

## SCIENTIFIC NOTE

### OVIPOSITION OF *Aedes aegypti* AND *Aedes albopictus* ON OVI TRAPS IN DRY AND RAINY SEASONS IN SOUTHERN VIETNAM

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**ABSTRACT.** Ovitrap traps were set inside and outside of 15 households in December 2012 (dry season) and August 2013 (rainy season) in 2 communes (An Thanh and Phu Hoa) of Binh Duong Province in southern Vietnam. Eggs laid in the ovitraps were collected after 4 days, dried, and soaked in water. Hatched larvae were transferred to cups and reared to adulthood to identify the species. The rate of positive ovitraps did not differ between December and August for *Aedes aegypti*, but it was lower in December for *Ae. albopictus*. The number of eggs laid per ovitrap by *Ae. aegypti* did not differ between December and August, while that for *Ae. albopictus* decreased significantly in December in both communes. Moreover, *Ae. albopictus* laid eggs in ovitraps placed outside the households. Therefore, it is necessary for future studies to investigate whether the major source containers for oviposition had dried in the dry season or *Ae. albopictus* entered diapause, leading to these observations.

**KEY WORDS** *Aedes aegypti*, *Aedes albopictus*, ovitrap, Vietnam

Dengue, chikungunya, and Zika viruses are transmitted primarily by the yellow fever mosquito, *Aedes aegypti* (L.), and the Asian tiger mosquito, *Aedes albopictus* (Skuse) (Kraemer et al. 2015). Although *Ae. aegypti* and *Ae. albopictus* are dominant in southern and northern Vietnam, respectively, both species also coexist in many areas of Vietnam (Higa et al. 2010). The abundance of these species is influenced by the egg-laying behavior of females and their temporal and spatial distribution, both of which largely depend on the local climate (Serpa et al. 2013). *Aedes aegypti* cannot enter diapause (Denlinger and Armbruster 2014), except for 1 local population (Fischer et al. 2019). *Aedes albopictus* is regarded as a nondiapause species in South-east Asian countries (Hawley et al. 1987), but most *Ae. albopictus* from Hanoi enter diapause during winter (Tsunoda et al. 2015), and little is known about the diapause in *Ae. albopictus* from southern Vietnam.

Ovitrap traps are useful tools for studying oviposition activity of *Aedes* mosquitoes (Service 1993). Ovitrap surveillance provides a better assessment of the infestation density of *Ae. aegypti* than conventional surveys (Regis et al. 2008) because juvenile and adult surveys are labor-intensive and influenced by the abilities of the investigators. Therefore, we investigated the oviposition activity of *Ae. aegypti* and *Ae.*

*albopictus* in southern Vietnam using ovitrap surveillance in dry and wet seasons.

Eggs were collected from ovitraps set in 2 communes, An Thanh of Thuan An district and Phu Hoa of Thu Dau Mot district, of Binh Duong Province, southern Vietnam, in December 2012 and August 2013. A preliminary study revealed that *Ae. albopictus* was dominant in the Phu Hoa commune, while *Ae. aegypti* was dominant in An Thanh.

In each commune, we visited 15 houses, each with an unroofed garden, and set an ovitrap both inside and outside each house for 4 days. We later visited the same houses in each season. However, we were unable to revisit a few households owing to the absence of house owners or their refusal to allow us entry.

Each ovitrap was composed of a red bucket (diam: 25 cm; height: 20 cm) with 4 holes (ø: 5 mm) and was placed at height of 12 cm with 6 filter paper sheets (30 cm × 8 cm) hanging from the bucket's edge. We filled one-third of the bucket with water from a well or a concrete tank. Later, filter papers from the field were collected in plastic bags and brought to the laboratory. We examined these filter papers for the presence of eggs, dried them for a day, and kept them at 25°C in the laboratory. After a week, the papers were placed in a metal container (40 cm × 30 cm × 7 cm) covered with a cotton cloth with rubber at the edge (to prevent contamination) and dipped in dechlorinated water up to 2 cm in depth. We examined larvae posthatching for 2 months.

Multivariate stepwise logistic regression analysis was conducted to analyze the relative importance of statistically significant variables (place and season) for the presence or absence of *Ae. aegypti* and *Ae. albopictus* eggs in the ovitraps. Zero-inflated regression with a log link model was used to analyze the

