

## Dispersal of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in an Urban Endemic Dengue Area in the State of Rio de Janeiro, Brazil

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*Experimental releases of female Aedes (Stegomyia) aegypti and Aedes (Stegomyia) albopictus were performed in August and September 1999, in an urban area of Nova Iguaçu, State of Rio de Janeiro, Brazil, to estimate their flight range in a circular area of 1,600 m where 1,472 ovitraps were set. Releases of 3,055 Ae. aegypti and 2,225 Ae. albopictus females, fed with rubidium (Rb)-marked blood and surgically prevented from subsequent blood-feeding, were separated by 11 days. Rb was detected in ovitrap-collected eggs by atomic emission spectrophotometry. Rb-marked eggs of both species were detected up to 800 m from the release point. Eggs of Ae. albopictus were more numerous and more heterogeneously distributed in the area than those of Ae. aegypti. Eggs positively marked for Rb were found at all borders of the study area, suggesting that egg laying also occurred beyond these limits. Results from this study suggest that females can fly at least 800 m in 6 days and, if infected, potentially spread virus rapidly.*

Key words: *Aedes aegypti* - *Aedes albopictus* - behavior - dispersal - rubidium - dengue - Rio de Janeiro - Brazil

Female mosquitoes may disperse to find mates, food or oviposition sites. Dispersal to seek a host is epidemiologically important as the mechanism whereby female mosquitoes acquire and disseminate pathogens. Additionally, dispersal for oviposition is relevant to disease propagation. Females of the classic dengue vector *Aedes aegypti* (Linnaeus) distribute their eggs among several oviposition sites (Christophers 1960, Reiter et al. 1995) and frequently take multiple blood meals within a single gonotrophic cycle (McClelland & Conway 1971, Trpis & Hausermann 1986, Scott et al. 1993), which increases dispersal of their progeny. According to Trpis and Hausermann (1986), *Ae. aegypti* also disperse to search for carbohydrates and a mate.

Studies on the dispersal of *Ae. aegypti* show females generally fly 100-500 m (McDonald 1977, Trpis & Hausermann 1986, Muir & Kay 1998). This range is short compared to other mosquitoes, such as *Aedes taeniorhynchus* (Wiedemann) which disperse up to 10 km (Provost 1957). Some authors believe that *Ae. aegypti* fly only a short distance from their emergence sites (Reuben et al. 1975, Trpis & Hausermann 1986, Trpis et al. 1995, Service 1997). However, a study conducted in urban San Juan, Puerto Rico, showed that female *Ae. aegypti* could,

in a few days, travel at least 441 m from a releasing point (Reiter et al. 1995). Recapture studies of marked female *Aedes albopictus* (Skuse), another potential dengue vector (Schatzmayer 2000), showed a maximum dispersal of 400-600 m (Rosen et al. 1976, Niebylski & Craig 1994). This same species flew no more than 200 m in studies in Hawaii and Japan (Bonnet & Worcester 1946, Mori 1979).

Despite its importance to dengue transmission, dispersal of *Ae. aegypti* and *Ae. albopictus* in urban Brazil has not been examined. In order to assess and compare their pre-oviposition dispersal, we fed adult females on blood enriched with rubidium chloride (RbCl) a method developed by Kimsey and Kimsey (1984). The release of Rb-marked females was carried out in an endemic dengue area of Southeastern Brazil, and dispersal was measured by the detection of Rb-labeled eggs in ovitraps.

### MATERIALS AND METHODS

*Study area* - Nova Iguaçu is a municipality of the State of Rio de Janeiro, Brazil, located at 22°45'S 43°27'W, 25 m altitude, with an area of 566.6 km<sup>2</sup> (Fig. 1). Atlantic forest is still present in 35% of this area, although it is predominantly urban. The climate is hot and humid, with annual temperature and rainfall averages of 22°C and 2,100 mm, respectively. Nova Iguaçu has an approximately population of 850,000 inhabitants, with an average density of 1,414 inhabitants/km<sup>2</sup> (Prefeitura Municipal de Nova Iguaçu 1999, Honório 1999, Honório & Lourenço-de-Oliveira 2001). The release point was chosen as the center of a circle with an 800 m radius that encompassed seven districts of Nova Iguaçu: Ambaí, Bela Vista, Boa Esperança, Figueira, Jardim Ocidental, Miguel Couto and Parque Flora (Fig. 1). These districts are essentially residential areas with small shops, have a limited sewage system, and sparse

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solid waste refuse collection. Figueira is unique among these in having a rural area, with low population density (Fig. 1).

