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# Ovitrap Monitoring of *Aedes aegypti* and *Aedes albopictus* in Two Selected Sites in Quezon City, Philippines

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**In recent years, *Aedes* mosquitoes have become a serious health threat in the Philippines, causing a dramatic increase in dengue incidence. To design and implement adapted vector control measures, knowledge of vector composition and abundance is essential. Surveillance of mosquito populations using ovitraps was conducted for about two years (from April 2018–February 2020) in two selected sites (Sitio Payong and Villa Beatriz in Old Balara, Quezon City). These areas were identified as potential sites for small-scale pilot trials of the sterile insect technique. *Aedes aegypti* and *Ae. albopictus* were present in both sites, but *Ae. aegypti* was about 20 times more abundant than *Ae. albopictus*. There was a significant difference between the two sites in the number of eggs collected weekly but not in the OI. The number of *Aedes* spp. eggs collected were found to positively correlate with the maximum daily temperature. These data provide comprehensive, evidence-based information that will help in the design and implementation of *Aedes* control measures, as part of wider public health interventions for prevention and control.**

Keywords: climatological parameters, dengue vector, ovitrap index, population monitoring, sterile insect technique

## INTRODUCTION

Mosquitoes (Diptera: Culicidae) are vectors of a large variety of pathogens ranging from viruses, bacteria, protozoans, and even worms – and, therefore, are a significant and contemporary health issue for both humans and animals worldwide. *Aedes aegypti* and *Ae. albopictus* are major vectors of dengue, chikungunya, yellow fever, and Zika viruses (WHO 2021a). The immature stages of *Ae. aegypti* are found in water-filled habitats, mostly artificial containers closely associated with human dwellings and often indoors (Manrique-Saide *et al.* 2015), whereas *Ae. albopictus* is primarily a forest species that have adapted to rural, suburban, and urban human environments (Li *et al.* 2014).

In the Philippines, dengue incidence has dramatically increased over the past two years. The Department of Health (DOH) reported a 42% increase, an additional 216,190 dengue positive cases nationwide in 2018 over those reported in 2017 (DOH 2018). Moreover, between January–August 2019, there was a 95% increase in

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dengue-positive cases compared to the same period in 2018, as well as 1,107 deaths (DOH 2019). Dengue cases seem to be dropping significantly in 2021 with 6,614 dengue cases, including 20 deaths, reported as of February 2021 (WHO 2021b). However, it might be possible that patients with dengue are not going to hospitals due to the fear brought about by the current COVID-19 pandemic, which started in the Philippines in January 2020.

Historical data reveals that cases of chikungunya have been reported in the Philippines since the 1950s, mostly in the center and south of the country, but the first major outbreak occurred in 2012 in San Pablo City, Laguna Province (Ballera *et al.* 2015). In 2018, 282 chikungunya cases were reported nationwide between 01 January–31 March, 6% higher than those in the same period in 2017 (266 cases) (DOH 2018).

With no specific treatments or vaccination available for most mosquito-borne diseases, vector control plays a significant role in controlling and preventing transmission (Lees *et al.* 2014). With the current vector control methods generally giving limited and short-term successes, innovative and complementary control tools and strategies such as the sterile insect technique (SIT) are urgently needed as part of an integrated vector management approach (Stone 2013).

The Philippines is considering including SIT as a complementary control method to be used synergistically in an integrated management framework against dengue vectors. However, deploying rational, appropriate, and timely response measures such as the SIT relies on a thorough knowledge of vector species composition, abundance, and seasonal dynamics. This knowledge can also help to forecast disease outbreaks. For instance, Duncombe *et al.* (2013) used double-sticky ovitraps to describe and project the spatial and temporal distribution of *Aedes* populations in a village in Muntinlupa City, Philippines. A study by Mistica *et al.* (2019), on the other hand, assessed the species composition of mosquitoes collected from ovitraps in selected schools of Metro Manila, Philippines, and showed that the infestation rate was 49.69% – of which 88.94% was *Ae. aegypti*, whereas the remaining 11.06% was *Ae. albopictus*. Ovitraps were a more sensitive, economical, and reliable tool for detecting *Ae*des spp. compared to larval surveys (Nascimento *et al.* 2020; Rozilawati *et al.* 2015). Resources for entomological surveillance and control are limited in the Philippines, and efforts are generally focused on epidemics when the transmission is difficult to control (Duncombe *et al.* 2013).

Implementation of effective mosquito control measures should be guided by reliable surveillance data on mosquito diversity, distribution, and abundance. However, baseline information is only available from limited studies (like those indicated above) in only a few areas of the Philippines. Since the Philippines is assessing the feasibility of applying the SIT as a component of area-wide integrated pest management (AW-IPM), this study aimed to explore the presence of both *Ae. aegypti* and *Ae. albopictus*, their relative abundance, and population fluctuations over two years – encompassing both dry and rainy seasons – in two selected areas in Quezon City, the most populated city of the Philippines. These sites are under consideration as potential sites for a feasibility trial of the SIT.

