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Observations on *Haemagogus janthinomys* Dyar (Diptera: Culicidae) and other Mosquito Populations within Tree Holes in a Gallery Forest in the Northwestern Region of Sao Paulo State, Brazil

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ABSTRACT - In 2000, an outbreak of sylvatic yellow fever possibly occurred in gallery forests of the Grande river in the Paraná basin in the northwestern region of São Paulo state. The aim of this study was to obtain information on the bionomics of *Haemagogus* and other mosquitoes inside tree holes in that area. Eighteen open tree holes were sampled for immature specimens. Adults were collected twice a month in the forest in Santa Albertina county from July 2000 to June 2001. The seasonal frequency of fourth instars was obtained by the Williams geometric mean (Mw), while the adult frequency was estimated either by hourly arithmetic or the Williams' means. Cole's index was applied to evaluate larval inter-specific associations. Among the ten mosquito species identified, the most abundant was *Aedes terrens* Walker followed by *Sabethes tridentatus* Cerqueira and *Haemagogus janthinomys* Dyar. Larval and adult abundance of these species was higher in summer than in winter. Although larval abundance of *Hg. janthinomys* peaked in the rainy season, correlation with rainfall was not significant. Six groups of larval associations were distinguished, one of which the most positively stable. The *Hg. janthinomys* and *Ae. terrens* association was significant, and *Limatus durhamii* Theobald was the species with most negative associations.

KEY WORDS: *Sabethes tridentatus*, *Aedes terrens*, *Limatus durhamii*, sylvatic yellow fever; immature seasonality; adult abundance

The first outbreak of sylvatic yellow fever (SYF) occurred in Brazil in 1908. In the thirties, the disease spread into the States of Goias, Minas Gerais and Mato Grosso. Soper (1936) used the term "jungle yellow fever" to differentiate the sylvatic disease from the rural form transmitted by *Aedes aegypti* Linnaeus. Further studies (Soper *et al* 1932, Lane 1936, Antunes & Whitmani 1937, Causey & Kumm 1948, Kumm 1950) described the diurnal sylvatic mosquitoes incriminated in the transmission of the SYF and among these *Haemagogus janthinomys* Dyar, the primary vector. The epidemy spread throughout the western region of the State of São Paulo, finally reaching the northwestern region of the state of Paraná. Lane (1936) identified *Hg. janthinomys* in localities with SYF in the western region of São Paulo, but those collections were performed as surveys in the counties. These surveys reported the occurrence of species but they did not add information about the environment or diel mosquito activity.

Studies on adult mosquito activity related to the influence of rainfall were addressed by Kumm (1950) in the locality of Passos, Minas Gerais. Although Dégallier *et al* (1992) introduced the concept of gallery forests as a phytoclimatic zones, gallery forests were better characterized by Forattini and Gomes (1988) as shelters for *Haemagogus* mosquitoes and non-human primates involved in maintaining the virus cycle and bridges for propagation along their course, in modified environments for agricultural practices. These sparsely forested areas are nowadays recognized as fragmented areas in the savannah biome.

In 2000, sixty-four years after the first record of *Hg. janthinomys*, two cases of SYF occurred in the northwestern region of São Paulo, one in the Santa Albertina county and another in the Ouroeste county. There is not much information about the immatures of sylvatic mosquitoes bred inside tree holes that could help to clarify the SYF epidemiology. The aim of this work was to describe the seasonal frequency of immature and adult *Hg. janthinomys* forms and of other mosquitoes occurring inside tree holes in a gallery forest in Santa Albertina County located in the southeastern region of the savannah, a potential area of SYF transmission.

Material and Methods

The Rancho do Vale (50º 48' W, 19º 58'S) is a patch of gallery forest situated in the Santa Albertina county, Sao Paulo state, on the left margin of the Grande river. Santa Albertina county is located in the northwestern region of Sao Paulo State, Brazil and covers an area of 274 km^2, most of which is rural. This county belongs to the eleventh region of the state. The climate is of Aw type, alternately dry and humid in that region, according to Koeppen. The mean values of rainfall and temperature oscillate between 150-1000 mm and 14-31ºC in the dry and wet seasons, respectively (Troppmair 1975). Gallery forests are corridors of evergreen mesophytic vegetation present in the savannah (Tubaki *et al* 2004).

