CHAPTER 9

VERTICAL OVIPOSITION ACTIVITY OF MOSQUITOES IN THE ATLANTIC FOREST OF BRAZIL WITH EMPHASIS ON THE SYLVAN VECTOR, *HAEMAGOGUS LEUCOCELAENUS* (DIPTERA: CULICIDAE)

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Jeronimo Alencar
Laboratório de Diptera, Instituto Oswaldo Cruz (Fiocruz), Manguinhos, Rio de Janeiro, Brazil

Cecilia Ferreira de Mello
Laboratório de Diptera, Instituto Oswaldo Cruz (Fiocruz), Manguinhos, Rio de Janeiro, Brazil
Programa de Pós-graduação em Biologia Animal, Instituto de Biologia, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil

Hélcio R. Gil-Santana
Laboratório de Diptera, Instituto Oswaldo Cruz (Fiocruz), Manguinhos, Rio de Janeiro, Brazil

Anthony Érico Guimarães
Laboratório de Diptera, Instituto Oswaldo Cruz (Fiocruz), Manguinhos, Rio de Janeiro, Brazil

Sergio Antonio Silva de Almeida
Laboratório de Diptera, Instituto Oswaldo Cruz (Fiocruz), Manguinhos, Rio de Janeiro, Brazil
Programa de Pós-graduação em Biologia Animal, Instituto de Biologia, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil

Raquel M. Gleiser
Centro de Relevamiento y Evaluación de Recursos Agrícolas y Naturales-Instituto Multidisciplinario de Biología Vegetal (Consejo Nacional de Investigaciones Científicas y Técnicas—Universidad Nacional de Córdoba, Facultad de Ciencias Agropecuarias and Cátedra de Ecología, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina

ABSTRACT: This study aimed to assess the vertical patterns of oviposition and temporal changes in the distribution of mosquito species in an area of the Atlantic Forest in Rio de Janeiro State, Brazil, and in particular, the behavior and oviposition of potential yellow fever virus vectors. Mosquito samples were collected from the Ecological Reserve Guapiaçu (REGUA, Brazil), which includes a somewhat disturbed forest, with a large diversity of plants and animals. In all, 5,458 specimens (ten species from seven genera) were collected. *Haemagogus leucocelaenus* was the most frequently captured species, representing 73% of the specimens collected. Species richness and diversity were the highest in the samples

collected from the ground-level ovitraps and decreased with height. Species composition also differed significantly among heights. The largest species differences were detected between ovitraps set at the ground level and those set at 7 m and 9 m; *Hg. leucocelaenus*, *Limatus durhamii*, and *Limatus paraensis* contributed most to these differences. Sampling month and climatic variables had significant effects on species richness and diversity. Species diversity and richness decreased with height, suggesting that the conditions for mosquito breeding are more favorable closer to the ground. Species composition also showed vertical differences.
KEYWORDS: Culicidae, *Haemagogus*, acrodendrophily, oviposition, yellow fever virus.

INTRODUCTION

Species belonging to the genera *Haemagogus* Williston, 1896 and *Sabethes* Robineau-Desvoidy, 1827 are the most important biological vectors of yellow fever in the forested areas of the American continent. Comprehensive analysis of the bioecology of *Haemagogus* spp. and other sylvan mosquitoes is of primary importance because they are vectors for other arboviruses, such as those that cause dengue and Mayaro fever (Marcondes and Alencar 2010). *Haemagogus* spp. are essentially sylvan mosquitoes that show diurnal activity and acrodendrophilic habits (Arnell 1973). Pinheiro et al. (1978) and Dégallier et al. (1992) suggested that these species mostly inhabit dense and gallery forest areas of Brazil. Their acrodendrophilic behavior might vary across different regions, hence, their vertical distribution. In French Guiana, for example, the ground level activity of *Haemagogus janthinomys* Dyar, 1921 occurred at different times of the year (Pajot et al. 1985). Furthermore, in Iquitos, Peru, *Hg. janthinomys* were frequently captured from forest canopies during human-landing collections (Ramirez et al. 2007). In Parque Nacional da Serra dos Órgãos, Rio de Janeiro, Brazil, *Hg. leucocelaenus* (Dyar & Shannon, 1924) and *Haemagogus capricornii* Lutz, 1904 captured during human-landing collections showed clear acrodendrophilic preference (Guimarães et al. 1985). Some mosquitoes have been shown to have height preferences for oviposition on phytotelma species (Alencar et al. 2013).

