Mosquito and Vector Control Association of California, 1 Capitol Mall, Suite 800, Sacramento, CA 95814
Phone: (916) 440-0826 | Fax: (916) 444-7462 | Email: mvcac@mvcac.org | Website: www.mvcac.org

MVCAC

ISSN 1554-4974 Volume 89 No.1

89

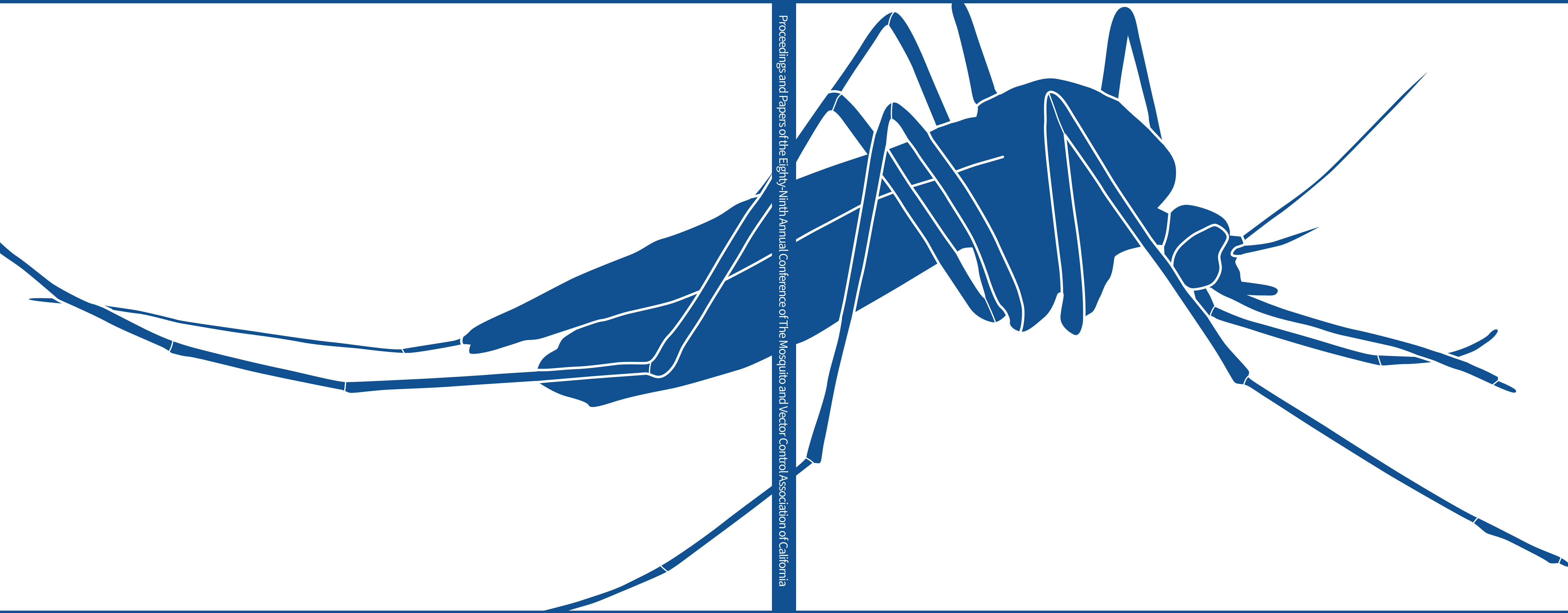
Proceedings and Papers of the Eighty-Ninth Annual Conference of The Mosquito and Vector Control Association of California

MVCAC

Proceedings and Papers

OF THE EIGHTY-NINTH ANNUAL CONFERENCE OF THE
Mosquito and Vector Control Association of California

October, 2021



ISSN 1554-4974 Volume 89 No.1

Mosquito and Vector Control Association of California, 1 Capitol Mall, Suite 800, Sacramento, CA 95814
Phone: (916) 440-0826 | Fax: (916) 444-7462 | Email: mvcac@mvcac.org | Website: www.mvcac.org

PROCEEDINGS AND PAPERS

OF THE

Eighty-Ninth Annual Conference of the Mosquito and Vector Control Association of California

February 1 – 3, 2021

Virtual

Editor: William K. Reisen, Ph.D.
Professor Emeritus
Department of Pathology
Microbiology and Immunology
School of Veterinary Medicine
University of California
Davis, CA 95616
wkreisen@gmail.com

Layout and Editorial Assistance: Katelyn Peyser, MVCAC

Mosquito and Vector Control Association of California
1 Capitol Mall, Suite 800
Sacramento, California 95816
Phone: 916-440-0826 Fax: 916-444-7462
Email: mvcac@mvcac.org
www.mvcac.org
Published October 2021

Mosquito and Vector Control Association of California

Robert Achermann, *Executive Director*

BOARD OF DIRECTORS

President: Truc Dever
President-Elect: Ken Klemme
Vice President: Wakoli Wekesa
Past President: Peter Bonkrude
Sacramento Valley Region Representative: Joel Buettner
Coast Region Representative: Phil Smith
Northern San Joaquin Region Representative: Rhiannon Jones
Southern San Joaqin Region Representive: Conlin Reis
Southern Region: Michelle Brown
Trustee Representative: Tamara Davis

CORPORATE MEMBERS

Coastal Region
Alameda County MAD
Alameda County VCSD
Contra Costa MVCD
Marin/Sonoma MVCD
Napa County MAD
No. Salinas Valley MAD
San Benito County Agricultural Commission
San Francisco Public Health, Environmental Health Section
San Mateo County MVCD
Santa Clara County VCD
Santa Cruz County MVCD
Solano County MAD

Sacramento Valley Region
City of Alturas
Burney Basin MAD
Butte County MVCD
Colusa MAD
Durham MAD
El Dorado County Environmental Management
Glenn County MVCD
Lake County VCD
Nevada County Community Development Agency
Oroville MAD
Pine Grove MAD
Placer MVCD
Sacramento-Yolo MVCD
Shasta MVCD
Sutter-Yuba MVCD
Tehama County MVCD

North San Joaquin Valley Region
East Side MAD
Merced County MAD
Saddle Creek Community Services District
San Joaquin County MVCD
Turlock MAD

South San Joaquin Valley Region
Consolidated MAD
Delano MAD
Delta VCD
Fresno MVCD
Fresno Westside MAD
Kern MVCD
Kings MAD
Madera County MVCD
South Fork Mosquito Abatement District MAD
Tulare Mosquito Abatement District (TMAD)
West Side MVCD

Southern California Region
Antelope Valley MVCD
City of Blythe
City of Moorpark/VC
Coachella Valley MVCD
Compton Creek MAD
Greater LA County VCD
Imperial County Vector Control
June Lake Public Utility District
Long Beach Vector Control Program
Los Angeles West Vector and Vector-borne Disease Control District
Mammoth Lakes MAD
Mosquito and Vector Management District of Santa Barbara County
Northwest MVCD
Orange County Mosquito and Vector Control District
Owens Valley MAP
Pasadena Public Health Department
Riverside County, Dept of Environmental Health VCP
San Bernardino County MVC
San Diego County Dept. of Environmental Health, Vector Control
San Gabriel Valley MVCD
Ventura County Environmental Health Division
West Valley MVCD

2021 SUSTAINING MEMBERS

ADAPCO

Aerial Services

AMVAC

Central Life Sciences

Clarke

Frontier Precision, Inc.

Leading Edge Associates, Inc.

MGK

SCI Consulting Group

Target Specialty Products

Valent BioSciences

Vector Disease Control International

Veseris

Reeves New Investigator Award

Poster Session

Challenges and Opportunities in Vector Borne Disease

Mosquito Biology and Disease

Community Engagement and Education

Public Policy, Governance & Operations During the COVID crisis

Operations & Technology

Pacific Southwest Center of Excellence in Vector Borne Diseases

In Control: A Look at Operational Tools & Equipment

Innovation & Discovery in Mosquito Control

Invasion of the Body Biters: Statewide Efforts Against *Aedes*

William C. Reeves New Investigator Award

The William C. Reeves New Investigator Award is given annually by the Mosquito and Vector control Association of California in honor of the long and productive scientific career of Dr. William C. Reeves.

The award is presented to the outstanding research paper delivered by a new investigator based on the quality of the study, the manuscript, and the presentation at the MVCAC Annual Conference.

Year	Award Winner	Title of Paper
1988	Vicki L. Kramer	A comparison of mosquito population density, developmental rate and ovipositional preference in wild versus white rice fields in the Central Valley
1989	Truls Jensen	Survivorship and gonotrophic cycle length in *Aedes melanimon* in the Sacramento Valley of California
1990	Gary N. Fritz	Polytenes, isozymes and hybrids: deciphering genetic variability in *Anopheles freeborni*
1991	David R. Mercer	Tannic acid concentration mediates *Aedes sierrensis* development and parasitism by *Lambornella clarki*
1992	Darold P. Batzer	Recommendations for managing wetlands to concurrently achieve waterfowl habitat enhancement and mosquito control
1993	Jeffery W. Beehler	The effect of organic enrichment and flooding duration on the oviposition behavior of *Culex* mosquitoes
1994	Merry-Holliday-Hanson	Size-related cost of swarming in *Anopheles freeborni*
1995	Margaret C. Wirth	Multiple mechanisms cause organophosphate resistance in *Culex pipiens* from Cyprus
1996	No award	
1997	John Gimnig	Genetic and morphological characterization of the *Aedes* (*Ochlerotatus*) *dorsalis* group
1998	Yvonne Ann Offill	A Comparison of mosquito control by two larvivorous fishes, the stickleback (*Gasterosteus aculeatus*) and the mosquitofish (*Gambusia affinis)*
1999	Parker D. Workman	Adult spatial emergence patterns and larval behavior of the "Tule Mosquito," *Culex erythrothorax*
2000	Jason L. Rasgon	Geographic distribution of *Wolbachia* in California *Culex pipiens* complex: infection frequencies in natural populations
2001	Christopher Barker	Geospatial and statistical modeling of mosquito distribution in an emerging focus of La Crosse virus
2002	No award	
2003	Laura Goddard	Extrinsic incubation period of West Nile virus in four California *Culex* (Diptera: Culicidae) species
2004	No award	
2005	Troy Waite	Improved methods for identifying elevated enzyme activities in pyrethroid-resistanct mosquitoes
2006	Lisa J. Reimer	Distribution of resistance genes in mosquitoes: a case study of *Anopheles gambiae* on Bioko Island
2007	Carrie Nielson	Impact of climate variation and adult mosquito control on the West Nile virus epidemic in Davis, California during 2006
2008	John Marshall	The impact of dissociation on transposon-mediated disease control strategies
2009	Win Surachetpong	MAPK signaling regulation of mosquito innate immunity and the potential for malaria parasite transmission control
2010	Tara C. Thiemann	Evaluating trap bias in bloodmeal identification studies
2011	Sarah S. Wheeler	Host antibodies protect mosquito vectors from West Nile virus infection
2012	Brittany Nelms	Overwintering biology of *Culex* mosquitoes in the Sacramento Valley, California
2013	Kimberly Nelson	The effect of red imported fire ant (*Solenopsis invicta* Buren) control on neighborhoods in Orange County, California
2014	Thomas M. Gilbreath, III	Land Use Change and the Microbial Ecology of *Anopheles gambiae*
2015	Jessica M. Healy	Comparison of the efficiency and cost of West Nile virus surveillance methods in California
2016	Mary Beth Danforth	The impacts of cycling temperature on West Nile virus transmission in California's Central Valley
2017	Nicholas A. Ledesma	Entomological and Socio-behavioral Components of Dog Heartworm (*Dirofilaria immitis*) Prevalence in Two Florida Communities
2018	Kim Y. Hung	House Fly (*Musca domestica L.)* Attraction to Insect Honeydew
2019	Matteo Marcantonia	Revising alkali metals as a tool for mark-recapture studies to characterize patterns of mosquito (Diptera: Culicidae) dispersal and oviposition
2020	Adena Why	Semiochemicals associated with the Western mosquitofish, *Gambusia affinis*, and their effect on the oviposition of *Culex tarsalis*
2021	Vanessa Hill	Evaluation of residential property types for *Ades aegypti* habitats in Placer County, California

Evaluation of residential property types for *Aedes aegypti* habitats in Placer County, California

Vanessa Hill*, Mario Boisvert, Meagan Luevano, Mary A. Sorensen, Joel Buettner

Placer Mosquito & Vector Control District, Roseville, CA 95678

*Corresponding author: vanessahill555@gmail.com

Abstract

The invasive container breeding mosquito *Aedes aegypti* was first discovered in Placer County in August 2019. It was anticipated that this mosquito would again be present and active in Placer County in 2020. However, the timing and extent of activity was not possible to predict. Our project gathered data and provided experience to prepare our operational procedures and staff for *Ae. aegypti* response. No *Ae. aegypti* mosquitoes were found after 92 residential property inspections, but the data collected revealed which container types were most prevalent at residential properties in Placer County that could potentially produce *Ae. aegypti*. Yard drains, found in newer, larger homes, and flowerpot saucer dishes, found in older, larger homes, were the most frequently observed types of containers. New homes were defined as properties built after 2000, and large homes were lot sizes greater than an acre. Larger lots typically had more containers than smaller lots; however, house size used as a surrogate for family income and age of the home did not significantly correlate with the number of containers present. Therefore, if *Ae. aegypti* were to be found in Placer County, they would most likely would be found in either yard drains of new, large residential properties or flowerpot saucer dishes at old, large residential properties. If *Ae. aegypti* were not found in yard drains or flowerpot saucer dishes, they would most likely be found in a container of a home with a large lot size.

INTRODUCTION

In September 2017, CDC updated the estimated distributional maps for *Aedes aegypti* and *Ae. albopictus* mosquitoes by using a model that predicts possible geographic ranges for these species in the contiguous United States (Centers for Disease Control. 2018). The model used county-level records, historical records, and suitable climate variables to predict the likelihood (very low, low, moderate, or high) that these species could survive and reproduce if introduced into an area during the months when local mosquitoes are locally active. Based on these maps, Placer County was identified as an area where it is "very likely" that *Ae. aegypti* would be able to live and reproduce.

Although it was once predicted that neither *Ae. aegypti* or *Ae. albopictus* could persist in the northern latitudes of California due to dry conditions and cool winters (Washburn and Hartmann. 1992), the recent spread of these mosquitoes has proven otherwise (Powell and Tabachnick. 2013). The successful dispersal of *Ae. aegypti* into more northern parts of California may mean that the original assessments of their ecological requisites were incorrect, or possibly that the mosquitoes were able to survive in microclimates created by human modification or adapt to different conditions.

On 27 Aug 2019, an *Ae. aegypti* was found for the first time in Placer County through surveillance activities of the Placer Mosquito and Vector Control District (Placer MVCD). Over the course of two subsequent months, four adult female mosquitoes were found in four different surveillance traps (a Fay-Prince trap, two BG Sentinels, and a BG Counter), all in a residential south Placer County neighborhood east of Auburn Boulevard at Interstate 80. The California Department of Public Health confirmed the identification of these specimens. Placer MVCD coordinated surveillance and operations with the Sacramento-Yolo Mosquito and Vector Control District (Sac-Yolo MVCD), because *Ae. aegypti* were trapped on both sides of the Placer/Sacramento County border. The Sac-Yolo MVCD found at least 50 adult mosquitoes in this neighborhood on their side of the county line during the initial detection period. The Placer MVCD response was door-to-door property inspections to determine the infestation level. The data collected during these inspections only encompassed the single neighborhood where *Ae. aegypti* was found. To prepare for the possible return of *Ae. aegypti*, more information regarding available habitats and container types was needed.

METHODS

Residential property inspections for *Ae. aegypti* potential habitats were conducted from June to August 2020, and were concentrated near the Placer County border with Sac-Yolo MVCD where *Ae. aegypti* were detected in 2019 (Fig. 1, Table 1). Properties were selected to represent diverse types: new vs old, large vs small by lot size representing

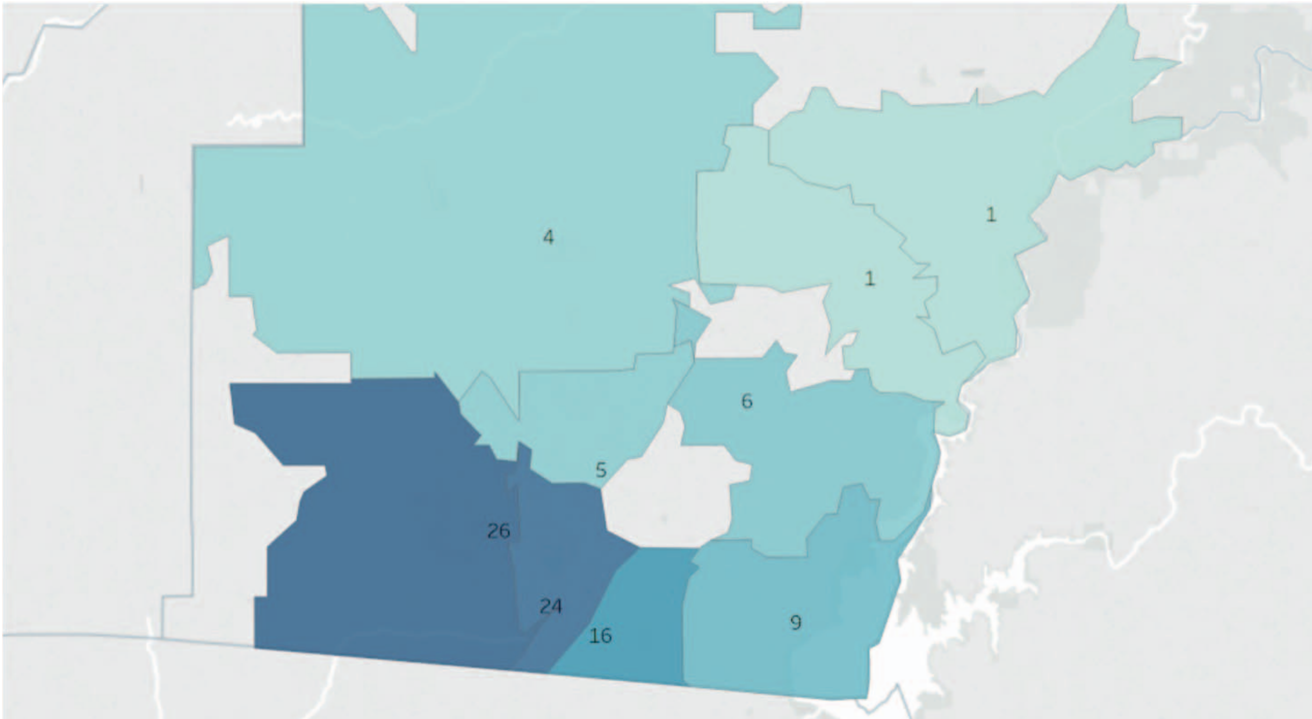


Figure 1.—Number of *Aedes* property inspections done in each Placer County, CA zip code from June to August 2020.

high and low income, respectively. 'Old' properties were houses built before the year 2000, and large properties had a lot size over an acre.