## MATERIALS AND METHODS

### Study Sites

The study was conducted in Quezon City, a highly urbanized and the most populous city in the Philippines. The selected sites – Sitio Payong (SP) (14°40'10.43" N, 121°5' 51.34" E) and Villa Beatriz (VB) (14°40'26.08" N, 121°4' 36.77"E) – are both located in Old Balara (Figure 1), a locality with total dengue cases of 233, 254, and 190 in 2017, 2018, and 2019, respectively and was listed in 2019 as the village with the 6$^{th}$ highest dengue cases in Quezon City (Philstar 2019). Both sites have similar environmental and social conditions, proven presence of both *Aedes aegypti* and *Ae. albopictus*, and dengue cases were identified as potential SIT pilot trial sites by the DOST-NRCP (Department of Science and Technology–National Research Council of the Philippines) in 2013. The request to set up traps in the selected sites was made through the Quezon City Health Office, and traps were placed with the assistance of the Old Balara Health Centre Health workers.

SP is located about 4–5 km from the capital city along the Marikina River, whereas VB is about 2 km from the capital city. The sites are separated by a highway and a distance of 2.7 km (Figure 1). SP covers 4 hectares with about 450 households comprising a population of approximately 3,000 inhabitants and is characterized by typical small to medium rural houses (between 4–20 m$^2$ with 1–2 bedrooms) made of wood with a corrugated metal roof. VB covers 3 hectares with about 235 households. Some people use mosquito coils and mosquito nets to prevent mosquito bites because windows and doors are not screened. Alarming rates of dengue fever cases were reported in SP and VB before the study. Hence, they were identified as the study sites for the SIT pilot trial (Figure 1).

As defined by the PAGASA (Philippine Atmospheric, Geophysical, and Astronomical Services Administration) under the DOST, the region has two seasons: the dry