In an eastern Amazon area, epizootic and epidemic diseases were found to be more prevalent during the beginning of the rainy season when mosquito densities are higher owing to the increase in forest rains (Dégallier et al. 2006). Although humid conditions are assumed to influence the flight and feeding height of mosquitoes, whether weather conditions affect their acrodendrophilic or ground preferences is not clear (Guimarães et al. 1985). This study aimed to assess the vertical pattern of oviposition and the temporal changes in the distribution of mosquito species in the Atlantic Forest of Rio de Janeiro State, Brazil, in particular, the yellow fever virus vectors.

MATERIALS AND METHODS

Study area

Mosquitoes were collected from the Guapiaçu Ecological Reserve (REGUA), Cachoeiras de Macacu municipality, Rio de Janeiro, Brazil. REGUA is a Private Natural Heritage Reserve that was established in 1996; it includes about 7,385 ha of dense rain forest and a remarkable diversity of flora and fauna. Freitas et al. (2005) suggested that the vast majority of forest fragments are located at 100–200 m above sea level (a.s.l.) within farms having areas ranging from 19 to 200 ha.

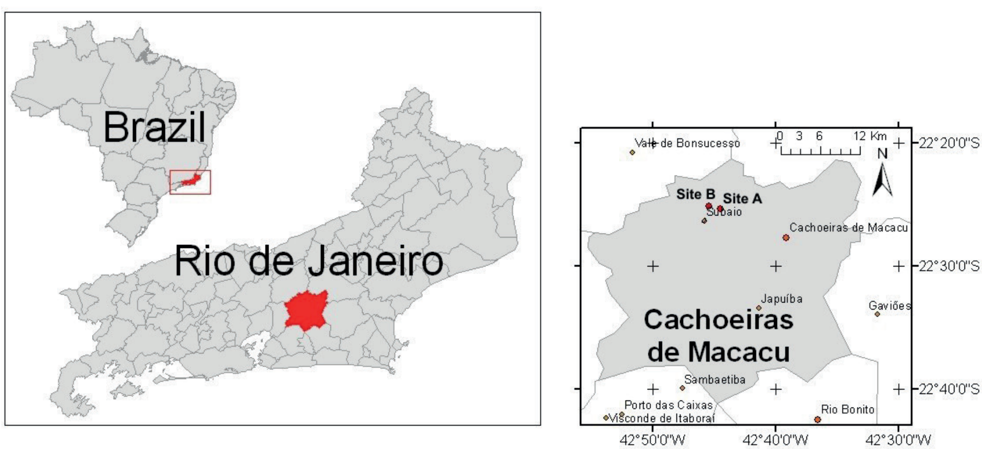

Figure 1. Study site location in Guapiaçu Ecological Reserve (REGUA), Brazil.

DATA COLLECTION

Mosquitoes were sampled once every two months from December, 2012 to March, 2014. Two sampling sites were established: Sites A (22°25′25.4″ S; 42°44′32.4″ W; 242 m a.s.l.) and B (22°25′96.9″ S; 42°45′26.8″ W; 223 m a.s.l.) (Figure 1). The two sites were located in the submontane zone that is characterized by hilly and rugged terrain. These sites comprise three strata: emergent trees (having height of about 45 m), main canopy (height, 5–10 m), and understory vegetation.

Mosquitoes were monitored with oviposition traps in order to standardize conditions and avoid (or reduce) habitat typeheight interactions. Oviposition traps were matte black pots (1 liter) without lids containing four 2.5 x 14 cm vertical plywood panels (Eucatex® boards) attached using paper clips (Silver 2008). A natural ecosystem-like environment was created by adding fresh water and leaf litter to the pots. The oviposition traps were installed on trees by using a fishing sinker (diameter approximately 4 cm) and secured using a nylon rope. The ovitraps were set at five heights (ground level, 3 m, 5 m, 7 m, and 9 m) in the forest and monitored biweekly by replacing the panels with new ones; ten ovitraps were set for each height. All panels within each ovitrap were sequentially numbered, placed in a humid container, and transferred to the Diptera Laboratory of the Oswaldo Cruz Institute. In the laboratory, the eggs were hatched by immersing them in transparent glass pans filled with MiliQ® water (Alencar et al. 2014), and the larvae were reared using standard entomological techniques to obtain adults for taxonomic identification by the direct observation of morphological characters under a stereomicroscope. The dichotomous keys by Arnell (1973) and Forattini (2002) were used for species identification. After identification, all specimens were deposited at the Entomological Collection of the Oswaldo Cruz Institute (Coleção Entomológica do Instituto Oswaldo Cruz). Mosquito genera and subgenera abbreviations are those proposed by Reinert (2009). The monthly relative humidity and temperature (maximum, minimum, and average) and monthly rainfall data were obtained from the National Meteorology Institute of Brazil (INMET).