Originally, this project was planned as a door-to-door outreach effort to gain resident participation. However, due to the COVID-19 pandemic, personal contact was kept to a minimum for public health safety. Staff used other means to contact residents to schedule inspections including NextDoor posts, door hangers, and calling residents who previously had requested inspections from the District.

The data collected during residential property inspections included the type of containers and if they were dry, wet, and/or held mosquito larvae. Containers included flowerpot saucers, flowerpots, yard drains, bird baths, wheelbarrows, pet water dishes, ornamental ponds, trash cans, utility boxes, tires, unmaintained pool, rain barrels, toys, uncovered boats, watering cans, fountains, small containers (<12"), large containers (>12", not otherwise listed), and other containers that could potentially sustain *Ae. aegypti* larvae. To determine the source of water for wet containers, the following water sources were documented: timed sprinklers, manual sprinklers, hand watering hose or watering can, and rain. Other property characteristics were documented for future analysis of indicators that may predict the presence of habitat or hosts included: was the backyard landscaped vs natural, were toys present, was the property unmaintained, was it forested vs open, was there a water feature, was the property dry vs lush, was the property new construction, was it an older home, was a BBQ present, was there a grass lawn, was it weedy vs manicured, was there much dirt, was the harborage artificial or vegetation, and was there outdoor seating and/or a shade structure. These latter property characteristics were not analyzed as part of the current study.

Table 1.—Total property types inspected in Placer County, CA from June to August 2020. New homes were defined as properties built after 2000, and large homes were lot sizes greater than an acre.

Old, large	Old, small	New, large	New, small	Other	Grand Total
25	34	16	16	1	92

When a container was found to be producing any species of mosquito larvae, the following were recorded: container type and size, species, mosquito life stages and numbers, water depth, water temperature, exposure to the sun, and appearance of water (color, organic material). Samples of the mosquito larvae were taken back to the laboratory to identify whether *Ae. aegypti* had been found before dumping or treating the container source.

After the residential property inspections were completed each day, the handwritten data sheets were inputted into an excel spreadsheet that was linked to a Tableau report for continuous monitoring of results. The data collected were intended to allow calculation of the Container Index (containers positive for *Ae. aegypti* larvae or pupae per 100 containers inspected), House Index (houses positive for *Ae. aegypti* larvae or pupae per 100 houses inspected), and/or Breteau Index (containers positive for *Ae. aegypti* larvae or pupae per 100 houses inspected) (Bowman et al. 2014).

Because this study was designed as a preliminary descriptive survey, statistical analysis was not possible to

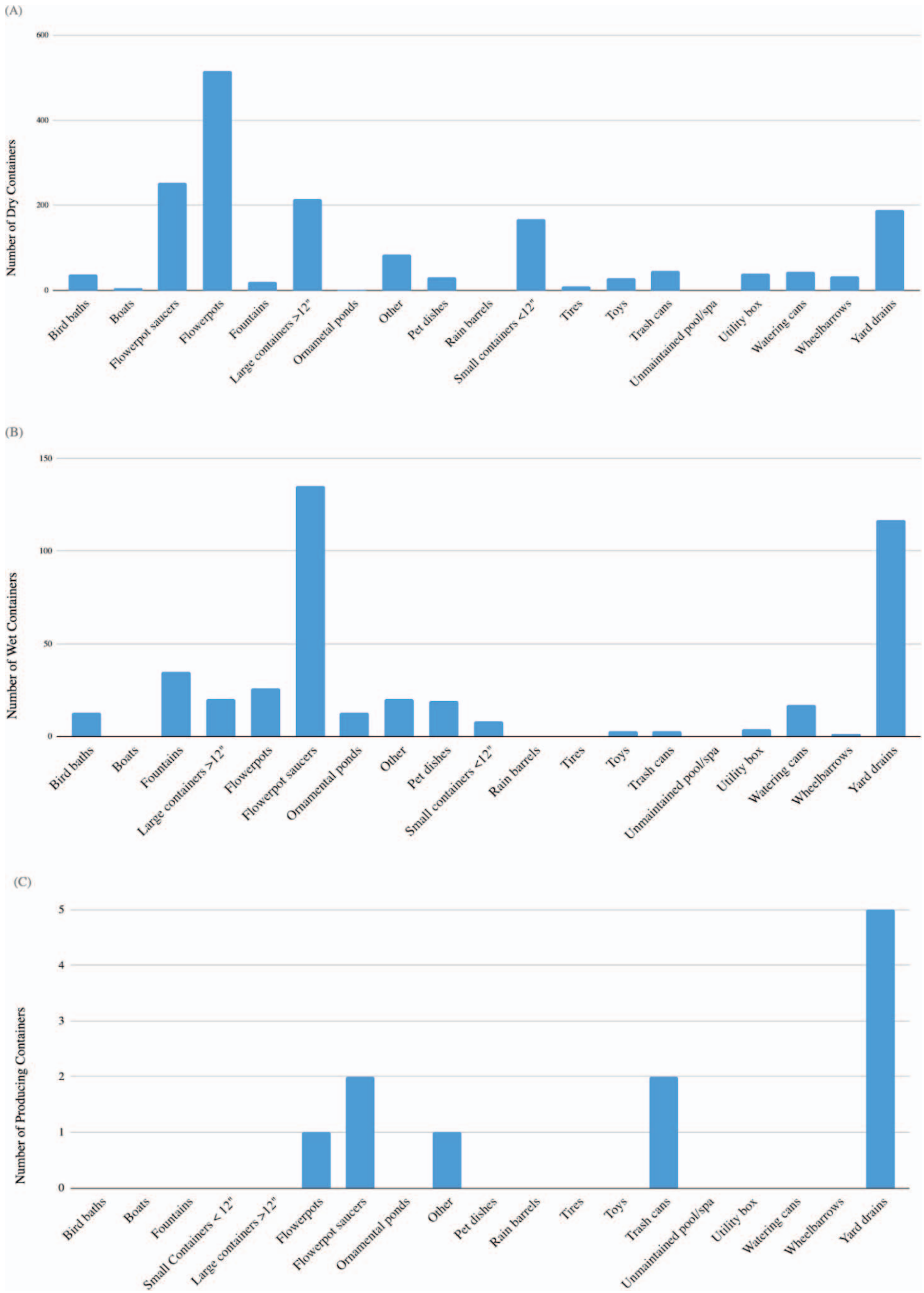


Figure 2.—Number of container types that were recorded as (A) dry, (B) wet, and (C) producing non-*Aedes aegypti* mosquito larvae during inspections for potential container *Aedes* done in Placer County, CA properties from June to August 2020.

Table 2.—Water sources by property type recorded during inspections for potential container *Aedes* habitat in Placer County, CA backyards from June to August 2020.

	Old, large	Old, small	New, large	New, small
Irrigation System	19	27	16	11
Hand-Watering	22	33	17	15
Hose Sprinkler(s)	8	0	1	0
Rain	0	0	0	0
Other Source (Describe)	0	0	0	0

compare neighborhood categories (large, small, old, and new), or container types. Total containers were regressed as a linear function of house size and age.

RESULTS

No *Ae. aegypti* eggs, larvae, or adults were found at any of the 92 properties surveyed. However, the data collected for containers on residential properties, as well as property types with possible larval sources, was analyzed to describe the potential *Ae. aegypti* habitats in Placer County.

Residential container habitats that potentially could support *Aedes aegypti* in Placer County

Among 92 residential properties inspected during summer 2020, a total of 1,723 containers were recorded as dry, but potentially could hold water. The most common dry container type was flowerpots (Fig. 2a), followed by flowerpot saucer dishes, large miscellaneous containers, yard drains, and small miscellaneous containers. Although these containers were dry at the time of inspection, they potentially could hold standing water and produce mosquitoes.

In addition, there were 434 wet containers found during this project, of which, flowerpot saucer dishes and yard drains were the most common type recorded (Fig. 2b). It is important to note that there was no rainfall during the study period, so the sources of water in containers were limited to timed or manual sprinkler systems, as well as hand watering (Table 2). The standing water in these recorded wet containers could potentially produce mosquitoes.

Only 11 containers were found to contain water with mosquito larvae. Although no *Ae. aegypti* larvae were found during this project, these containers were found to be producing *Culex* and could potentially produce *Ae. aegypti* as well. The most common container type positive for *Culex* mosquito larvae were yard drains, followed by trash cans and flowerpot saucer dishes (Fig. 2c).

Container types examined as a function of housing type

After determining that yard drains and flowerpot saucer dishes are the most common container types that could potentially produce *Ae. aegypti*, we determined the property types where these containers were found in greatest quantity. The most likely property types to produce *Ae. aegypti* were determined by calculating the average

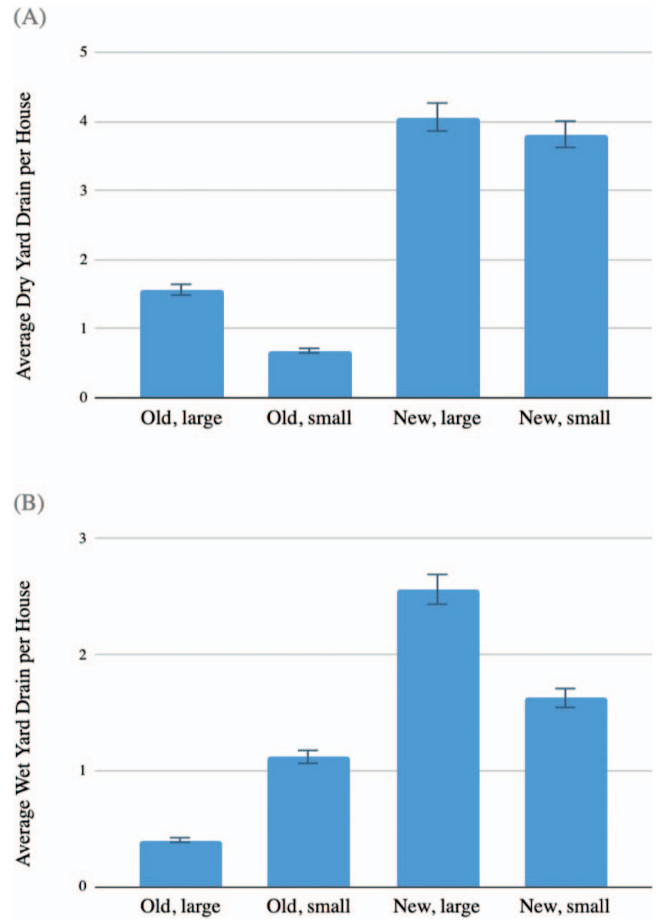


Figure 3.—Average number of (A) dry and (B) wet yard drains recorded per residential home by property type during inspections for potential container *Aedes* habitat in Placer County, CA backyards from June to August 2020.

number of each dry and wet yard drain and flowerpot saucer dish per home by property type. Newer homes, regardless of lot size, had on average more yard drains (dry or wet) per house when compared to older homes (Fig. 3). Among newer homes, large properties averaged more wet yard drains than small homes.

In contrast, more flowerpot saucer dishes (dry or wet) were found in older than newer homes regardless of lot size (Fig. 4). Among the older homes, the average number of dry and wet flowerpot saucers per house was greater in larger than small homes.

If *Ae. aegypti* were to be found in flowerpot saucer dishes in Placer County, the most likely property type where they would be found was in older and larger residential properties. For inspections of older and larger residential properties, it is critical that flowerpot saucers be prioritized because these are one of the most common containers in older homes of Placer County that could breed *Ae. aegypti*.

Residential properties having the most total containers

Larger residential properties, regardless of the year the homes were built, tended to have more dry, wet, and mosquito producing containers (Fig. 5). That is, larger

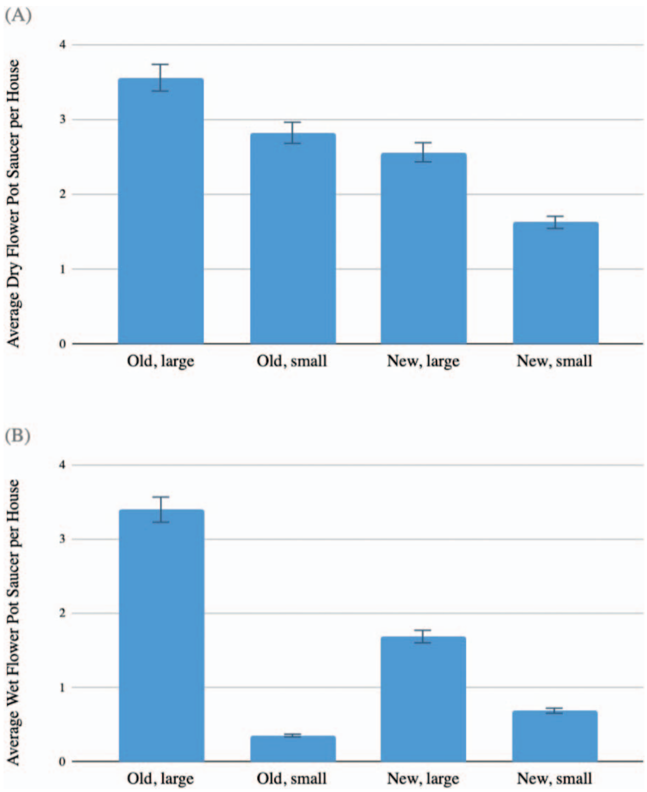


Figure 4.—Average number of (A) dry and (B) wet flowerpot saucer dishes recorded per residential home by property type during inspections for potential container *Aedes* habitat in Placer County, CA backyards from June to August 2020.

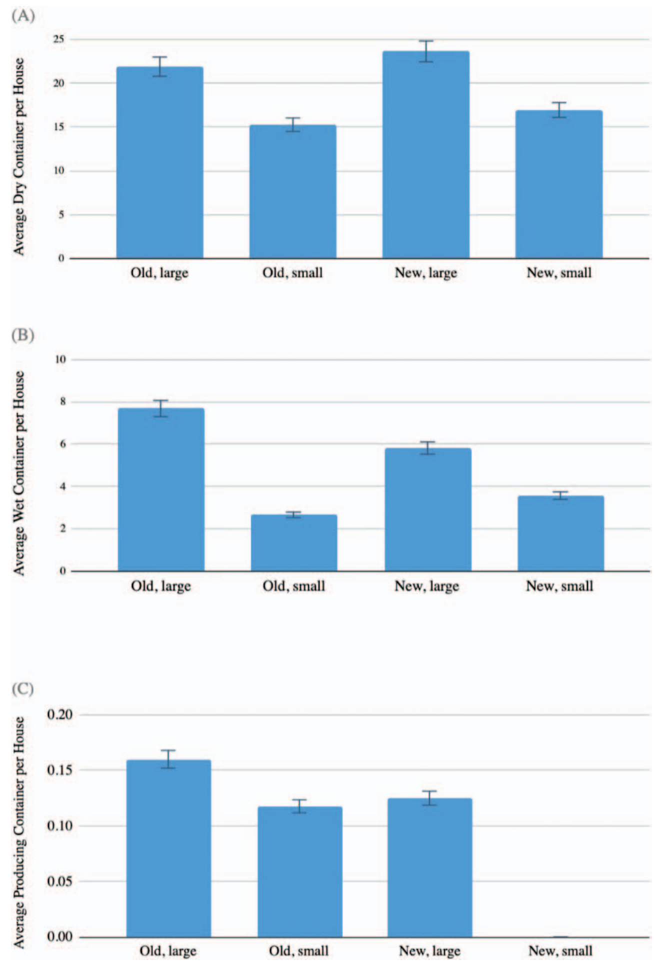


Figure 5.—Average number of total (A) dry, (B) wet, and (C) producing containers recorded per residential home by property type during inspections plotted as a function of the year built for residential properties inspected for potential container *Aedes* habitat in Placer County, CA backyards from June to August 2020.

properties have more space and therefore more containers. When inspecting large properties, it is important to be prepared to find more containers.

