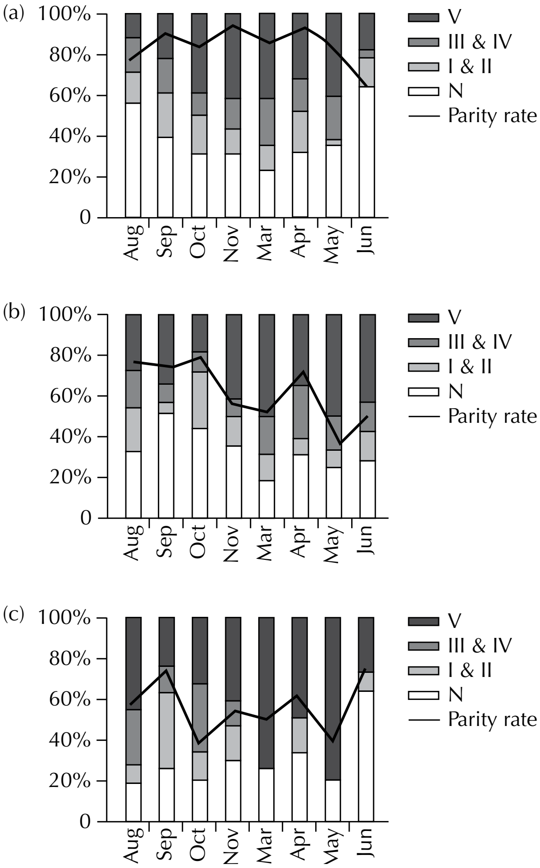

Figure 2. Parity rates of *Cx. Quinquefasciatus*. Slum (A), suburbia (B) and middle-class (C) areas, Rio de Janeiro, Southeastern Brazil, July 2008 to July 2009.

Parity rates in the suburbia area ranged from 36.4% (May/09) to 78.5% (October/08), while in the slum they varied from 40.0% (May/09) to 73.3% (October/08). There was no evident correlation of the ovarian development stage and parity rates with meteorological data in the slum. There was a higher parity rate in the suburbia site during August-November/08 than in March-June/09 (χ^2 = 5.28, p > 0.05), but no differences were apparent in the slum (χ^2 = 3.18, p > 0.05).

Assuming a gonotrophic cycle of three days, there were high values of daily survival, especially for the middle-class neighborhood, which reflected high longevity for females (Table 2). Longevity estimates reinforced the differences in the three neighborhoods. Eight days after a potential infection of WNV, between 30.4 and 84.7% *Cx. quinquefasciatus* would be alive in the middle-class site. In the suburbia and slum areas the proportion of females surviving eight days after infection would vary between 6.6 and 53.3% and from 7.6 to 48.1%, respectively.

Cx. quinquefasciatus longevity was greater in the middle-class neighborhood, with an average life expectancy ranging from 6.7 to 48.2 days. A lower average life expectancy was obtained in the other two sites, ranging from 2.93 to 12.7 days in the suburbia and from 3.2 to 10.9 in the slum.

Table 2. Survival rates of *Cx. quinquefasciatus* and the proportion of females that survived eight days after a potential WNV infection under field conditions based on Probability of Daily Survival.[8] Middle-class, suburbia and slum sites of Rio de Janeiro, Southeastern Brazil, August 2008 to June 2009.

Month/year	Middle-class		Suburbia		Slum	
	Survival rate	Proportion alive at 8th day	Survival rate	Proportion alive at 8th day	Survival rate	Proportion alive at 8th day
Aug/08	0.921	0.515	0.904	0.448	0.843	0.256
Sep/08	0.965	0.754	0.901	0.432	0.901	0.432
Oct/08	0.947	0.648	0.924	0.533	0.724	0.076
Nov/08	0.979	0.847	0.824	0.213	0.804	0.175
Mar/09	0.954	0.690	0.814	0.193	0.794	0.157
Apr/09	0.976	0.824	0.892	0.401	0.843	0.256
May/09	0.935	0.589	0.711	0.066	0.737	0.087
Jun/09	0.862	0.304	0.794	0.157	0.912	0.481

DISCUSSION

The variation in vector biology among the areas was remarkable, especially concerning survival and longevity estimates. The results may indicate that *Cx. quinquefasciatus* biology depends on the urban characteristics of the site where the mosquito is distributed, although we worked in three neighborhoods in a city of around 8 million people. The ecological plasticity of *Cx. quinquefasciatus* may be useful in the determination of a cost-effective control policy.

There was a moderate negative correlation between mosquito density and maximum temperature in the same week of collection. No significant correlation, positive or negative, was established when the number of mosquitoes was compared with meteorological data one or two weeks before vector collection. However, a higher proportion of nulliparous females was observed during the dryer months, especially in Valqueire and Tubiacanga. There were more gravid (stage V) females in rainy months. This suggests that the larval habitats were close to the capturing points, which might have biased the collection of newly emerged females. Although the collection was homogeneously performed throughout the three study sites, this potential bias must be considered. Mosquito density in the three neighborhoods of Rio de Janeiro was directly influenced neither by temperature nor by rainfall.