DATA ANALYSES

Species richness at each height was estimated using Chao1- bc (a bias-corrected form of Chao1 (Chao 2005)) and the non- parametric estimator ACE-1 (a modified abundance-based coverage estimator for highly heterogeneous communities (Chao and Lee 1992)) by using SPADE software (Chao and Shen 2010) for 200 bootstrap replications. Rare species were defined as those for which fewer than ten individuals were collected. The degree of heterogeneity among species was estimated using the squared coefficient of variation (CV) of species abundance, which equals zero when all species have equal abundances and is positively correlated with the degree of heterogeneity (Chao and Shen 2003). Further, Shannon's Index and the associated effective number of species (Shannon diversity, based on the Chao and Shen (2003) estimator) and Simpson's index and the associated effective number of species (Simpson diversity, based on a minimum variance unbiased estimator (MVUE)) were estimated. General linear mixed models (GLMs) were used to assess the differences in diversity (for each diversity estimate) among heights and among sampling months by using Infostat software (InfoStat versión 2014). Site was included as a random effect in these models. Fisher's least significant difference (LSD) was used for pairwise comparison. The significance level was set at $p < 0.05$.

Principal component analysis (PCA) was used to determine the global relationships among species assemblages and ovitrap heights. For this, the numbers of specimens for each species were converted to percentages (percentage of total number of specimens collected at a given height from the total number of specimens collected at all the heights). Since raw counts might reflect the local sampling milieu at a particular site and date and thus mask the fundamental differences among assemblages at different heights, the relative frequency (frequency of individuals at a given height from all positive samples for a given species) was also calculated. Species associations (C_{AB}) were analyzed using Cole's index (Cole 1949) with a 2 × 2 contingency table and $\chi2$ test. The elevation, but not temporal segregation, between a pair of species was evaluated by considering the dates when both the species were detected.

The differences in species composition between the sampling months and heights were analyzed using a non- parametric multivariate analysis of variance (PERMANOVA); data transformed to ln(n+1) based on Bray–Curtis distances and having 10,000 permutations. The multivariate patterns among observations were visualized by performing nonmetric multidimensional scaling (nMDS) on the Bray–Curtis distances (Past software, http://palaeo-electronica.org). Samples holding no mosquitoes were excluded to facilitate visual interpretation. Where differences were noted, similarity percentages (SIMPER) were calculated to determine the species that most contributed to the dissimilarities (Clarke and Warwick 2001). Relationships between the average monthly climatic variables and diversity measures or proportion of eggs for each height were assessed using multiple stepwise regression analysis (Stepwise InfoStat software) by using $p \leq 0.15$ as the criterion for retaining variables.

Table 1. Numbers of specimens collected using ovitraps (in parenthesis expressed as percentages per species) at each of the five heights above the ground level in Guapiaçu Ecological Reserve (REGUA), Rio de Janeiro, Brazil.

Species	Height above ground				
	0 m	3 m	5 m	7 m	9 m
Aedes albopictus	7 (100)	0	0	0	0
Aedes terrens	0	8 (50)	12 (50)	0	0
Culex sp.	52 (100)	0	0	0	0
Haemagogus leucocelaenus	122 (2.8)	623 (14.4)	1,540 (35.5)	1,190 (27.7)	845 (19.6)
Limatus durhamii	428 (85.4)	61 (10.9)	24 (3.7)	0	0
Limatus flavisetosus	55 (77.8)	15 (20.8)	1 (1.4)	0	0
Limatus paraensis	192 (58.8)	92 (28.4)	22 (8.0)	0	12 (4.8)
Limatus pseudomethysticus	79 (89.2)	14 (10.8)	0	0	0
Toxorhynchites mariae	7 (23.8)	6 (42.9)	12 (33.3)	0	0
Wyeomyia sp.	28 (95.7)	0	2 (4.3)	0	0

RESULTS

Of the 5,458 specimens obtained, 5,376 were identified to the species level (Table 1). The specimens belonged to the following seven genera: *Haemagogus* (72.5%), *Limatus* (17.5%), *Culex* (0.9%), *Wyeomyia* (0.5%), *Toxorhynchites* (0.4%), *Aedes* (*Ochlerotatus)* (0.3%), and *Aedes* (*Stegomyia*) (0.1%). In all, ten species were captured; the most frequently captured species was *Hg. leucocelaenus* (72.5%), followed by *Limatus durhamii* Theobald 1901 (9.4%). The number of days when specimens were obtained varied among species; *Aedes albopictus* Skuse 1895, *Aedes terrens* Walker 1856, and *Toxorhynchites mariae* (Bourroul, 1904) were infrequent, whereas *Hg. leucocelaenus*, *Li. durhamii*, and *Limatus paraensis* Theobald 1903 were frequently detected on most dates, but there were no clear seasonal patterns (Figure 2).

