



Logistics of a 2,000 trap mosquito surveillance program

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Abstract

The Puerto Rico Vector Control Unit (PRVCU) was established through a cooperative agreement between the Centers for Disease Control and Prevention (CDC) and the Puerto Rico Science, Technology, and Research Trust, to monitor and control the mosquito *Aedes aegypti*, the principal vector for dengue, Zika, and chikungunya in Puerto Rico. From 2018-2020, the PRVCU has established a long term and large-scale surveillance system using the Autocidal Gravid Ovitrap (AGO) in Puerto Rico across eight municipalities distributed over 82 surveillance sites. In less than two years, the PRVCU has deployed 1,772 AGO, which aims to monitor the prevalence of mosquito density of *Aedes aegypti* and the detection of active arboviral transmission in the Island. Like an industrialized process, the PRVCU has developed an efficient, quick, and easy method to handle a large number of traps every week, separating the most time-consuming parts: trap servicing, mosquito ID, and mosquito pooling for arboviral testing. This system has allowed the PRVCU to collect an average of 12,000-15,000 *Aedes aegypti* females/week and conduct arboviral testing for 800 pools/week. Historical data has shown a high density of *Aedes aegypti* population in Puerto Rico all year round with seasonal peaks during rainy seasons. Having a unique mosquito surveillance system like this has been vital to demonstrate the importance of mosquito control programs in arbovirus endemic countries to stakeholders and public health agencies.

Methodology

- From 2018-2020, the PRVCU established the *Aedes aegypti* Mosquito Surveillance System (MoSS) in Puerto Rico using AGOs (Figure 1).
- MoSS started in municipality of San Juan and then expanded to Bayamon, Carolina, Dorado, Guaynabo, Caguas and Ponce, Puerto Rico.
- Study areas were selected based on historical arboviral data (dengue and Zika) from Puerto Rico and/or through governmental, federal and local agencies collaborative agreements.
- Using ArcGIS 10.8.1 (Esri), municipalities were divided in zones and the zones were subdivided in 250m grids to suggest the trap location.
- AGOs were installed in patios, gardens or open garages. Every 7 days, the AGO capture chamber is pick up and delivered to the PRVCU Laboratory for mosquito identification. Every 8 weeks, the AGO bucket and lid are cleaned with fresh water. The sticky board, 30g of hay packet, and screen are replaced with clean pieces.
- Once the capture chambers are in the laboratory, traps are processed in subdivided activities: mosquito extraction from sticky board, mosquito identification using a stereoscope, mosquito pools (2-20 *Ae. aegypti* female per 2 ml vial/trap) and real-time data entry into the customized PRVCU Mosquito Surveillance System App (Javascript, Node.js, React.js software).
- Ae. aegypti* female mosquito pools are tested for Zika, chikungunya, dengue and dengue serotypes through mosquito maceration (TissueLyser, Qiagen), RNA extraction (MagnaPure 96, Roche) and RT-PCR amplification (CFX96 Touch, Biorad).
- Each trap have an unique QR-Code (Zebra Printer, Multisystem) that allows chain of custody from the deployment date through arboviral testing report.