Larval collections were carried out fortnightly on the Rancho do Vale gallery forest from July 2000 to June 2001. Trees were examined every 5 m, eighteen open tree holes per tree on average. Samples of larvae and pupae were transported to the laboratory and kept alive until the imago state. Adult were then killed and immature ecdyses preserved and mounted for identification. The tree holes with continuous larval production were chosen for analysis and the seasonal abundance of the most frequent larval mosquito species found in the tree holes was estimated by using Williams' mean (Haddow 1960) and standard errors to standardize results.

The adult collections followed larval sampling on two subsequent days. Two men (previously immunized against YF) carried out catches for 15 min every hour. Collections were undertaken on humans in movement, with the aim of improving the efficiency in collecting *Hg*. *janthinomys* females through constant human intrusion into the forest. The adult collection period ranged from 9:00h to 16:00h and an hourly average per month was calculated with standard errors to standardize results.

Daily rainfall records were obtained from the Casa da Agricultura in Santa Albertina whereupon monthly records were compared with decennial ones (July 1989 through June 1999) by the non-parametric Man-Whitney (U) test to verify whether they were anomalous at the period of study. Rainfall was accumulated over four weeks prior each larval collection and the relationship between species abundances and accumulated rainfall, was tested for Spearman correlations.

An evaluation of the association between tree hole mosquito species was done to provide information on those species sharing tree holes with *Hg*. *janthinomys* in the Rancho do Vale gallery forest. Cole's coefficient of interspecific association (C_{AB}) (1949) takes into consideration the occurrence of two coexistent species in collections. The values change from a perfect negative association (-1), when habitat requirements are distinct or one species has the tendency to exclude the other, to a perfect positive association (+1), when the species have both overlapping habitats and requirements or interact to favor mutual presence. A value of zero represents random or neutral association. The Cole index was tested for significance by using the Fisher test when values were equal or lower than 5.

Results

Rainfall data. The records of rainfall over the study period were compared with those of decennial one whereby they showed similar curves (U = 60.0; P > 0.05), thereby suggesting that the distribution of rainfall was typically seasonal during the period. There was a total rainfall of 1, 412, 6 mm throughout with a peak in January (Fig 1).

Larval abundance. A total of 10 mosquito species were identified in the larval survey (Table 1). The most abundant species (50%) was *Aedes terrens* Walker with 154 specimens (males and females) followed by *Sabethes tridentatus* Cerqueira (24.4%) and *Hg*. *janthinomys* (8.8%) (Table 1).

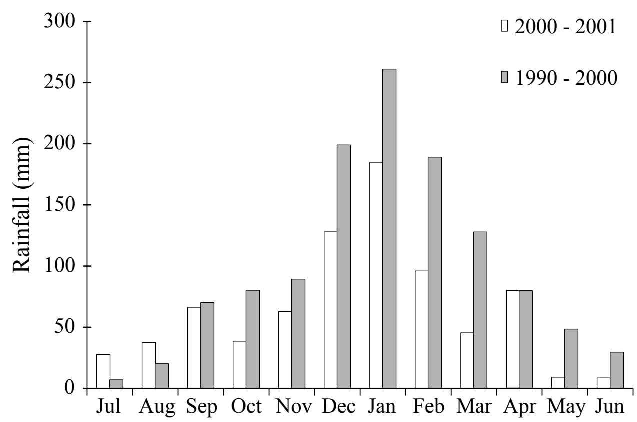

Fig 1 Comparison of rainfall distribution to decenial data (July 1989 to June 1999) and period of study (July 2000 through June 2001), Santa Albertina county, SP, Brazil.

Table 1 Frequency of species from larval sampling in tree holes at Rancho do Vale, S. Albertina, SP, Brazil, July 2000 through July 2001 (n = number of individuals).