The overall richness and diversity estimates for each height are shown in Table 2. At all the heights assessed, the number of species observed matched the expected species richness as per the Chao1-bc and ACE-1 estimates, and sample coverage was 1 (or close to 1). Thus, the samples obtained were considered to provide adequate representations of species diversity at the different heights. Species richness showed an overall (total data) significant negative correlation with height ($R^2 = 0.79$; $p < 0.05$; Figure 3a); according to the monthly data, species richness was the highest in ground-level ovitraps ($F_{4,54} = 28.8$; $p < 0.0001$). Species diversity showed similar patterns, with the highest diversities observed in ground-level ovitraps, which decreased with height (effective number of species based on Shannon index [$F_{4,54} = 30.5$; $p < 0.0001$] and Gini-Simpson index [$F_{4,54} = 23.2$; $p < 0.0001$]; Figure 3 b and c, respectively). The CV values for each height were relatively high (except for ovitraps set at 7 m, where only one species was detected), indicating high heterogeneity in species abundances. Sampling month had a significant effect on species richness and diversity; the highest values were recorded in August, 2013 and the lowest in May and October, 2013, but these two factors did not interact with height.

The first two components of the PCA of relative abundance explained 97% of the data variability (Figure 4). The first component clearly separated species such as *Aedes albopictus*, *Culex* spp., and *Limatus pseudomethysticus* (Bonne-Webster and Bonne 1920) (positive values) that were usually collected at ground level from those that were most frequently collected from greater heights. The second component separated *Hg. leucocelaenus*, which was found at a broad range of heights, but more frequently in ovitraps set at 5 m or higher, from other species found predominantly in ovitraps installed 3 to 5 m from the ground. A similar pattern was observed when presence data were considered (data not shown).

Species composition significantly differed between heights ($F = 5.27$; $p < 0.001$) and months ($F = 4.27$; $p < 0.001$) as revealed by PERMANOVA, but no significant interactions ($F = 0.86$; $p = 0.76$) were noted between height and month. Ground-level (0 m) ovitraps were clustered separately from those installed at 7 and 9 m in the two-dimensional ordination space when species abundances from different months and sites were analyzed using NMDS (Figure 5). SIMPER between ground-level ovitraps and ovitraps placed at a height of 7 m and 9 m above the ground showed that *Hg. leucocelaenus*, *Li. durhamii*, and *Li. paraensis* contributed the most to the dissimilarities in species abundances (Table 3).

Analysis of species associations considering only those dates when both the species of a pair were present showed significant associations that were consistent with their main height occurrence, such as negative associations between *Hg. leucocelaenus* and *Li. pseudomethysticus* or *Tx. mariae* (found at distinct heights) and positive associations between *Li. pseudomethysticus* and *Li. durhamii*, or *Li. durhamii* and *Li. paraensis* (found at similar heights) (Table 4). However, although *Li. durhamii* was frequently found at ground level ovitraps, it was negatively associated with *Ae. albopictus*. Diversity measures and climatic variables mostly showed weak and non-significant correlations. Significant relations were detected only for traps placed at heights (combining collections from ovitraps placed at 7 and 9 m). Both Shannon and Simpson diversities and the proportion of eggs collected from traps placed at heights were significantly negatively related to the maximum temperature of the previous month ($p < 0.05$) and positively related to the minimum or mean temperature of the same month (Table 5). The proportion of eggs according to height of ovitraps, Shannon diversity, and Simpson diversity models explained 37%, 37%, and 51% of the variation in the data, respectively.

Table 2. Mosquito diversity estimates for ovitraps placed at five heights above the ground in Guapiaçu Ecological Reserve, Cachoeiras de Macacu, Rio de Janeiro, Brazil, from December 2012 to March 2014 (bootstrap mean; 95% confidence intervals are shown in parentheses).

