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Original Article

Extremely High Biting Densities of *Aedes albopictus* (Skuse) (Diptera: Culicidae) at a University Campus in Nagasaki, Japan

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SUMMARY: *Aedes albopictus* (Skuse) transmits several arboviral diseases. This mosquito was the vector responsible for both the past and recent dengue outbreaks in Japan. Sites with a high density of *A. albopictus* are at risk of outbreaks of arboviral diseases. This study describes extremely high biting densities of *A. albopictus* at a campus of Nagasaki University, southwestern Japan. In August of 2015 and 2016, an 8 min human-bait-sweep collection obtained on average 33.4 and 38.9 females, respectively. In both 2015 and 2016, the highest and the second highest biting densities were observed at sites densely shaded by trees and covered with a large number of understory plants. In addition, major *A. albopictus* breeding sites were identified near these locations in 2016. A predaceous larval mosquito, *Lutzia vorax* Edwards, appeared to strongly suppress the breeding of *A. albopictus* in catch basins near the site with the highest adult density, although its effect was insufficient to maintain *A. albopictus* density at a low level. After the catch basins had been cleaned, *A. albopictus* immatures became more abundant, especially in shallow catch basins in shaded sites.

INTRODUCTION

Aedes albopictus (Skuse) is one of the most common mosquitoes in Japan and is known to transmit several pathogenic viruses such as the dengue, chikungunya, and Zika viruses. It was the vector responsible for the dengue outbreak in Tokyo in 2014 (1) and was also responsible for past large-scale outbreaks in several cities in western Japan from 1942 to 1944 (2). Before the 1980s, the distribution of *A. albopictus* ranged from Madagascar to temperate and tropical Asia and Hawaii (3). In recent years, however, this species has invaded other areas such as North and South America, Europe, and Africa (4). Dengue epidemics due to the invading *A. albopictus* have been reported from several temperate European countries such as France (5,6), Croatia (7), and Spain (6). It has also caused outbreaks of chikungunya fever in Italy (8,9).

As international traffic has increased, so have imported cases of dengue, chikungunya, and Zika (10). Although the areas where these diseases are endemic are limited to tropical regions, temporal autochthonous transmission of these diseases can also occur in any non-endemic region where *A. albopictus* is present, either tropical or temperate, in the seasons when this species is active. Since vaccines for these diseases are currently not available, mosquito populations should be maintained at low levels to prevent future outbreaks. Moreover, when resources for mosquito control are limited, efforts should be targeted at controlling areas where mosquito density is high. Therefore, it is essential to understand the environmental settings that allow a high density of *A. albopictus*.

The present study reports extremely high biting densities of *A. albopictus* observed at a university campus in Nagasaki, southwestern Japan, and describes the environmental settings that might affect the abundance of this species, such as availability of shade, understory vegetation, breeding sites, and occurrence of a larval-stage predator.

METHODS

To evaluate the potential risk of dengue outbreaks, the author conducted a human-bait-sweep collection at the peak of the *A. albopictus* population (mid to late August) (11) in 2015 in Sakamoto Campus 1 of Nagasaki University (32°48′24″ N, 129°52′09″ E). The campus is located in a residential area of Nagasaki City. The elevation ranged from 18 m to 54 m and the campus covered an area of approximately 9 ha. Vegetation with large trees created shaded areas in several places within the campus. The predominant tree species was *Cinnamomum camphora* (L.) J. Presl. An adult mosquito collection was performed at 9 sites from August 12–14, 2015. Similarly, a collection was performed at 11 sites from August 16–18, 2016. Sites for adult collection were selected from the areas lightly or densely shaded by trees. At each site, a single collector caught mosquitoes that landed on his body or were flying around him using a 30 cm diameter hand insect net for 8 min (12) during the afternoon (16:00–18:00). The collected mosquitoes were killed by placing in a freezer (−20°C) for 30 min and identified using the taxonomic key by Tanaka et al.

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(13).

After the adult mosquito collection in 2016, the major breeding sites of *A. albopictus* were surveyed on the campus. The breeding sites containing a large number (> 50) of *Aedes* larvae were recorded and mapped. The 12 catch basins (length and width, 35 cm and 45 cm; depth, 39–70 cm) near the sites where the highest adult density was observed were intensively surveyed because they appeared to be the most productive breeding sites in the campus. Mosquito larvae were collected from the 12 catch basins from August 19–25, 2016. Length and width of the opening, depth to the water surface, and water level were measured for each catch basin.

