Short Communication

Evidence for *Aedes aegypti* (Diptera: Culicidae) Oviposition on Boats in the Peruvian Amazon

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ABSTRACT Dengue vector *Aedes aegypti* L. is invading peri-urban and rural areas throughout Latin America. Our previous research in the Peruvian Amazon has shown that river boats are heavily infested with immature and adult *Ae. aegypti* mosquitoes, likely playing a major role in their long-distance dispersal and successful invasion. However, the presence of immature mosquitoes provides no information about the timing of oviposition, and whether it took place in the boats. Here, we used baited ovitraps deployed on river boats to test the hypothesis that *Ae. aegypti* oviposition occurs during boat travel. We deployed 360 ovitraps on 60 different barges during August and October of 2013, and February 2014 (with 20 barges sampled during each month). We found that *Ae. aegypti* mosquitoes in 22 individual ovitraps from 15 of the 60 barges (premise index 25%) across all sampling dates. Further, the distribution of *Ae. aegypti* egg abundance was highly aggregated: 2.6% of traps ($N = 7$) were responsible for 71.8% of eggs found, and 1.5% of traps ($N = 4$) were responsible for all (100%) of the larvae found. Similarly, 5% of boats were responsible for the 71.47% of eggs. Our results provide strong evidence that *Ae. aegypti* oviposition commonly occurs during boat travel. Baited ovitraps could represent a cost-effective means of monitoring and controlling mosquito populations on boats.

KEY WORDS *Aedes aegypti*, oviposition, boat, ovitrap

Dengue vector *Aedes aegypti* L. is an invasive mosquito—originally West African in origin, it is widely accepted that *Ae. aegypti* was transported to the Americas via trade ships in the 17th–19th centuries (Gubler 1997). Following the waning of a Pan American Health Organization (PAHO) yellow fever control program in the mid-1900s, *Ae. aegypti* began reinvading urban centers throughout Latin America (PAHO 1986). Recent reports from Argentina and Peru exemplify *Ae. aegypti* geographic expansion from urban to peri-urban and rural areas (Troyes et al. 2006, Diaz-Nieto et al. 2013).

Ae. aegypti mosquitoes are highly adapted to human environments: females feed almost exclusively on humans, prefer to rest in dark, cool areas (usually indoors) (Halstead 2008), and adult female mosquitoes lay their eggs on the inner walls of water-filled artificial containers found in and around the home such as vases, plastic buckets, bird baths, water storage tanks, and discarded refuse and tires. These artificial containers are dependent on biotic factors such as bacteria, fungi, algae, as well as abiotic factors including pH, temperature, dissolved solids, and dissolved oxygen. A wide variety of microbes have been identified in *Ae. aegypti* breeding containers (Ponnusamy et al. 2008a). These microbes play important roles in larval nutrition as well as attraction and oviposition stimulation of gravid female mosquitoes (Kaufman et al. 1999; Ponnusamy et al. 2008b, 2010). This mosquito's adaptation to human environments, coupled with the longevity and resistance of its eggs to desiccation (Sota and Mogi 1992, Juliano et al. 2002), contribute to the vector's passive spread to new areas via human transportation networks (Soper 1967).

Our previous research demonstrated infestation of immature and adult *Ae. aegypti* on large barges in and around the Amazonian city of Iquitos, Peru

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(Guagliardo et al. 2015; Morrison et al. 2006). Results from these studies showed that Amazonian barges provide ideal conditions for all stages of the mosquito life cycle: there are abundant oviposition sites (in the form of floor puddles in cargo holds), ample human hosts for blood meals, and dark, humid resting sites for adult mosquitoes. Despite persistent *Ae. aegypti* infestation of boats, several questions about the infestation process remain (discussed in further detail, Guagliardo et al. 2015). In this Short Communication, we address the question: does *Ae. aegypti* oviposition occur during boat travel? The mere presence of immature mosquitoes on barges does not offer any information about the timing of oviposition. In other words, two possibilities exist: 1) port populations of gravid females may fly aboard docked barges in search of oviposition sites and 2) *Ae. aegypti* females on barges may oviposit during transit. We tested the hypothesis that *Ae. aegypti* oviposition occurs during boat travel by setting baited ovitraps on barges prior to departure from Iquitos, and collecting and examining the traps upon return to Iquitos.

Findings from this study have implications for the control of *Ae. aegypti* spread. If oviposition occurs while boats are docked, then vector control authorities could effectively apply larvicides and adulticides while boats are docked in the city of Iquitos. However, if *Ae. aegypti* oviposition occurs during boat travel, this implies that mosquito populations colonize and thrive on boats, thus presenting more challenges to mosquito population control.

To our knowledge, this is the first study that has used traps to monitor *Ae. aegypti* populations aboard vehicles. Such an approach may represent an innovative and cost-effective means for monitoring mosquito populations on vehicles.

Materials and Methods

Large barges were monitored for *Ae. aegypti* oviposition during August and October 2013 and February 2014, with 20 barges sampled during each month. Barges were selected for the study on the basis of owners' willingness to participate and departure time (only barges departing within 1 d were included). Congruent with previous protocols, ovitraps were red plastic cups filled three-fourths water (volume = 56.5 in^3) and lined with paper (Guagliardo et al. 2014). Ovitraps were baited with a mixture of bio-active bacterial attractant (composed of four species of bacteria, Ponnusamy et al. in review) in calcium alginate beads and spinosad larvicide (Wesson et al. in review). The composition of the mixture in each ovitrap was 100 mg of attractant and 240 mg of spinosad granules (Natular G, Clarke, Roselle, IL). Six ovitraps were placed within each barge in a dark secluded area to maximize the probability of oviposition.