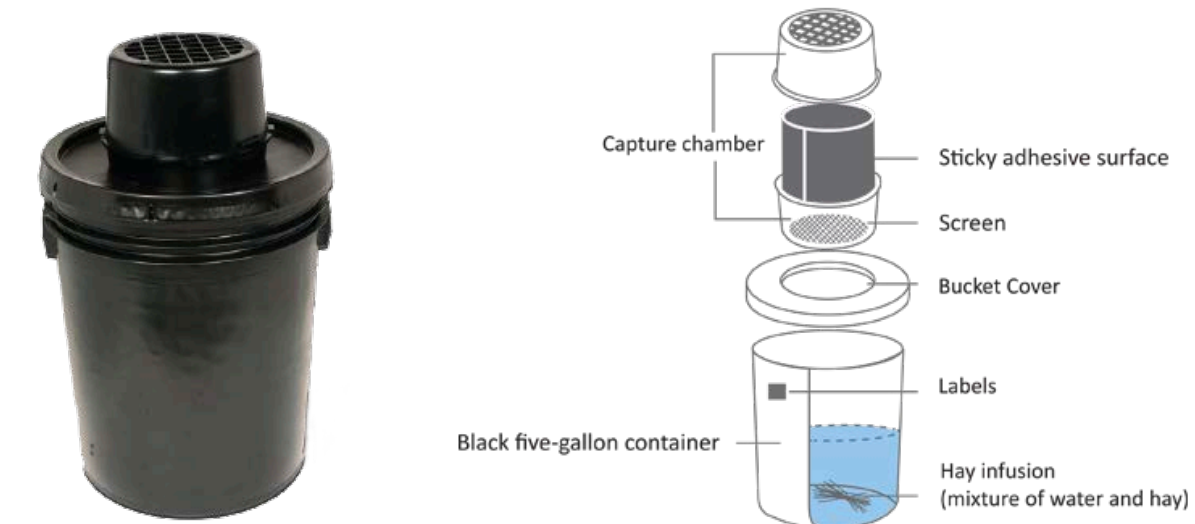


Figure 1. Autocidal Gravid Ovitrap components

Methodology

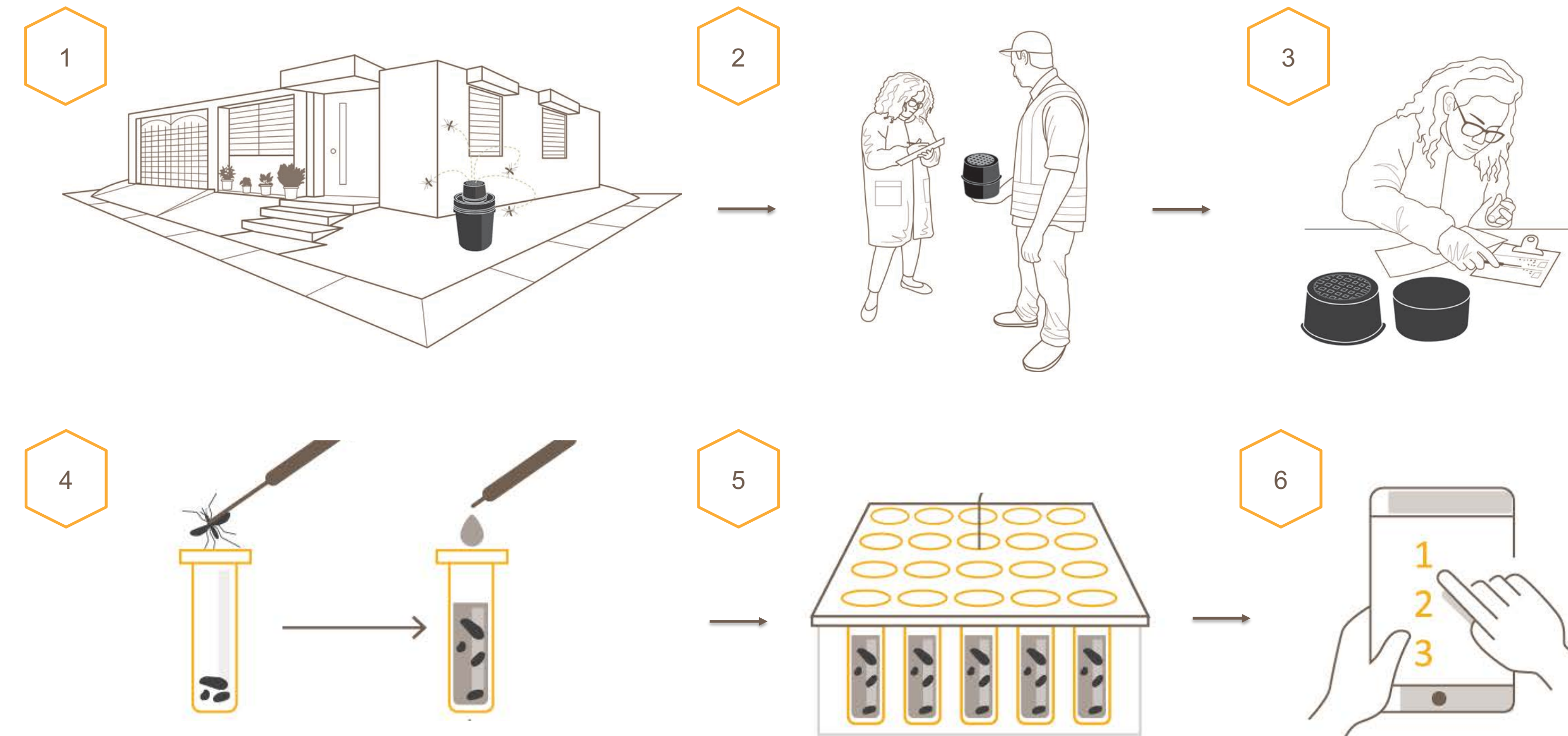


Figure 2. Flowchart of the PRVCU AGO processing method 1) AGO are placed in patios; 2) Every 7 days capture chambers are delivered to the PRVCU Laboratory; 3) Laboratory technician removes mosquitoes from sticky board; 4) *Ae. aegypti* females are pooled into 2-20 females per vial/trap; 5) Mosquito pools go through arboviral testing; 6) Real-time data entry



Figure 3. Laboratory technician processing AGO traps. A) Using a teasing needle mosquitoes are removed from sticky board; B) Using the unique QR-Code mosquitoes are placed in a napkin by genus, specie, and sex; C) *Ae. aegypti* females are pooled into 2ml vials.