All the mosquitoes were collected from these basins by draining all the water using a dipper, a plastic tray, and a piece of sponge (the first larval collection). Predaceous mosquito larvae (*Lutzia vorax* Edwards) were counted and separated at the collection sites to prevent predation on other collected larvae. As the adult collection showed that *A. albopictus* was the sole member of the subgenus *Stegomyia* of the genus *Aedes* in the adult samples, the present study refers to all *Aedes* (*Stegomyia*) larvae as *A. albopictus*. Several pupae were reared to adults for easier identification. After the immatures had been collected, all water, detritus, and sediments were removed from the 12 catch basins. A few days after the larval collection, heavy rain filled the basins with water, resulting in the hatching of *Aedes* larvae. These larvae were collected and counted from September 1–2, 2016 (the second larval collection).

The correlation between the abundance of *A. albopictus* immatures and basin characteristics (depth to the water surface, water level, and water volume) was examined with Spearman's rank correlation coefficient. The correlation of the abundance at the first and second collection times was also examined with Spearman's correlation coefficient. The relationships between the presence of *Lt. vorax* and the abundance of *A. albopictus* immatures at the first and second collection times were examined using a generalized linear model that assumes residuals of negative binomial distribution. In the model, the abundance of *A. albopictus* immatures at the first and second collection times was set as the dependent variable. The presence [1] or absence [0] of *Lt. vorax* immatures at the first larval collection was set as the independent variable for both models. These statistical analyses were conducted using R ver 3.4.5 statistical software (14).

RESULTS

In 2015, a human-bait-sweep collection obtained a total of 301 female and 207 male *A. albopictus* adults from 9 sites (Table 1; Fig. 1A; mean ± SD, 33.4 ± 27.5 and 23.0 ± 22.3 for females and males, respectively). The highest adult female density was observed on the slope of Gibiroga Hill, where 73 females and 9 males were collected in 8 min. The second highest density was observed near the garbage area, with 65 females and 46 males. In 2016, 428 adult females and 236 adult males of *A. albopictus* were collected from 11 sites (Table 1; Fig. 1B; mean ± SD, 38.9 ± 61.0 and 21.5 ± 26.1 for females and males, respectively). The slope of Gibiroga Hill again presented the highest density (210 females and 51 males / 8 min), followed by the same site near the garbage area, with 69 females and 57 males / 8 min. No other mosquito species was caught during the human-bait-sweep collection in either year.

Table 1 compares the availability of shade, understory vegetation that can be adult resting sites, and nearby major breeding sites. Sites with large numbers of *A. albopictus* adults in 2016 (sites 1, 9, 10, and 11) tended to be shaded by a dense canopy, covered with abundant understory plants, and close to major breeding sites. In contrast, sites with smaller numbers of *A. albopictus* in 2016 (sites 2, 4, 5, 7, and 8) were without nearby breeding sites. In addition to the 12 catch basins near Gubiroga Hill, the following breeding sites were confirmed as presenting large numbers (> 50) of *A. albopictus* larvae: various discarded containers in the garbage area (Fig. 1B a), discarded tires (Fig. 1B b, c), cement tanks at the top of Gubiroga Hill (Fig. 1B d), a stone basin, and flower pots on Gubiroga Hill (Fig. 1B f).

Larvae and/or pupae of the predaceous mosquito

Table 1. Characteristics of the adult collection sites

Site	Canopy[1]	Understory plants[1]	Breeding sites[2]	No. of A. albopictus captured (/ 8 min / person)			
				2015		2016	
				Female	Male	Female	Male
1	+++	++	+	65	46	69	57
2	++	+	–	37	10	6	6
3	+	+	+	ND	ND	23	8
4	+++	+	–	15	36	7	5
5	+	–	–	4	7	2	1
6	+	–	–	2	0	17	14
7	++	+	–	37	59	4	3
8	–	++	–	ND	ND	4	2
9	++	++	+	9	0	56	74
10	+++	++	+	73	9	210	51
11	++	++	+	59	40	30	15
Total				301	207	428	236

[1]: Coverage: +++, ≥ 80%; ++, 50-80%; +, 10-50% ; –, < 10%.

[2]: +, breeding sites identified within 30 m; –, no breeding sites identified within 30 m.

ND, no data.