	Height above ground				
	0 m	3 m	5 m	7 m	9 m
Number observed Individuals	999	819	1,771	1,199	857
Species	9	7	7	1	2
Individuals, rare species	14	14	1	0	0
Rare species	2	2	1	0	0
Estimated sample coverage	1	1	0.99	1	1
Estimated CV	1.15	1.78	2.09	0	0.97
Chao 1-bc	9.0 (9.0, 9.9)	7.0 (7.0, 7.0)	7.0 (7.0, 7.0)	1 (1.0, 1.0)	2.0 (2.0, 2.0)
ACE-1	9.0 (9.0, 9.9)	7.0 (7.0, 7.0)	-	-	-
Shannon index	1.64 (1.59, 1.70)	0.87 (0.80, 0.95)	0.53 (0.48, 0.58)	0.0 (0.0, 0.0)	0.07 (0.04, 0.11)
Shannon diversity*	5.18 (4.89, 5.46)	2.39 (2.21, 2.57)	1.70 (1.61, 1.78)	1.0 (1.0, 1.0)	1.08 (1.04, 1.11)
Simpson index	0.26 (0.15, 0.37)	0.60 (0.04, 1.15)	0.76 (-1.13, 2.66)	1.0 (-1.77, 3.77)	0.97 (-1.69, 3.63)
Simpson diversity**	3.86 (3.43, 4.30)	1.67 (0.74, 2.61)	1.31 (-1.17, 3.78)	1.0 (-1.77, 3.77)	1.03 (-1.71, 3.77)

Shannon based on Horvitz-Thompson estimator and sample coverage method (Chao and Shen 2003). Estimated standard error is based on a bootstrap method; Simpson estimator is minimum variance unbiased estimator;* Diversity of order 1; ** Diversity of order 2.

Table 3. Percentage contribution of each mosquito species to the observed dissimilarities between pairs of samples collected using ovitraps placed at the ground level and 7 m and 9 m above the ground in Guapiaçu Ecological Reserve (REGUA), Rio de Janeiro, Brazil. Species contributing >10% are shown in bold font.

Taxon	Mean abundance			% Contribution	
	0 m	7 m	9 m	0-7 m	0-9 m
Haemagogus leucocelaenus	1.94	44.15	28.37	27.37	24.27
Limatus durhamii	13.59	0	0	21.83	21.98
Limatus paraensis	7.67	0	0	17.15	16.09
Limatus pseudomethysticus	1.89	0	0	7.34	7.39
Limatus flavisetosus	0.90	0	0	4.47	4.50
Wyeomyia sp.	0.80	0	0	3.94	3.97
Culex sp.	0.64	0	0	2.71	2.73
Toxorhynchites mariae	0.18	0	0	1.32	1.33
Aedes albopictus	0.18	0	0	1.32	1.33

Table 4. Species associations (Cole's index (Cole 1949)) estimated for pairs of species considering the sampling dates when both species were detected.

Species	*Ae. alb.*	*Ae. ter.*	*Cx. sp.*	*Hg. leu.*	*Li. dur.*	*Li. fla.*	*Li. par.*	*Li. pse.*	*Tx. mar.*
Aedes terrens	-1.00 ± 1.90								
Culex sp.		NS							
Haemagogus leucocelaenus	0.25 ± 0.16	NS	NS						
Limatus durhamii	-1.00 ± 0.35	NS	0.20 ± 0.10	NS					
Limatus flavisetosus		NS	0.17 ± 0.15	NS	0.70 ± 0.18				
Limatus paraensis	1.00 ± 0.52	NS	0.14 ± 0.07	NS	0.80 ± 0.10	0.28 ± 0.09			
Limatus pseudomethysticus		-1.00 ± 0.92	0.37 ± 0.11	-0.50 ± 0.22	1.00 ± 0.19	0.84 ± 0.17	0.83 ± 0.22		
Toxorhynchites mariae	1.00 ± 0.32	1.00 ± 0.32	NS	-0.32 ± 0.27	0.58 ± 0.37	NS	0.12 ± 0.11	NS	
Wyeomyia sp.	1.00 ± 0.32	0.26 ± 0.16	1.00 ± 0.32	NS	0.67 ± 0.21	NS	0.68 ± 0.20	0.25 ± 0.19	1.00 ± 0.32