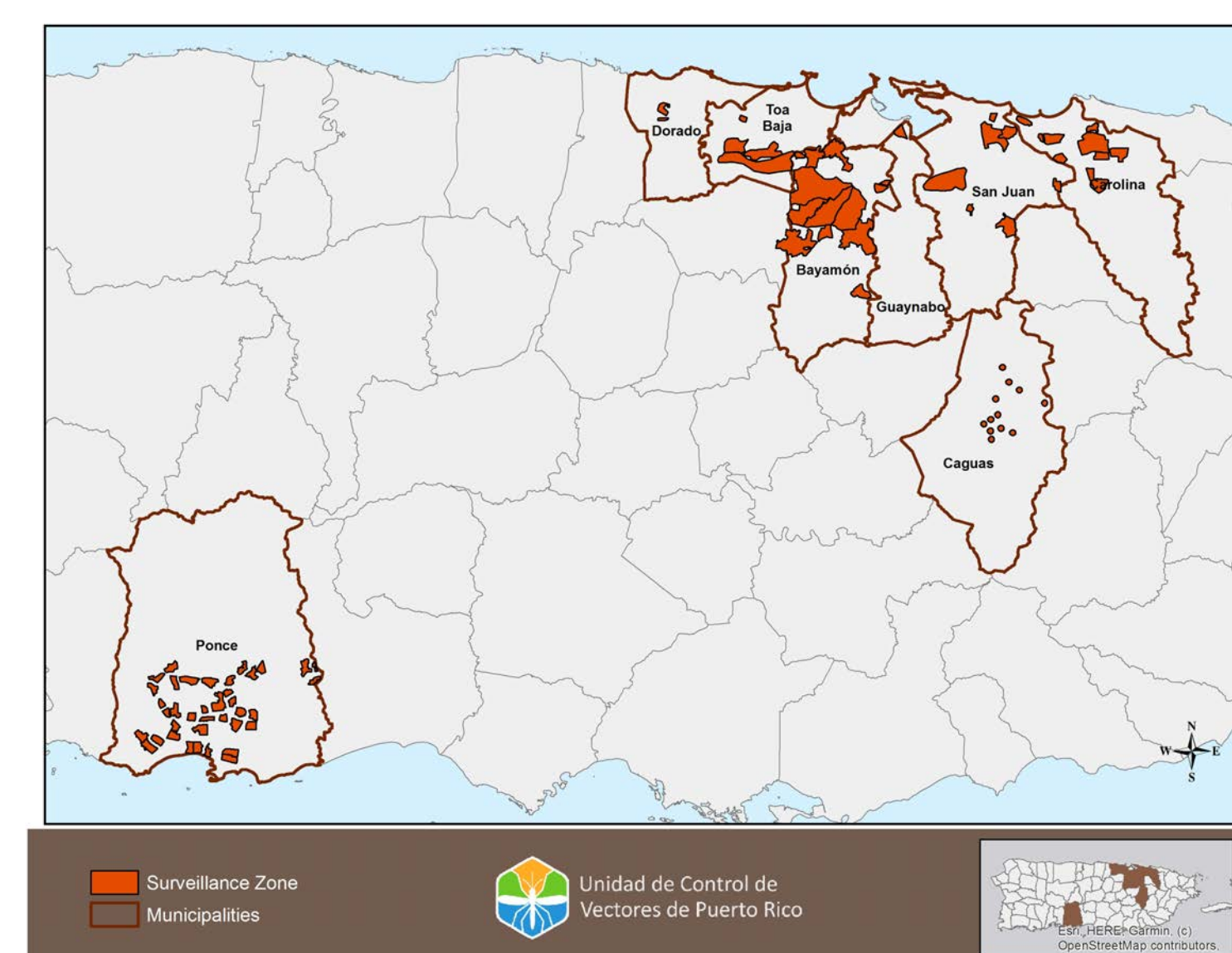


Figure 4. Municipalities participating in the PRVCU *Ae. aegypti* Mosquito Surveillance System.

