

## SCIENTIFIC NOTE

### COMPARATIVE ANALYSIS BETWEEN SAMPLING METHODS FOR IMMATURE MOSQUITOES IN AN ATLANTIC FOREST FRAGMENT IN BRAZIL

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**ABSTRACT.** In this study, traps were set out to improve mosquito monitoring, study their viability, and determine the most suitable traps for female mosquito species of epidemiological importance during oviposition. The effectiveness of 3 types of traps (bamboo traps, tire traps, and ovitraps) were compared at 2 sampling sites. A total of 24 traps were installed on the ground at elevations of 3 m, 6 m, and 9 m in a fragment of the Atlantic Forest in the municipality of Nova Iguaçu, Rio de Janeiro State, Brazil. The samplings took place every 2 wk from July 2017 to June 2018. A total of 1,854 mosquitoes belonging to 16 different species were identified, of which 2 species are involved in the transmission of arbovirus: *Haemagogus leucocelaenus* (Dyar and Shannon) and *Aedes albopictus* Skuse. Larval tire traps were the most effective at trapping females laying eggs, followed by ovitraps. The results were consistent with the usual habitats of certain species that were found in tire traps, which are artificial objects commonly found in human environments.

**KEY WORDS** Atlantic Forest, Culicidae, immature mosquitoes, sylvatic habitat, traps

Studies on faunistic diversity contribute to monitoring, population control, and identification efforts concerned with mosquitoes. In addition, they can help to explain the impact of current environmental changes resulting from human activity. For example, excessive population growth leads humans to encroach on natural ecosystems and construct anthropogenic environments in which unexpected biotic interactions result in certain species transmitting infectious diseases (Guimarães et al. 2000, Montes 2005).

Exploring the distribution and abundance of mosquitoes in the remaining characteristic areas of the Atlantic Forest of Brazil is crucial for understanding the eco-epidemiology of mosquito-borne diseases. The Atlantic Forest biome has a high diversity of flora and vertebrate and invertebrate fauna, providing a wide range of niches for the development of mosquito species. In the Rio de Janeiro State, studies have focused on the presence of infectious disease vectors, primarily in urban areas (Alencar et al. 2011).

Entomological traps are important tools in studying geographic distribution patterns and biological cycles. They also contribute to studies on biodiversity, bioindicators of environmental change, patterns and control of ecological dynamics, and taxonomy of insect vectors. Collecting mosquitoes helps to

characterize the species that make up an ecosystem, providing information on their richness, dominance, abundance, and diversity (Rafael 2002).

The objective of this study was to assess and compare different sampling methods of a mosquito community in a fragment of the Atlantic Forest belonging to the Associação dos Taifeiros da Armada (ATA), located in the municipality of Nova Iguaçu, Rio de Janeiro State, Brazil. The following 2 sampling sites were established in ATA: site 1 (22°37'50.3"S, 43°27'13.8"W; elevation 68 m) and site 2 (22°37'27.6"S, 43°27'18.5"W; elevation 193 m). Samplings took place every 2 wk from July 2017 to June 2018. Ovitrap (OT), bamboo traps (BT), and tire traps (TT) were set up at 2 different sampling sites. At each site, each type of trap was installed at a different elevation (ground level, 3 m, 6 m, and 9 m), totaling 24 traps. All the containers were filled with water from streams in the study site. The sampling points have contrasting characteristics, mainly in terms of vegetation. Site 1 is about 200 m from an inhabited area with more disturbed vegetation, recent reforestation, many small seedlings, and few large trees, allowing constant penetration of light, heat, and wind. This sampling site is near a stream, and bromeliad species are present. Site 2 is located further into a secondary forest with more well-preserved vegetation, many larger trees, and a dense canopy, making it difficult for light and wind to penetrate. The moisture levels are higher than at site 1; there is also a series of waterfalls nearby. The oviposition traps were set up as follows: 1) TT with reservoir made of one-third cross section of a motorcycle tire filled with 500 ml of water, which was enough to fill it to two-thirds; 2) BT made with pieces of bamboo with internodes as separators