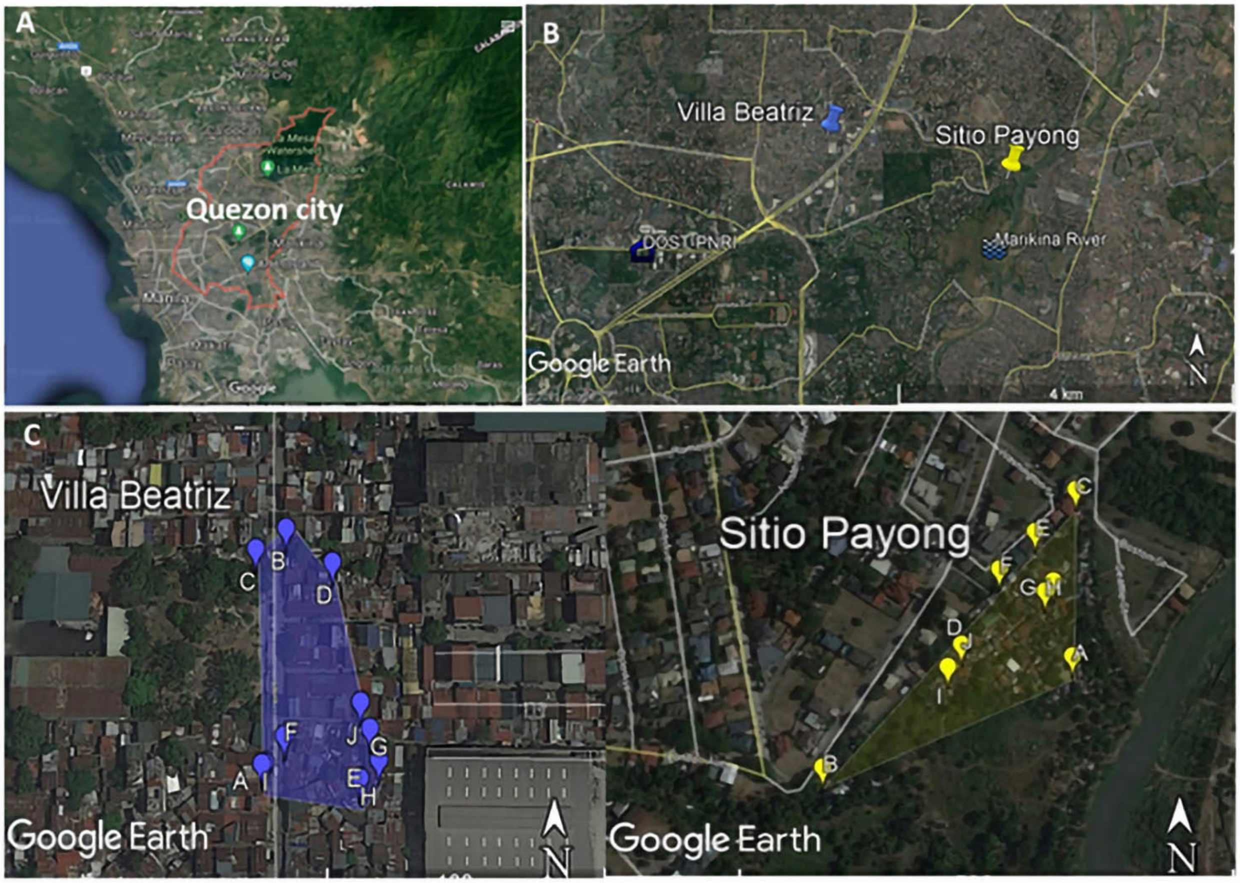


**Figure 1.** Aerial view and location of the study area (A), study sites (B), and ovitrap sampling points (C).

season that runs from December–May and the wet season that extends from June–November.

**Field Setup of the Ovitraps**

The population monitoring was performed using ovitraps, the most common and cost-effective *Aedes* invasive mosquito surveillance and monitoring method that is widely used in various settings (Velo *et al.* 2016; Wint *et al.* 2020). Ovitraps used in this study (9.0-cm diameter, 12.3-cm height, 500-mL capacity) were adapted from a model of ovicidal-larvicidal traps developed by the DOST to attract female dengue mosquitoes to lay eggs on the cup's surface or the paddle placed inside the cup. This trap was modified by replacing the pellet and paddle with seed germination paper, which has been found to be the most suitable oviposition substrate (Velo *et al.* 2016).

The modified ovicidal-larvicidal traps (thereafter ovitraps) were set in 10 different locations per site through the assistance of the health worker assigned in the area and with permission from the house owners: 10 ovitraps per site and one ovitrap per house from April 2018–February 2020 (23 mo) in SP and VB. Ovitraps were placed across each area – an average of 94.78 ± 8.44 m from each other – in discrete shaded areas away from sprinklers, rainfall, and direct sunlight to attract gravid mosquitoes. Weekly inspections were done with the assistance of the barangay health workers after informing house owners. During weekly inspections, seed germination papers were removed from the ovitraps and placed in individual plastic bags, and water samples were transferred to bottles. The inside surfaces of ovitraps were thoroughly cleaned by washing with clean water then refilled with clean tap water, and new seed germination papers were added for the next weekly collection. All samples were brought to the Entomology Mosquito Laboratory of the PNRI (Philippine Nuclear Research Institute).

**Laboratory Processing of Field-collected Eggs**

Eggs papers were placed in covered trays for one day and air-dried for about 5–6 days, counted using the stereomicroscope (Olympus SZG1TR w/ 0.67–4.5 x magnification), then submerged in water for hatching. After the eggs hatch, larvae were transferred to rearing cups for development. The water collected from each ovitrap was checked for the presence of eggs and immature mosquitoes. Larvae from water samples were counted and placed in rearing containers or cups and fed with a small amount of diet (85% cat food and 15% brewer's yeast). The pupae were collected and transferred to separate 12-oz plastic cups for emergence. The emerging adults were stored in the freezer and identified using taxonomic keys from Rueda (2004). *Ae. albopictus* has a narrow-median longitudinal white stripe in the scutum, whereas *Ae. aegypti* has a pair of submedian-longitudinal white stripes or with a lyre-shaped marking and without median-longitudinal white stripe (Figure 2).

### Statistical Analysis

Species distribution % corresponds to the proportion of each species in a site, computed based on the % of each species (*Ae. aegypti* or *Ae. albopictus)* over the total number of *Aedes* spp. Egg hatch was measured from the eggs present on the seed germination paper only. All the other parameters were determined based on seed germination paper and water samples from the ovitraps combined. The ovitrap index (OI) was calculated for each site using the following formula:

$$Ovitrap\ index = \frac{Number\ of\ ovitraps\ positive\ for\ oviposition}{Total\ number\ of\ ovitraps} \times 100$$

Monthly OI referred to the pooled results of all the ovitraps retrieved weekly from the 10 ovitraps per site from the same month. It reflects the overall vector situation for the month. Over the 23 mo of monitoring, a total of 974 and 990 ovitrap collections were made in households for VB and SP, respectively.

For the data on comparison of two sites, all data passed the normality tests except for % adult emergence in SP and male: female ratio in SP. In addition, all data passed the homogeneity tests of variances except for the OI, number of eggs, and % adult emergence. For the data on comparing two *Aedes* species, data on the number of adults collected and sex ratio passed the normality tests but failed to pass the homogeneity tests of variances. Meanwhile, the data on % adult emergence and % species distribution passed the homogeneity tests of variances but failed to pass the normality tests. Satterthwaite method of two independent sample t-test was done on data with unequal variances while pooled for data with equal variances. The alpha level of statistical analyses was 0.05 in all analyses.

Spearman's rank correlation was used for the analysis of the relationship between the observed parameters and the climatological data obtained from the DOST-PAGASA Science Garden Weather Station in Quezon City.