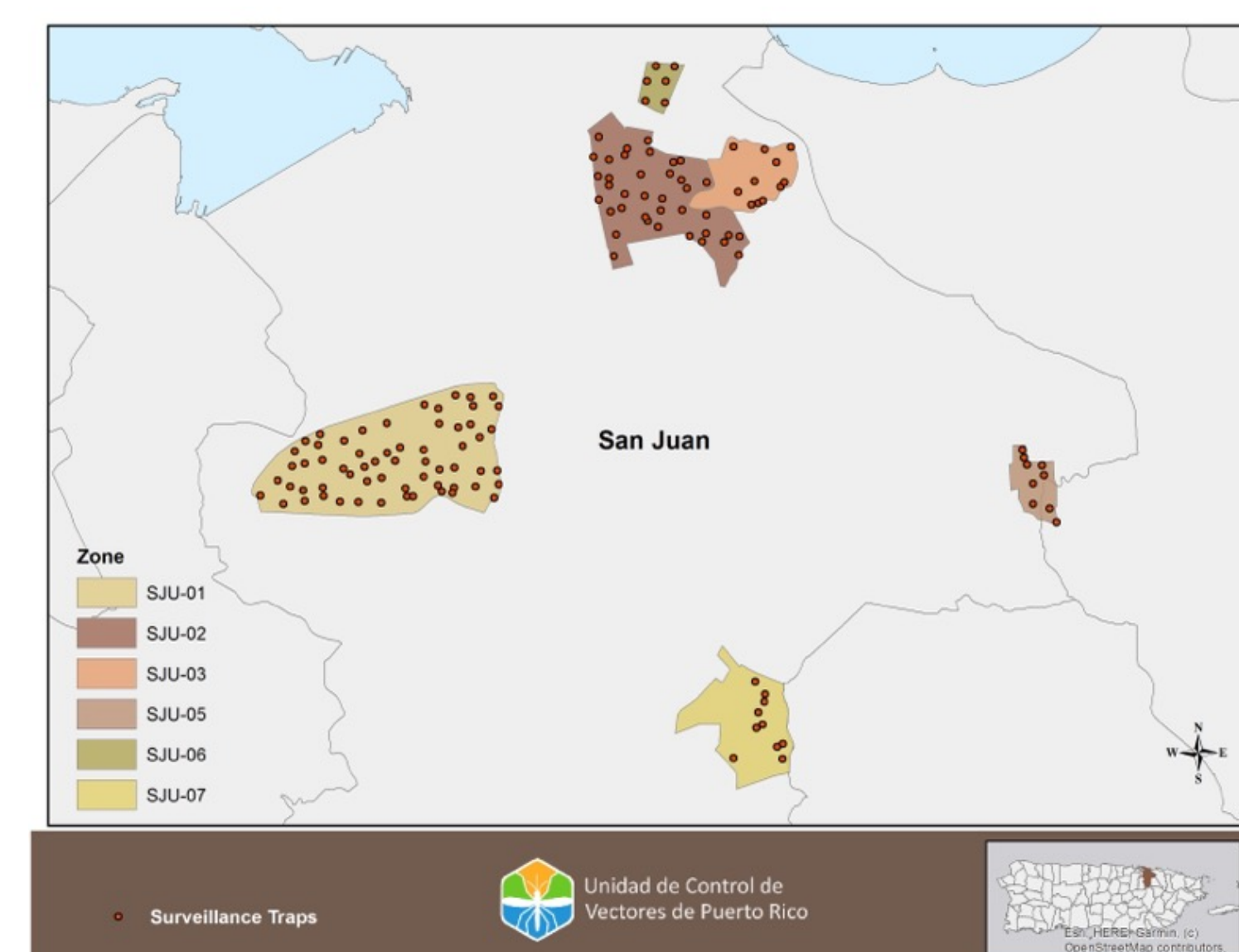


Figure 5. Surveillance zones within the municipality of San Juan, Puerto Rico. Red dots represents AGO location using 250m grids.