*Entomological inspection* - Two weeks before the release of Rb-marked mosquitoes, all houses (nearly 5,000) located in the 1,600 m diameter study area were inspected for containers containing immature mosquitoes which were identified and counted. Containers yielded 5,280 immatures identified as 3,055 *Ae. aegypti* and 2,225 *Ae. albopictus*. Most of the small containers that represented potential breeding sites for *Aedes* mosquitos were eliminated or removed by collectors, and the others, particularly large ones such as water reservoirs, were treated with Temephos® (20g/1000l of water) to kill the larvae. These

procedures were done to avoid increases in mosquito density attributable to oviposition by released females.

*Oviposition traps* - 1,472 ovitraps were placed in shaded peridomestic areas located at ground level and protected from the rain. In order to distribute the ovitraps homogeneously, the 800 m radius circular area was divided in to five concentric areas of 0-100 m radius (23 ovitraps), 100-200 m (69 ovitraps), 200-400 m (276), 400-600 m (460) and 600-800 m (644), respectively (Fig. 1). Each ovitrap contained 270 ml of tap water and 30 ml of a 10% by weight aqueous hay infusion that had been previously incubated for 7 days. A 12 cm x 5 cm wooden paddle was placed in each ovitrap. On the third day after setting all paddles were collected and replaced with new ones.