## RESULTS

### Positive Ovitrap Index

The monthly OI varied from 70–94% in VB and 47.5–100% in SP. The overall mean OI was 85.64 ± 1.44% and 83.17 ± 2.68% for VB and SP (Table 1); no significant difference was found between both study sites.

### Egg Density, Egg Hatch, and Pupation Percentages

A total of 59,548 and 39,220 *Aedes* spp. eggs were collected from the ovitraps throughout the study period in VB and SP, respectively. Although Figure 3 shows that egg density per ovitrap varied between months; there was, however no trend of seasonal variation across wetter and drier months. As for VB, the monthly mean number of eggs collected per ovitrap was higher in the first year of collection than in the second year; for SP, the trend was similar across years. From April 2018–February 2020, a significant difference ($F$ = 17.63, $p$ = 0.001) was found in the mean number of *Aedes* spp. eggs collected per ovitrap and per week between the two sites (mean ± SE; VB = 58.83 ± 5.07 and SP = 39.07 ± 2.34) (Figure 3). There was no significant difference in mean weekly egg

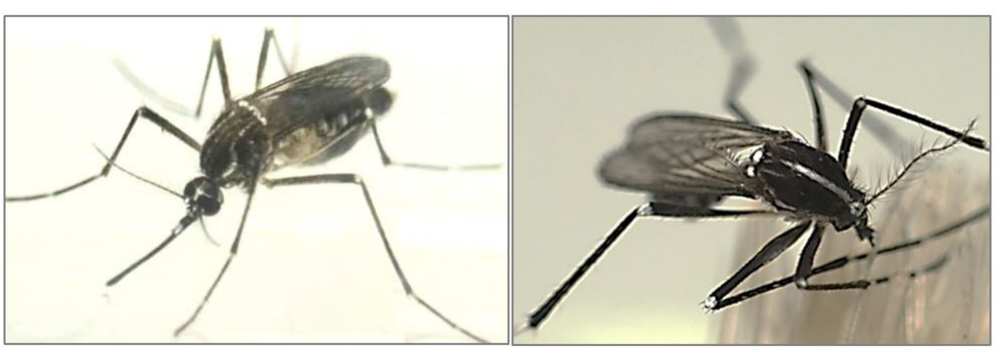

**Figure 2.** Taxonomic characters of adult *Aedes aegypti* with lyre-shaped white bands on the thorax (left) and *Aedes albopictus* with one line in the middle of the thorax (right). (photo not on-scale; photo credit: G.B. Obra).

**Table 1.** Comparison of Sitio Payong and Villa Beatriz mosquito populations based on ovitrap index, no. of eggs, hatch, pupation, and adult emergence of *Aedes* spp. [SE] standard error; [DF] degrees of freedom; [s] significant; [ns] not significant.

| Parameter | Villa Beatriz mean ± SE | Sitio Payong mean ± SE | DF | Variances | *t*-value | Pr(>(>\|t\|) | Sig. |
|---|---|---|---|---|---|---|---|
| Ovitrap index | 85.64 ± 1.44 | 83.17 ± 2.68 | 33.68 | Unequal | –0.81 | 0.4225 | ns |
| No. of eggs (per trap per week) | 58.83 ± 5.07 | 39.07 ± 2.34 | 30.95 | Unequal | –3.54 | 0.0013 | s |
| % hatch | 80.01 ± 1.10 | 82.24 ± 1.30 | 44.00 | Equal | –1.31 | 0.1961 | ns |
| % pupation | 84.69 ± 1.34 | 86.63 ±1.34 | 44.00 | Equal | 1.18 | 0.2449 | ns |
| % adult emergence | 96.22 ± 0.47 | 95.12 ±1.36 | 27.27 | Unequal | 27.27 | 0.4505 | ns |
| Sex ratio (male: female) | | | | | | | |
| *Ae. aegypti* | 0.51:0.49 | 0.50:0.50 | 44 | Equal | –/+1.56 | 0.1256 | ns |
| *Ae. albopictus* | 0.51:0.49 | 0.44:0.56 | 39 | Equal | –/+1.80 | 0.0801 | ns |