Results

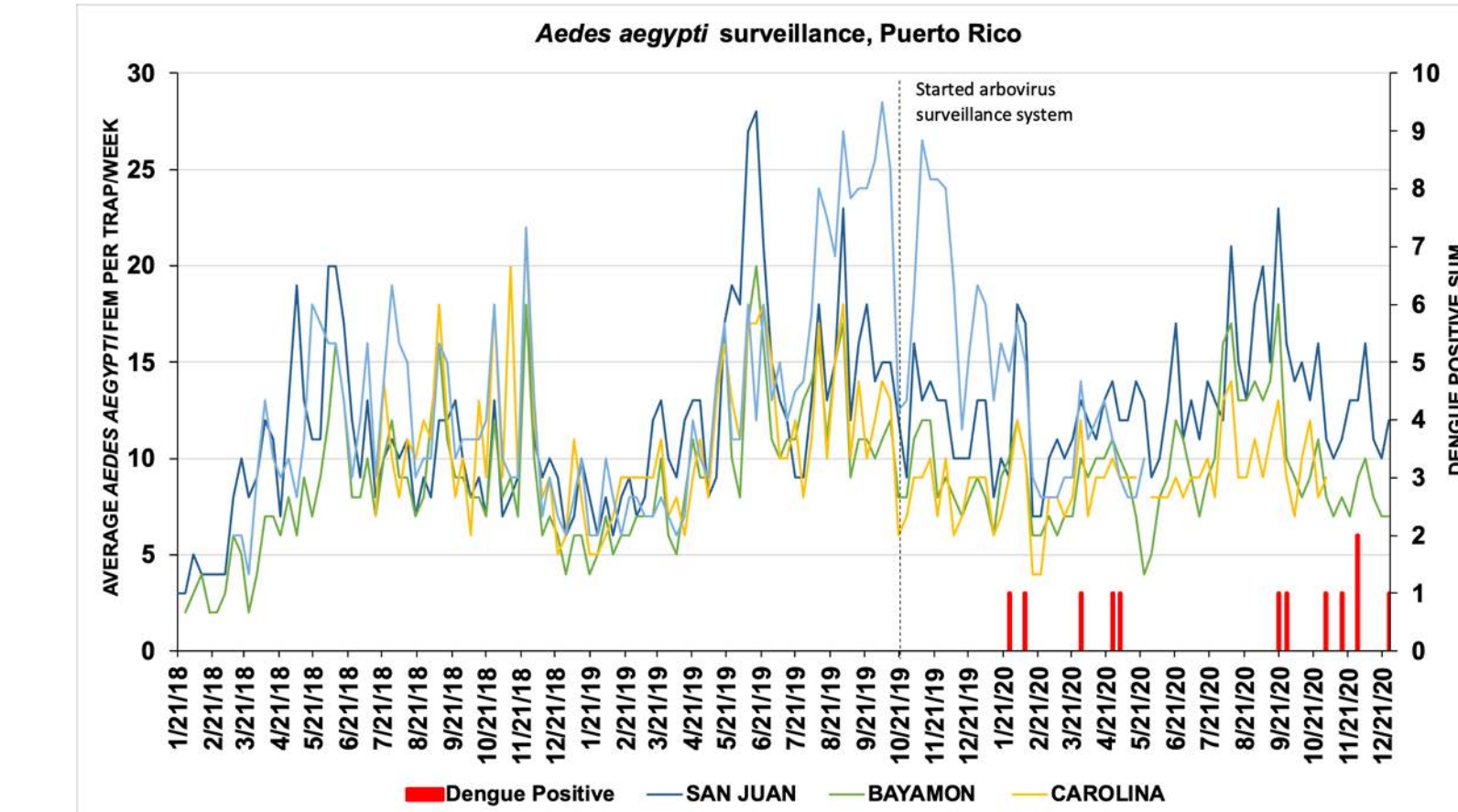


Figure 6. *Ae. aegypti* female weekly average per trap and total of dengue positive pools for San Juan, Carolina and Bayamon, 2018-2020. Dengue positive were isolated from San Juan during 2020.