The exact dates of trap deployment and collection were noted for each barge, and the total travel time was calculated. The number of days between ovitrap deployment and collection varied because of different travel routes and destinations. Therefore, an increasing duration of ovitrap deployment could result in greater probability of oviposition because of the accumulation of organic material in the water over time. To account for this, we used a *t*-test to compare the mean travel time in days for positive versus negative ovitraps.

Table 1. Proportion of positive boats by date

Month	No. positive boats	No. sampled	Premise index (%)
Aug. 2013	4	20	20
Oct. 2013	4	20	20
Feb. 2014	7	20	35
Overall	15	60	25

There were no significant differences in the proportion of boats with positive ovitraps by month (Fisher's exact test $P > 0.5$). The premise index describes the number of positive vehicles/number sampled × 100 (Morrison et al. 2006).

Sterile bags (Whirlpak, NASCO Plastics, New Hamburg, Ontario, Canada) were used to transport immature mosquitoes to the field laboratory for taxonomic identification to species, and ovitrap paper was thoroughly inspected under a microscope for the presence of eggs. The number of *Ae. aegypti* eggs was tallied by individual ovitrap and boat, and the proportions of positive traps were calculated by boat and by month. In some cases, ovitraps were knocked over or disappeared. To adjust for missing ovitraps, we calculated the proportion positive using a denominator that only included the number of intact traps at the time of collection.

All data analysis and graphs were produced using R statistical software (R Development Core Team 2008, Vienna, Austria). The premise index (positive vehicles/number sampled × 100) was calculated by date, and Fisher's exact tests were used to determine whether there were significant differences in the proportion of barges positive for *Ae. aegypti*. To measure abundance, we calculated the mean number of eggs, larvae, and pupae per trap by month.

Results

Approximately 75% of ovitraps that were set (271 traps of 360) were successfully recovered. The remaining 89 traps were either knocked over during transit or disappeared. The average trip duration (and thus the number of days between ovitrap deployment and collection) was 15.5 d (SD = 6.1), although trip duration did not differ for positive versus negative boats ($t = 1.55$; $P > 0.1$).

Among positive ovitraps, the overwhelming majority of mosquitoes found were *Ae. aegypti*. Two *Culex quinquefasciatus* Say larvae were found in a single trap during the month of February. Immature *Ae. aegypti* mosquitoes were found in 22 individual ovitraps from 15 of the 60 barges (premise index 25%) across all sampling dates (Table 1). The proportion of positive boats was highest in the month of May (35%) followed by August and October (20% each), although these observed differences were not statistically significant (Fisher's exact test $P > 0.5$).

The distribution of *Ae. aegypti* egg abundance was highly aggregated: 2.6% of traps ($N = 7$) were

Table 2. Number of *Ae. aegypti* eggs and larvae per trap by month

Month	Mean eggs per trap (*N*)	Mean larvae per trap (*N*)	Mean pupae per trap (*N*)	Collected traps
Aug. 2013	0.91 (86)	0	0	95
Oct. 2013	4.06 (325)	0	0	80
Feb. 2014	2.07 (199)	0.10 (10)	0.03 (3)	96
Overall	2.25 (610)	0.04 (10)	0.01 (3)	271

The greatest number of eggs was found in October, while the least number of eggs was collected in August.

responsible for 71.8% of eggs found, and 1.5% of traps ($N = 4$) were responsible for all (100%) of the larvae found. Similarly, 5% of boats were responsible for the 71.47% of eggs. The greatest abundance of eggs was found during the month of October ($N = 325$, 4.1 eggs per collected trap), while the least number of eggs were collected in August ($N = 86$, 0.9 eggs per trap; Table 2). Larvae and pupae were only found during the month of February.

Discussion

Our results provide strong evidence that *Ae. aegypti* oviposition occurs during barge travel throughout the year. It is probable that barges can support entire *Ae. aegypti* populations: barges contain dark, secluded areas, abundant oviposition sites, and ample human hosts for blood meals. Although oviposition occurs during barge travel, this does not eliminate the possibility that oviposition may also occur when port populations of mosquitoes invade boats in search of oviposition sites.

Our previous research has shown that ground puddles formed in the bottom of cargo holds serve as productive and common *Ae. aegypti* immature habitats. Therefore, it is likely that our estimates of oviposition frequency are conservative, as our ovitraps compete with "natural" habitats in the cargo holds. Even so, in comparison with other collection methods such as aspiration of adults, ovitraps represent a cost-effective and an easy way to monitor mosquito populations. Local health authorities and vector control programs could use this approach to monitor the presence of *Ae. aegypti* on vehicles, and ultimately use this information to slow the spread of this invasive vector.

To complement our findings that a few boats are responsible for most mosquito production and movement over long distances, we now add the observation that a few oviposition sites are responsible for much of the larval production. Such information is relevant to the targeted control of larval habitats within boats.

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