Species	♀(n)	♂(n)	Total ♀+♂(n)
Aedes aegypti	5	2	7
Ae. albopictus	8	–	8
Ae. terrens	142	12	154
Culex dolosus affinis	–	–	–
Cx. quinquefasciatus	4	2	6
Culex (*Culex*) sp.	1	–	1
Cx. (*Melanoconion*) sp.	2	1	3
Haemagogus janthinomys	16	11	27
Limatus durhamii	8	–	8
Sabethes glaucodaemon	1	–	1
Sa. tridentatus	64	11	75
Toxorhynchites theobaldi	17	1	18
Total	268	40	308

Abbreviations follow Reinert (1975, 1982, 1991).

Larval seasonal abundance of *Ae. terrens* and *Sa. tridentatus* were highest in the wet season between November and March, with Mw (Williams' means) values equal to 2.4 and 1.7, respectively. Their larval abundance also peaked in the dry season and reached Mw values of 2.4 for *Ae. terrens* and 3.5 for *Sa. tridentatus*. The larval abundance of *Hg. janthinomys* was only slightly correlated with rainfall, but with no statistical support ($r_s = 0.03$, $P = 0.8$). The larval abundances of *Sa. tridentatus* ($r_s = 0.18$, $P = 0.2$) were not correlated with rainfall at all (Fig 2).

Adult seasonal abundance. Adult abundance of *Ae. terrens*, *Sa. tridentatus* and *Hg. janthinomys* was also highest during the rainy season, with *Hg. janthinomys* abundance being twice as high the abundance of the other two species. *Aedes terrens* and *Sa. tridentatus* adult abundance peaked in February whereas in *Hg. janthinomys* this occurred in January. Values obtained as arithmetic means were almost twice of those of the Williams' mean. Differences between the arithmetic mean and Williams' mean were explained by the amount of hours considered in the calculation (Table 2).

Cole's larval species associations. A comparison of interspecific association coefficient values (C_{AB}) among nine larval species from the tree holes allowed for the discrimination of six association groups. As shown in Table 3, there were four pairs of perfectly negatively associated species (-1 ± 2.46 to -1.18). *Limatus durhamii* Theobald was the species with the most negative associations (5) from all pairs compared. Completely negative associations occurred between *Sa. tridentatus* and *Ae. aegypti*, besides *Li. durhamii* and *Ae. aegypti*, *Ae. albopictus* Skuse, *Sa. tridentatus* and *Cx. quinquefasciatus* Say. The second group was slightly negatively associated (-0.25 ± 0.34 to -0.04 ± 0.30). In this group, *Tx. theobaldi* (Dyar and Knab) was negatively associated with both *Hg. janthinomys* and *Ae. terrens*. The negative association between *Tx. theobaldi* and *Ae. terrens* was significant ($P < 0.05$). Twelve pairs of species were included in the third group (0.01 ± 0.14 to 0.16 ± 0.45) whose associations were considered neutral. In this group, *Hg. janthinomys* was only slightly associated with *Cx. dolosus*