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Fig. 1. Artificial traps used in this study in Nova Iguaçu, Rio de Janeiro State, Brazil: (a) traps installed on the ground; (b) traps installed at the height of 3 m. BT, bamboo trap; TT, tire trap; OT, ovitrap.

forming a container about 30 cm tall with an opening of 25 cm in diam and filled with 500 ml of water; 3) OT made from open matte black containers with a 400-ml capacity and 4 pieces of plywood (Eucatex® boards; Sao Paulo, Brazil) measuring  $2.5 \times 14$  cm and laid vertically inside the trap using clips (Fig. 1).

The containers were filled with water from streams in the study area. Larvae and pupae found in the traps were collected, using a fine brush (pipette) and transferred into 250-ml plastic bags (Whirl-Pak® bags; BioQuip®, Compton, CA), and transported to the Diptera Laboratory at the Instituto Oswaldo Cruz, Rio de Janeiro, Brazil, where they were kept in small polyethylene cups containing water from the same trap from which they were collected. The water was topped up periodically with distilled water when evaporation occurred. The cups were kept in a drying oven with a controlled thermoperiod and photoperiod at a temperature of  $28 \pm 1^\circ\text{C}$ , 75% relative humidity, and photoperiod of 12 h light and 12 h dark.

Larvae were fed with TetraMin® (Tetra, Blacksburg, VA) fish food and monitored daily until the immature mosquitoes reached the adult stage. The pieces of plywood were kept in a moist chamber to be transported to the laboratory, where the eggs on the plywood were allowed to hatch in basins containing chlorinated water. The adults were identified using specific keys and descriptions from the literature (Lane 1953, Forattini 2002). All of the specimens were then deposited at the Entomological Collection of the Instituto Oswaldo Cruz (Fiocruz), Rio de Janeiro, Brazil, under the title “ATA Sampling” (“Coleção ATA”).

Throughout the study, 1,854 specimens belonging to 16 different species were identified (Table 1). Site 2 had a higher species richness and relative

population abundance (A%), with 1,006 individuals over site 1 that had 848 specimens. There was no significant difference in species diversity at the 2 sites, using the Shannon’s diversity index ( $t$ -test,  $P < 0.05$ ).

The TT showed the highest A%, followed by OT and BT. At site 1, *Trichoprosopon digitatum* Rondoni had the highest A% in BT and the lowest in OT. *Culex iridescens* (Lutz) had a much lower A% at site 1, with TT presenting the highest A%. *Haemagogus leucocelaenus* (Dyar and Shannon) had the highest A% in OT and the lowest in TT. *Limatus pseudomethisticus* (Bonne-Wepster and Bonner) showed the highest A% in TT and the lowest in BT, while *Li. durhamii* Theobald had high A% in all traps, with the highest values in TT. At site 2, *Tr. digitatum* followed the same pattern as at site 1, with the highest A% in BT and the lowest in OT.

*Culex iridescens* had the highest A% in TT and lowest in BT, and *Hg. leucocelaenus* had the highest A% in OT and the lowest in BT. *Limatus pseudomethisticus* had the highest A% in TT and the lowest in BT, while *Li. durhamii* was highly abundant in all traps, with the highest A% in TT. Regarding the vertical distribution of species at site 1, *Li. durhamii* was found at all levels with the highest abundance in traps at ground level. On the other hand, *Cx. iridescens* was found only in traps set at 3 m. *Limatus pseudomethisticus* was found in traps at ground level, 3 m, and 9 m.