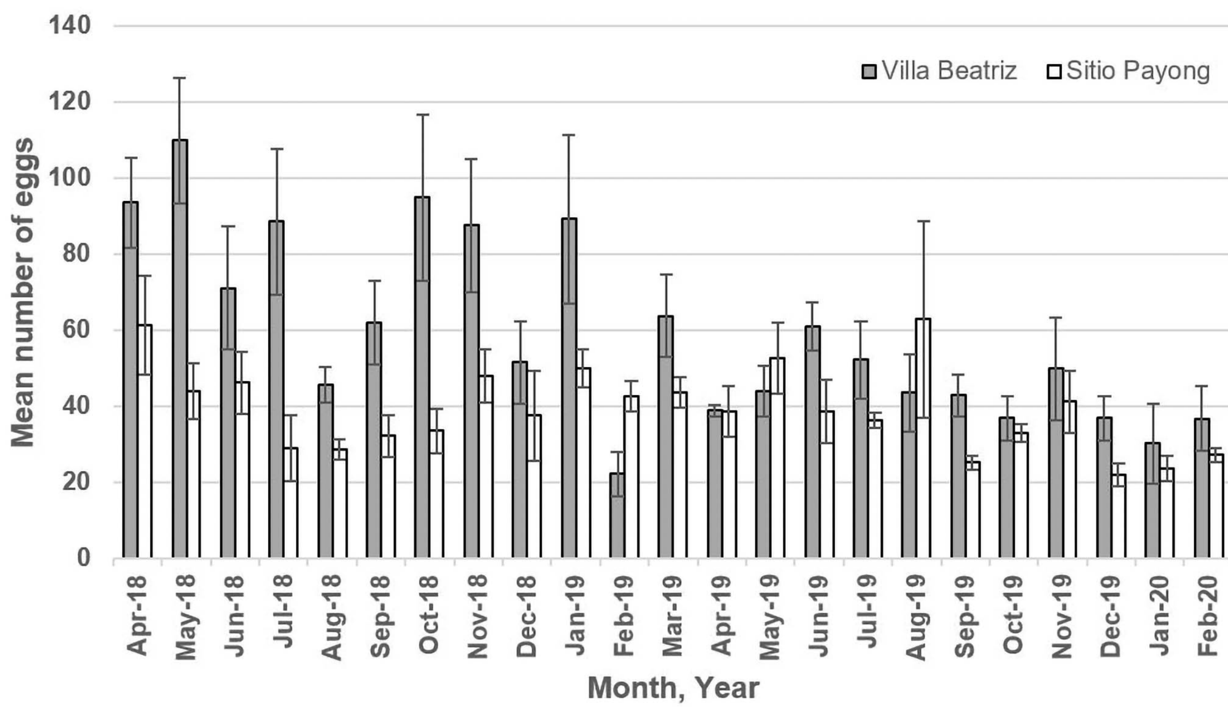


**Figure 3.** Monthly average number of *Aedes* spp. eggs per ovitrap in Villa Beatriz and Sitio Payong. Data shown as an average of all ovitraps per site; error bars denote standard error.

hatch: 82.24 ± 1.10% and 82.34 ± 1.30% for VB and SP, respectively (Table 1).

The percentage of pupae collected from larvae reared decreased slightly towards the end of the year. The mean pupation percentage was 84.69 ± 1.34% for VB and 86.63 ± 1.34% for SP. There was no significant difference in mean weekly pupation between sites; rates were lower than typically observed in the laboratory, with an average of 98.33 ± 0.29% across both sites.

The percentage of adult emergence for both sites and both species is presented in Tables 1 and 2. A difference in adult emergence of *Aedes* spp. was noted between sites. A total of 32,485 adults *Ae. aegypti* emerged from collections in VB and 21,420 in SP. Meanwhile, for *Ae. albopictus*, 972 adults emerged from collections in VB and 1,425 in SP. *Ae. aegypti* was predominant in both study sites (Table 2; Figure 4). From December–May when no rains occur (Figure 5), the adult population of *Ae. albopictus* was very low or absent in both sites (Figure 4). However; *Ae aegypti* is present in higher proportion throughout the year, the mean male: female sex ratio for both sites

**Table 2.** Comparison of *Aedes aegypti* and *Ae. albopictus* populations in Sitio Payong and Villa Beatriz combined based on no. adults that emerged, adult emergence, and male sex ratio. [SE] standard error; [DF] degrees of freedom; [s] significant; [ns] not significant.

| Parameter | *Ae. aegypti* mean ± SE | *Ae. albopictus* mean ± SE | Variances | DF | *t*-value | Pr(>(>\|t\|) | Sig. |
|---|---|---|---|---|---|---|---|
| No. of adults (per trap per week) | 26.73 ± 1.72 | 1.18 ± 0.93 | Unequal | 22.57 | 14.79 | < 0.001 | s |
| % adult emergence | 95.67 ± 0.79 | 97.63 ± 0.84 | Equal | 43 | –1.70 | 0.0969 | ns |
| % species distribution | 95.02 ± 0.68 | 4.98 ± 0.86 | Equal | 44 | 74.33 | < 0.001 | s |
| Sex ratio | 0.50: 0.50 | 0.49: 0.51 | | | | | |
| Male | 0.50 ± 0 | 0.49 ± 0.02 | Equal | 43 | 0.64 | 0.5256 | ns |
| Female | 0.50 ± 0 | 0.51± 0.02 | Equal | 43 | –0.64 | 0.5256 | ns |