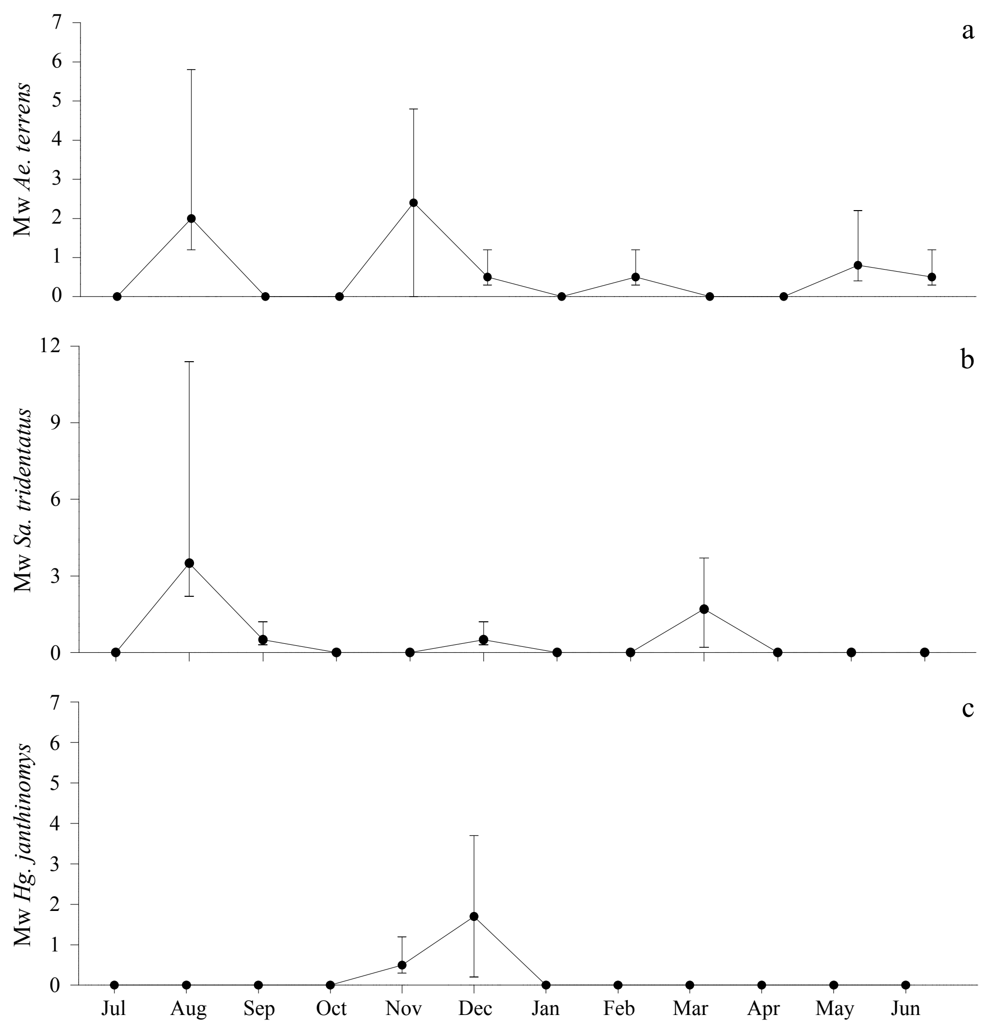

Fig 2 Seasonal larval abundance of the most abundant species found in tree holes: a) *Aedes terrens*, b) *Sabethes tridentatus* and c) *Haemogogus janthinomys* in Santa Albertina, SP, July 2000 to June 2001. Full circles represent Williams´ mean (Mw) and bars are standard errors.

Table 2 Hourly and Williams's means of the most abundant adult mosquito species collected in Rancho do Vale, S. Albertina, SP, Brazil, from July 2000 to June 2001.

Species	*Aedes terrens*		*Sabethes tridentatus*		*Haemagogus janthinomys*	
Month	Mean (SE)	Mw	Mean (SE)	Mw	Mean (SE)	Mw
July	–	–	0.2	0.12	–	–
August	–	–	0.4 (0.1)	0.17	–	–
September	–	–	–	–	0.4 (0.1)	0.23
October	–	–	0.5	0.11	0.5	0.11
November	–	–	0.7 (0.1)	0.87	0.5	0.11
December	0.5	0.11	–	–	1.9 (0.9)	1.15
January	1.7 (0.5)	0.50	1.3 (0.3)	0.31	4.8 (3.0)	2.52
February	2 (0.6)	1.21	2.3 (1.8)	0.45	1.0	0.77
March	0.8 (0.2)	0.90	1.8 (0.6)	1.22	0.6 (0.1)	0.52
April	0.5	0.31	0.3	0.23	0.3	0.85
May	–	–	–	–	–	–
June	–	–	–	–	–	–

Table 3 Values of Cole Index (C_{AB}) among tree holes mosquito species collected at Rancho do Vale, Santa Albertina, SP, Brazil.