We originally hypothesized that old homes would have on average more containers than new properties of similar size, because the owners would accumulate containers over time. In part, the data supported this premis. Instances where newer homes had a greater number of containers than older homes were dry containers among large residential properties (Fig. 5a) and wet containers among small residential properties (Fig. 5b).

Unlike lot size, the relationship between home age and number of containers found was not significant (Fig. 6).

Income did not predict the numbers of containers

The square footage of each residential home was recorded as a part of the property inspections and was assumed to roughly correlate with household income. However, there was not a significant relationship when total containers was regressed as a function of house square footage. (Fig. 7).

DISCUSSION

There was no rainfall recorded during the summer of 2020, which was normal for the summer season in California, so therefore all containers with water were filled by human irrigation activities. By carefully recording the container types found on representative residential properties, we were able to determine which container types most commonly were dry and could hold standing water, currently held standing water, and currently were holding water and producing mosquito larvae. Yard drains and saucer dishes under flowerpots were the most common containers to hold or potentially hold standing water. Yard drains are most common in newer, large residential properties, whereas flowerpot saucer dishes are most common in older, large residential properties. These two container types should be prioritized when conducting property inspections due to their increased likelihood of potentially producing *Ae. aegypti*.

In contrast to our predictions, new houses (regardless of size) typically had equal numbers of dry and wet containers that could potentially produce *Ae. aegypti* compared to old houses. Therefore, both new and old properties must be considered when inspecting for *Ae. aegypti*. Similarly, large houses, representative of greater family income, had

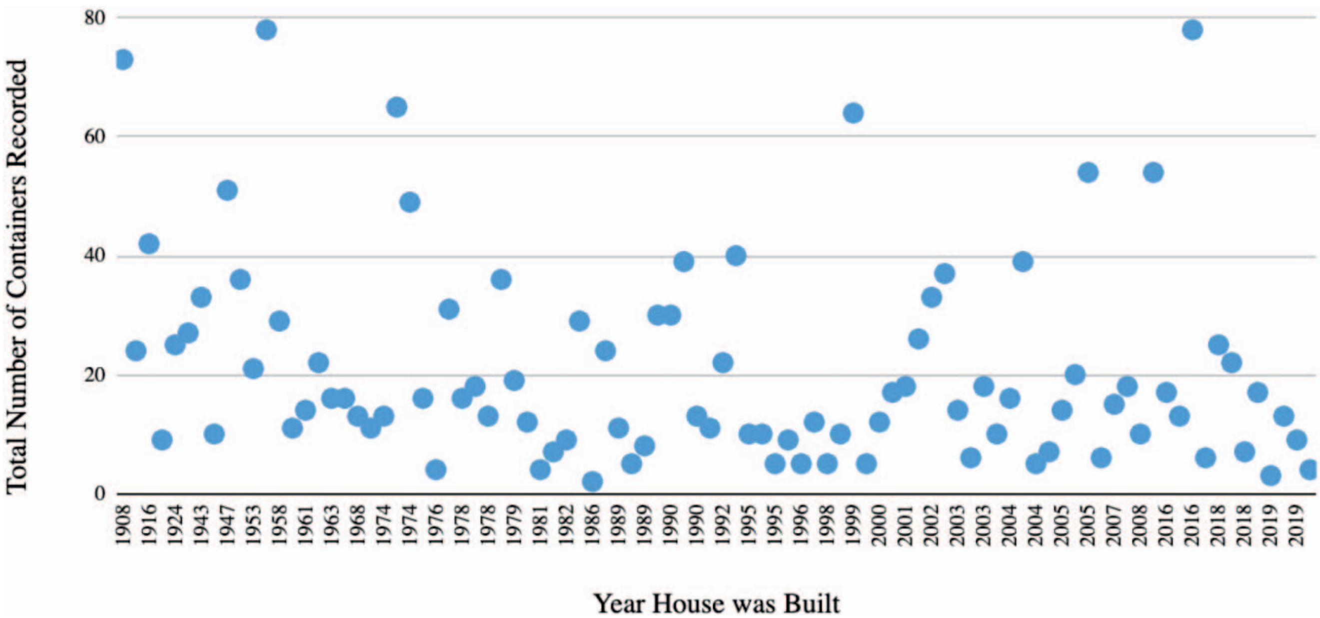


Figure 6.—The total number of containers found on the property that could potentially hold standing water during inspections for potential container *Aedes* habitat in Placer County, CA backyards from June to August 2020.

equal numbers of dry and wet containers that could potentially produce *Ae. aegypti* compared to homes of lower family income. Both higher and lower income homes must be considered when inspecting for *Ae. aegypti*.

Larger residential properties had on average more containers than smaller properties. When inspecting for *Ae. aegypti*, local mosquito control agencies may wish to consider prioritizing larger residential properties as well as allocating more time for inspecting the greater number of containers expected to be present.

It would be beneficial to investigate which of these properties and containers are exploited compared to our predictions should *Ae. aegypti* re-infest Placer County in the future.

ACKNOWLEDGMENTS

We thank the residents of Placer County that gave us access to their properties for inspection. Special thanks to

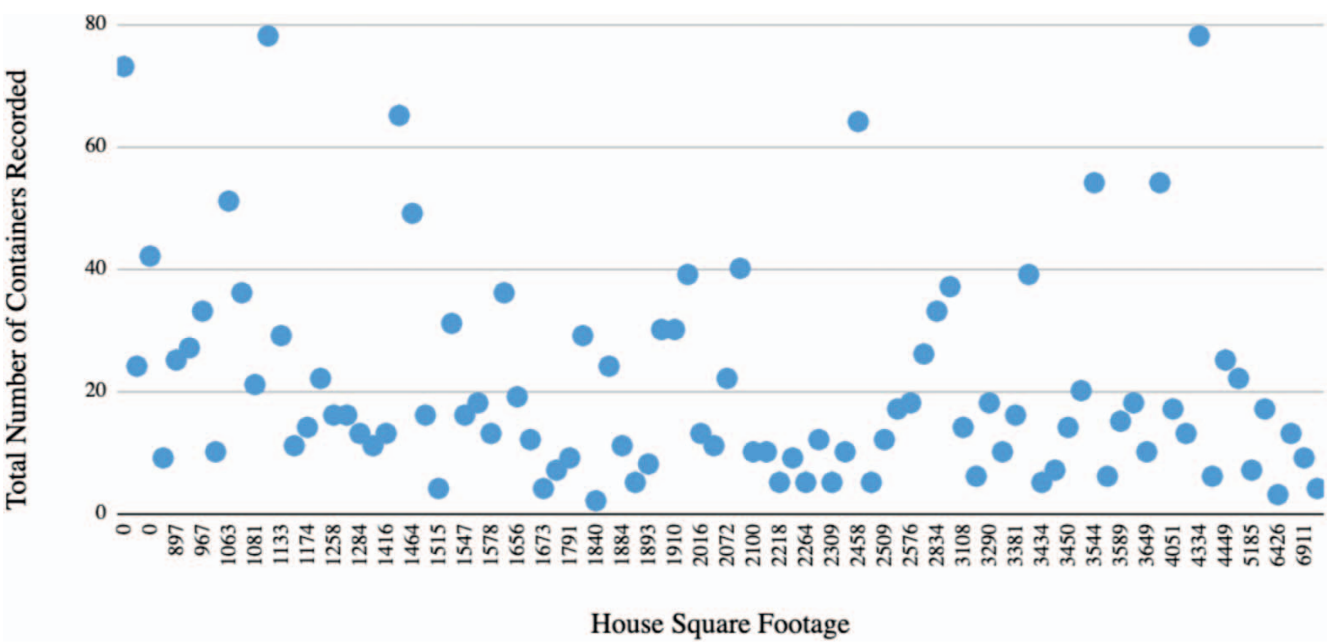


Figure 7.—The total number of containers that could potentially hold standing water found during inspections for potential container *Aedes* habitat plotted as a function of house square footage (representative of family income) of residential properties in Placer County, CA backyards from June to August 2020.

the trained mosquito control technicians that assisted during inspections: Michael Ashley, Steve Crosby, Tim Garner, Brent Geeve, Brian Ghilarducci, Everardo Ortiz, Jason Smith, and Ted Williams.

REFERENCES

Bowman, L. R., S. Runge-Ranzinger, and P.J. McCall. 2014. Assessing the relationship between vector indices and dengue transmission: A systematic review of the evidence. PLoS Negl. Trop. Dis. 8: 1-11.

(CDC) Centers for Disease Control. 2018. Estimated range of *Aedes aegypti* and *Aedes albopictus* in the US. www.cdc.gov/zika/vector/range.html. 11 September 2020.

Powell, J. R., and W. J. Tabachnick. 2013. History of domestication and spread of *Aedes aegypti*–a review. Mem. Instit. Oswaldo Cruz. 108: 11-17.

Washburn, J. O., and E. U. Hartmann. 1992. Could *Aedes albopictus* (Diptera: Culicidae) become established in California tree holes? J. Med. Entomol. 29: 995-1005.

Spatio-temporal impacts of aerial adulticide applications on populations of West Nile virus vector mosquitoes**

Karen M. Holcomb[1]*, Robert C. Reiner[2], and Christopher M. Barker[1]

[1]Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, California, USA, 95616
[2]Institute for Health Metrics and Evaluation, University of Washington, Seattle, Washington, USA, 98121

*Corresponding author: kmholcomb@ucdavis.edu

**The full version of this manuscript was published in Parasites and Vectors (Holcomb, Reiner, and Barker 2021).

Introduction

Aerial applications of insecticides are used during periods of epidemic risk to rapidly reduce the abundance of infectious mosquitoes in proximity to humans to reduce the risk of zoonotic disease outbreaks (Rose 2001). A common method for estimating the efficacy of these aerial spray events, called "Mulla's formula", compares the ratios of trap counts before and after spraying in the treatment zone vs. an adjacent unsprayed control zone (Reisen 2010). A change in the ratios is attributed to the treatment. However, this method does not take into account the spatio-temporal structure of the mosquito population as well as stochastic variation in trap-counts unrelated to treatment, thus leading to widely varying estimates between spray events (Lothrop et al. 2007, Macedo et al. 2007, Nielsen et al. 2007).

To remove the bias in estimating the average efficacy of aerial spraying on the individual-by-individual event basis, we used twelve years of surveillance and control data (2006-2017) from the Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) to develop statistical models to estimate the effect of aerial spraying on mosquito abundance after accounting for baseline population dynamics. We developed models for each *Culex tarsalis* and *Culex pipiens* populations, the primary West Nile virus (WNV) vector mosquitoes in California.

Methods

Surveillance & Control Data

We obtained surveillance and control records from SYMVCD for 2006-2017, which comprised 24,344 CO_2-baited trap collections and 930 unique aerial spray events. The spray records included the target zone and pesticide product used.

Model Structure and Analysis

We chose a negative binomial generalized additive model (GAM) framework to relate aerial spraying to nightly female abundance of either *Cx. tarsalis* or *Cx. pipiens*. A GAM is a form of regression that uses smooth functions to capture non-linear relationships of the covariates with the outcome (Hastie and Tibshirani 1986).

We quantified the impact of aerial spraying through a two-fold approach to capture the spatial and temporal impacts. For traps that fell within the spatial boundaries of an aerial spray, we calculated spray coverage as the proportion of the 5-km buffer that fell within the target spray zone. The buffer size was chosen based on the flight range of *Cx. tarsalis* and *Cx. pipiens* (Bailey et al. 1965, Reisen et al. 1991) to capture marginal effects of aerial sprays along the treatment boundaries. To capture any lagged effects from repeated spraying, we determined the temporal sequence of spray events in the preceding four weeks, on a weekly basis, before the respective night of trapping. For each sequence, we characterized the spray history as either employing all pyrethrin or pyrethroid products or using at least one organophosphate product.

To capture population abundance dynamics in the absence of aerial spraying, we considered a variety of smooth functions of covariates in each GAM. We chose a spatio-temporal surface to define broad inter-annual variation in the spatial pattern and seasonality curves by land cover type (urbanized, crop, and other natural) to capture intra-annual trends. Land type categories represented the availability of different larval habitats. We used two-week average temperature to capture developmental rates and the deviation from average temperature on the night of trapping to reflect mosquito activity.

To arrive at the final model for each species, we used backward selection guided by a reduction in AIC (Aike 1974). We then estimated the mean change (95% CI) in abundance from the baseline unsprayed population for each species. We assessed change given the average spatial coverage of the trap collection area and temporal sequence of sprays preceding trapping, producing a species-specific spatio-temporal surface of the effect of aerial spraying. All analysis was performed with R (version 3.3.2; R Core Team 2020) and model fitting used the *mgcv* package (Wood 2006, 2011).

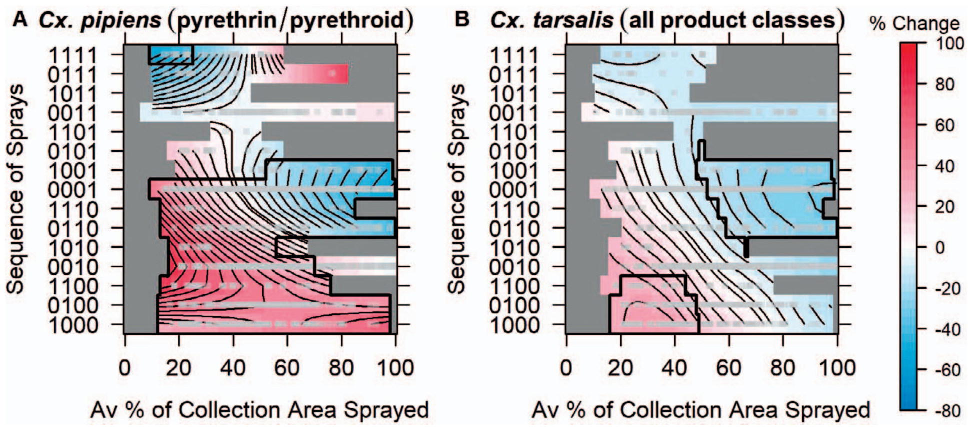


Figure 1.—Mean change in *Culex* mosquito abundance following aerial spraying, as compared to no-spray baseline. Estimates for (A) *Cx. pipiens* when all sprays used pyrethrin or pyrethroid products and (B) *Cx. tarsalis* populations with any combination of product class. Horizontal axes represent the average proportion of the 5km-radius area surrounding a trap covered by a spray event and the vertical axes represent the temporal sequence of aerial sprays during the four weeks preceding the trap. Presence (1) or absence (0) of spray in the 1, 2, 3, and 4 weeks (R to L) prior to trap indicated by the 4-digit sequence. Estimates are truncated to the range present in the data (grey squares indicate points present in data). Areas enclosed in a black border represent a portion of the spatio-temporal surface with significant estimates ($P < 0.05$).

Results and Discussion

We estimated the spatio-temporal impacts of aerial spraying after adjusting for the expected abundance in the absence of spraying (Fig 1). Combinations of spatial coverage and temporal sequence of sprays absent from the data were not estimated. For both species, we observed a general trend of larger magnitudes of reductions in populations with high average spatial coverage vs. those towards the outside fringes of sprays as well as within one to two weeks of spraying. We estimated an increase in abundance (i.e. population rebound) for both species at longer time lags. The dichotomization of product class was only significant in the final model for *Cx. pipiens*.

Our results indicated that aerial spraying effectively reduced the abundance of both *Cx. tarsalis* and *Cx. pipiens* in the short-term. We estimated a larger magnitude of reduction for *Cx. pipiens* than for *Cx. tarsalis*. For example, within a week of spraying with any combination of pyrethrins and pyrethroids, we estimated a -52.4% (95% CI: -65.6, -36.5%) change in *Cx. pipiens* populations at 100% spatial coverage (Fig 1A). The presence of at least one organophosphate product resulted in an additional 48.9% reduction in *Cx. pipiens* abundance across the whole spatio-temporal surface, which equated to a -76.2% (95% CI: -82.8, -67.9%) change in abundance for a population completely covered by spraying within the last week. In contrast, for any combination of pesticide products, we estimated a -30.7% (95% CI: -54.5, 2.5%) change in *Cx. tarsalis* abundance within one week for populations at 100% spatial coverage (Fig 1B).

The observed variation in effects between species is likely related to their respective ecology and dispersal patterns. *Cx. pipiens* is largely an urban species across our study area, with localized populations (Reisen and Reeves 1990). Therefore, an aerial spray event would impact a large proportion of the population and there would be only low immigration from surrounding unsprayed segments of the population, leading to a sustained reduction in the local population. On the other hand, *Cx. tarsalis* populations are generally larger in number and spatial extent (Reisen and Reeves 1990), spanning productive larval habitat in irrigated agricultural areas as well as urban areas into which adults may fly looking for bloodmeals (Bailey et al. 1965, Wekesa et al. 1996). Due to financial and logistic constraints, an aerial spray event would only impact a small proportion of the total population in the area. Rapid immigration from surrounding unsprayed locations (Dow et al. 1965) and emergence from productive larval habitats would replace adults killed during the spray events at a faster rate than in *Cx. pipiens* populations.

Our estimates are concordant with previously published estimates from the Sacramento Valley using Mulla's formula (Macedo et al. 2007, 2010, Nielsen et al. 2007, Elnaiem et al. 2008). However, these estimates exhibit heterogeneity in the magnitude of reduction (*Cx. pipiens*: 39-77% reduction; *Cx. tarsalis*: 25-57% reduction), highlighting the variation introduced when not accounting for underlying population dynamics. Additionally, previous studies used different timescales (1-7 days) to compare abundance pre- to post-treatment so direct comparisons between estimates are not possible.