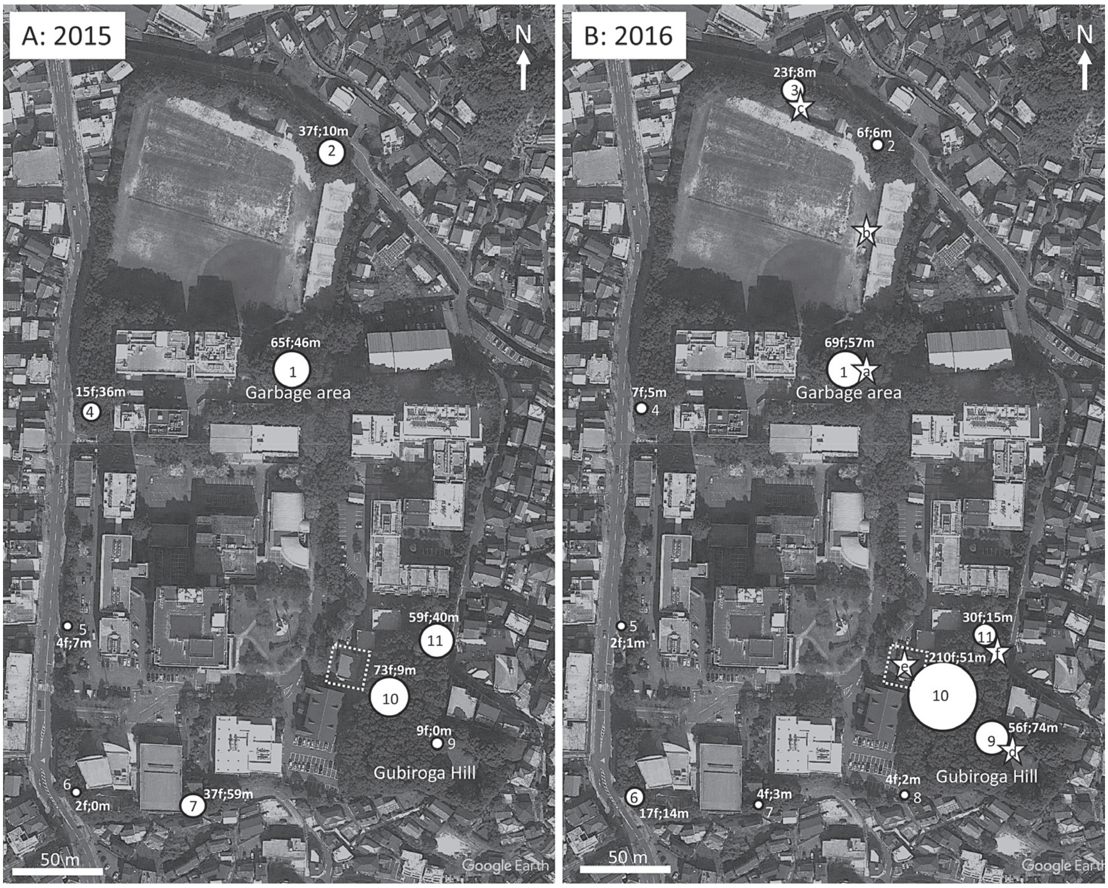

Fig. 1. Numbers of females (f) and males (m) of *A. albopictus* adults caught by human bait sweeping collection in 9 and 11 sites in Sakamoto Campus 1 of Nagasaki University in 2015 and 2016, respectively. The size of the circle indicates the number of females. The stars indicate major breeding sites: a, discarded containers; b, c, tires; d, cement tanks; e, catch basins; f, a stone basin and flower vases. The detailed distribution of the 12 catch basins within the rectangle with white dotted line is presented in Fig. 2.

Table 2. Number of mosquito immatures (larvae + pupae) collected from the 12 catch basins near Gubiroga Hill in Sakamoto Campus 1 of Nagasaki University