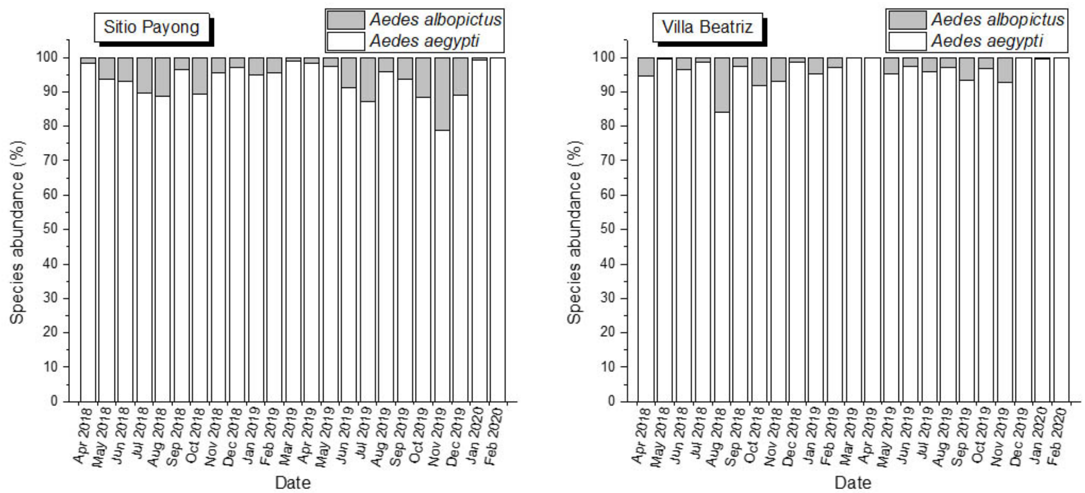


**Figure 4.** Relative abundance and population variation of *Aedes albopictus* and *Aedes aegypti* in Sitio Payong (left) and Villa Beatriz (right). Data shown as total abundance in all ovitraps at each site each month.

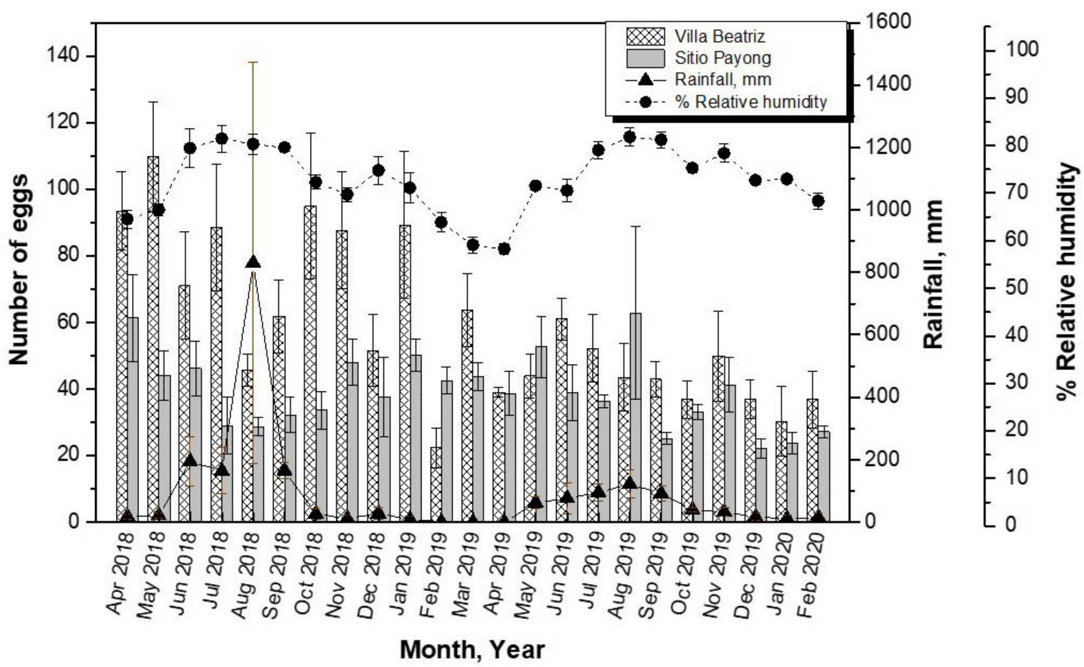


**Figure 5.** Temporal variation in the monthly mean number of *Aedes* spp. eggs collected per weekly trap in relation to rainfall and % relative humidity in Villa Beatriz and Sitio Payong from April 2018 to February 2020.

was 50: 50 for *Ae. aegypti* and 49: 51 for *Ae. albopictus* (Table 2). Although a slightly female-biased population was observed in *Ae. albopictus*, no significant difference was calculated between species (Table 2), and between sites or species (Table 1).