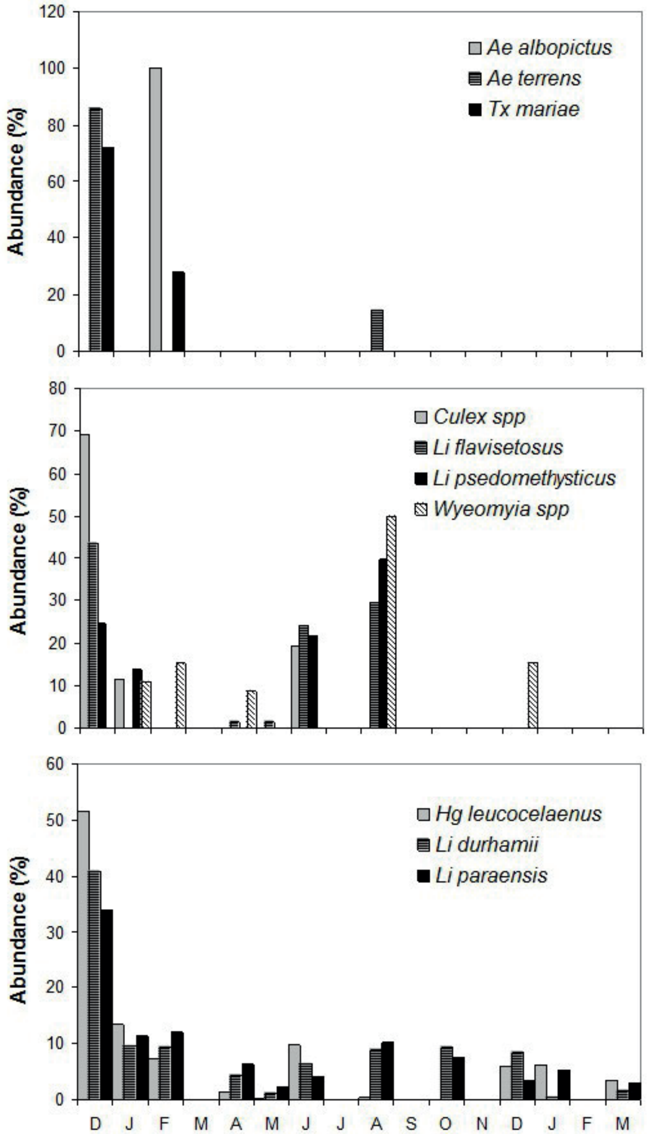

Figure 2. Temporal pattern of species abundance (expressed as percentages) per ovitrap elevation above ground level.

Table 5. Results of stepwise multiple linear regression analyses of mosquito egg proportion per height and measures of diversity as functions of temperature (monthly maximum and minimum), rainfall, and relative humidity in the same or previous month (only significant models are shown).

Variable	Parameter	T	P
	High ovitraps (7 and 9 m)		
Proportion at high traps			
Intercept	0.49 ± 0.23	2.14	0.048
T_{med}	0.03 ± 0.01	3.24	0.005
T_{max} (previous)	-0.02 ± 0.01	-2.35	0.032
Shannon diversity			
Intercept	1.36 ± 0.22		<0.0001
T_{max} (previous)	-0.02 ± 0.01		0.012
T_{min}	0.01 ± 0.01		0.043
Simpson diversity			
Intercept	1.30 ± 0.18	7.24	<0.0001
T_{max} (previous)	-0.02 ± 0.01	-2.82	0.012
Te_{min}	0.01 ± 0.01	2.19	0.043

Only variables with $p < 0.05$ were retained. T_{max} (previous) = Monthly maximum temperature of the previous month; T_{min} = Monthly minimum temperature of the same month.

DISCUSSION

Knowledge of community biodiversity of mosquitoes in the Atlantic Forest is relevant for the assessment of the possible changes in their behavior and adaptations according to the diverse environmental conditions of this region or where the environment has undergone or is undergoing anthropic disturbances. Of the species captured in this study, two are notable for their efficiency in transmitting various arboviruses: *Aedes albopictus* and *Haemagogus leucocelaenus* (Arnell 1973, Gratz 2004). Arbovirus diseases such as dengue and yellow fever are common in Brazilian populations (Vasconcelos 2010).

REGUA is known to have a high diversity of mosquitoes. Parallel surveillance of adult mosquitoes using light traps within the reserve detected 48 species belonging to 14 genera (Alencar et al. 2015). In all, ten mosquito species were detected in the ovitraps in our study, a species richness higher than for the four species captured in a previous study performed in the more degraded Atlantic Forest patches near the Minas Gerais State boundary (Alencar et al. 2013). It also reflects a high mosquito diversity, considering that only one type of larval habitat (a container) was surveyed, although there are many other habitats available for mosquitoes in the Atlantic Forest. Negative correlations of species richness and diversity with above-ground height suggest that the conditions for mosquitoes are generally better closer to the ground. Climatic conditions might also play a role in the vertical selection of oviposition sites because eggs were laid at higher locations when the average temperatures were high and might influence the humidity required for embryogenesis (Impoinvil et al.

2007). Yanoviak (1999) investigated the diversity of macroorganisms in natural and artificial tree-holes of Panama and indicated that species richness was lower in the canopy than at midstory and understory heights, which was partly attributed to the frequent drying of tree-holes and higher temperatures at tree crowns.