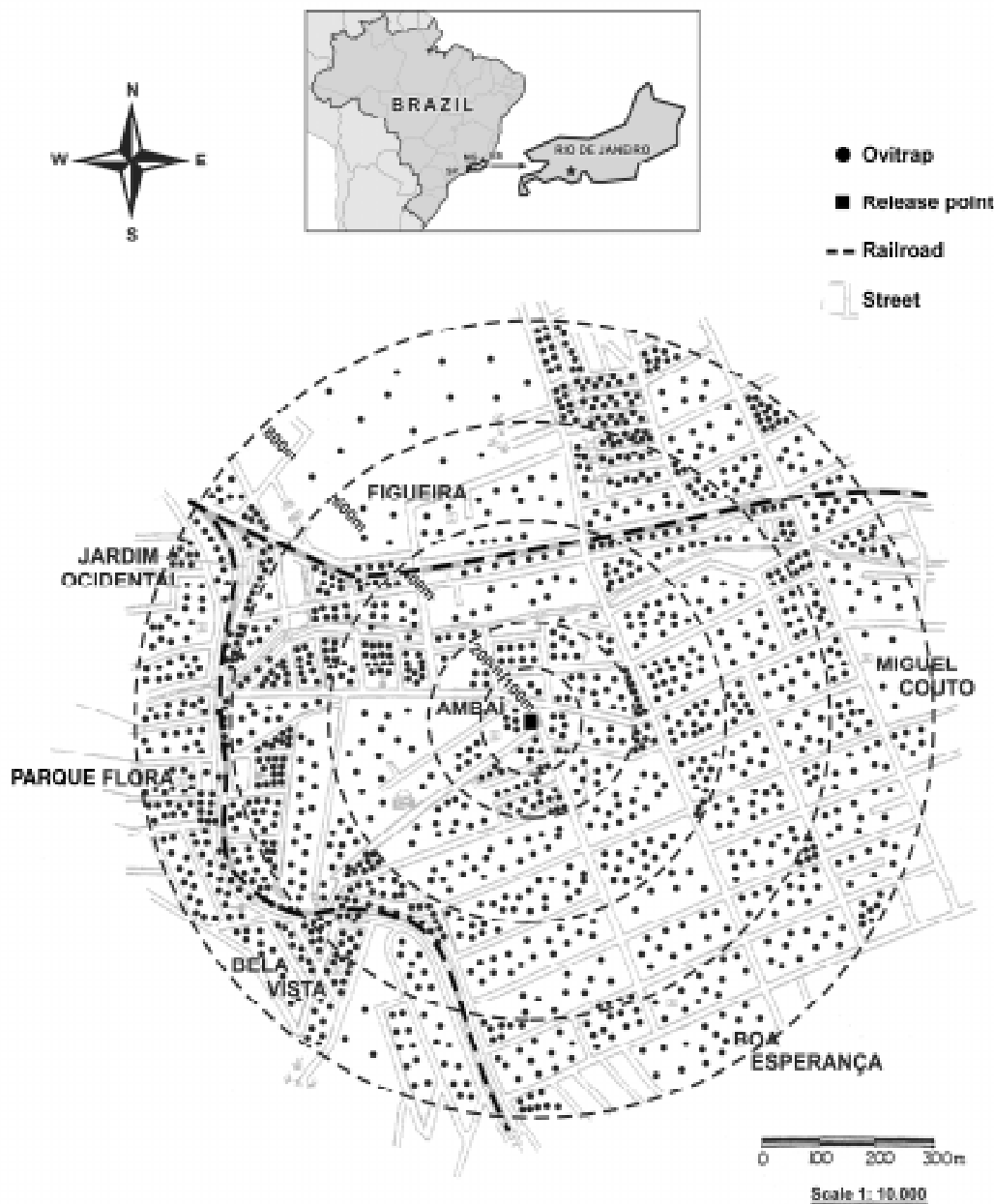


Fig. 1: municipality of Nova Iguaçu, State of Rio de Janeiro, Brazil, where field work was conducted. Each dot represents an ovitrap placed within a circular area of 1,600 m diameter.

Throughout the study, missing ovitraps were replaced with new traps.

**Mosquitoes** - *Ae. aegypti* and *Ae. albopictus* mosquitoes used in the experiments were F<sub>3</sub> from laboratory colonies derived from large numbers of eggs collected in Ambaí. Mosquito colonies were maintained in an insectary at 27 ± 1°C and 80 ± 10% humidity. Larvae were fed with fish food (Tetramin®) and reared according to Consoli and Lourenço-de-Oliveira (1994). Adults of both species were provided with sucrose solution from emergence to one day before blood feeding.

**Blood meal mixed with RbCl** - Four to six day old F<sub>3</sub> females were separated from males in cardboard cylindrical cages (9 cm height, 9 cm diameter). These females were offered defibrinated sheep blood containing 0.025 M RbCl (Reiter et al. 1995). Rb is an alkali metal, non-toxic to plants, insects and other animals, that is incorporated into ovarian follicles and accumulates in mosquito eggs if it is present in the blood meal (Kimsey & Kimsey 1984, Anderson et al. 1990). Eggs containing Rb were detected by atomic emission spectrophotometry (Reiter et al. 1995). A Rb-marked blood meal was offered to mosquito females in a feeding apparatus described by Rutledge (1964). Females that fed to repletion were kept in cages (60 x 60 x 60 cm) in the insectary. Twenty-four hours after the Rb-blood meal, the tip of proboscises of all *Ae. aegypti* and *Ae. albopictus* females were cut off with a dissection scissors to prevent subsequent feeding. This procedure prior to release was recommended by the institutional ethics committee to avoid the risk of increased virus transmission in the dengue endemic area. Available evidence suggests that proboscis amputation should not affect post-feeding dispersal for oviposition (Shirai et al. 2000).