Basin No.[1]	Length and width (cm)	Depth to water[2] (cm)	Water Level (cm)	Water volume[3] (L)	First collection[4]				Second collection[5]	
					Lutzia vorax	*Aedes albopictus*	*Culex Pipiens* group	*Aedes japonicus*	*Aedes albopictus*	*Aedes japonicus*
1	35 × 35	37.8	13.2	16.1	13	0	9	0	1,989	0
2	35 × 35	37.5	11.5	14.4	37	0	172	0	1,218	0
3	35 × 35	27.9	12.1	14.8	0	2,637	0	0	1,996	0
4	35 × 35	26.2	20.8	25.5	12	0	2	0	3,054	0
5	35 × 35	25.7	5.3	18.8	32	42	2	0	2,701	0
6	35 × 35	39.8	16.2	19.8	12	118	1	0	393	35
7	45 × 45	38.5	14.5	29.4	0	205	0	0	486	0
8	35 × 35	56.9	14.1	17.3	8	6	0	25	170	0
9	35 × 35	35.2	12.8	15.7	26	15	0	0	442	0
10	35 × 35	24.2	14.8	18.1	0	2,113	0	0	913	0
11	45 × 45	40.2	12.8	25.9	12	0	104	0	903	0
12	45 × 45	39.3	14.7	29.8	23	0	0	0	400	0
Total					175	5,136	290	25	14,665	35

[1]: labelled arbitrarily.

[2]: from the opening to the water surface.

[3]: length × width × water level.

[4]: August 19-25, 2016.

[5]: September 1-2, 2016 (after the heavy rain).

Lt. vorax were found in 9 (75%) of the 12 catch basins inspected (Table 2). In total, 5,136 *A. albopictus* immatures were obtained at the first larval collection (Table 2); importantly, 4,955 (96.5%) of these were collected from the 3 basins where *Lt. vorax* was absent. In the 9 basins containing *Lt. vorax*, it was noted that *A. albopictus* immatures were either absent (5 basins) or less abundant (4 basins; ranging from 6 to 118). In addition to *Lt. vorax* and *A. albopictus*, species from the *Culex pipiens* group (*C. pipiens pallens* Coquillett and *C. pipiens* form *molestus* Forskal were not distinguished) and *Aedes japonicus* (Theobald) were also observed in the catch basins. Unlike *A. albopictus*, larvae of the *C. pipiens* group and *A. japonicus* occurred only in the basins where *Lt. vorax* was present. The association of the presence of *Lt. vorax* and the abundance of *A. albopictus* immatures was evaluated using a generalized linear model that assumes residuals of the dependent variable (in this case, counts of *A. albopictus* immatures) follow a negative binomial distribution (Table 3). During the first larval collection, the presence of *Lt. vorax* had a significant negative effect on *A. albopictus* immatures. The number of *A. albopictus* immatures at the first collection time was not significantly correlated with depth to the water surface (Spearman's rank correlation, $\rho = -0.340$, $P = 0.278$), water level ($\rho = 0.102$, $P = 0.753$), or water volume ($\rho = -0.145$, $P = 0.653$).

After the heavy rains, *A. albopictus* larvae were

Table 3. Summary of negative binomial regression analysis to examine the relationship between the occurrence of *Lt. vorax* immatures and the abundance of *A. albopictus* immatures in the catch basins at the first and the second collection times

	First collection			Second collection		
	Estimate	SE	*P*	Estimate	SE	*P*
Coefficients						
Intercept	7.41	1.23	< 0.001	7.03	0.453	< 0.001
Presence of *Lt. vorax*[1)]	− 4.408	1.425	0.002	0.101	0.523	0.847
Dispersion parameter	0.2195			1.6259		

[1)]: The presence of *Lt. vorax* at the first collection time was set as the independent variable for both models.