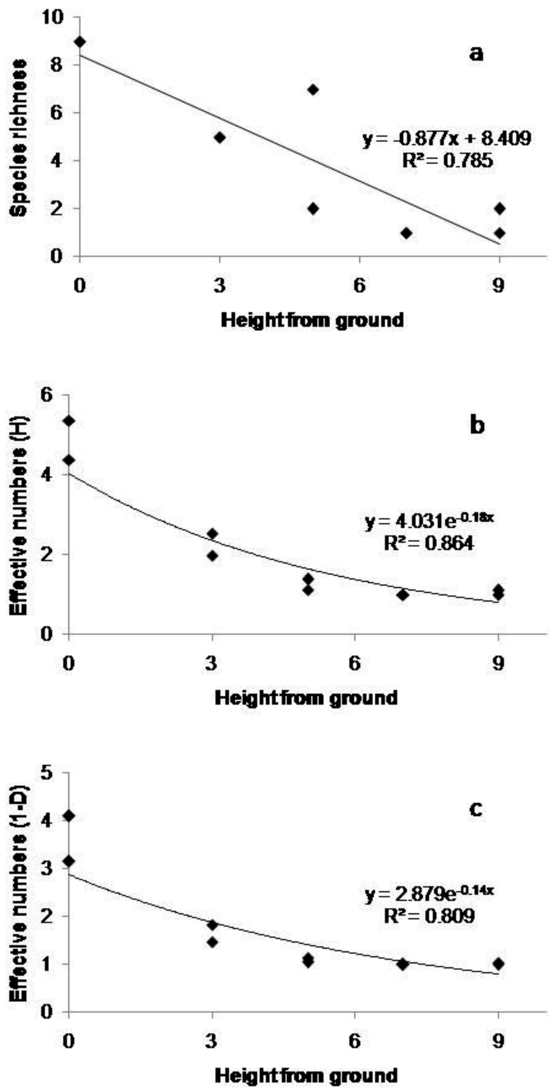

Figure 3. Regressions between height and a. observed species richness (S); b. effective number of species (based on Shannon-W); c. effective number of species (based on Simpson 1-D index).

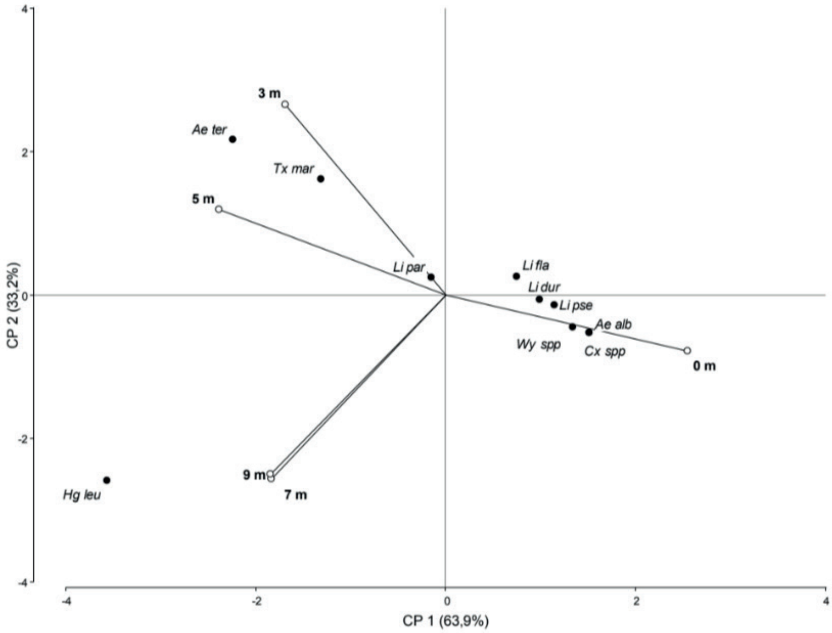

Figure 4. Principal components analysis of species abundances (transformed to percentage per species) at each of the five heights above the ground level.