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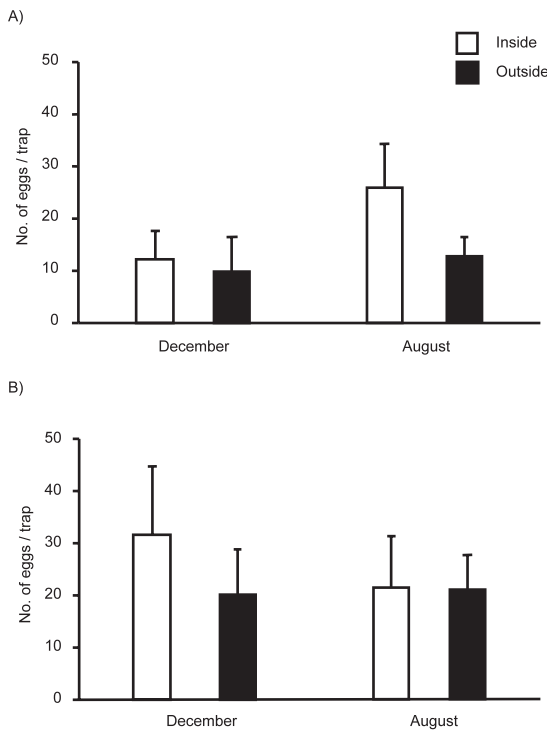


Fig. 1. Number of *Aedes aegypti* eggs collected from ovitraps set inside and outside the houses in December 2012 and August 2013. (A) An Thanh, (B) Phu Hoa.

number of eggs with seasons (December and August) and place (inside and outside). The best-fit model was selected to compare the zero-inflated Poisson model with the original Poisson model or zero-inflated negative bimodal model using the Vuong non-nested test.

The abundance of *Ae. aegypti* eggs recovered in December (total = 316, average  $\pm$  SD = 22.6  $\pm$  34.0 in An Thanh; total = 773, average  $\pm$  SD = 51.5  $\pm$  58.0 in Phu Hoa) was not significantly different from that in August (total = 577, average  $\pm$  SD = 38.5  $\pm$  10.7 in An Thanh; total = 636, average  $\pm$  SD = 42.4  $\pm$  46.2 in Phu Hoa). However, the number of *Ae. albopictus* eggs recovered in December was significantly lesser than that in August in Phu Hoa (total = 30, average  $\pm$  SD = 2.0  $\pm$  4.1 in December; total = 1409, average  $\pm$  SD = 93.9  $\pm$  65.2 in August) but not in An Thanh (total = 78, average  $\pm$  SD = 5.6  $\pm$  9.4 in December; total = 352, average  $\pm$  SD = 23.5  $\pm$  27.9 in August).

The rates of ovitraps positive for *Ae. aegypti* were not significantly different between December and August in An Thanh (41.4% in December and 66.7% in August) and Phu Hoa (56.7% in December and 70.0% in August). However, the rate of ovitraps, placed outside the households, that were positive for *Ae. albopictus* was lower in December (27.6% in An Thanh; 26.7% in Phu Hoa) than in August (66.7% in

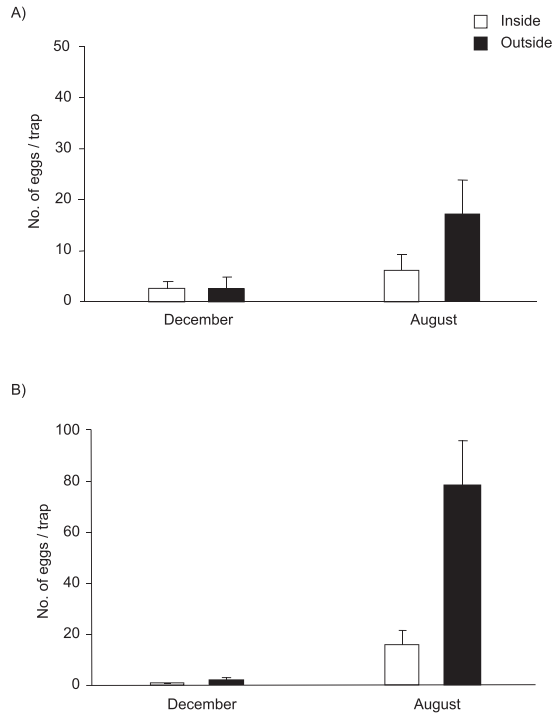


Fig. 2. Number of *Aedes albopictus* eggs collected from ovitraps set inside and outside the houses in December 2012 and August 2013. (A) An Thanh, (B) Phu Hoa.

An Thanh; 80.0% in Phu Hoa). The logistic regression model revealed significant interaction between seasons and place in *Ae. albopictus* in An Thanh (estimate = -1.66,  $z$  = -2.12,  $P$  < 0.05). In Phu Hoa, the percentage of positive ovitraps for *Ae. albopictus* was 83.3% lower in December than that in August (estimate = -1.79,  $z$  = -2.15,  $P$  < 0.05).