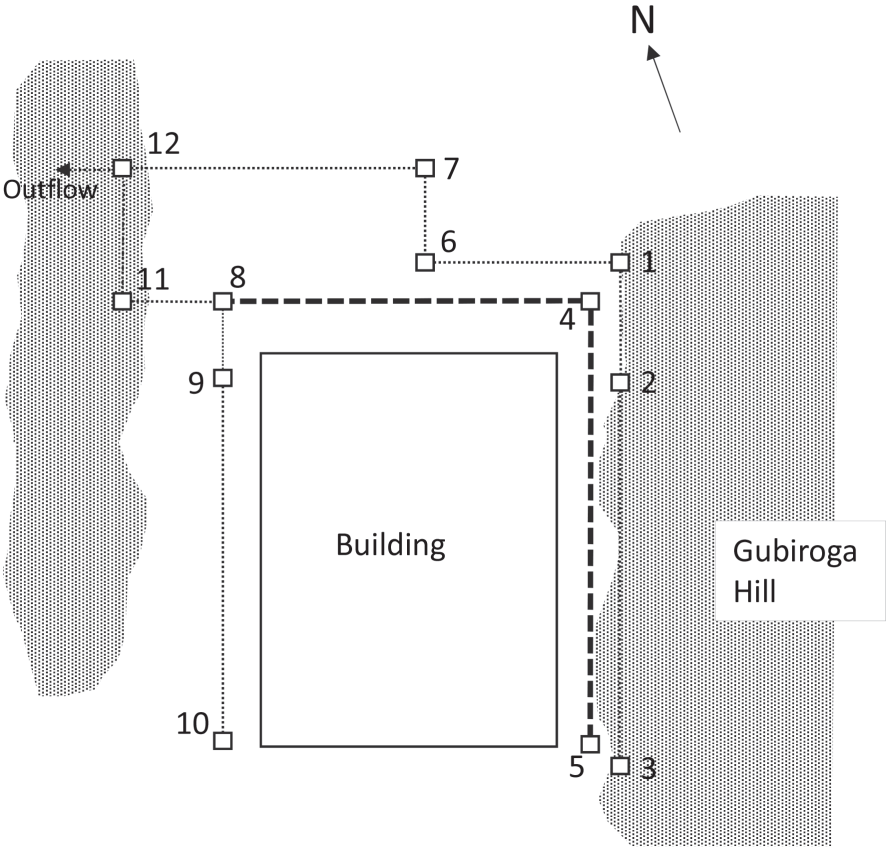

Fig. 2. Location of the 12 catch basins near Gubiroga Hill. The numbers are correspondent to the basin number in Table 2. Shaded areas are covered with trees. Thin broken lines indicate underground pipes that connect catch basins (the arrow indicates outflow). Thick broken lines indicate ditches.

found in all 12 basins. Total abundance (14,695) was considerably greater than that at the first larval collection. The larval density was high, especially in basins that were located on the east side and densely shaded by the trees on Gubiroga Hill (basins 1–5; range: 1,218–3,054; Fig. 2, Table 2). In contrast, the catch basins in sunlit areas (basins 6–8) tended to harbor smaller numbers of immatures (range: 170–486). The abundance of *A. albopictus* immatures in the second collection was negatively correlated with the depth to the water surface (Spearman's rank correlation, $\rho = -0.741$, $P < 0.01$), whereas it was not correlated with water level ($\rho = 0.035$, $P = 0.914$) or water volume ($\rho = -0.224$, $P = 0.485$). There was no significant correlation between the abundance of *A. albopictus* immatures at the first and the second collection times ($\rho = -0.0435$, $P = 0.893$). The presence of *Lt. vorax* at the first collection time also did not significantly affect the abundance of *A. albopictus* immatures at the second larval collection time (Table 3). In addition to *A. albopictus*, *A. japonicus* larvae were also found in one basin at the second collection time (Table 2).

DISCUSSION

The highly elevated biting density of *A. albopictus* in the campus of Nagasaki University was investigated in the present study. From the author's experience, the highest biting density observed (site 10; 210 females and 51 males / 8 min in 2016) was an extreme level. It was higher than the results reported by Nakano (15) (103 females/10 min/person in a vegetation area and 161 females/10 min/person in a cemetery) in Tokyo in 2005. According to the mosquito survey carried out shortly after the confirmation of the first autochthonous dengue infection around Yoyogi Park and Shinjuku Central Park, Tokyo, in 2014 (12), the mean densities were 7.13 and 11.43 females/8 min/person, respectively. The mean biting densities observed in the current study area (33.4 and 38.9 females/8 min/person for 2015 and 2016, respectively) were markedly higher than those reported in Yoyogi Park and Shinjuku Central Park, where the case of dengue transmission occurred.

There is a monument at the top of Gubiroga Hill that was constructed in memory of the atomic bomb victims and is occasionally visited by the local population. Although no quantitative study on mosquito bites has been performed, it is likely that people who visit the hill during the seasons with high mosquito activity will have a significantly higher chance of suffering a high number of mosquito bites if using insufficient repellents for personal protection. Compared to other city areas, universities in general likely have more people traveling to/from countries where transmission of dengue, chikungunya, and Zika viruses can occur continuously. If people coming or returning from these countries are infected with a virus and are bitten by an *A. albopictus* mosquito, autochthonous transmission may take place. The risk may be comparatively more serious for dengue because although approximately 75% of infected people present with inapparent symptoms (mild ambulatory or asymptomatic) (16), they still have the potential to infect mosquito vectors (17). Consequently, a high *A. albopictus* density in a university campus is a public health concern.