In models for both species, seasonality by land type accounted for the largest impact on the magnitude of the expected abundance in the absence of spraying, followed by the spatio-temporal surface, indicating that intra-year trends drove abundance patterns over year-to-year variation. Average temperature and anomalies in temperature on the night of trapping modulated the expected abundance by a much smaller, but still significant, degree. All smooth functions were highly significant in establishing baseline abundance ($P < 0.0001$).

Our estimates were limited by the density and placement of mosquito traps. Spatially, sparse trapping in agricultural areas reduced our ability to define the baseline population dynamics and thus the full impact of aerial spraying in these areas. Additionally, due to a lack of temporal coverage of trapping across spray events, we were unable to estimate the impact of multi-night spray events (i.e., one vs two vs three consecutive nights), choosing instead to use a weekly time-step to aggregate spray events.

Conclusions

In conclusion, we found that aerial spraying effectively reduced the abundance of the two WNV vector mosquito species in the one to two-week timeframe post-spraying, achieving the epidemiological goal of reducing the number of infected mosquitoes in proximity to humans. Heterogeneity in the magnitude and duration of effect between species is likely related to their respective ecology and dispersal patterns.

Accurate estimation of the efficacy of aerial spraying is vital for effective implementation of vector control to protect human health. Our approach expanded upon conventional single-point estimates of control efficacy by taking into account the spatio-temporal population structure, habitat, and dispersal of vector mosquitoes as well as weather to produce robust estimates of population impacts.

Acknowledgements

We would like to thank Ruben Rosas, Marcia Reed, Sarah Wheeler, Samer Elkashef and Gary Goodman from Sacramento-Yolo Mosquito & Vector Control District for providing trapping and aerial treatment data used in this study and for their insight on mosquito control practices. KH acknowledges funding support from the Floyd & Mary Schwall Fellowship in Medical Research at UC Davis and the National Center for Advancing Translational Sciences, National Institutes of Health, through grant number UL1 TR001860 and linked award TL1 TR001861. KH and CB acknowledge support from the Pacific Southwest Center of Excellence in Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516).

References Cited

Aike, H. A. I. 1974. A new look at the statistical model identification. IEEE Trans. Automat. Contr. 19: 716–723.

Bailey, S. F., D. A. Eliason, and B. L. Hoffmann. **1965**. Flight and dispersal of mosquito *Culex tarsalis* Coquillett in Sacramento Valley of California. Hilgardia. 37: 73–113.

Dow, R. P., W. C. Reeves, and R. E. Bellamy. **1965**. Dispersal of female *Culex tarsalis* into a larvicided area. Am J Trop Med Hyg. 14: 656–670.

Elnaiem, D. A., K. Kelley, S. Wright, R. Laffey, G. Yoshimura, M. Reed, G. Goodman, T. Thiemann, L. Reimer, W. K. Reisen, and D. Brown. **2008**. Impact of aerial spraying of pyrethrin insecticide on *Culex pipiens* and *Culex tarsalis* (Diptera: Culicidae) abundance and West Nile virus infection rates in an urban/suburban area of Sacramento County, California. J Med Entomol. 45: 751–757.

Hastie, T., and R. J. Tibshirani. **1986**. Generalized additive models. Stat. Sci. 1: 297–318.

Lothrop, H., B. Lothrop, M. Palmer, S. Wheeler, A. Gutierrez, H. Lothrop, B. Lothrop, M. Palmer, S. Wheeler, A. Gutierrez, P. Miller, D. Gomsi, and W. K. Reisen. **2007**. Evaluation of pyrethrin aerial ultra-low volume applications for adult *Culex tarsalis* control in the desert environments of the Coachella Valley, Riverside county, California. J Am Mosq Control Assoc. 23: 405–419.

Macedo, P. A., C. F. Nielsen, M. Reed, K. Kelley, W. K. Reisen, G. W. Goodman, and D. A. Brown. **2007**. An evaluation of the aerial spraying conducted in reponse to West Nile virus activity in Yolo county. Proc. Pap. Mosq. Vector Control Assoc. Calif. 75: 107–114.

Macedo, P. A., J. J. Schleier, M. Reed, K. Kelley, G. W. Goodman, D. A. Brown, and R. K. D. Peterson. **2010**. Evaulation of efficacy and human health risk of aerial ultra-low volume applications of pyrethrins and piperonyl butoxide for adult mosquito management in response to West Nile virus activity in Sacramento county, California. J. Am. Mosq. Control Assoc. 26: 57–66.

Nielsen, C. F., W. K. Reisen, V. Armijos, S. Wheeler, K. Kelley, and D. Brown. **2007**. Impact of climate variation and adult mosquito control on the West Nile virus epidemic in Davis, California during 2006. Proc Pap Mosq Vector Control Assoc Calif. 75: 125–130.

R Core Team. **2020**. R: A language and environment for statistical computing. https://www.r-project.org.

Reisen, W. K. 2010. Using "Mulla's Formula" to estimate percent control, pp. 127–138. *In* Atkinson, P.W. (ed.), Vector Biol. Ecol. Control. Springer Science+Business Media B.V., New York.

Reisen, W. K., M. M. Milby, R. P. Meyer, A. R. Pfuntner, J. Spoehel, J. E. Hazelrigg, and J. P. Webb. **1991**. Mark-release-recapture studies with *Culex* mosquitoes (Diptera: Culicidae) in southern California. J Med Entomol. 28: 357–371.

Reisen, W. K., and W. C. Reeves. **1990**. Epidemiology and control of mosquito-borne arboviruses in California, 1943-1987., pp. 254–329. *In* Reeves, W.C. (ed.), California Mosquito and Vector Control Association, Inc., Sacramento, California.

Rose, R. I. 2001. Pesticides and public health: integrated methods of mosquito management. Emerg Infect Dis. 7: 17–23.

Wekesa, J. W., B. Yuval, and R. K. Washino. **1996**. Spatial distribution of adult mosquitoes (Diptera: Culicidae) in habitats associated with the rice agroecosystem of northern California. J Med Entomol. 33: 344–350.

Wood, S. N. 2006. Generalized additive models: an introduction with R. Chapman & Hall/CRC, Boca Raton, FL.

Wood, S. N. 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. J. R. Stat. Soc. Ser. B-Statistical Methodol. 73: 3–36.

Association between pyrethrum knock down time and sodium channel genotypes in California *Aedes aegypti*.

Lindsey K. Mack[1]* and Erin Taylor Kelly[1]*, Yoosook Lee[2], Katherine Brisco[3], Kaiyuan Victoria Shen[1], Aamina Zahid[1], Tess van Schoor[1], Anthony J. Cornel[3], Geoffrey M Attardo[1]**

[1]Department of Entomology and Nematology, College of Agriculture and Environmental Sciences, University of California, Davis, California
[2]University of Florida – Florida Medical Entomology Laboratory, Vero Beach, Florida
[3]Mosquito Control Research Laboratory, Kearney Agricultural Center, Department of Entomology and Nematology, University of California, Davis, California

**Corresponding author: gmattardo@ucdavis.edu

*These authors contributed equally to the preparation of this manuscript.

Introduction

Since their detection in 2013, *Aedes aegypti* has become a widespread urban pest in California. The availability of cryptic larval breeding sites in residential areas and resistance to insecticides pose significant challenges to control efforts. Resistance to pyrethroids is largely attributed to mutations in the voltage gated sodium channels (VGSC), the pyrethroid site of action. However, past studies have indicated that VGSC mutations may not be entirely predictive of the observed resistance phenotype. the overall aim of our study.

Methods

To investigate the frequencies of VGSC mutations and the relationship with pyrethroid insecticide resistance phenotypes in California, we sampled *Ae. aegypti* collected during the summer of 2018 from four locations in the Central Valley (Dinuba, Clovis, Sanger and Kingsburg), and the Greater Los Angeles (LA) area. Mosquitoes from each population (80-95 females) were subjected to individual pyrethrum bottle bioassays using a diagnostic dose of pyrethrum (15.6 ug/ml) to determine knockdown times. Immediately following either knockdown or termination of the assay at 120 minutes, mosquitoes were cold anesthetized and homogenized in lysis buffer from the Zymo Quick DNA/RNA miniprep kit and stored at 4°C. Genomic DNA was extracted from between 58-67 individuals from each population with half representing individuals from the lower quartile (most susceptible) of the knockdown time distribution and the other half representing the upper quartile (most resistant). The DNA for these individuals was sent to the UC Davis Veterinary Genetics Laboratory for iPLEX Single nucleotide polymorphism (SNP) analysis. This analysis facilitated determination of the composition of 8 single nucleotide polymorphism (SNP) loci within the VGSC gene.

Results

The distribution of knockdown times for each of the five Californian populations sampled was non-parametric with bimodal distributions. One group succumbed to insecticidal effects after 35-45 minutes, whereas the second group survived beyond the termination of the assay (120+ minutes). We detected 5 SNPs polymorphic within California populations. One was potentially new and alternatively spliced (I915K), and four were known and associated with resistance: F1534C, V1016I, V410L and S723T. The Central Valley populations (Clovis, Dinuba, Sanger and Kingsburg) were fairly homogenous, with only 5% of the mosquitoes showing heterozygosity at any given position. In the Greater LA mosquitoes, 55% had at least one susceptible allele at any of the five SNPs. The known resistance allele F1534C was detected in almost all sampled mosquitoes (99.4%). We observed significant heterogeneity in individuals with identical VGSC haplotypes, suggesting that the presence of additional undefined resistance mechanisms such as metabolic resistance may account for the unexplained variance.

Conclusions

Resistance associated VGSC SNPs were prevalent, particularly in the Central Valley. Interestingly, among mosquitoes with all 4 resistance associated SNPs, we observed heterogeneity in bottle bioassay profiles suggesting that other factors/mechanisms are important to the individual resistance of *Ae. aegypti* in California.

Acknowledgements

We would like to thank the California Department of Public Health, Vector-borne Disease Section for their consultation.

Morphological variance of *Aedes aegypti* mosquito populations in Northern Tulare County

Jesse Erandio*, Crystal Grippin, Mark Nakata, Javier Valdivias, Mir Bear-Johnson, Mustapha Debboun

Delta Vector Control District, PO Box 310, Visalia, CA 93279

*Corresponding author: jerandio@deltavcd.com

The yellow fever mosquito, *Aedes aegypti* (L.), is commonly identified by its contrasting black and white coloration and the distinct lyre on its thorax. In 2019, Delta Vector Control District identified a mosquito specimen that closely resembled *Ae. aegypti,* but with almost entirely pale yellow-scaled terga. Previous findings have shown different morphological forms of *Ae. aegypti* across tropical and subtropical regions based on the density of scales on the terga. This poster presentation discusses the morphological variations of *Ae. aegypti* that have recently been identified in northern Tulare County. Both variant and standard *Ae. aegypti* mosquitoes were mailed to San Mateo County Mosquito and Vector Control District for genetic analysis using a cytochrome c oxidase subunit I (COI) barcoding polymerase chain reaction. The sequence of the COI gene was compared to other *Ae. aegypti* sequences available in the GenBank using the Basic Local Alignment Search Tool (BLAST) under default parameters. Additionally, one variant of *Ae. aegypti* was sent to Dr. Leopoldo Rueda, a taxonomist from the Smithsonian Biosystematics Unit, for species confirmation and morphological identification. BG-Sentinel traps were set where variant *Ae. aegypti* previously werecollected to acquire additional adult specimens and photograph morphological differences. Ovicups also were placed in these areas to collect mosquito eggs for rearing. Sequencing results for the COI gene for the variant *Ae. aegypti* species showed >99% matching identity to *Ae. aegypti*. Furthermore, Dr. Rueda confirmed the dried specimen as *Ae. aegypti*. It is important to be aware that *Ae. aegypti* with pale yellow-scaled terga exists in northern Tulare County. Genetic and physiological analyses of the variant *Ae. aegypti* are required to further understand its biology and potential ramifications for pesticide resistance and mosquito-borne virus transmission.

Identifying potential mammalian reservoir hosts for *Rickettsia* 364D, an emerging tick-borne pathogen in California

Vincent Mai[1]*, Kerry Padgett[2] Chris Paddock[3], Robert S. Lane[4], Megan Saunders[2], Sarah Billeter[2], Andrea Swei[1]

[1]San Francisco State University, 1600 Holloway Ave, San Francisco, CA 94132
[2]California Department of Public Health, 850 Marina Bay Pkwy, Richmond, CA 94804
[3]Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, Georgia 30333
[4]University of California, Berkeley, 101 Sproul Hall Berkeley, CA 94720

*Corresponding author: vmai1@mail.sfsu.edu

INTRODUCTION

Pacific Coast tick fever (PCTF) is a recently described and understudied tick-borne, zoonotic disease in California caused by a spotted fever group rickettsia currently designated *Rickettsia* 364D and transmitted by the Pacific Coast tick, *Dermacentor occidentalis*. Because *D. occidentalis* is one of the most widely distributed tick species in California, it is frequently encountered by humans in savannah, chaparral, and coastal scrub habitats. Since the first confirmed human PCTF case in 2008, 13 additional human cases have been diagnosed in California, with more than half of them in children (Padgett et al. 2016). All cases presented with one or more pathognomonic necrotic lesions known as eschars and common symptoms of the disease including fever, headache, and lymphadenopathy. Four patients required hospitalization (Padgett et al. 2016, Shapiro et al. 2010).

Knowledge regarding the transmission cycle and geographic distribution of *Rickettsia* 364D is limited (Lane et al. 1981, Philip et al. 1981, Padgett et al. 2016, Paddock et al. 2018). In the present study, molecular investigations were conducted to determine the potential vertebrate hosts involved with the natural history of this pathogen in California. Common host species for juvenile stages of *D. occidentalis* that were targeted included ground squirrels (*Otospermophilus beecheyi*), deer mice (*Peromyscus* spp.), and woodrats (*Neotoma lepida*). For molecular analysis, a recently developed qPCR assay amplifying an intergenic region between the *nusG* and *rplK* gene was utilized (Karpathy et al. 2020). We also performed MaxEnt species niche modeling using existing tick occurrence records and *Rickettsia* 364D infection status.

METHODS

Testing for *Rickettsia* 364D using digital droplet PCR

A CDC qPCR protocol was validated and optimized for the detection of *Rickettsia* 364D (Karpathy et al. 2019) on a digital droplet PCR platform (BioRad QX200). Preliminary tests on control samples confirmed that the protocol was able to detect R364D at single digit concentration (8 copies/20uL well or 0. 4 copies/uL) in tick and mammal 'spiked-in' samples. Pathogen screening was conducted on ear-tissue biopsies obtained from several potential reservoir hosts, namely *N. lepida* (n=1), *Peromyscus* spp, (n=4) and *O. beecheyi* (n=55) as well as from blood samples from these three rodent species (n=60). In addition, pathogen screening was conducted on 164 *D. occidentalis* ticks collected from Kern County in the southern San Joaquin Valley region.

Species Distribution Modeling

Species distribution models (SDM) for *D. occidentalis* were generated using tick occurrence records and *Rickettsia* 364D infection status gathered from published studies (Paddock et al. 2018; Padgett et al. 2016). We georeferenced CDPH records based on zip code and county metadata associated with the tick samples by joining CDPH tick occurrence records with a 1999 US Census Bureau zip code gazetteer (CivicSpace Labs, 2004). Redundant occurrence points that were within a 10 km radius of each other were removed to reduce sampling bias prior to MaxEnt environmental niche modeling using the package Wallace *(v1.0.5)* run in R *(v4.0.4)*. Preliminary models for predicting *D. occidentalis* distribution and infected *D. occidentalis* distributions were produced using eight environmental data layers provided through the Bioclim database (Bio1, 2, 4, 8, 9, 15, 18, 19) (Lippi et al. 2020) (Figure 1).