*Haemagogus leucocelaenus* tended to colonize traps set at 9 m high; its abundance was recorded in traps at all other levels. At site 2, *Cx. iridescens* was not found in ground-level traps, preferring to oviposit in traps at 3 m, 6 m, and 9 m. *Limatus pseudomethisticus* preferred to oviposit in traps located at

Table 1. Absolute values (Total), relative abundance (A%), specific richness (S), and Shannon diversity index (H') for 2 sampling sites sampled between July 2017 and June 2018 in the Associação dos Taifeiros da Armada site, Nova Iguaçu, Rio de Janeiro State, Brazil.<sup>1</sup>

Species	Site 2					Site 1					Total				
	BT	TT	OT	Total	A%	BT	TT	OT	Total	A%	BT	TT	OT	Total	A%
<i>Aedes albopictus</i>	0	0	5	5	0.50	4	18	5	27	3.20	4	18	10	32	1.70
<i>Ae. terrens</i>	0	0	0	0	0.00	5	1	1	7	0.80	5	1	1	7	0.40
<i>Culex davisi</i>	2	0	0	2	0.20	0	0	0	0	0.00	2	0	0	2	0.10
<i>Cx. iridescens</i>	16	158	44	218	21.70	0	15	0	15	1.80	16	173	44	233	12.60
<i>Cx. mollis</i>	9	5	7	21	2.10	2	1	0	3	0.40	11	6	7	24	1.30
<i>Cx. pleuristriatus</i>	0	1	0	1	0.10	0	0	0	0	0.00	0	1	0	1	0.10
<i>Cx. urichii</i>	1	0	0	1	0.10	0	0	0	0	0.00	1	0	0	1	0.10
<i>Culex sp.</i>	0	0	0	0	0.00	3	0	2	5	0.60	3	0	2	5	0.30
<i>Haemagogus leucocelaenus</i>	11	15	33	59	5.90	3	0	8	11	1.30	14	15	41	70	3.80
<i>Limatus durhamii</i>	73	210	62	345	34.30	93	275	122	490	57.80	166	485	184	835	45.00
<i>Li. pseudomethisticus</i>	6	133	69	208	20.70	3	162	53	218	25.70	9	295	122	426	23.00
<i>Sabethes identicus</i>	0	0	0	0	0.00	0	0	1	1	0.10	0	0	1	1	0.10
<i>Sa. undosus</i>	12	0	0	12	1.20	0	0	0	0	0.00	12	0	0	12	0.60
<i>Trichoprosopon digitatum</i>	90	36	1	127	12.60	56	9	1	66	7.80	146	45	2	193	10.40
<i>Tr. pallidiventer</i>	3	0	1	4	0.40	1	0	0	1	0.10	4	0	1	5	0.30
<i>Toxorhynchites sp.</i>	0	0	1	1	0.10	0	3	1	4	0.50	0	3	2	5	0.30
<i>Wyeomyia aporonomia</i>	1	0	1	2	0.20	0	0	0	0	0.00	1	0	1	2	0.10
Total	224	558	224	1,006	100.00	170	484	194	848	100.00	394	1,042	418	1,854	100.00
Overall total				1,006					848					1,854	
Richness (S)				14					12					17	
Diversity (H')				1.679					1.233						

<sup>1</sup> BT, bamboo trap; TT, tire trap; OT, ovitrap.

ground level, 3 m, and 6 m; however, it was not found in traps at 9 m. *Trichoprosopon digitatum* and *Li. durhamii* were found in traps at all levels. The similarity between the species composition found in traps at different levels at the 2 sites was analyzed using the Sørensen index (SI). At site 2, similar species composition was observed between ground level and 3 m (SI = 0.67), ground level and 6 m (SI = 0.67), 3 m and 6 m (SI = 0.77), 3 m and 9 m (SI = 0.67), and 6 m and 9 m (SI = 0.67). Traps at ground level and 9 m had dissimilar species composition (SI = 0.43). At site 2, we also observed similar species composition between ground level and 3 m (SI = 0.67), ground level and 6 m (SI = 0.67), 3 m and 6 m (SI = 0.77), 3 m and 9 m (SI = 0.67), and 6 m and 9 m (SI = 0.67). Traps at ground level and 9 m had dissimilar species composition (SI = 0.43).