In order to assess both timing of oviposition and effectiveness of Rb detection, 12 females of each species received an Rb-blood meal and were housed in the laboratory in individual glass cylinders (7 x 2.5 cm) containing filter paper and dechlorinated water at the bottom. Eggs were recovered up to five days after blood feeding and tested for the presence of Rb as described below. Rb was detected in 9/12 (75%) *Ae. aegypti* and 11/12 (91%) *Ae. albopictus*. These frequencies were not significantly different from one another ( $P = 0.771$ , Fisher exact test).

**Release of Rb-marked females** - One day after a Rb-blood meal, gravid females were released at dusk (1700-1900 h) at the center of the study area (Fig. 1). Two Rb female releases were performed, one for each species. A total of 3,055 *Ae. aegypti* and 2,225 *Ae. albopictus* females were released on August 17 and 28, 1999, respectively. The 11 day interval between releases was considered sufficient to distinguish between the species because the chances of *Ae. aegypti* laying eggs more than 13 days after a single blood meal, and deprived of additional blood meals, is very small (Christophers 1960).

**Ovitraps collections and Rb-marked eggs detection** - On the third day after releases, the wooden paddles in each ovitrap were collected and replaced by fresh paddles. The replacement paddles were subsequently collected after an additional three days. The number of eggs collected per paddle was estimated after examination with a stereomicroscope. All eggs from a positive paddle were

gently transferred to a 10 ml glass flask to which 2 ml of nitric acid were added. The flasks were heated to 100°C on a hot plate for 30 min, followed by the addition of 1 ml distilled water. Each sample was analyzed by atomic emission spectrophotometry (Perkin Elmer model 5500). Eggs from laboratory colonies of *Ae. aegypti* and *Ae. albopictus* were used as controls for each assay.

**Statistical analysis** - Data were analyzed by chi-square and Fisher exact tests using SPSS for Windows (8.0). Chi-square tests were used to compare frequencies of Rb positive ovitraps in different portions of the study area.

## RESULTS

**Egg trap collection for Rb detection** - Of the 1,472 ovitraps placed in the study area, 1,347 were recovered on the third day after the release of Rb-marked *Ae. aegypti* females. On the sixth day 1,411 out of 1,472 ovitraps were recovered. The remaining 186 paddles (6.3%) were missing. For *Ae. albopictus*, on the third day after release, 1,433 of 1,472 ovitraps were recovered and on the sixth day, 1,381 of 1,472 ovitraps. In total, 120 paddles (4.1%) were missing. For both species, more Rb-eggs were detected on the sixth day (Tables I, II).

**Dispersal of *Ae. aegypti*** - Fifty-one ovitraps (17 on day 3 and 34 on day 6) were found with Rb-marked *Ae. aegypti* eggs (Table I, Fig. 2). Ambaí and Jardim Ocidental were the districts with the highest number of Rb-positive eggs (28 positive ovitraps out of 1,400 set), followed by Miguel Couto (8), Figueira (6), Boa Esperança (5) and Bela Vista and Parque Flora (4). *Ae. aegypti* Rb-marked eggs were found up to 800 m from the release point. None of the 23 ovitraps placed up to 100 m from the release point was positive for Rb-marked *Ae. aegypti* eggs. In the 100-200 m area, where 118 paddles were recovered, only 2 (1.7%) were positive (Table III). In the 200-400 m area, 12 had Rb-marked *Ae. aegypti* eggs (or 2.3% of the 515 recovered). Out of the 850 paddles collected in the 400-600 m, 20 (2.3%) were positive while in the 600-800 m area there were 17 (1.3%). The percentages of positive paddles were not significantly different among areas divided either as concentric circles ( $\chi^2 = 3.3$ , df = 4,  $P = 0.503$ ; Table III), pie-shaped wedges of equivalent size ( $\chi^2 = 4.76$ , df = 7,  $P = 0.688$ ; Table IV) or by districts ( $\chi^2 = 2.37$ , df = 4,  $P > 0.05$ ; Table I).