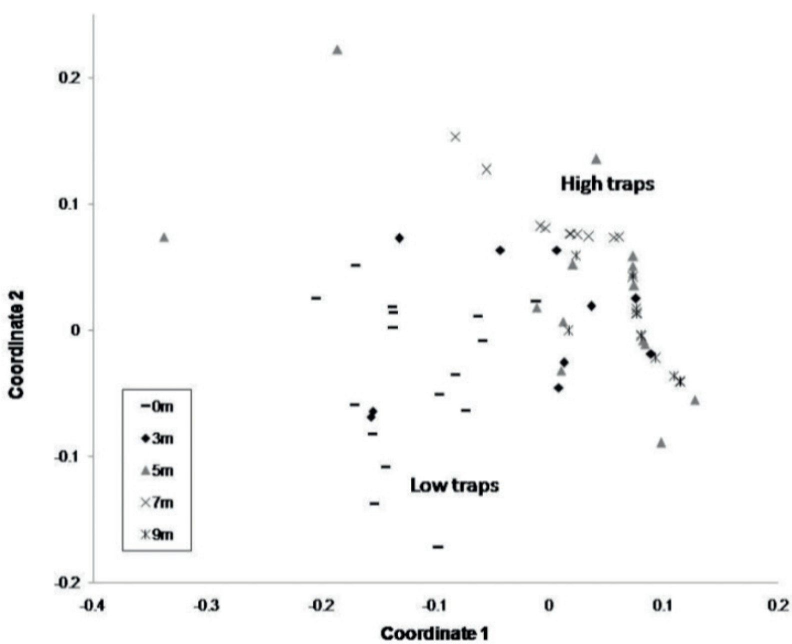

Figure 5. Non-parametric multidimensional scaling plot of ten mosquito species collected bimonthly at five heights above the ground (0, 3, 5, 7, and 9 m) between December, 2012 and February, 2014. Stress is 0.16, indicating a good representation of the data in the two- dimensional ordination plot.

Haemagogus leucocelaenus were found in the greatest densities throughout the seasons and colonized traps installed at all heights, suggesting that they are generalists. These results are consistent with those of Alencar et al. (2013) who found a higher frequency of *Hg. leucocelaenus* during all sampling periods and most often at the highest levels in southeastern Brazil. Eggs of *Haemagogus* species can resist desiccation for up to seven months (Galindo et al. 1955). Hatchability of eggs depends on several factors that might be related to the height of larval habitat, such as seasonal differences, individual conditions of females, and decreased oxygen concentrations in water (Forattini 1965). Forattini (1965) suggested that female *Haemagogus* predominantly search for a blood meal at the highest levels of a tree canopy. However, *Hg. leucocelaenus* eggs were collected from a broad range of heights. The selection of sites at greater heights for oviposition might be a strategy to reduce interspecific competition, since *Hg. leucocelaenus* was the only species that laid eggs in ovitraps set at 7 and 9 m.

In the present study, the numbers of *Ae. albopictus* were low in February, with eggs only collected from ground-level ovitraps. Alencar et al. (2013) also showed that *Ae. albopictus* was more frequent at heights of <3 m, although the eggs were collected from the highest level of tree strata (7 m), particularly during April, when the highest number of eggs were collected. Oviposition preferences of mosquitoes were studied in Sri Lanka by using oviposition traps made of canes of giant bamboo. Although 23% of these traps installed at 7 m were positive, accounting for 17% of the eggs collected, more eggs and occurrences of *Ae. albopictus* were recorded at the ground-level traps (Amerasinghe and Alagoda 1984). The occurrence of *Ae. albopictus* only in low ovitraps might be attributed to the high availability of potential larval habitats at this height, since Obenauer et al. (2009) suggested that more opportunistic and variable oviposition behavior was caused by a potential decrease in habitat availability.

Toxorhynchites species are obligate predators (Lounibos 1985). In the southern United States, *Aedes triseriatus* inhabiting treeholes avoid excessive contact with predaceous *Toxorhynchites* larvae by limiting oviposition to deep, rot-holes of trees (Copeland and Craig 1990). Interestingly, *Tx. mariae* was positively associated with most mosquito species found in the ovitraps established at low heights. Since the other culicids are potential prey for *Tx. mariae*, the positive associations could likely be the result of the oviposition preferences of females for sites with more potential food, since the oviposition behavior of adult mosquitoes is responsible for the spatial occurrence of larvae (Lounibos 1985). Determining the oviposition sequences of different species would be necessary to confirm whether *Tx. mariae* deposits its eggs at sites where eggs from other species are present or whether other culicid species are indifferent to the presence of *Toxorhynchites* eggs.

Species associations were mostly consistent with their main height occurrences. There were negative associations between species occurring at different heights and positive associations for species at the same heights. However, *Ae. albopictus* and *Li. durhamii,* both detected mainly at ground level ovitraps, were negatively associated. These two species were also found to be negatively associated in tree holes in gallery forests of Sao Paulo State, Brazil (Tubaki et al. 2010). Since ovitrap conditions were similar (within the same heights), the possibility of behavioral avoidance should be further explored.

The information about the oviposition behavior of Culicidae species having an acrodendrophilic behavior might be useful for obtaining biological data for yellow fever surveillance programs. Our study findings provide relevant information for the surveillance and control operations of medically important vectors of arboviruses, including the yellow fever arbovirus, such as *Ae. albopictus* and *Hg. leucocelaenus*.

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