The highest acceptance for oviposition by female mosquitoes occurred in TT, followed by OT. This observation is in line with a previous study that assessed the effectiveness of different trapping methods, with TT showing higher rates of capture than OT (Silva et al. 2009). Lopes (1997) reported that the disposal by humans of containers capable of retaining water favors the breeding of groups of individuals from a population of mosquitoes with genetic plasticity that allows their development in these artificial breeding sites. Another study showed that OT, in addition to being more attractive even in the presence of natural breeding areas, were more effective than larval traps (Marques et al. 1993). In contrast to Marques et al. (1993), this study found that OT were the second most accepted by female

mosquitoes for oviposition, and the TT traps were the least efficient.

*Limatus durhamii* is a species that inhabits natural environments, though it adapts to anthropogenic environments with considerable ease (Marcondes 2006). Lopes (1997) reported that *Li. durhamii* reproduced preferentially in tires and plastic pots, but the regular appearance of these mosquitoes in tins and bamboo showed their acceptance also for these types of sites. Similar results were found by Lopes et al. (1985), who showed that *Li. durhamii* predominated in plastic containers. In contrast, in the present work, this species was more abundant in TT.

Zequi et al. (2005) carried out a study on immature mosquitoes in artificial breeding environments. The authors found that many mosquito species favored tires over other traps, a behavior that was most frequent in *Li. durhamii*. Similarly, the highest A% of *Li. durhamii* individuals was found in TT in this study. Species of *Trichoprosopon* exhibit a preference for natural breeding environments and are found predominantly in bamboo containers (Lopes 1997). This study echoed the findings of Lopes (1997), with *Tr. digitatum* showing its highest abundance in the BT.

*Haemagogus leucocelaenus* had no particular preference for oviposition, with individuals present in all of the sampling methods used; however, the highest A% was found in OT at 9 m, although it was also present at the other levels in the tree canopy (ground, 3 m, and 6 m), with less relative abundance. Alencar et al. (2008) reported higher numbers of *Hg. leucocelaenus* individuals in the canopies of trees,

with a tendency toward acrodendrophily. However, this result was not found by Mondet et al. (2002), who detected the highest abundance for *Hg. leucocelaenus* at ground level. Gomes et al. (2008) found that the abundance of this species was higher near to the level of tree canopies, which seems to be due to their affinity for nonhuman primates, which suggests a link between the natural source of arbovirus and human settlements located at the edges of the species' range.

Of the species collected in this study, the following were dominant at both sampling sites: *Li. durhamii*, *Li. pseudomethisticus*, *Cx. iridescens*, *Tr. digitatum*, and *Hg. leucocelaenus*. In addition, specimens were more abundant in the TT at all sampling points surveyed. It is important to highlight that *Li. durhamii* was more abundant throughout the sampling period. In the observations made by Zequi et al. (2005) in the Mata Daher Natural Reserve in Londrina, Paraná, Brazil, *Li. durhamii* was also predominant in tires. Beier et al. (1983) reported that the predilection for some species of mosquitoes to colonize tires could be explained by the similarity of these containers with tree hollows.

The artificial containers used as traps in the study area were colonized by a diverse assemblage of species. Given that the mosquito community heavily colonized the traps used in the ATA site, potential containers discarded in the area may cause an increase in mosquito populations. Although the study area is reduced to a fragment of the Atlantic Forest, itself a fragmented biome, the mosquito fauna showed the presence of *Hg. leucocelaenus*, a species of high epidemiological relevance in the transmission of the yellow fever virus. Since there are confirmed cases of the disease in humans in the area surrounding ATA, active and constant entomological surveillance is recommended in the region.

Based on the present results, this study concludes that the sampling methods for collecting immature mosquitoes are important indicators that should be included in efforts to monitor biological vectors. Tire traps showed the highest colonization rates, followed by OT.

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