**Weather Data and Correlation with Entomological Parameters**

During the study period, the daily average temperature ranged from 20.9 ˚C (February 2019) to 34.5 ˚C (May 2018), whereas the daily average relative humidity varied from 56% (March 2019) to 84% (June 2018). The wettest month was August, during which the highest rainfall was 70 mm, whereas the driest month with the lowest rainfall of about 3 mm was February. Weather parameters significantly correlated with the number of collected eggs. The maximum temperature was positively correlated with the number of eggs laid in both sites ($r_s = 0.28$, $p = 0.005$; $r_s = 0.26$, $p = 0.009$) for VB and SP (Figure 6). Relative humidity was negatively correlated with the number of eggs laid in SP ($r_s = -0.28$, $p = 0.005$) (Figure 5).

## DISCUSSION

SP and VB have previously been identified as a suitable pair of study sites to test new *Aedes* control interventions due to their high reported dengue cases, proximity to production and irradiation facilities, and similar characteristics. The objective of the study was to determine the *Aedes* population in these areas over time, which would help in the planned SIT pilot trial. If the mosquito population is high, then there is a need to initially reduce the population using other methods prior to the release of sterile males. The release may also be timed during months when the population level is low. The lower the population before release, the better since it would be less costly in terms of production in the mosquito-rearing facility. SIT is not a stand-alone technology; it is best integrated or complementary with other control methods. Based on the results, the two sites have similar mosquito population dynamics. Since *Ae. aegypti* is the predominant species, SIT can be first done to control *Ae. aegypti*. The OI indices obtained in VB and SP suggest that gravid females were present and abundant in both sites, and dengue outbreaks were likely. This population density would be classified as a level four dengue risk category (40% or above OI, the highest category) based on the classification of Cheung and Fok (2009) designed for operational dengue surveillance in Hong Kong. At this level, space spraying of insecticides at the resting places of adult mosquitoes is recommended to contain the mosquito problem. Indeed, according to Lee (1992), an OI above 10% for *Aedes* species in an area may already indicate a possible risk of dengue transmission, though Wijegunawardana *et al.* (2019) noted that the OI is more useful in monitoring the population of *Aedes* mosquitoes than in predicting dengue outbreaks. OI is useful to quantify the distribution of the *Aedes* mosquito and in quantifying the level of infestation of the *Aedes* mosquito in the target area. It gives an overall idea of the risk of disease transmission. It can be useful as an indicator to stakeholders and decision-makers of the need to initiate

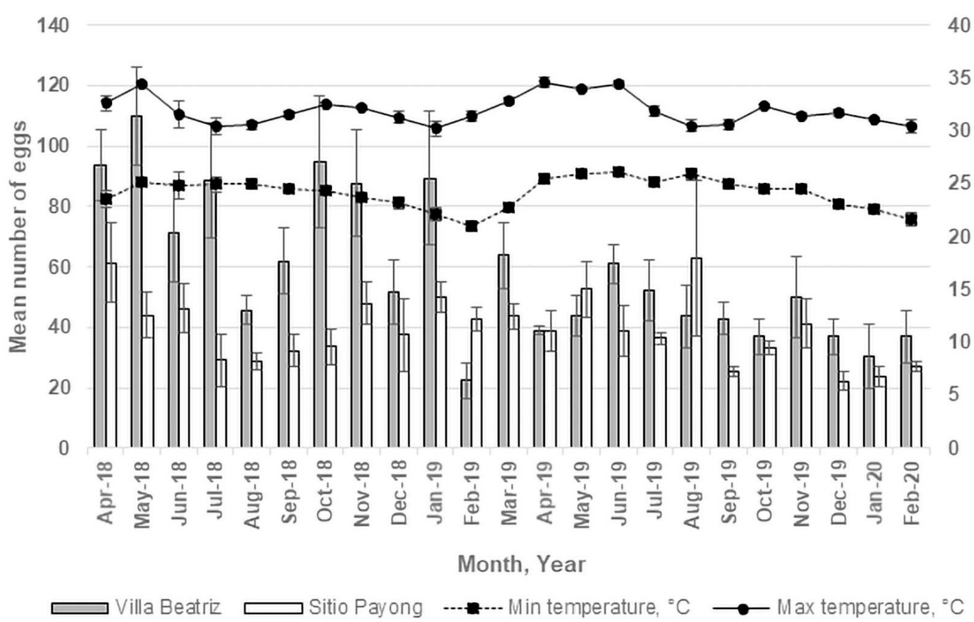


**Figure 6.** Temperature (°C) and number of *Aedes* eggs collected during weekly trap inspection in Villa Beatriz and Sitio Payong from April 2018–February 2020.

mosquito control intervention, including the SIT.

Hatch rate from field-collected eggs was similar to those reported by Vitek and Livdahl (2006) between the laboratory and field-collected eggs. Pupal recovery (from eggs) was slightly lower than in the laboratory, where the authors typically observe a pupation rate of 87.36 ± 1.24 in colonies established locally (Obra and Javier-Hila 2021). This may be due to insufficient food availability and exposure to varying weather and climatic conditions in the field compared to laboratory conditions.