Coefficient of association values (C_{AB}) among tree holes mosquito species					
Species	*Aedes aegypti*	*Aedes albopictus*	*Culex dolosus aff.*	*Culex quinquefasciatus*	*Haemagogus janthinomys*
	C_{AB}	C_{AB}	C_{AB}	C_{AB}	C_{AB}
Ae. aegypti	–	–	–	–	–
Ae. albopictus	0.46 ± 0.18	–	–	–	–
Cx. dolosus aff.	-0.15 ± 0.97	-1 ± 1.68	–	–	–
Cx. quinquefasciatus	1 ± 0.26	0.46 ± 0.18	-1 ± 2.46	–	–
Hg. janthinomys	0.14 ± 0.01	1 ± 0.47	0.08 ± 0.14	0.14 ± 0.10	–
Li. durhamii	-1 ± 2.46	-1 ± 1.68	-1 ± 1.68	-1 ± 2.46	0.17 ± 0.45
Ae. terrens	0.05 ± 0.06	0.10 ± 0.21	-0.17 ± 0.21	0.05 ± 0.06	0.44 ± 0.17
Sa. tridentatus	-1 ± 1.18	0.04 ± 0.12	0.04 ± 0.12	0.04 ± 0.12	0 ± 0.22
Tx. theobaldi	1 ± 1.03	-0.07 ± 0.70	0.01 ± 0.14	0.06 ± 0.06	-0.25 ± 0.34
Species	*Limatus durhamii*	*Aedes terrens*	*Sabethes tridentatus*	*Toxorhynchites theobaldi*	
	C_{AB}	C_{AB}	C_{AB}	C_{AB}	
Ae. aegypti	–	–	–	–	
Ae. albopictus	–	–	–	–	
Cx. dolosus aff.	–	–	–	–	
Cx. quinquefasciatus	–	–	–	–	
Hg. janthinomys	–	–	–	–	
Li. durhamii	–	–	–	–	
Ae. terrens	0.03 ± 0.21	–	–	–	
Sa. tridentatus	-1 ± 0.93	-0.17 ± 0,26	–	–	
Tx. theobaldi	1 ± 0.70	-0.2 ± 0.33	-0.05 ± 0.30	–	

affinis Lynch Arribalzaga and occurred in three of four pairs of associations, which were similar to those associations with *Cx. quinquefasciatus* and *Ae. aegypti*. The fourth group was characterized by positive associations (0.44 ± 0.17 to 0.46 ± 0.18) and revealed less variation when compared with the other groups. In this group. *Hg. janthinomys* was significantly associated with *Ae. terrens* ($P < 0.001$). The fifth group was constituted by four pairs with perfect positive associations *Ae. aegypti* and *Cx. quinquefasciatus*, *Ae. aegypti* and *Tx. theobaldi*, *Ae. albopictus* and *Hg. janthinomys*, *Tx. theobaldi* and *Li. durhamii* (Table 3).

Fig 3 shows the distribution of the Cole's indexes to mosquito species associated to the two highly synantropic (*Ae. aegypti* and *Ae. albopictus*) and to the two sylvatic aedine species (*Ae. terrens* and *Hg janthinomys*). Species indexes associated to the most synantropic species are shown in C_{AB1}, and their values are not repeated in C_{AB2}. The variation of the coefficient values was larger in C_{AB1} than in C_{AB2}.