RESULTS

Testing for *Rickettsia* 364D using digital droplet PCR

Host pathogen testing identified three individual *O. beecheyi* as PCR positive for *Rickettsia* 364D; two whole blood and one ear-tissue specimens harbored detectable DNA. These samples were collected from Los Angeles County and Santa Barbara County, respectively. Confirmatory testing using an *omp*A protocol (Roux *et al*. 1996)

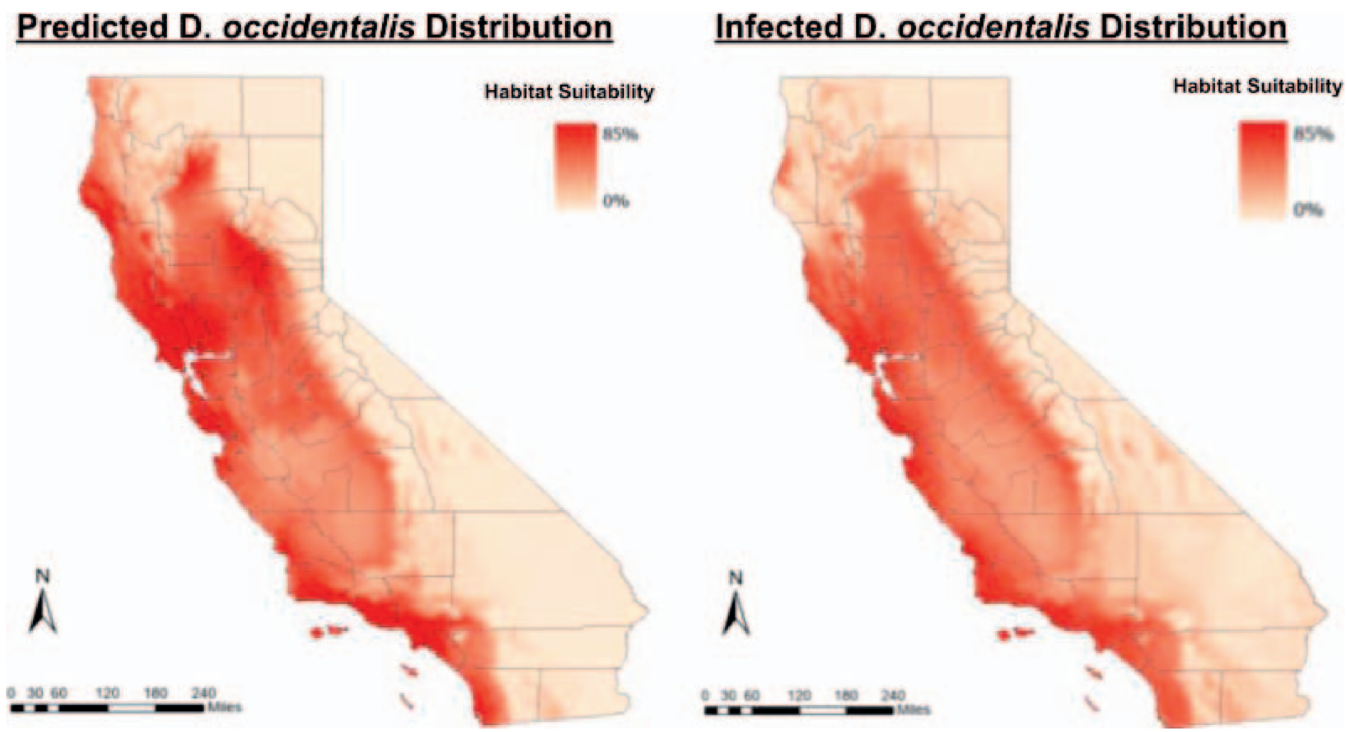


Figure 1.—Preliminary MaxEnt models for predicted *D. occidentalis* distribution and infected *D. occidentalis* distribution was made using occurrence records of *D. occidentalis* from Paddock et al. (2018) and Padgett et al. (2016) and environmental data from Bioclim (Bio1, 2, 4, 8, 9, 15, 18, 19) (*Lippi* et al. 2020) using the package Wallace (*v1.0.5*) run in R (*v4.0.3*)

and sequencing is ongoing. *Rickettsia* 364D DNA was not detected in ticks collected from Kern County.

Species Distribution Modeling

Preliminary species niche modeling showed areas of high suitability along the coast and in the foothills of the Sierra Nevada for predicted *D. occidentalis* distribution as well as predicted niche of *Rickettsia* 364D-infected *D. occidentalis*. The predicted distribution of infected *D. occidentalis* is a subset of the overall predicted distribution of *D. occidentalis*. These findings support the hypothesis that an amplifying host supports higher infection prevalence in the infected tick regions compared to other areas of the state.

CONCLUSION

Results from this preliminary study suggest that *O. beecheyi* could either be a vertebrate reservoir or amplifying host for *Rickettsia* 364D. Previously published research also suggests that the black-tailed jackrabbit (*Lepus californicus*) may be a potential amplifying host of *Rickettsia* 364D or related spotted fever group rickettsiae (Lane et al. 1981). Hence, that lagomorph warrants further examination as do other small mammals present throughout the known geographic distribution of *Rickettsia* 364-infected *D. occidentalis* ticks. Additionally, confirmatory tests for the positive *O. beecheyi* samples are still ongoing. After refining the preliminary SDMs, the distributions of suspected vertebrate hosts can be compared to the predicted niche of infected *D. occidentalis*.

ACKNOWLEDGEMENTS

The authors thank Dr. Marco Metzger, California Department of Public Health, for assistance with sample collection. The authors acknowledge funding support from the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516).

References

Lippi, C.A., HD Gaff, AL White, HK St. John, AL Richards, and SJ Ryan. 2020. Exploring the niche of *Rickettsia montanensis* (Rickettsiales: Rickettsiaceae) infection of the American Dog Tick (Acari: Ixodidae), using multiple species distribution model approaches, Journal of Medical Entomology. Dec 2020 :tjaa263. doi: 10.1093/jme/tjaa263. Epub ahead of print.

Karpathy, S. E., A. Espinosa, M. H. Yoshimizu, J. K. Hacker, K. A. Padgett, and C. D. Paddock. 2019. A novel TaqMan assay for detection of *Rickettsia* 364D, the etiologic agent of Pacific Coast tick fever. J. Clinical Microbiology ***58:*** e01106-19. https://doi.org/10.1128/JCM.01106-19

Lane RS, Emmons RW, Dondero DV, Nelson BC. 1981. Ecology of tick-borne agents in California. I. Spotted fever group rickettsiae. Am. J. Trop.l Med. Hyg. **30**:239-52. doi: 10.4269/ajtmh.1981.30.239.

Lane, R.S., Philip, R.N., and Casper, E.A. 1981. Ecology of tickborne agents in California. II. Further observations on rickettsiae. Pages 575-584 *in* Burgdorfer, W., and R.L. Anacker (eds). Rickettsiae and rickettsial diseases. Academic Press, New York.

Paddock CD, Yoshimizu MH, Zambrano ML, Lane RS, Ryan BM, Espinosa A, Hacker JK, Karpathy SE, and Padgett KA. 2018. *Rickettsia* Species Isolated from *Dermacentor occidentalis* (Acari: Ixodidae) from California. J. Med. Entomol. **55:** 1664. https://doi.org/10.1093/jme/tjy149

Padgett, K. A., D. Bonilla, M. E. Eremeeva, C. Glaser, R. S. Lane, C. C. Porse, M. B. Castro, S. Messenger, A. Espinosa, J. Hacker, A. Kjemtrup, B. Ryan, J. J. Scott, R. J. Hu, M. H. Yoshimizu, G. A. Dasch, and V. Kramer. 2016. The eco-epidemiology of Pacific Coast Tick Fever in California. PLoS Neglected Trop. Dis.. **10**:e0005020. doi: 10.1371/journal.pntd.0005020.

Philip, R.N., Lane, R.S., and Casper, E.A. 1981. Serotypes of tick-borne spotted fever group rickettsiae from western California. Am. J. Trop.. Med. Hyg. 30:722-727.

Roux, V., Fournier, P. E., & and Raoult, D. 1996. Differentiation of spotted fever group rickettsiae by sequencing and analysis of restriction fragment length polymorphism of PCR-amplified DNA of the gene encoding the protein rOmpA. J. Clinical Microbiol. 34: 2058–2065. https://doi.org/10.1128/JCM.34.9.2058-2065.

Shapiro, M. R., C. L. Fritz, K. Tait, C. D. Paddock, W. L. Nicholson, K. F. Abramowicz, S. E. Karpathy, G. A. Dasch, J. W. Sumner, P. V. Adem, J. J. Scott, K. A. Padgett, S. R. Zaki, and M. E. Eremeeva. 2010. Rickettsia 364D: A newly recognized cause of eschar-associated illness in California. Clinical Inf. Dis. **50**:541-8. doi: 10.1086/649926. PMID: 20073993

Pyrethroid resistance and spread of *Aedes aegypti* in California

Erin Taylor Kelly

University of California, Davis, CA

Corresponding author: etkelly@ucdavis.edu

Invasive *Aedes aegypti* were established in the Central Valley in 2013. Since then, they have moved across the state and have now been detected in 22 of the 58 counties in California. The high levels of pyrethroid insecticide resistance in these mosquitoes has made control difficult. Using an assay developed for the iPLEX MassARRAY system, which detects selected single nucleotide polymorphisms (SNPs), we have gained insight on the resistance profiles and California origins of mosquitoes in the state. The assay includes 5 mutations in the voltage gated sodium channel (VGSC), the pyrethroid site of action, and 31 location related SNPs. Previously, using mosquitoes collected in Clovis, Sanger, Kingsburg, Dinuba, and the Greater Los Angeles (LA) area, we determined that the VGSC SNPs do not definitively determine the level of insecticide resistance in a population, but target site resistance does play a part. We also determined that those mosquitoes collected from the Greater LA area were significantly different in their location based-SNP profile than those from the Central Valley populations. Using this assay, we can place mosquitoes into one of these two general populations in California. Since this initial study, we have used this assay on mosquitoes collected in other areas of California to provide mosquito abatement districts with information on the origin of these introduced mosquitoes, as well as some information on their resistance status. We plan to continue to collect mosquitoes from counties with recent introductions to trace from where these mosquitoes may have originated.

Reducing *Aedes aegypti* production among residents with a history of continued production

Crystal Grippin*, Javier Valdivias, Jesse Erandio, Mark Nakata, Mir Bear-Johnson, Mustapha Debboun

Affiliation: Delta Vector Control District, PO Box 310, Visalia, CA 93279

*Corresponding author: clgrippin@deltavcd.com

Analysis of property inspection data from 2017 to 2019 showed that among properties with a history of *Aedes aegypti* (L.) production, 33% continued to produce mosquitoes during a subsequent year. These properties represent an increased risk of re-infesting the surrounding areas and contributing to the continued spread of *Ae. aegypti*. Our study examined the effectiveness of a pre-mosquito season door hangers in reducing continued *Ae. aegypti* production among residents with a history of mosquito production. Properties that had *Ae. aegypti* larval sources in 2019 were assigned to a control or intervention group using a randomized block design. The intervention group received a color door hanger in May 2020 that reminded residents to empty water holding containers weekly and scrub them with bleach to remove any oviposited eggs. Properties in both groups were later inspected for breeding larval habitats when a service request was received or when at least 10 female *Ae. aegypti* were caught in a single trap night in the area. There was no significant difference between intervention and control groups in this study. Developing an effective method to reduce continued *Ae. aegypti* production across multiple mosquito seasons has the potential to reduce overall control costs and slow the spread of this invasive species.

Aedes aegypti in 2020: Potential source preference

Crystal Grippin*, Javier Valdivias, Jesse Erandio, Mark Nakata, Mir Bear-Johnson, Mustapha Debboun

Delta Vector Control District, PO Box 310, Visalia, CA 93279

*Corresponding author: clgrippin@deltavcd.com

Aedes aegypti (L.) ecology and container preference varies widely based on local environment, human behavior, and container availability. Understanding regional differences within individual districts may help with selecting the most cost-effective control options and refining outreach efforts. Our poster presents the primary *Ae. aegypti* larval habitats and their contribution to overall abundance within the Delta Vector Control District (District) boundaries using data collected during property inspections. In 2020, the District identified potential larval sources and recorded the presence or absence of mosquito larvae during property inspections. Larval samples were taken from various source types and identified to species. Results showed that potted plant trays contributed 23.5% of the overall *Ae. aegypti* production while yard drains and fountains contributed 12.8% and 11.7%, respectively. Although often cited as a common source, tires contributed only 0.3% to the overall *Ae. aegypti* larval production within the District. Focusing public education and outreach efforts on controlling the most productive household containers is likely to be more cost-effective than broad messages, especially if they incorporate a concrete 'call-to-action' for residents. More effective homeowner engagement and control of key container sources can greatly reduce the costs of control efforts by Districts.

Rickettsial infections in fleas of southern California

Jia Li[1], Xiaoming Wang[1,2], Daisy Rangel[2], Laura Krueger[2], Kiet Nguyen[2], and Robert Cummings[2]

[1]Program in Public Health, University of California, Irvine, Irvine, CA 92697
[2]Orange County Mosquito and Vector Control District, Garden Grove, CA 92843

*Corresponding author: rcummings1026@gmail.com

INTRODUCTION

Rickettsia typhi and *R. felis* are flea-borne bacterial pathogens which cause an acute undifferentiated febrile illness, flea-borne rickettsiosis (formerly, murine typhus), throughout the world. After implementation of US Public Health Service-sponsored flea and rodent control programs from 1946 through the 1960s, cases declined substantially in endemic regions of California and the US (CDPH 1950, Woodward et al. 1973, Anstead 2021). However, since the 1970s, southern California has experienced several focal outbreaks and a recent (2001-2020) area-wide reemergence of flea-borne rickettsial disease, with most cases (1,142 of 1,185) occurring in Los Angeles (932) and Orange (210) Counties (CDPH 2020). Documenting the distribution of *R. typhi* and *R. felis* across different species of flea vectors among different host animals is necessary to elucidate the epidemiology of flea-borne rickettsiae and the apparent regional distribution of human disease (Azad et al. 1997). From 2014 to 2019, the Orange County Mosquito and Vector Control District (OCMVCD) conducted disease investigations by collecting fleas from captured mammalian wildlife and domestic pets at putative human exposure sites. The Los Angeles County Department of Public Health also provided flea and mammal specimens collected from several flea-borne rickettsiosis outbreak areas in Los Angeles County to OCMVCD for testing and were included in this study. The current study was a retrospective analysis of flea data for rickettsial infection associations among nine flea species (N = 3,700 fleas) (Table 1) and their mammalian hosts (14 species; N = 302 animals) (Table 2) collected from 2014 – 2019. Our current study extends earlier work by Rangel et al. (2019), which focused on developing a duplex PCR for testing *R. typhi* and *R. felis* simultaneously, and Penick et al. (2020), which examined flea assemblages and flea/mammalian host associations. Collectively, understanding flea indices and assemblages on their animal hosts with rickettsial infection rates by flea and animal species could help OCMVCD determine how to minimize the risk of flea-borne pathogen transmission in Orange County.

METHODS

Host animals and their fleas were collected and processed according to the methods outlined previously in Penicks et al. (2020). Briefly, Tomahawk traps (107×30×30 cm) (Tomahawk Live Trap Co., Tomahawk, WI) were used to live-trap wild animals at case homes in Orange and Los Angeles Counties. In Orange County, pet cats and dogs were combed for fleas, and their sleeping areas inspected, with owner's permission at the home. Fleas were tested individually for rickettsial infection from each animal by real time polymerase chain reaction (qPCR) using an Applied Biosystems™ 7500 Fast Real-Time PCR System for detecting rickettsial DNA. The primers and probe used to detect *R. typhi* are specific for the outer membrane protein B (ompB) gene (Henry et al. 2007), whereas the primers and probe used for detecting *R. felis* are specific for the protein phosphatase gene (Leulmi et al. 2014). Fleas collected from 2014 – 2017 were tested for *R. typhi* and *R. felis* separately in a singleplex assay, whereas fleas collected from 2018 – 2019 were tested using a duplex PCR reaction for both rickettsial species simultaneously, as described previously by Rangel et al.

Table 1.—Fleas collected off mammals in Los Angeles and Orange Counties, 2014 - 2019.

Flea Species	Common Name
Ctenocephalides felis	Cat flea
Diamonus montanus	Ground squirrel flea
Echinophaga gallinacea	Sticktight flea
Hoplopsyllus anomalus	Rodent flea
Leptopsylla segnis	Mouse flea
Nosopsyllus fasciatus	Northern rat flea
Orchopeas howardii	Grey squirrel flea
Pulex irritans/simulans	Human/false human flea
Xenophyllus cheopis	Oriental rat flea

Table 2.—Mammalian hosts of fleas collected in Los Angeles and Orange Counties, 2014 - 2019.

Mammal Species	Common Name
Canis latrans	Coyote
Canis familiaris	Domestic dog
Didelphis virginiana	Virginia opossum
Felis catus	Domestic cat
Homo sapiens	Human
Mephitis mephitis	Striped skunk
Neotoma bryanti	Bryant's woodrat
Otospermophilus beecheyi	Calif. ground squirrel
Oryctolagus cuniculus	European rabbit
Procyon lotor	Raccoon
Rattus norvegicus	Norway rat
Rattus rattus	Roof rat
Sylvilagus audubonii	Cottontail rabbit
Sciurus niger	Fox squirrel

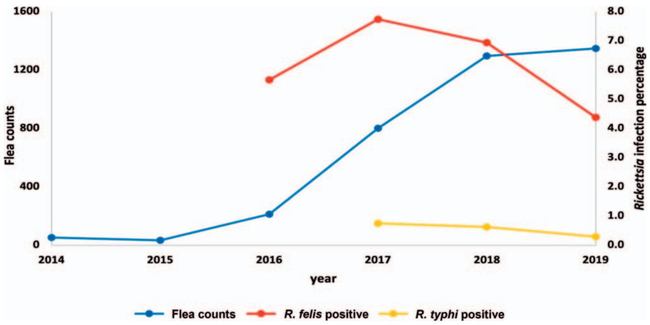


Figure 1.—Flea counts collected from 2014 to 2019 and *R. typhi/ R. felis* infection prevalence from 2016 to 2019.

(2019). Fleas with cycle thresholds (Ct) < 36.0 were considered positive for either rickettsial species. Data from a total of 3,700 tested fleas and their mammalian hosts ($N = 302$) were evaluated in this study.