**Dispersal of *Ae. albopictus*** - A total of 304 ovitraps were recovered with Rb-marked *Ae. albopictus* eggs: 95 on day 3 and 209 on day 6 after release of Rb-marked females (Table II, Fig. 2). As for *Ae. aegypti*, Ambaí and Jardim Ocidental were the districts with the most Rb-marked eggs (127 positive ovitraps), followed by Miguel Couto (79), Figueira (47), Bela Vista and Parque Flora (28) and Boa Esperança (23). *Ae. albopictus* Rb-marked eggs were also found up to 800 m from the release point. A total of 8 ovitraps placed up to 100 m from the release point were positive for Rb-marked eggs (18.2%). In the 100-200 m area, where 127 paddles were recovered, 21 (16.5%) were positive. In the 200-400 m area, 50 had Rb-marked eggs (9.5% of the 525). Out of the 875 paddles collected in the 400-600 m area, 82 (9.3%) were positive and in the 600-800 m area there were 143 (11.4%) positive. *Ae. albopictus* Rb positive eggs were found in all blocks in the experi-

mental area, even in those located as far as 800 m for the release point. The percentages of positive paddles were significantly different among areas divided either as concentric circles ( $\chi^2 = 10.08$ ,  $df = 4$ ,  $P = 0.039$ ; Table III), pie-shaped wedges of equivalent size ( $\chi^2 = 32.24$ ,  $df = 7$ ,  $P < 0.001$ ) or by districts ( $\chi^2 = 10.43$ ,  $df = 4$ ,  $P = 0.034$ ; Table II).

## DISCUSSION

In the preliminary laboratory experiment in which females were kept individually in glass vials, 75% of *Ae. aegypti* and 91% of *Ae. albopictus* females laid Rb-marked eggs. Although this difference between species was not statistically significant, the trend corresponds to the

TABLE I

Number of wooden paddles set and recovered with or without eggs and numbers of paddles with rubidium (Rb)-marked eggs, in districts of the study area in Nova Iguaçu, State of Rio de Janeiro, Brazil, after the release of 3,055 Rb-marked blood fed *Aedes aegypti* females on August 17, 1999. Egg collections performed on August 20 (first collection) and 23 (second collection)

District	Set	Without eggs collection		With eggs collection		With Rb-marked eggs collection	
		1st	2nd	1st	2nd	1st	2nd
Ambaí and Jardim Ocidental	700	651	665	22	51	7	21
Bela Vista and Parque Flora	198	164	183	4	10	2	2
Boa Esperança	117	106	96	6	19	1	4
Figueira	221	196	188	8	17	2	4
Miguel Couto	236	175	165	15	17	5	3
Total	1,472	1,292	1,297	55	114	17	34
	$\chi^2_{(4)} = 2.37$					$P > 0.05$	

$\chi^2$ : test for significant frequency differences of Rb-positive paddles among districts

TABLE II

Numbers of wooden paddles set and recovered with or without eggs and numbers of paddles with rubidium (Rb)-marked eggs, in districts the study area in Nova Iguaçu, State of Rio de Janeiro, Brazil, after the release of 2,225 Rb-marked blood fed *Aedes albopictus* females on August 28, 1999. Egg collections performed on August 31 (first collection) and September 3 (second collection)

District	Set	Without eggs collection		With eggs collection		With Rb-marked eggs collection	
		1st	2nd	1st	2nd	1st	2nd
Ambaí and Jardim Ocidental	700	580	408	185	261	45	82
Bela Vista and Parque Flora	198	151	149	37	45	11	17
Boa Esperança	117	77	81	32	32	10	13
Figueira	221	112	131	59	79	17	30
Miguel Couto	236	123	85	87	110	12	67
Total	1,472	1,043	854	400	527	95	209
	$\chi^2_{(4)} = 10.43$					$P < 0.05$	

$\chi^2$ : test for significant frequency differences of Rb-positive paddles among districts

TABLE III

Dispersal distances of *Aedes aegypti* and *Aedes albopictus* females in Nova Iguaçu, State of Rio de Janeiro, Brazil, August and September 1999