Discussion

The composition of immature species inside open tree holes is restricted to only a few species and is quite specific, having been previously described in a survey on natural microhabitats undertaken in Tremembe County (Gomes *et al* 1992), another phytogeographical region in the State of Sao Paulo. *Haemagogus janthinomys* has not been recorded in Sao Paulo since Lane's reports in 1936. At that time, Lane identified *Hg. janthinomys* in the Alta Araraquarense, a region that included Santa Albertina County and was formerly named Tanaby. *Haemagogus janthinomys* and *Haemagogus capricornii* Lutz are very much alike, they have the same range of distribution in the southeastern region of Brazil and are only distinguishable by the male genitalia. We confirmed the specific status of *Hg. janthinomys* by rearing the larvae collected in the tree holes until the imago stage and by identifying the specimens by using characters of the male genitalia. *Haemagogus janthinomys* adult seasonal activity in Santa Albertina was similar to that observed in Trinidad (Chadee 1992), with a small number of females in the dry season and high abundance in the wet season. If *Hg. janthinomys* number of females collected monthly in Chaguaramas Forest (Chadee 1992) is reduced proportionally to 15 min, the results are similar to those reported in this study. The diel activity of *Hg. janthinomys* adults is not described in our study although the period of collection varied between 9:00h and 16:00h, coinciding with the period of activity reported for *Hg. janthinomys* and close related species (Forattini & Gomes 1988, Chadee 1992, Camargo-Neves *et al* 2005). Our data also suggest that *Hg. janthinomys* is to be found in drier areas than *Hg. capricornii* in Sao Paulo, in accordance with the distribution of sylvatic yellow fever risk areas (Dégallier *et al* 1992).

The correlation of mosquito larval abundance in ephemeral breeding sites with rainfall was tentatively shown in our study area. This relationship is currently pursued in the investigations to determine the risk of YF transmission. Dégallier *et al* (2006) inferred a time lapse of 11 to 12 days between egg hatching induced by rainfall and adult emergence. They also pointed out the occurrence of YF cases two months after heavy rains corroborating Chadee (1992).

Haemagogus janthinomys biting activity peaked in

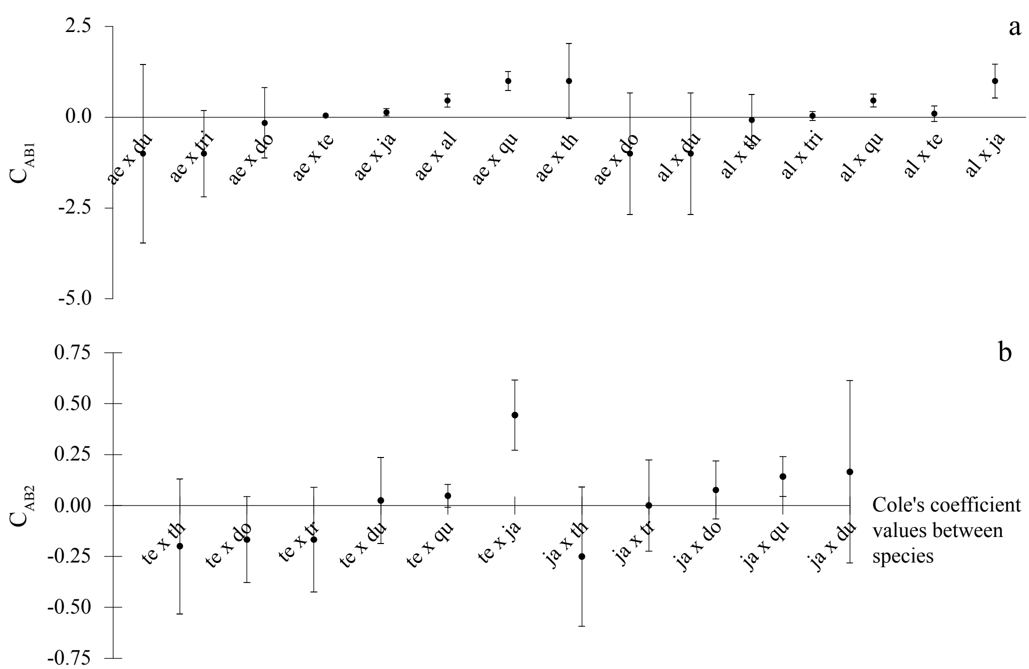

Fig 3 Distribution of Cole Index of larval species association with *Aedes aegypti* and *Ae. albopictus*. b) *Ae. terrens* and *Haemagogus janthinomys* in tree holes in S. Albertina county, SP. Full circles represent Cole Index values and bars are standard errors. Legend: Ae – *Ae. aegypti*, al – *Ae. albopictus*, do – *Culex dolosus*, du – *Limatus durhamii*, ja – *Hg. janthinomys*, te – *Ae. terrens*, th – *Toxorhynchites theobaldi*, tr – *Sabethes tridentatus*, qu – *Cx. quinquefasciatus*.