All statistical analyses were conducted with RStudio (2020). The associations between flea species, host species, flea sex, collection year, and collecting county were evaluated using a Chi-squared test or Fisher's exact test depending on the size of the infected samples. Proportion trend test was used to see if there was an increasing/ decreasing trend of *R. typhi/R. felis* infection over the years. The Mantel Haenszel test was used to test the association between two variables after adjusting the third variable. Odds ratios were generated from the Epitools library (RStudio 2020). Associations between *R. typhi/R. felis* infection and variables tested were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

We observed that *R. felis* infection prevalence increased significantly from 2016 to 2019 (Chi-squared test, $\chi^2 = 6.97$, $P = 0.008$) (Figure 1), especially with the increasing number of *R. felis*-infected *C. felis* fleas over the years of the study. In contrast, we observed a decrease in *R. typhi* infection prevalence from 2017 to 2019, which was not statistically significant (Chi-squared test, $\chi^2 = 2.21$, $P = 0.14$). Our data indicated a significant prevalence of *R. felis* in multiple species of fleas collected in Los Angeles and Orange Counties (Figure 2); in contrast, the highest *R. typhi* infection prevalence was detected in oriental rat fleas (6.8%), with infected fleas collected mainly from Norway rats and secondarilyfrom cats (Figure 3). For *R. felis*, 97% (230/236) of the positive fleas were detected from cat fleas, and 2% (5/236) came from sticktight fleas. A significant percentage of cats and opossums were infested with *R. felis*-positive fleas (14.5% and 12.0%, respectively), whereas coyotes had a lower infestation rate at 3.6% (Chi-squared test, $\chi^2 = 143.55$, $P < 0.001$) (Figure 2). Interestingly, our data showed that male fleas had a significantly higher odds ratio of being infected with *R. felis* than female fleas, with $OR = 1.91$ (Chi-squared test, $\chi^2 = 20.70$, $P < 0.001$) (Figure 2). Theoretically, female fleas would have a higher chance of contracting *Rickettsia* from host animals, because they feed more often and require more blood due to their larger body size and egg production. The reasons for the differences require further investigation.

Los Angeles and Orange Counties accounted for 78.5% and 18.0%, respectively, of all flea-borne rickettsiosis cases in California from 2001 to 2019 (CDPH 2020). Of the fleas tested, Orange County was 2.65 times more likely to have *R. felis*-positive fleas compared with Los Angeles County (Chi-squared test, $\chi^2 = 25.79$, $P < 0.001$, Figure 2). Los Angeles County was found to have a similar odds ratio of having *R. typhi*-positive fleas compared to Orange County (Fisher's exact test, $P = 0.60$, Figure 3).

CONCLUSIONS

A significant increasing trend of prevalence of *R. felis* from 2016 to 2019 in parallel with an increased number of

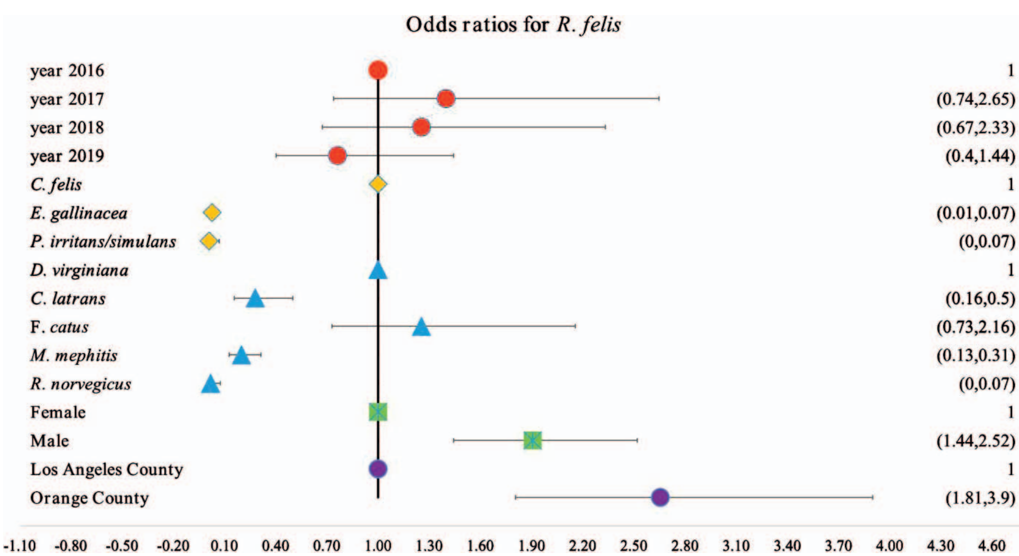


Figure 2.—Forest plot for a summary of odds ratios of *R. felis* infections for five variables tested in this study. Year 2016, *C. felis*, *D. virginiana*, female, Los Angeles County, were the reference groups for the respective variables. Odds ratios and confidence intervals are displayed for each level.

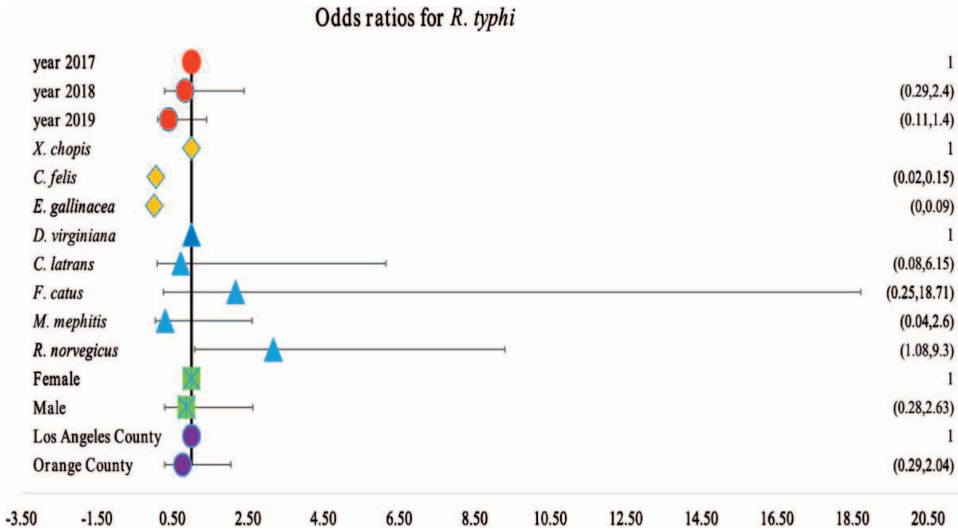


Figure 3.—Forest plot for a summary of odds ratios of *R. typhi* infections for five variables tested in this study. Year 2017, *C. felis*, *X. cheopis*, female, and Los Angeles County were the reference groups for the respective variables. Odds ratios and confidence intervals are displayed for each level.

reported cases of flea-borne rickettsiosis in southern California was found, with a non-significant trend of decreasing prevalence of *R. typhi* from 2017 to 2019. Cats and opossums were found to have a significantly higher rate of being infested by *R. felis*-positive fleas, whereas Norway rats had a significantly higher rate of being infested by *R. typhi*-positive fleas. The cat flea was found to have a significantly higher rate of being infected with *R. felis*, whereas the oriental rat flea had a significantly higher rate of infection with *R. typhi*. Male fleas had a significantly higher rate of infection with *R. felis*, but a non-significant lower rate of being infected with *R. typhi* than females. Lastly, Orange County was found to have a significantly higher risk of detecting *R. felis* positive fleas. The findings from both flea and host species further support the roles of the cat flea and its primary mammalian hosts, opossums and cats, in the suburban transmission cycle and the rat-oriental rat flea-rat urban transmission cycle, respectively (Adams et al. 1970, Eremeeva et al. 2012).

ACKNOWLEDGEMENTS

We would like to thank OCMVCD field staff for collecting these samples and to acknowledge the support from UC Irvine's student practicum program.

REFERENCES

Adams, W. H, R. W. Emmons, and J. E. Brooks. 1970. The changing ecology of murine (endemic) typhus in southern California. Am. J. Trop. Med. Hyg. 19:311–318.

Anstead, G. M. 2021. History, rats, fleas, and opossums. II. The decline and resurgence of flea-borne typhus in the United States, 1945–2019. Trop. Med. Infect. Dis. 6:2. https://doi.org/10.3390/tropicalmed6010002

Azad, A. F., S. Radulovic, J. A. Higgins, B. H. Noden, and J. M. Troyer. 1997. Flea-borne rickettsioses: ecologic considerations. Emerg. Infect. Dis. 3:319-27.

CDPH (California Department of Public Health). 1950. Typhus fever in California 1916-1948 (PDF). https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/Flea-borneTyphusCaseCounts.pdf. Accessed April 20, 2020.

CDPH (California Department of Public Health). 2020. Human flea-borne typhus cases in California, Vector-Borne Disease Section, California Department of Public Health. http://www.cdph.ca.gov/healthinfo/discond/pages/typhus.aspx. Accessed March 21, 2021.

Eremeeva, M. E., S. E. Karpathy, L. Krueger, E. K. Hayes, A. M. Williams, Y. Zaldivar, S. Bennett, R. Cummings, A. Tilzer, R. K. Velten, N. Kerr, G. A. Dasch, and R. Hu. 2012. Two pathogens and one disease: detection and identification of flea-borne Rickettsiae in areas endemic for murine typhus in California. J. Med. Entomol. 49:1485-1494.

Henry, K. M., J. Jiang, P. J. Rozmajzl, A. F. Azad, K. R. Macaluso, and. A. J. Richards. 2007. Development of quantitative real-time PCR assays to detect *Rickettsia typhi* and *Rickettsia felis*, the causative agents of murine typhus and flea-borne spotted fever. Mol. Cell. Probes. 21:17-23.

Leulmi, H., C. Socolovschi, A. Laudisoit, G. Houemenou, B. Davoust, I. Bitam, D. Raoult, and P. Parola. 2014. Detection of *Rickettsia felis*, *Rickettsia typhi*, and *Bartonella* species and *Yersinia pestis* in fleas (Siphonaptera) from Africa. PloS Negl. Trop. Dis. E3152.

Penicks, A., L. Krueger, J. Campbell, C. Fogarty, D. Rangel, K. Nguyen, and R. Cummings. 2020. Flea abundance, species composition, and prevalence of rickettsiosis, from urban wildlife in Orange County, California, 2015-2019. Proc. Vertebr. Pest Conf. 29:e7. https://escholarship.org/uc/vertebrate_pest_conference/29/29.

Rangel, D., M. Pecolar M., C. Fogarty, J. Campbell, L. Krueger, T. Morgan, and R. Cummings. 2019. The development and use of a duplex real-time PCR for the detection of *Rickettsia typhi* and *Rickettsia felis* in fleas collected in Orange and Los Angeles Counties, California. Proc. Calif. Mosq. Control Assoc. 87:221-225.

RStudio. 2020. Version 1.2.5042-1. https://www.rstudio.com/

Woodward, T. E. 1973. A historical account of the rickettsial diseases with a discussion on unsolved problems. J. Infect. Dis. 127:583-594.

Habitat suitability of underground storm drain systems for *Aedes aegypti*

Tanya M. Posey*, Harold Morales, Ryan Amick

Greater Los Angeles County Vector Control District, Santa Fe Springs, CA 90670

*Corresponding author: tposey@glacvcd.org

Introduction

Los Angeles County's Underground Storm Drain Systems (USDS) consists of more than 2,650 miles of storm drains which can produce large quantities of mosquitoes (Kluh et al. 2001). Anecdotal reports from operational staff suggested the presence of *Aedes aegypti* in the USDS, which if confirmed, would necessitate changes in the District's approach to the treatment and control of mosquitoes in the USDS. The main objective of our brief study was to determine whether water samples taken from USDS sources were capable of sustaining *Ae. aegypti* larval and pupal development without the addition of nutrients or additional water.

Methods

For this study, nine USDS sites were selected. Each site was trapped with one unbaited EVS trap and upon retrieval, a 1 liter water sample was taken, stored in an amber jar, and transported back to the laboratory in an insulated cooler to control any potential exposure to light, heat, or cold which could alter the presence of organic matter within each water sample. The water samples were then placed in plastic containers, *Ae. aegypti* eggs introduced, and the containers placed in rearing cages contained within a dark enclosure in the insectary. Two plastic containers with mosquito eggs flooded in 1 liter of tap water and 12 ml of a liver powder slurry per container also were placed in the insectary and served as positive controls. The insectary was maintained at 23.9° C (75° F) with 69% +/- 2% humidity for the duration of the project. Larval development was monitored, and adult emergence was recorded.

Results and Discussion

Four of the nine traps deployed collected *Ae. aegypti*, and two of those collected both males and females. Of the nine water samples taken, one developed a film and failed to produce any *Ae. aegypti* larvae, whereas the remaining eight samples had varying rates of larval development. Six water samples contained sufficient nutrients to support development through adult emergence, while two of the samples could not sustain development through the pupal stage. Only 32 adults emerged out of the 1,436 eggs introduced to the nine water samples taken (0.02% emergence rate). The control groups had much greater emergence, with 263 adults emerging from the 343 eggs for an emergence rate of 77%.

Conclusion

The emergence of adult *Ae. aegypti* mosquitoes from the water samples taken from the USDS confirmed the belief that the Underground Storm Drain Systems provide a suitable habitat for *Ae. aegytpi* and their development. Given the expansive USDS within Los Angeles County, it is of utmost importance to definitively determine the role of the storm drains, catch basins, and manhole chambers in the proliferation of *Ae. aegypti*.

Acknowledgements

The authors would like to thank Yessenia Curiel and Apolinar Estrada for their assistance and collaboration.

Reference Cited

Kluh, S., P. De Chant, J.E. Hazelrigg, M. W. Shaw, and M. B. Madon. 2001. Comparison of larvicidal application methods in the underground storm drain systems in urban Los Angeles. Proc. Calif. Mosq. Vector Control Assoc. 69: 48-53.

Similarities in environmental exposure to the causative agent of hantavirus pulmonary syndrome in two Mono County human cases, 2019.

Joseph Burns*, Renjie Hu, Marco Metzger, Sarah Billeter

California Department of Public Health, Vector-Borne Disease Section, Ontario, CA 92358

*Corresponding Author: Joseph.Burns@cdph.ca.gov

In 2019, CDPH Public Health Biologists conducted environmental investigations in response to two hantavirus pulmonary syndrome (HPS) cases reported from Mono County, one of which was fatal. Our presentation will describe the importance of environmental investigations in determining the likely points or locations of exposure to Sin Nombre virus, the causative agent of HPS, to reduce the risk of further virus transmission. In both cases, forced-air heating systems compromised by rodents may have played a role in the infections.

Environmental investigation and response to a human plague case, South Lake Tahoe, 2020

Bryan T. Jackson,*[1] Mark Novak,[1] Gregory Hacker,[1] Stefan Sielsch,[2] Vicki Kramer[1]

[1]Vector-Borne Disease Section, California Department of Public Health, Sacramento, CA
[2]El Dorado County Vector Control District, South Lake Tahoe, CA

*Corresponding author: Bryan.Jackson@cdph.ca.gov

In August of 2020, a human plague case associated with exposure in South Lake Tahoe was identified. In response, the California Department of Public Health Vector-Borne Disease Section (VBDS), in collaboration with El Dorado County Vector Control, launched an environmental investigation. Risk assessments were conducted at seven locations visited by the case patient, and evidence of recent plague activity was identified at five locations. Plague prevention signage and other educational efforts were enhanced at these five locations. Based on rodent and flea surveillance, a temporary closure for flea control at Tallac Point, U.S. Forest Service Lake Tahoe Basin Management Unit, was recommended by VBDS. DeltaDust® (0.05% deltamethrin) was applied to rodent burrows over approximately 17 acres and 80 rodent bait-stations treated with Suspend® PolyZone® (0.06% deltamethrin) were placed around the parking area and adjacent hiking trails. A post-treatment assessment demonstrated a significant decrease in flea abundance on rodents in the treatment area, which helped to decrease human plague risk.

Surveillance for mosquito-borne encephalitis virus activity in California, 2020

Tina Feiszli[1]*, Kerry Padgett[1], Robert E. Snyder[2], Leslie Foss[1], Ying Fang[3], Jody Simpson[3], Christopher M. Barker[3], Sharon Messenger[1], and Vicki Kramer[2]

[1]California Department of Public Health, Richmond, CA 94804
[2]California Department of Public Health, Sacramento, CA 95899
[3]Davis Arbovirus Research and Training, University of California, Davis, CA, 95616

*Corresponding author: Tina.Feiszli@cdph.ca.gov

Abstract

In 2020, the California surveillance program for mosquito-borne encephalitis virus activity tested humans, horses, dead birds, mosquitoes, and sentinel chickens to detect arbovirus activity. West Nile virus (WNV) activity was reported from 40 out of 58 counties in California, and St. Louis encephalitis virus (SLEV) activity was reported from 9 counties. A total of 263 human WNV infections were reported, and enzootic WNV activity was detected among horses, dead birds, mosquitoes, and sentinel chickens. Six human cases of SLEV disease were identified in four counties, and enzootic SLEV activity was detected in mosquitoes collected from nine counties.

INTRODUCTION

The California Arbovirus Surveillance program is a cooperative effort between the California Department of Public Health (CDPH), the University of California Davis Arbovirus Research and Training (DART) laboratory, the Mosquito and Vector Control Association of California (MVCAC), local mosquito abatement and vector control agencies, county and local public health departments, and physicians and veterinarians throughout California. Additional local, state, and federal agencies collaborated on, and contributed to, the West Nile virus (WNV) component of the arbovirus surveillance program. In 2020, the surveillance program included the following: 1) Diagnostic testing of specimens from human patients who exhibited symptoms compatible with WNV disease as well as blood bank and organ donor screening for WNV infection, 2) Monitoring mosquito abundance and testing mosquitoes for the presence of WNV, St. Louis encephalitis virus (SLEV), western equine encephalitis virus (WEEV), and other arboviruses as appropriate, 3) Serological monitoring of sentinel chickens for WNV, SLEV, and WEEV antibodies, 4) Reporting and testing of dead birds for WNV, 5) Weekly reporting of arbovirus test results to ArboNET, the national arbovirus surveillance system, 6) Weekly reporting of arbovirus activity in the CDPH Arbovirus Surveillance Bulletin and on the California WNV website: www.westnile.ca.gov, and 7) Data management and reporting of non-human data through the CalSurv Gateway, the California arbovirus surveillance system.