Distance (m)	<i>Aedes aegypti</i>		<i>Aedes albopictus</i>	
	Number of paddles recovered	Number of rubidium (Rb)-positive paddles (%)	Number of paddles recovered	Number of Rb-positive paddles (%)
0 - 100 m	43	0/0	44	8/18.2
100 - 200 m	118	2/1.7	127	21/16.5
200 - 400 m	515	12/2.3	525	50/9.5
400 - 600 m	850	20/2.3	875	82/9.3
600 - 800 m	1,232	17/1.3	1,253	143/11.4
	$\chi^2_{(4)} = 3.33$	$P > 0.05$	$\chi^2_{(4)} = 10.08$	$P < 0.05$

$\chi^2$ : test for significant frequency differences among concentric rings

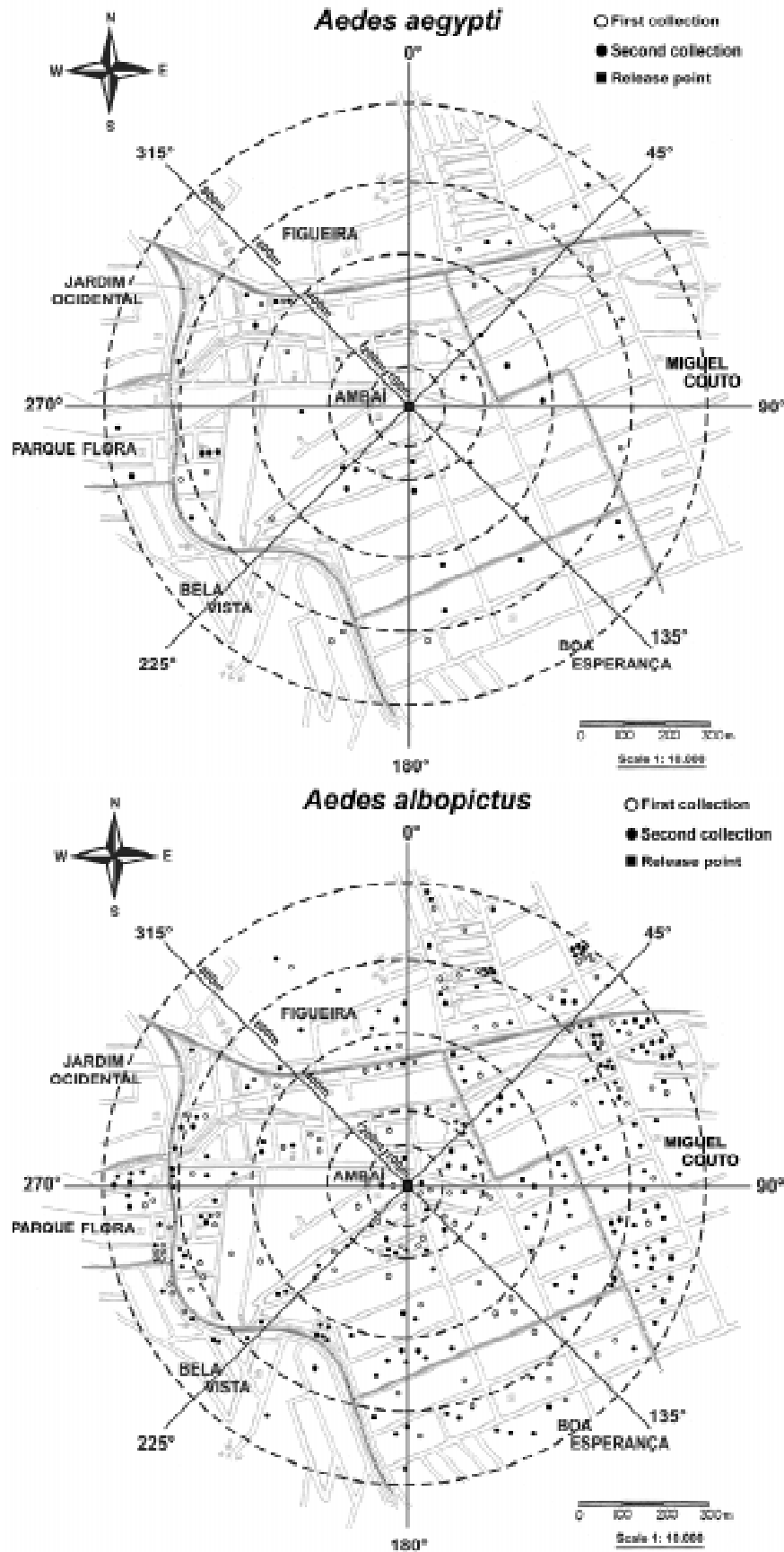


Fig. 2: distribution of collections of rubidium-marked eggs of *Aedes aegypti* and *Aedes albopictus* in a circular area of 1,600 m diameter in the municipality of Nova Iguaçu, State of Rio de Janeiro, Brazil, according to districts, and dispersal distances and directions.

poorer recovery of Rb-positive *Ae. aegypti* in the field experiment. Although more *Ae. aegypti* females were released in the field than *Ae. albopictus*, more Rb-positive paddles of the latter were collected.

The flight of mosquitoes is influenced by factors such as species-specific traits, oviposition site availability, climate (e.g., wind, humidity, temperature, rainfall), terrain, vegetation, housing characteristics (for synanthropic species) and blood source (Forattini 1962). Our experiment changed the number and location of available oviposition sites in the study area, and we cannot discount the possibility that these alterations influenced the dispersal patterns of the released mosquitoes.

Rb-marked *Ae. aegypti* eggs were found as far as 800 m from the release point (Table I, Fig. 2). These results indicate a greater flight range for *Ae. aegypti* than those estimated by other authors who suggest  $\leq 200$  m (Morlan & Hayes 1958, Sheppard et al. 1969, Reuben et al. 1975, Trpis & Hausermann 1986).