January when rainfall was more intense and the beginning of symptoms in humans in March, all according to data of the epidemiological vigilance service, seem to be in agreement with Dégallier's hypothesis. This might also suggest that the filling of tree holes by rainfall would be simultaneous with and synchronize the egg hatching and larval development towards immediate imago formation as in other ephemeral breeding sites. Nevertheless, rainfall might cause the flooding of open tree holes, with a subsequent loss or decrease in *Hg. janthinomys*, *Ae. terrens* and *Sa. tridentatus* populations. The difficulty to assign a sampling program adjusted to day-lags and rainfall would explain the low larval abundance and the ineffective attempts to correlate it with rainfall.

The abundance of *Sa. tridentatus* immatures, increased in the dry and post-wet seasons when compared with the wet season. Nevertheless, the abundance of *Sa. tridentatus* females in the dry and wet seasons was in agreement with the pattern in *Sabethes chloropterus* (Von Humboldt) in Trinidad (Chadee 1990). As shown in a prior evaluation (Rocco *et al* 2003), *Sa. tridentatus* does not seem to be associated with the transmission of YF.

Aedes terrens is quite adaptive occurring in several habitats (Lopes *et al* 2004). It occupied natural breeding sites as observed in this study, but may also dwell in artificial containers, mainly in patches of riparian forest in modified environments (Zequi *et al* 2005).

The Cole index of insterspecific association as related to the two synanthropic species suggests that the associations between *Ae. aegypti* and *Ae. terrens*, *Ae. aegypti* and *Hg. janthinomys, Ae. aegypti* and *Ae. albopictus* were casual, whereas the same situation is presented in associations between *Ae. albopictus* and *Tx. theobaldi*, and *Ae. albopictus* and *Sa. tridentatus* (Fig 3a). On considering the sylvatic species, casual associations should be suggested between *Cx. quinquefasciatus* and *Ae. terrens, Ae. terrens* and *Li. durhamii* and *Hg. janthinomys* and *Sa. tridentatus* (Fig 3b). Although the invasive *Ae. aegypti* was found within the tree holes, it has been pointed out that *Ae. aegypti* activity made it more vulnerable to predation when compared with other species (Grill & Juliano 1996) from the Aedine tribe. It is likely that some associations with *Ae. aegypti* are usual and stable compared to others, and might be explained by feeding habits or unknown competitive interactions.

The slight variation in the Cole index obtained for sylvatic species shows that *Hg. janthinomys* and *Cx. quinquefasciatus,* and specially *Ae. terrens* and *Hg. janthinomys* associations are usual, and possibly the species shared the same requirements in terms of physical environmental conditions and food abundance in tree holes. The associations *Ae. albopictus* and *Ae. terrens* on one hand and *Ae. albopictus* and *Hg. janthinomys* on the other were positive and stable. It also indicates the restrained behavior of the invasive *Ae. albopictus* in exploring natural breeding sites as opposed to the synanthropic species (Lounibos *et al* 2001, Braks *et al* 2003). Special concern should also be given to the ubiquitous behavior of *Ae. albopictus*. This observation suggests that positively associated species may not have the same obligatory feeding behavior, but are flexible, depending on the variation in food abundance, be it regulated by predators (Alto *et al* 2005, Merritt *et al* 1992) or by the quality of tree hole environment. Flexible habits could possibly explain the nature of mosquito larval associations. Although mosquito species relationships are poorly understood in natural containers, experimental investigation gives evidence of these associations although restricted to invasive species, such as *Ae. albopictus* and *Ae. aegypti* (Juliano 2009).

Even if the attempt to correlate rainfall and the larval abundance for the most frequent species was ineffective, we presume that the wet season exerted a generally positive effect on the abundance of *Hg. janthinomys* and other tree hole mosquitoes, thereby suggesting that greater epidemiological vigilance should be exercised in YF risk during the wet season.

Acknowledgments

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