Reports indicate the effect of seasonality on mosquito density, but the density peak period seems to vary geographically. In Puerto Rico, *Cx. quinquefasciatus* was more abundant in the spring than in the fall.[25] After modeling mosquito population dynamics, Morin & Comrie[19] reported that *Cx. quinquefasciatus* populations have a dramatic growth during spring in California, mainly due to the increase in rainfall during January. Mosquitoes were abundant throughout the year because of sufficient precipitation in Florida,[19] but some reports also showed that *Cx. quinquefasciatus* density peaks occurred during the winter.[20,21] More *Cx. quinquefasciatus* were collected in the winter in India.[20] If there is any influence of meteorological variables on mosquito density, it is probably low and caused by the nearby presence of potential breeding sites. Female individuals may have a higher response to microclimatic variations than to outside rainfall and temperature. *Cx. quinquefasciatus* is well adapted to human dwellings, where the influence of outside temperature is low. All three areas have nearby polluted or sewage water, such as streams, open or leaking sewage systems or the Guanabara Bay. These sites are permanent yearlong, i.e., they support immature mosquito populations continuously throughout the year and, consequently, their availability is not influenced by rainfall. In this particular situation, the effect of meteorological variables on *Cx. quinquefasciatus* density is masked by the presence of permanent mosquito habitats within the surroundings. Rio de Janeiro has an average winter temperature of 20-22°C, which reduces the potential effects a harsh cold might have on vector density.

The parity rates in Rio de Janeiro were higher than in other regions of Brazil, namely, in the city of Manaus (Northern Brazil)[4] and in an ecological park located in the State of São Paulo.[15] Collections by Laporta and Sallum[15] were conducted outdoors, which provided several nulliparous specimens. The majority of reports indicate parity rates that are similar to those in our study. Chandra et al[3] observed a parity rate of 0.87 in Calcutta, India, while in the study carried out by Garcia-Rejon et al,[10] it varied daily, reaching a maximum value of 0.90. We estimated the monthly instead of the daily parity rate, as Garcia-Rejon et al[10] did. The field strategy of estimating parity rates on a monthly basis may cause a loss in the natural variation of parity rates in field populations. However, due to personal field limitations and to the low number of captured females during some months, we had to assess mosquitoes collected during 3-4 weeks. Estimating the parity rates of a natural population may provide substantial information on its dynamics. When the proportion of nulliparous females in a field population is unexpectedly high, one might assume that this population has suffered a drastic reduction in density for a few weeks, caused by, for example, an insecticide campaign or severe and unfavorable meteorological conditions.[19]

Daily vector survival rates may be calculated by either mark-release-recapture (MRR) techniques or by dissection of female insects to assess parity rates,[8] followed by the application of the Davidson[7] equation. Discrepancies in survivorship estimations between Davidson's method and linear regression of captures as a function of time in MRR experiments are not known. Theoretically, comparisons between methods are unusual due to sampling bias and population fluctuation over time. To estimate survival rate as the proportion of parous females in a population using Davidson's method, recruitment must be constant throughout the sampling period. The percentage of parous females can be severely reduced by a spike in mosquito emergence, lowering survival estimates.[9] Estimates based on MRR experiments are strongly influenced by losses due to mosquito death or to emigration from the study site, a crucial difference that is undetectable under conventional MRR procedures.[16] Only one study compared survival estimates based on Davidson's method and linear regression of captures, and it provided similar values for both techniques.[23]

Survival estimates calculated by Davidson's method are strongly influenced by the duration of the gonotrophic cycle. Small variations in the length of the gonotrophic cycle can influence survival rates. We assumed a gonotrophic cycle of three days for *Cx. quinquefasciatus* based on previous reports with very similar estimates.[9,10,15] The high survival rates for *Cx. quinquefasciatus* may have an important consequence on vectorial capacity and disease transmission, since in a long-lived vector population females have several opportunities to blood feed, which maximizes the contact rate between a potentially infected mosquito and a susceptible human host. High survivorship implicates high longevity. More than 50% of the females would have been able to transmit WNV under field conditions in the middle-class area, with the exception of the month of June/2009. This proportion is higher than that for other arboviruses, such as *Ae. aegypti*-dengue[6,16] and *Aedes camptorhyncus*-Ross River virus.[2] However, before an alarmist scenario is created, one should test the vector competence of Brazilian *Cx. quinquefasciatus* populations to WNV infections and host-feeding preferences of the native mosquitoes.

The significant differences in survival and longevity estimates in the three neighborhoods are puzzling and may be connected with the availability of artificial containers, socioeconomic levels and human demography. Despite the possible difficulties in disentangling these effects from each other, it is possible to say that the availability of container types is highly correlated with socioeconomic level in Rio de Janeiro.[6,16] Containers used for water storage are abundant in areas with irregular water distribution, such as the slum and suburbia. When piped water distribution is regular, containers such as metal drums and water tanks are almost inexistent, and domestic rains and plastic pots become productive breeding sites.[17] The high abundance of large containers with clean water storage may have influenced the oviposition pattern of *Cx. quinquefasciatus* in the slum and suburbia areas, strengthening larval competition in containers with high amounts of organic matter. Additionally, districts with lower socioeconomic statuses tend to have higher availability of containers, mainly due to the accumulation of small abandoned objects in the yard and surroundings. Mosquito survival and longevity varied inversely to human demography, i.e., higher survivorship was observed in the middle-class district, where human density is more than six times lower than in the slum. The human defensive behavior may kill mosquitoes during their bite or rest. Thus, the absolute number of mosquitoes being killed by the human defensive behavior would be lower in low human density areas, allowing higher population survival.

We evaluated some aspects of *Cx. quinquefasciatus* bionomics in three neighborhoods with very distinct socioeconomic and infrastructure statuses in which there were different vector survival rates, an important component of vectorial capacity. Understanding *Cx. quinquefasciatus* population biology under different urban scenarios may provide an advantage for controlling this potential vector species.

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