In Brazil, female *Ae. aegypti* were released inside a house in an urban area of the State of Bahia and the maximum recovery distance after 24 h was 120 m (Shannon et al. 1930). In a second experiment, the maximum dispersal distance was 300 m for two individuals (Shannon & Davis 1930). Because of the difficulties in recapture of *Ae. aegypti*, owing to its escape ability and behavior when approaching for blood (Christophers 1960), both values should be considered underestimates of the true flight range of the species. In a third experiment, 12,000 females were marked with methylene blue and released in a boat anchored in the sea 1 km from the coast (Shannon & Davis 1930). Only eight specimens were recovered on land, indicating that dispersal of up to 1 km is possible for *Ae. aegypti*. Conditions were also unnatural for gravid *Ae. aegypti* females released in the desert of Israel (Wolfinschon & Galun 1953), a zone free of *Ae. aegypti* with no houses that could act as adult attractants. Under these conditions, oviposition of *Ae. aegypti* was recorded as far as 2,500 m from the release point.

In our field study, paddles were collected on days 3 and 6 after release, that correspond to 4 and 7 days after the blood meal, respectively. Paddles with Rb-positive eggs were more numerous on day 6. Because *Ae. aegypti* generally lays eggs 3 to 5 days after a blood meal (Christophers 1960), some females may not have been ready to oviposit before the first paddle-collection.

The number of ovitraps with Rb-positive eggs was highest for *Ae. aegypti* at Ambaí and Jardim Ocidental (Table I), but the number of ovitraps set there was also greater, as these districts encompass most of the study area (Fig. 1). Positive ovitraps were also common along the railroad that crosses Parque Flora and Figueira, which are mostly poor neighborhoods of rudimentary housing in an undulating terrain. In these districts the water supply is irregular, which leads inhabitants to store water in containers. It is likely that these containers are used as oviposition sites by container dwelling mosquitoes.

The recovery of marked *Ae. aegypti* eggs was not significantly different among 5 concentric circles up to 800 m from the release point. The overall low recovery of Rb-positive paddles for *Ae. aegypti* contributed to the ab-

sence of detectable differences in dispersal of this species among districts or sectors of the study area.