HUMAN DISEASE SURVEILLANCE

Serological testing of human specimens for WNV and other arboviruses was conducted by local public health laboratories, commercial laboratories, and the CDPH Viral and Rickettsial Disease Laboratory (VRDL). Laboratories tested for WNV using an IgM enzyme immunoassay (EIA) and/or an IgM immunofluorescence assay (IFA). Specimens with inconclusive results or from counties with enzootic SLEV activity were further tested by plaque reduction neutralization tests (PRNT) by VRDL. Additional WNV infections were identified by nucleic acid amplification tests and serologic assays performed by blood and organ donation centers.

In 2020, a total of 235 symptomatic and 28 asymptomatic infections with WNV were identified (Tables 1 and 3). Of the 235 symptomatic cases, 179 (76%) were classified as West Nile neuroinvasive disease (WNND) (i.e., encephalitis, meningitis, acute flaccid paralysis, or other neurologic dysfunction) and 56 (24%) were classified as non-neuroinvasive disease; 11 (4.7%) cases were fatal. Cases were residents of 26 (45%) counties and 149 (63%) were male. In 2020, WNV incidence in California was 0.59 cases per 100,000 persons. Incidence was highest (6.3 cases per 100,000 persons) in Stanislaus County, whereas Los Angeles County reported the most cases (90, 38% of total) (Figure 1, Table 3). The median age of those with WNND was 61 years (range, 9 to 91 years), and among cases with non-neuroinvasive disease the median age was 60 years (range, 23 to 93 years). The median age of the 11 WNV-associated fatalities was 68 years (range, 37 to 89 years).

Table 1.—West Nile virus activity in California by county, 2020. Humans include asymptomatic infections detected through blood bank and organ donor screening. NT = None tested

County	Humans	Horses	Dead Birds	Mosquito Pools	Sentinel Chickens
Alameda	0	0	8	0	0
Alpine	0	0	1	NT	NT
Amador	0	2	NT	NT	NT
Butte	4	1	4	28	23
Calaveras	0	0	NT	NT	2
Colusa	0	0	NT	NT	0
Contra Costa	4	0	22	13	7
Del Norte	0	0	NT	NT	NT
El Dorado	1	0	0	NT	NT
Fresno	12	0	5	322	NT
Glenn	1	1	NT	3	2
Humboldt	0	0	0	NT	NT
Imperial	1	0	NT	3	NT
Inyo	0	0	NT	0	NT
Kern	10	0	0	83	NT
Kings	2	1	NT	87	NT
Lake	2	0	0	12	2
Lassen	0	0	NT	NT	NT
Los Angeles	98	0	102	437	38
Madera	6	0	NT	77	NT
Marin	0	0	1	0	NT
Mariposa	0	0	NT	NT	NT
Mendocino	0	0	NT	NT	NT
Merced	14	1	2	42	14
Modoc	0	1	NT	NT	NT
Mono	0	0	NT	NT	NT
Monterey	0	0	0	NT	NT
Napa	0	0	1	2	NT
Nevada	0	1	0	NT	0
Orange	19	0	47	326	NT
Placer	2	0	3	58	NT
Plumas	0	0	NT	NT	NT
Riverside	12	2	2	64	NT
Sacramento	9	1	91	115	4
San Benito	0	0	NT	0	1
San Bernardino	4	1	0	13	NT
San Diego	1	0	1	2	NT
San Francisco	0	0	0	0	NT
San Joaquin	4	4	4	260	NT
San Luis Obispo	0	0	0	0	NT
San Mateo	0	0	1	0	0
Santa Barbara	0	0	0	1	0
Santa Clara	1	0	7	8	NT
Santa Cruz	0	0	0	0	0
Shasta	2	0	0	25	3
Sierra	0	0	NT	NT	NT
Siskiyou	0	0	NT	NT	NT
Solano	1	1	3	8	4
Sonoma	0	0	0	0	NT
Stanislaus	38	3	4	351	NT
Sutter	1	0	0	20	26
Tehama	2	0	NT	NT	5
Trinity	0	0	NT	NT	NT
Tulare	7	0	5	189	10
Tuolumne	0	0	0	NT	NT
Ventura	0	0	0	0	1
Yolo	5	0	29	77	1
Yuba	0	0	0	2	1
State Totals	**263**	**20**	**343**	**2,628**	**144**

Table 2.—St. Louis encephalitis virus activity in California by county, 2020. NT = None tested

County	Humans	Mosquito Pools[1]	Sentinel Chickens
Fresno	3	233	NT
Imperial	0	10	NT
Kern	0	31	NT
Kings	0	11	NT
Madera	1	17	NT
Riverside	0	159	NT
San Joaquin	1	2	NT
Stanislaus	1	2	NT
Tulare	0	45	0
Totals	**6**	**510**	**0**

[1] Positive mosquito pools included *Cx. quinquefasciatus* (322 pools), *Cx. tarsalis* (178 pools), *Cx. pipiens* (5 pools), and *Cx. stigmatosoma* (5 pools)

Dates of symptom onset ranged from June 2 to December 21, with the peak occurring during epidemiological week 36 (August 30 - September 5), when 30 (13%) symptomatic infections were reported.

Six symptomatic cases of SLEV infection also were identified in 2020. Five (83%) presented with neuro-invasive disease and one fatality (17%) was reported. Cases were residents of four counties (Table 2) and five (83%) were male. The median age was 75 years (range, 45 to 90 years) and dates of symptom onset ranged from July 20 to October 1.

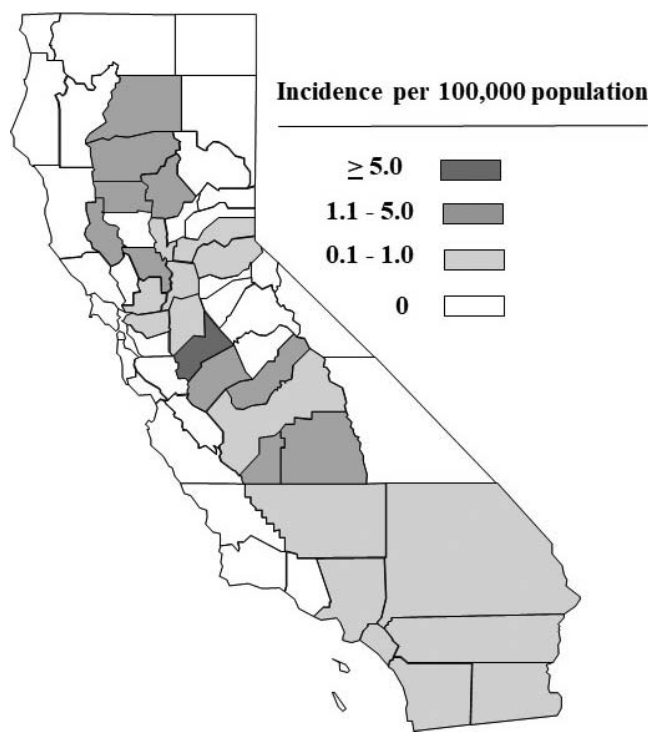


Figure 1.—Incidence of human cases of West Nile virus in California, 2020.

Table 3.—Reported West Nile virus human cases by county of residence and year, California, 2011 – 2020.

County	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2020 incidence per 100,000 person-years	Ten-year incidence per 100,000 person-years
Alameda	0	2	0	1	0	0	1	0	1	0	0.00	0.03
Alpine	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Amador	1	0	0	0	0	1	0	1	1	0	0.00	1.06
Butte	3	10	24	24	53	21	4	12	5	4	1.90	7.61
Calaveras	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Colusa	0	3	2	3	1	2	0	0	1	0	0.00	5.48
Contra Costa	3	4	5	5	1	4	4	4	1	4	0.35	0.30
Del Norte	0	0	0	0	0	0	0	0	0	0	0.00	0.00
El Dorado	1	0	1	0	0	1	0	0	0	1	0.52	0.21
Fresno	9	24	8	43	8	14	13	14	51	10	0.98	1.90
Glenn	1	7	9	10	19	6	0	2	0	1	3.40	18.71
Humboldt	0	0	0	0	0	0	0	1	0	0	0.00	0.08
Imperial	0	1	0	1	1	0	3	0	3	1	0.53	0.53
Inyo	0	0	0	0	0	0	4	0	0	0	0.00	2.15
Kern	18	25	25	11	11	17	30	13	28	8	0.87	2.03
Kings	1	3	1	4	0	8	5	0	3	2	1.30	1.76
Lake	0	1	0	1	2	1	0	1	0	2	3.12	1.25
Lassen	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Los Angeles	58	163	151	253	286	151	277	43	31	90	0.88	1.48
Madera	2	3	3	3	4	6	2	4	3	6	3.79	2.28
Marin	0	0	2	0	1	0	0	0	0	0	0.00	0.12
Mariposa	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Mendocino	0	0	0	1	2	0	0	0	0	0	0.00	0.34
Merced	1	13	0	1	1	0	10	2	10	12	4.23	1.76
Modoc	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Mono	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Monterey	0	1	0	0	0	1	0	1	0	0	0.00	0.07
Napa	0	0	1	0	0	0	0	1	0	0	0.00	0.14
Nevada	0	0	0	0	2	0	0	1	0	0	0.00	0.31
Orange	10	42	10	263	92	32	33	9	5	17	0.53	1.61
Placer	1	12	6	7	0	7	0	9	1	2	0.50	1.11
Plumas	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Riverside	7	19	35	14	127	11	32	15	12	10	0.41	1.15
Sacramento	4	29	11	10	4	25	6	15	4	7	0.45	0.74
San Benito	0	0	0	0	0	0	0	0	0	0	0.00	0.00
San Bernardino	4	33	13	21	54	8	57	9	7	3	0.14	0.96
San Diego	0	1	0	11	42	20	2	2	3	1	0.03	0.25
San Francisco	0	1	1	0	0	0	1	0	0	0	0.00	0.03
San Joaquin	5	13	8	9	2	13	14	14	7	2	0.26	1.12
San Luis Obispo	0	0	0	0	0	0	0	0	2	0	0.00	0.07
San Mateo	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Santa Barbara	1	0	1	0	0	0	0	0	0	0	0.00	0.04
Santa Clara	1	0	2	10	8	1	0	1	1	0	0.00	0.12
Santa Cruz	1	0	0	0	0	0	0	0	0	0	0.00	0.04
Shasta	0	1	1	2	3	1	1	1	0	2	1.12	0.67
Sierra	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Siskiyou	0	0	0	0	1	0	0	0	0	0	0.00	0.22
Solano	0	2	1	5	1	4	1	0	1	1	0.23	0.36
Sonoma	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Stanislaus	11	26	17	33	13	26	28	15	16	35	6.28	3.94
Sutter	0	8	10	8	2	12	3	1	1	1	0.99	4.57
Tehama	1	4	5	4	5	5	2	2	0	2	3.07	4.61
Trinity	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Tulare	11	7	5	21	13	10	12	8	24	7	1.46	2.46
Tuolumne	0	0	0	0	0	0	0	1	0	0	0.00	0.18
Ventura	0	7	2	1	6	7	1	2	2	0	0.00	0.33
Yolo	0	10	6	15	8	16	6	11	1	4	1.80	3.47
Yuba	3	4	13	6	10	11	1	2	0	0	0.00	6.34
Total WNV Cases	**158**	**479**	**379**	**801**	**783**	**442**	**553**	**217**	**225**	**235**	**0.59**	**1.07**
Asymptomatic Infections	18	48	54	91	77	41	47	26	18	28		
Total WNV infections	**176**	**527**	**433**	**892**	**860**	**483**	**600**	**243**	**243**	**263**	**0.65**	**1.19**

Table 4.—Results of mosquito and sentinel chicken testing for West Nile virus, California, 2020.

County	No. mosquitoes tested	No. mosquito pools tested	WNV + pools	No. flocks	No. chickens	No. WNV positive flocks	WNV + sera
Alameda	173	63	0	3	21	0	0
Butte	21,870	464	28	7	42	7	23
Calaveras	0			1	10	1	2
Colusa	0			1	10	0	0
Contra Costa	14,288	471	13	4	24	3	7
Fresno	86,440	2,602	322	0			
Glenn	812	17	3	1	6	1	2
Imperial	1,932	135	3	0			
Inyo	845	19	0	0			
Kern	30,816	775	83	0			
Kings	20,071	628	87	0			
Lake	12,195	580	12	2	12	1	2
Los Angeles	142,326	3,968	437	28	197	12	38
Madera	14,520	467	77	0			
Marin	632	42	0	0			
Merced	20,416	756	42	8	48	6	14
Napa	5,366	199	2	0			
Nevada	0			2	12	0	0
Orange	147,797	5,084	326	0			
Placer	35,854	2,022	58	0			
Riverside	194,849	5,640	64	0			
Sacramento	68,426	4,799	115	3	15	1	4
San Benito	464	28	0	1	8	1	1
San Bernardino	64,646	3,142	13	0			
San Diego	15,036	1,694	2	0			
San Francisco	156	15	0	0			
San Joaquin	83,747	2,664	260	0			
San Luis Obispo	4,950	108	0	0			
San Mateo	2,539	274	0	2	14	0	0
Santa Barbara	2,275	121	1	4	26	0	0
Santa Clara	21,609	1,937	8	0			
Santa Cruz	1,100	59	0	2	12	0	0
Shasta	21,870	690	25	6	40	2	3
Solano	16,029	530	8	3	21	1	4
Sonoma	9,159	346	0	0			
Stanislaus	81,834	2,235	351	0			
Sutter	9,200	257	20	5	35	5	26
Tehama	0			3	30	2	5
Tulare	102,401	3,114	189	1	10	1	10
Ventura	1,685	36	0	5	53	1	1
Yolo	47,901	2,167	77	2	10	1	1
Yuba	6,760	184	2	1	7	1	1
Total	**1,312,989**	**48,332**	**2,628**	**95**	**663**	**47**	**144**

MOSQUITO SURVEILLANCE

In 2020, mosquito testing was performed at DART and 12 local mosquito and vector control agencies. A total of 1,312,989 mosquitoes (48,332 pools) collected in 38 counties were tested by a real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) for SLEV, WEEV, and/or WNV viral RNA (Table 4). *Aedes aegypti* and *Ae. albopictus* mosquitoes also were tested for chikungunya, dengue, and Zika viruses at DART by a separate RT-qPCR.

West Nile virus was detected in 2,628 mosquito pools from 28 counties (Tables 1 and 4), and SLEV was detected in 510 mosquito pools from 9 counties (Table 2). Statewide, the annual minimum infection rate (MIR-defined as the minimum number of infected female mosquitoes per 1,000 tested) of WNV in all mosquitoes tested was 2.0. During California's peak transmission period (July – September) the statewide MIR in *Culex* mosquitoes was 3.5 and nine counties reported MIRs greater than 5.0, the epidemic threshold value (Figures 2 and 5) (California Department of Public Health, 2020).

West Nile virus was detected in pools from five different *Culex* species (*Cx. erythrothorax*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. stigmatosoma*, and *Cx. tarsalis)* (Table 5) and positive pools were collected from May 15 to November 13, with the peak occurring during epidemiological week 35 (August 23 – August 29). St. Louis encephalitis virus also was detected in pools from four *Culex* species (*Cx. pipiens*, *Cx. quinquefasciatus*, *Cx.*

Table 5.—Mosquito species tested for West Nile virus, California, 2020.

Culex species	No. Pools	No. mosquitoes	WNV +	MIR
Cx. erythrothorax	1,479	55,949	7	0.1
Cx. pipiens	9,664	180,857	462	2.6
Cx. quinquefasciatus	18,658	567,183	1,320	2.3
Cx. stigmatosoma	1,185	13,907	13	0.9
Cx. tarsalis	16,329	486,394	826	1.7
Cx. thriambus	75	149	0	0.0
Other *Culex*	3	18	0	0.0
All *Culex*	**47,393**	**1,304,457**	**2,628**	**2.0**
Anopheles species	Pools	No. mosquitoes	WNV +	MIR
An. franciscanus	14	110	0	0.0
An. freeborni	1	37	0	0.0
An. hermsi	5	96	0	0.0
All *Anopheles*	**20**	**243**	**0**	**0.0**
Aedes species	Pools	No. mosquitoes	WNV +	MIR
Ae. aegypti	503	2,272	0	0.0
Ae. albopictus	3	58	0	0.0
Ae. melanimon	7	62	0	0.0
Ae. nigromaculis	1	7	0	0.0
Ae. sierrensis	1	3	0	0.0
Ae. taeniorhynchus	1	30	0	0.0
Ae. washinoi	2	100	0	0.0
All *Aedes*	**518**	**2,532**	**0**	**0.0**
Other species	Pools	No. mosquitoes	WNV +	MIR
Culiseta incidens	322	4,362	0	0.0
Culiseta inornata	32	234	0	0.0
Culiseta melanura	1	7	0	0.0
Culiseta particeps	32	1,029	0	0.0
Unknown	14	125	0	0.0
All other	**401**	**5,757**	**0**	**0.0**

stigmatosoma, and *Cx. tarsalis)* collected from May 19 to October 29.

A total of 14,066 *Aedes aegypti* and 128 *Ae. albopictus* were tested for chikungunya, dengue, and Zika viruses; all were negative.