The zero-inflated negative bimodal regression model was selected for *Ae. aegypti* females from both communes, and their coefficients were not significant (Fig. 1A, 1B). Although the average number of *Ae. albopictus* eggs per ovitrap placed inside the house were less than those placed outside in December, they were not significant in An Thanh (Fig. 2A). In Phu Hoa, the same model revealed that the average number of *Ae. albopictus* eggs per ovitrap was 97.7% lower in December than that in August (estimate = -2.38,  $z$  = -4.89,  $P$  < 0.001). Furthermore, it was 4.38 times higher outside than on the inside in Phu Hoa (estimate = 0.95,  $z$  = 2.56,  $P$  < 0.05) (Fig. 2B).

Our results revealed that neither the rate of positive ovitraps nor the average number of eggs per ovitrap was different inside and outside of the houses, and between seasons in *Ae. aegypti*. In contrast, in *Ae. albopictus*, both parameters were significantly higher outside in August and significantly lower in December.

Mosquitoes thrive in Binh Duong throughout the year because of its tropical monsoon climate with an annual minimum temperature of 18°C or higher. Temperature below 17°C makes *Ae. aegypti* sluggish (Christophers 1960). However, due to the tropical monsoons, the weather of Binh Duong is hot with little rain from December to March (Binh Duong Statistical Office 2017). In northern Vietnam, *Ae. albopictus* prefers discarded rainwater containers for egg laying (Tsunoda et al. 2014). In December, being the dry season in the province, there are few containers for immature mosquitos to develop outside households, leading to fewer adult *Ae. albopictus* but with less pronounced effects on *Ae. aegypti*. Moreover, *Ae. albopictus* from Binh Duong Province might become quiescent. Therefore, the dormancy of *Ae. albopictus* in Binh Duong Province must be examined in the laboratory to explain these observations.

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#### REFERENCES CITED

- Binh Duong Statistical Office. 2017. *Statistical yearbook of Binh Duong 2016*. Binh Duong, Vietnam: Binh Duong Statistical Office.
- Christophers SR. 1960. *Aedes aegypti (L.) The yellow fever mosquito. Its life history, bionomics and structure*. Cambridge: Cambridge University Press.
- Denlinger DL, Armbruster PA. 2014. Mosquito diapause. *Annu Rev Entomol* 59:73–93.
- Fischer S, De Majo MS, Di Battista CM, Montini P, Loetti V, Campos RE. 2019. Adaptation to temperate climates: evidence of photoperiod-induced embryonic dormancy in *Aedes aegypti* in South America. *J Insect Physiol* 117:103887.
- Hawley WA, Reiter P, Copeland RS, Pumpuni CB, Craig GB Jr. 1987. *Aedes albopictus* in North America: probable introduction in used tires from northern Asia. *Science* 236:1114–1116.
- Higa Y, Yen NT, Kawada H, Son TH, Hoa NT, Takagi M. 2010. Geographic distribution of *Aedes aegypti* and *Aedes albopictus* collected from used tires in Vietnam. *J Am Mosq Control Assoc* 26:1–9.
- Kraemer MU, Sinka ME, Duda KA, Mylne AQ, Shearer FM, Barker CM, Moore CG, Carvalho RG, Coelho GE, Van Bortel W, Hendrickx G, Schaffner F, Elyazar IR, Teng HJ, Brady OJ, Messina JP, Pigott DM, Scott TW, Smith DL, Wint GR, Golding N, Hay SI. 2015. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. *Elife* 4:e08347.
- Regis L, Monteiro AM, Melo-Santos MA, Silveira JC Jr, Furtado AF, Acioli RV, Santos GM, Nakazawa M, Carvalho MS, Ribeiro PJ, Souza, WV. 2008. Developing new approaches for detecting and preventing *Aedes aegypti* population outbreaks: basis for surveillance, alert and control system. *Mem Inst Oswaldo Cruz* 103:50–59.
- Serpa LL, Monteiro Marques GR, De Lima AP, Voltolini JC, Arduino Mde B, Barbosa GL, Andrade VR, De Lima VL. 2013. Study of the distribution and abundance of the eggs of *Aedes aegypti* and *Aedes albopictus* according to the habitat and meteorological variables, municipality of São Sebastião, São Paulo State, Brazil. *Parasit Vectors* 6:321.
- Service MW. 1993. *Mosquito ecology: field sampling methods*. 2nd edition. London and New York: Elsevier Applied Science.
- Tsunoda T, Chaves LF, Nguyen GT, Nguyen YT, Takagi M. 2015. Winter activity and diapause of *Aedes albopictus* (Diptera: Culicidae) in Hanoi, northern Vietnam. *J Med Entomol* 52:1203–1212.
- Tsunoda T, Cuong TC, Dong TD, Yen NT, Le NH, Phong TV, Minakawa N. 2014. Winter refuge for *Aedes aegypti* and *Ae. albopictus* mosquitoes in Hanoi during winter. *PLoS One* 9:e95606.