In terms of the relative abundance of *Ae. aegypti* and *Ae. albopictus,* results are consistent with several studies in Southeast Asia reporting that *Ae. albopictus* was more abundant in rural (Mogi *et al.* 1988; Rozilawati *et al.* 2015) than in urban areas, where it was not observed regularly (Mogi *et al.* 1988). No *Ae. albopictus* were collected for 41 weeks in VB and 26 wk in SP, mainly from January–April, which coincided with low rainfall – thus indicating increased rain-dependency in *Ae. albopictus* (Mogi 1988). Meanwhile *Ae. aegypti* was present in both sites throughout the study period, suggesting that *Ae. aegypti* is more adapted to urban environments (Li *et al.* 2014; Mogi *et al.* 1988). Both sites are densely populated areas with congested households, where Ae. aegypti can be commonly found (Honório *et al.* 2009). Location and topography play an integral role in the existence of *Aedes* species. *Ae. aegypti* has been reported to be present in highly urbanized areas, whereas *Ae. albopictus* is a predominantly rural species (Lizuain *et al.* 2019; Braks *et al.* 2003) and prefers breeding places in shaded water bodies surrounded by vegetation (Kweka *et al.* 2019). SP had a slightly higher number of *Ae. albopictus* since it is situated near the Marikina River along with some vegetation. Both species co-exist in VB and SP, sub-urban areas where households are more congested, making them more favorable to *Ae. aegypti.* Mistica *et al.* (2019) observed the coexistence of both species in their four-month surveillance study in selected schools in Metro Manila but with a lower *Ae. aegypti* (89%) and a higher *Ae. albopictus* (11%) compared with our results.

*Ae. albopictus*, which is relatively more abundant outdoors than indoors, greatly depends on rainfall (Mogi *et al.* 1988). A similar finding was reported by Mogi *et al.* (1988), where egg density increased exponentially during the hottest month and peaked in the mid-rainy season. The monthly OI obtained in the study was not significantly associated with the weather parameters in both sites. Rainfall and RH were favorable to *Ae. albopictus*. *Ae. albopictus* eggs are more sensitive to desiccation than *Ae. aegypti* eggs (Juliano *et al.* 2002), possibly associated with their smaller chorionic egg pad (Dickerson 2007). The reduced egg hatch in *Ae. albopictus* relative to *Ae. aegypti* was more evident when eggs were laid after a period of no rainfall (Juliano *et al.* 2002). The findings show that although the two *Aedes* species population coexist in the area, *Ae. aegypti* remains the primary vector based on the relative abundance of the two species in the area. The mosquito population also fluctuates over time. In SIT program targeting *Ae. aegypti*, this period of low density might be the best time to release sterile males and have the biggest impact and when the population is abundant, a large number of sterile males should be released to overwhelm the wild male population.

Meanwhile, the maximum temperature was favorable for egg production (Figures 4a and b), probably more for *Ae aegypti*. At lower temperatures, there were more *Ae. albopictus* and fewer *Ae. aegypti* in the population in both VB and SP. Warm and dry climates favor the dominant species (*Ae. aegypti*) compared to the other species (*Ae. albopictus*) (Higa *et al.* 2010) and make possible the coexistence of the two species *via* differential egg mortality (Juliano *et al.* 2002). However, the climatological data were obtained from a weather station nearest (7.5 km) to the study sites, and it would have been preferable if these parameters had been measured on-site. The weather data can help predict the population dynamic during the mosquito season and, therefore, determine the optimal and cost-effective releases while accounting for the local seasonal climate.

## CONCLUSION

Information on vector species and abundance is fundamental to implementing a vector control measure effectively. This study aimed to establish baseline entomological information in two sites in Quezon City, Philippines, under consideration as pilot trial sites for the SIT. The ovitrap was shown to be a valuable monitoring tool for the surveillance of dengue mosquitoes, collecting eggs in all months of the two-year study period. *Aedes albopictus* and *Ae. aegypti* were present in both sites, with the latter being more abundant. However, *Ae. aegypti* resulted from collected eggs that were found throughout the whole study period, whereas *Ae. albopictus* were irregularly observed in the study sites (adults were absent or at a very low number at some time period). Knowledge of the initial fertility, species distribution, and dynamic is crucial to tailor releases over time and determine the impact on the SIT in the release area. Temperature and rainfall are both abiotic factors that influence vector abundance in these sites. The study's main limitation is that it was conducted using ovitraps only and should also be conducted using BG sentinel traps. Moreover, the density of trapping sites should be increased for a reliable estimation of the population abundance and seasonal dynamic. Before SIT pilot trial, future studies will include monitoring activities using ovitraps, BG sentinel traps, or human landing catch, and mark-release-recapture studies for better estimation of the population size and the performance of the released sterile males. However, these results provide useful information for the future planning and implementation of control interventions such as the SIT, as part of an integrated vector management program against *Aedes* spp. in an urban setting in the Philippines.

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