The biting density varied greatly among the collection sites. The Gubiroga Hill canopy, where the highest biting density was observed in both 2015 and 2016, was densely covered mainly by *C. camphora*, *Distylium racemosum* Sieb. et Zucc., *Quercus glauca* Thunb., and *Acer palmatum* Thunb. As the ground surface had not been mown, there were numerous shrubs and herbaceous plants, such as *Ficus erecta* Thunb., *Liriope muscari* (Decne.) L. H. Bailey, *Farfugium japonicum* (L.) Kitam., *Rubus buergeri* Miq., *Boehmeria nivea* (L.) Gaudich., *Oplismenus undulatifolius* (Ard.) Roem. et Schult., *Thelypteris acuminata* (Houtt.) C.V. Mortn, and *Dicranopteris linearis* (Burm.f.) Underw., that could be acting as resting sites for adult mosquitoes. In addition, the 12 catch basins presented large numbers of *A. albopictus* immatures during both collection times of 2016. Similar conditions were also seen at the site near the garbage area, where the second highest biting density was observed in both 2015 and 2016. This site was also densely shaded by large *C. camphora* trees. As the surface was not well mown, understory herbs dominated by *Rubus hirsutus* Thumb. were abundant. In addition, various garbage containers were temporarily stored and created breeding sites of *A. albopictus*. Therefore, shade, resting places close to the ground, and nearby suitable breeding sites appeared to support a high *A. albopictus* population density.

In this study, larval populations in catch basins near Gubiroga Hill were also investigated. Interestingly, predaceous *Lt. vorax* larvae occurred in a large proportion (75%) of the catch basins. Several studies have reported that habitat use by *Lutzia* spp. larvae overlapped with that of *A. albopictus* larvae (18–24), and that *Lutzia* larvae fed on *A. albopictus* larvae (25,26). However, no study has quantitatively evaluated the effects of predation by *Lutzia* spp. on *A. albopictus* larval populations. Although *A. albopictus* immatures were either absent or present in low numbers in basins that also contained *Lt. vorax* larvae, very large numbers (> 2,000) of *A. albopictus* immatures were found in 2 of the 3 basins without *Lt. vorax* in the first collection time. Furthermore, the abundance of *A. albopictus* larvae increased in all the basins after the basins had been cleaned and the *Lt. vorax* larvae removed. These results indicate that these catch basins were suitable breeding sites for *A. albopictus*, but the presence of *Lt. vorax* larvae had suppressed their numbers in the first collection time. To the best of the author's knowledge, this is the first study to provide evidence that predation by *Lutzia* spp. limits the abundance of *A. albopictus* larvae under natural conditions. If *Lt. vorax* was absent from the campus, the density of *A. albopictus* could have been considerably higher. The high frequency of *Lutzia* spp. occurrence in catch basins may be unique to the present study sites. Previous investigations carried out in Japan have not reported the occurrence of *Lutzia* spp. in catch basins (27,28), or reported it at low frequencies (23). When *Lutzia* spp. or other predators have strong impacts on *A. albopictus* populations, chemical or physical control measures should be used with caution to avoid creating predator-free conditions for *A. albopictus*. In the present study, cleaning the catch basins after the first collection removed *Lt. vorax* larvae but not *A. albopictus* eggs. After the heavy rains, the newly hatched *A. albopictus* larvae could develop without the pressure of predation and attained high densities. Such improper cleaning of catch basins may result in temporary *A. albopictus* outbreaks.

There are other factors that may limit larval abundance in catch basins. Although at the first collection 2 of the 3 basins without *Lt. vorax* (basins 3 and 10) harbored > 2,000 *A. albopictus* larvae, a relatively small number of *A. albopictus* immatures were found in basin 7, possibly because it was located in a sunlit area. Three basins (basins 6–8) in the sunlit area also showed a relatively low abundance of immatures at the second collection time. In addition, there was a tendency for a higher abundance of *A. albopictus* larvae in shallower basins at the second collection time. The gravid females may find water bodies more easily in shallower basins. Thus, the results of the present study suggest that the abundance of *A. albopictus* larvae increases in shallow catch basins in shaded areas. Mosquito control measures may be more efficient if these catch basins are targeted as a high priority.

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Conflict of interest None to declare.

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