Studies on *Ae. albopictus* dispersal have not been previously done in Brazil. In Missouri, USA, non-blood fed *Ae. albopictus* females marked with fluorescent dyes dispersed up to 525 m (female) and 225 m (male) (Niebylski & Craig 1994). We observed a dispersal range of at least 800 m for this species. In the 600-800 m area 143 ovitraps were positive for Rb-marked eggs, (11.4% of 1,253 paddles).

Egg distribution in the study area was significantly heterogeneous for *Ae. albopictus* but not for *Ae. aegypti* (Tables III, IV, Fig. 2). When *Ae. albopictus* were released, winds were blowing to the east, which could have contributed to biased dispersal. Although the number of *Ae. albopictus* released females (2,225) was lower than that of *Ae. aegypti* (3,055), more marked *Ae. albopictus* eggs were recovered in ovitraps (Table II). The highest frequency of Rb-marked eggs occurred in the 100-200 m area (Fig. 2). Frequencies of Rb-marked eggs of *Ae. albopictus* were significantly different among the districts, among concentric circles, and among pie-shaped wedges of equivalent size. 'Hot spots' of recovery were concentrated in Figueira and Miguel Couto, mainly at the 600-800 m area. The recovery of Rb-positive ovitraps was significantly higher in sectors 1 and 2 than in sectors 7 and 8 (Table IV). Unlike sectors 7 and 8, the terrain of sectors 1 and 2 is hillier, the houses have trees in the yards, and are separated by patches of pasture. This result agrees with known preferences of this species for rural and vegetated habitats (Hawley 1988).

The dispersal of *Ae. aegypti* and *Ae. albopictus* at least 800 m occurred in a dengue endemic area within a 6 day period. It is known that the extrinsic incubation period of dengue virus, the interval of time from acquisition of infectious blood to subsequent transmission, is approximately 11-14 days for *Ae. aegypti* (Siler et al. 1926). If a female feeding once on a viremic inhabitant can fly at least 800 m in 6 days, it can spread the virus very rapidly. This extensive flight range must be taken into account in focal insecticidal treatment in response to yellow fever or dengue outbreaks. A new dengue case could appear at 1 km distance from another in few days. This has been observed in previous dengue epidemics, when time for control action measures was minimal between the first confirmed cases and the rapid spread of the disease (Kuno 1995). Rapid dispersal would also apply to *Aedes* re-infestation of areas considered free of these vectors. Our results suggests that from an initial focus, in 6 days one might expect house infestation of almost 1 km<sup>2</sup> due to female dispersal independent of human activities, such as transport and trade. It is clear that female dispersal by flight has the potential to be very important for the spread of dengue vectors, either between adjacent cities or in urban centers such as Rio de Janeiro.

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TABLE IV

Dispersal directions of *Aedes aegypti* and *Aedes albopictus* females in Nova Iguaçu, State of Rio de Janeiro, Brazil, August and September 1999

Distance (m)	Arc (45°)	<i>Aedes aegypti</i>		<i>Aedes albopictus</i>	
		Number of paddles recovered	Number of rubidium (Rb)-positive paddles (%)	Number of paddles recovered	Number of Rb-positive paddles (%)
3	0 - 45°	388	8/2.1	391	45/11.5
1	45 - 90°	374	7/1.9	374	63/16.8
2	90 - 135°	316	5/1.6	328	47/14.3
4	135 - 180°	287	6/2.1	309	33/10.7
7	180 - 225°	311	4/1.3	319	26/8.1
6	225 - 270°	473	11/2.3	489	45/9.2
8	270 - 315°	451	10/2.2	451	28/6.2
5	315 - 360°	158	0/0	163	17/10.42
		$\chi^2_{(7)} = 4.76^a$	$P > 0.05$	$\chi^2_{(7)} = 32.24$	$P < 0.001$

a: unplanned test for homogeneity (Sokal & Rohlf 1981). For *Ae. albopictus*, sectors sharing common line are statistically homogeneous: 1 2 3 4 5 6 7 8

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