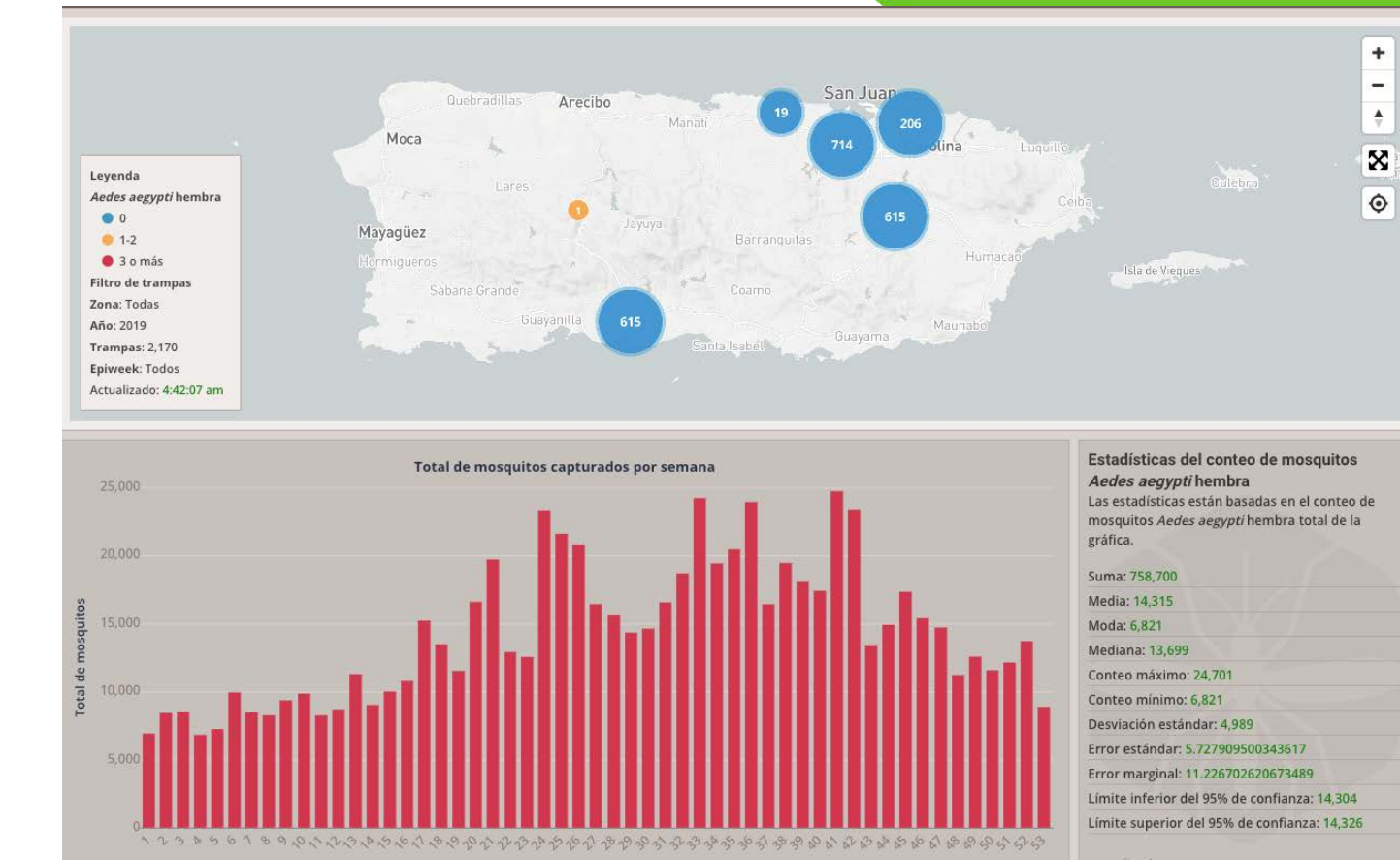


Figure 7. PRVCU integrated real-time *Ae. aegypti* surveillance dashboard. This figure shows the PRVCU dashboard that feeds daily and real-time through the PRVCU MoSS.

Table 1. Summary of PRVCU Surveillance System. This table include municipalities participating in the surveillance system, traps per municipality, study zones, year of deployment, total of *Ae. aegypti* captured from 2018-2020, quantity of pools tested for arbovirus during 2020, quantity of *Ae. aegypti* tested for arbovirus, quantity of dengue-1 positive during 2020.

Municipality	AGO (N)	Zones	Deployment Year	# <i>Ae. aegypti</i> fem 2018-2020	# Pool tested for arbovirus 2020	# <i>Ae. aegypti</i> fem tested for arbovirus	# Pool Positive DENV-1 2020
Bayamon	551	10	2018-2020	654806	2543	32406	3
Carolina	135	9	2018-2019	100012	2531	27549	0
Dorado	19	2	2019	17027	124	1774	0
Guaynabo	14	1	2019	4784	96	1030	0
San Juan	138	7	2018-2019	229707	4540	58972	9
Toa Baja	66	3	2020	13508	185	2256	0
Caguas	239	12	2019	485224	1632	21149	1
Ponce	610	38	2019	449125	6676	66797	1
Total	1,772	82		1,954,193	18,327	211,933	15

Conclusion

- While the AGO processing and mosquito identification can be conducted in the field, the PRVCU established an industrialized-like process as an alternative to have a broader capacity to process hundreds of traps and have results daily.
- Some of the benefits from this methodology related to field logistics is that reduces the time that a field technician is on the road and minimizes environmental hazards (sun, dog and mosquito bites). While in the laboratory logistics, the mosquito removal is facilitated for using a fully open sticky board and mosquito identification can be more accurate with the use of a stereoscope.
- In terms of time, an average field technician collects 40 traps in 3 hours (including driving) while a laboratory technician process 25 traps (extraction, id, pools and data entry) in 2 hours.
- In two years (2018-2020), the PRVCU has captured over 1.9 million *Ae. aegypti* females, process more than 212,000 AGO capture chambers, and tested 18,327 mosquito pools for arboviral detecting 15 dengue-1 positive pools.

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