CHICKEN SEROSURVEILLANCE

In 2020, 26 local mosquito and vector control agencies in 23 counties maintained 95 sentinel chicken flocks (Table 4). Blood samples were collected from chickens every other week and tested for antibodies to WNV, SLEV, and WEEV by an EIA at the CDPH Vector-Borne Disease Section Laboratory and one local agency. Presumptive positive samples were confirmed by IFA or western blot. Samples with inconclusive results were tested by PRNT at the VRDL.

Of 6,302 chicken blood samples tested, 144 seroconversions to WNV were detected among 47 (49%) flocks in 17 counties (Tables 1 and 4). Seroconversions to WNV occurred between July 2 and November 12, with the peak occurring during epidemiological weeks 36-37 (August 30 – September 12). No SLEV or WEEV seroconversions were detected in 2020.

DEAD BIRD SURVEILLANCE

In 2020, the WNV Dead Bird Call Center and website received a total of 5,850 dead bird reports from the public from 50 counties (Table 6). Oral swabs or tissue samples from dead bird carcasses were tested at DART or at one of 12 local agencies by RT-qPCR. Of the 1,685 bird carcasses that were deemed suitable for testing, WNV was detected in 343 (20%) carcasses from 21 counties (Tables 1 and 6). Twenty-one different bird species tested positive for WNV: 60% were American crows, 18% were California scrub-jays, 9% were other corvids, and 13% were non-corvid species. Positive birds were detected from February 3 to December 14, with the peak occurring during epidemiological week 35 (August 23 – August 29).

HORSES

Serum or brain tissue specimens from horses displaying neurological symptoms were tested for WNV at the California Animal Health and Food Safety Laboratory. In 2020, WNV infection was confirmed in 20 horses from 13 counties (Table 1). Six (30%) of the horses died or were euthanized.

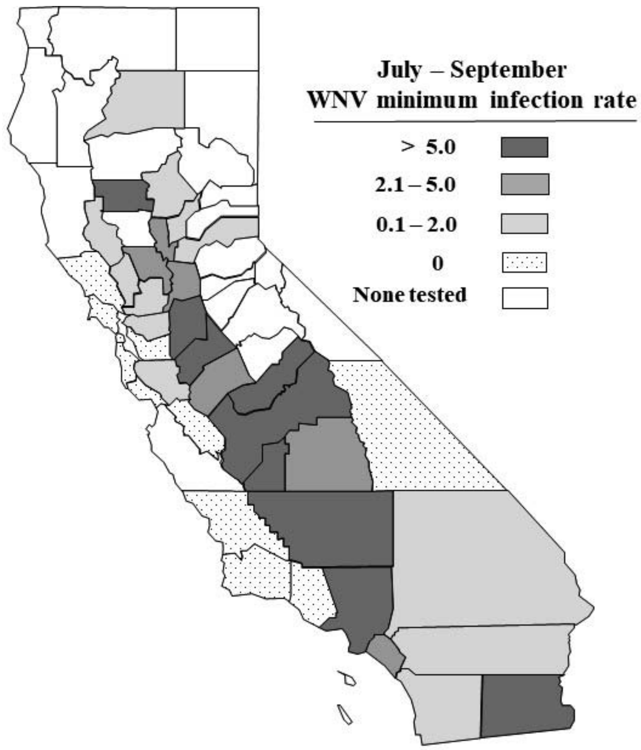


Figure 2.—West Nile virus minimum infection rate in *Culex* mosquitoes, by county, California, July – September, 2020. Minimum infection rate is defined as the minimum number of infected female mosquitoes per 1,000 tested.

DISCUSSION

In 2020, 40 (69%) out of 58 counties reported WNV activity. A total of 235 human cases were reported from 26 counties, which was among the lowest number of cases reported in California within the last 10 years (Figure 3, Table 3). Los Angeles County reported the most cases (N=90), but the incidence was highest in Stanislaus County, which reported more cases in 2020 (N=35) than in any other year since 2005 (Table 3; California Department of

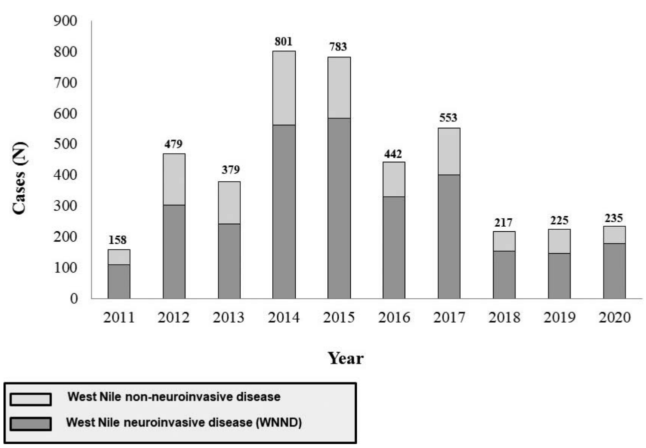


Figure 3.—Human cases of West Nile virus in California, by year, 2011 – 2020.

Table 6.—Dead birds reported, tested, and positive for West Nile virus, California, 2020.

County	Reported	Tested	Positive	Percent
Alameda	378	117	8	6.8
Alpine	2	2	1	50.0
Amador	4	0		
Butte	48	12	4	33.3
Calaveras	1	0		
Colusa	1	0		
Contra Costa	501	61	22	36.1
Del Norte	0			
El Dorado	31	13	0	0.0
Fresno	194	16	5	31.3
Glenn	1	0		
Humboldt	7	2	0	0.0
Imperial	1	0		
Inyo	0			
Kern	9	1	0	0.0
Kings	9	0		
Lake	12	7	0	0.0
Lassen	0			
Los Angeles	863	200	102	51.0
Madera	16	0		
Marin	32	3	1	33.3
Mariposa	0			
Mendocino	2	0		
Merced	73	5	2	40.0
Modoc	0			
Mono	1	0		
Monterey	19	4	0	0.0
Napa	13	3	1	33.3
Nevada	24	2	0	0.0
Orange	548	225	47	20.9
Placer	138	57	3	5.3
Plumas	2	0		
Riverside	150	31	2	6.5
Sacramento	709	318	91	28.6
San Benito	2	0		
San Bernardino	67	11	0	0.0
San Diego	197	111	1	0.9
San Francisco	51	6	0	0.0
San Joaquin	159	38	4	10.5
San Luis Obispo	16	2	0	0.0
San Mateo	244	85	1	1.2
Santa Barbara	23	4	0	0.0
Santa Clara	486	152	7	4.6
Santa Cruz	79	18	0	0.0
Shasta	20	1	0	0.0
Sierra	0			
Siskiyou	0			
Solano	83	28	3	10.7
Sonoma	62	7	0	0.0
Stanislaus	178	12	4	33.3
Sutter	37	11	0	0.0
Tehama	5	0		
Trinity	0			
Tulare	54	12	5	41.7
Tuolumne	1	1	0	0.0
Ventura	61	18	0	0.0
Yolo	211	88	29	33.0
Yuba	25	1	0	0.0
Totals	**5,850**	**1,685**	**343**	**20.4**

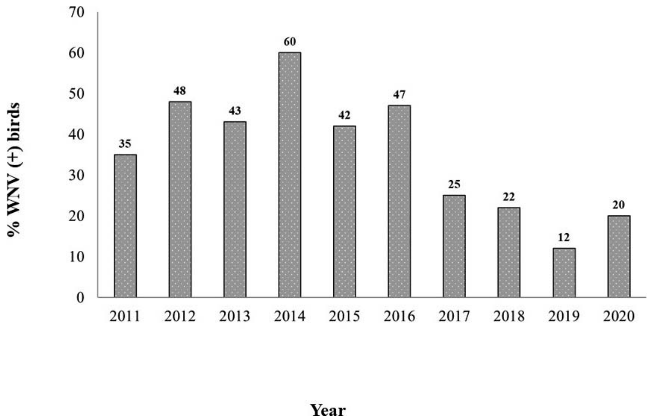


Figure 4.—Percentage of dead birds positive for West Nile virus in California, 2011 – 2020.

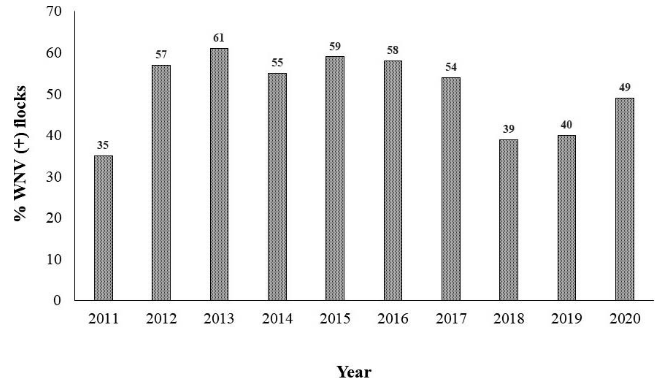


Figure 6.—Percentage of sentinel chicken flocks in California with one or more birds positive for antibodies to West Nile virus, 2011–2020.

Public Health). Although enzootic WNV activity was reported from 39 counties, the environmental indicators were also lower compared to previous years (Figures 4, 5 and 6). Nevertheless, the mosquito MIR during July through September still indicated epidemic conditions in Los Angeles County and several Central Valley counties, including Stanislaus County. Similar to previous years, surveillance results documented WNV activity throughout the year, but the vast majority of detections occurred from June through October, with peak activity occurring in late August.

For the sixth consecutive year, SLEV continued to co-circulate with WNV in many areas of the state. Outreach to local health departments was conducted in areas with enzootic detections of SLEV and medical providers were encouraged to include SLEV testing for suspect WNV cases. This resulted in the identification of six human SLEV cases, including one fatality, from four counties. A total of 510 SLEV-positive mosquito pools were reported from 9 counties, including those counties with reported human SLEV cases. No sentinel chickens tested positive for SLEV antibodies in 2020, but sentinel flocks were absent from almost all counties where SLEV was detected in mosquitoes (Table 2).

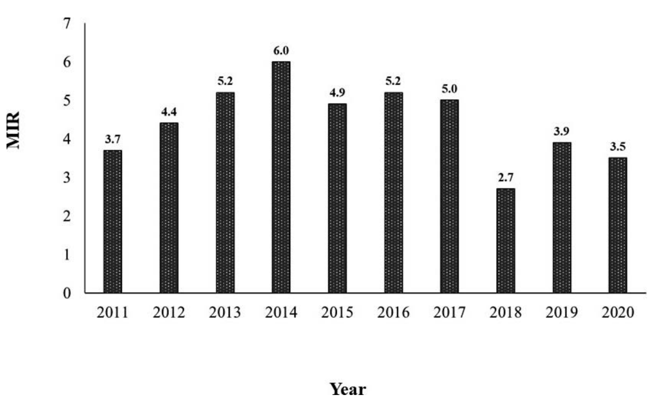


Figure 5.—Minimum infection rate (MIR) in females per 1,000 tested for West Nile virus in *Culex* mosquitoes in California, July – September, 2011–2020.

Although WEEV has not been detected in California since 2007, routine testing of mosquitoes and sentinel chickens for WEEV has continued in the event this historically endemic arbovirus reemerges.

CONCLUSIONS

Although WNV activity was lower in 2020 compared to many previous years, 263 human WNV infections, including 11 fatalities, were still identified, highlighting the public health importance for ongoing surveillance and awareness of potential human disease risk. Six human cases of SLEV disease were also identified in 2020, along with enzootic detections in nine counties. Environmental detections of both viruses often preceded the incidence of human cases, supporting the value of environmental surveillance to direct mosquito control efforts and decrease the risk of arboviral diseases in California.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the cooperation and assistance of the local mosquito and vector control agencies in the collection and submission of samples for testing and their financial support to the testing laboratories and WNV Dead Bird Call Center; the local public health and commercial laboratories which tested clinical samples; the many physicians and veterinarians who submitted specimens from suspect cases of arboviral disease, and the valuable contributions of the staff of MVCAC, DART (especially Sandra Garcia), the California Animal Health and Food Safety Laboratory, and the CDFA Animal Health Branch. From CDPH, we thank the VRDL Branch (especially Phacharee Arunleung, Theresa Brown, Teal Bullick, Lyndsey Chaille, Mojgan Deldari, Kim Hansard, Carl Hanson, Kristina Hsieh, Maria Liu, Ruth Lopez, Dominick Morales, Leo Oceguera, Peter Patiris, Chris Preas, Maria Salas, Pat Stoll, and Shigeo Yagi), the Veterinary Public Health Section (especially Curtis Fritz), the Infectious Diseases Branch (especially Allyx Nicolici), and VBDS (especially Ervic Aquino, Arielle Crews, Mary Beth Danforth, Margaret Kerrigan, Mary Joyce Pakingan, and the WNV Call Center staff).

This study was supported in part by the Epidemiology and Laboratory Capacity for Infectious Diseases Cooperative Agreement number 5 NU50CK000539-02-00 from the US Centers for Disease Control and Prevention.

REFERENCES CITED

California Department of Public Health. 2020. California Mosquito-Borne Virus Surveillance and Response Plan. http://westnile.ca.gov/resources.php

California Department of Public Health. California West Nile Virus Website. http://westnile.ca.gov/reports.php

West Nile virus-associated hospitalizations and costs, California, USA, 2004–2017

Robert Snyder*, Vicki Kramer, and Duc Vugia

Infectious Diseases Branch, Division of Communicable Disease Control, California Department of Public Health, Sacramento and Richmond, CA

*Corresponding author: Robert.Snyder@cdph.ca.gov

West Nile virus (WNV) is the most commonly reported mosquito-borne disease in the United States, and California reported more WNV disease cases than any other state. We analyzed WNV-associated hospitalizations in California Patient Discharge Data from 2004 through 2017 and described the epidemiology, disease incidence, and hospital charges from these hospitalizations. From 2004 through 2017, 3,109 Californians were hospitalized with WNV disease (median: 214 patients per year, range: 72 – 449 patients). These hospitalizations represented an annual median of 54% of all WNV disease cases that were reported to the CDPH between 2004 and 2017 (range: 25%-67% of cases each year). Hospitalization rates increased with increasing age; the highest annual median hospitalization incidence was in those $\geq$60 years old. Regionally, the highest median annual hospitalization rate was in California's Central Valley, followed by southern California. Most patients were hospitalized with West Nile neuroinvasive disease, and had at least one underlying condition, including hypertension, cardiovascular disease, diabetes, chronic kidney disease, or immunosuppression due to drugs or disease. WNV disease in California has been substantial and costly. Among the 2,726 patients with available charge data, the median inflation-adjusted charge per-patient was $142,321, with a median daily charge of $12,602. Mean annual WNV charge per patient was $59,905,762 / year.

Exploring twenty years of mosquito collection data from the Coachella Valley

Kim Y. Hung*[1], Melissa Snelling[1], Aviva Goldmann[2]

[1]Coachella Valley Mosquito and Vector Control District, Indio, CA
[2]San Gabriel Valley Mosquito and Vector Control District, West Covina, CA

*Corresponding Author: khung@cvmvcd.org

Introduction

The Coachella Valley Mosquito and Vector Control District (District) began storing its trap collection data with VectorSurv (formerly CalSurv) Gateway (https://ca.vectorsurv.org) in 2006, which was when the online database program began. Prior to this, all the collection data was stored in local hard drives and as paper copies in filing cabinets. This meant that there were trap collection datasheets in the cabinets dating as far back as the 1990s that were not in the system. Although the Gateway has been easy to use and convenient for analyses, there was an abundance of data that was missing from the system and could not be analyzed collectively. This abstract provides a brief overview of the mosquito abundance trends observed during the past 20 years.

Methods

In 2020, the rural zone CO_2 trap collections conducted prior to 2006 were added to VectorSurv. Currently, collections going back to the year 1997 have been entered, meaning there are at least 20 years of data to examine. During this period, the trap locations and trap design have not changed since established by Reisen and Lothrop (1999). The CO_2 traps used were CDC-style dry ice-baited traps set without light for one night. Trap collections in Sept 2006 from Duck Clubs and Aug 2000 from North Shore that ranged from 2 to 7 trap-nights were not included. The trap collections were grouped into four areas (Fig. 1): North Shore (trap nos. 54, 56, 57, 58); Agriculture (45, 121, 122, 123, 611), Duck Clubs (17, 33, 35, 48) and West Shore (2, 5, 6, 8). These locations were of interest because of their high mosquito abundance or consistent arbovirus detections. For this study, *Cx. tarsalis* collections were examined. A linear regression analysis was conducted in R (R Core Team 2020) to examine the relationship between mosquito abundance and time in years. The analysis was run in RStudio (RStudio Team 2020). Salton Sea water surface elevation was obtained from the USGS Surface-Water Monthly Statistics for the Salton Sea near Westmorland, CA (U.S. Geological Survey 2021) and local weather information was obtained from the California Department of Water Resources (CIMIS) weather station in Oasis (California Department of Water Resources 2021).

Figure 1.—Black boxes define the four trapping regions in the rural area of Coachella Valley.

Results and Discussion

At Agriculture sites (Fig. 2a), abundance increased over time ($F = 26.4$, $p<0.001$, slope=38.9, R^2=0.56), whereas at Duck Clubs (Fig. 2b) there was an increase of abundance over time (F=16.0, $p<0.001$, slope=12.8, R^2=0.43). At North Shore (Fig. 2c), there was no significant linear increase or decrease over the study period (F=2.04, $p>0.05$, slope=3.8, R^2=0.09). Abundance appeared to

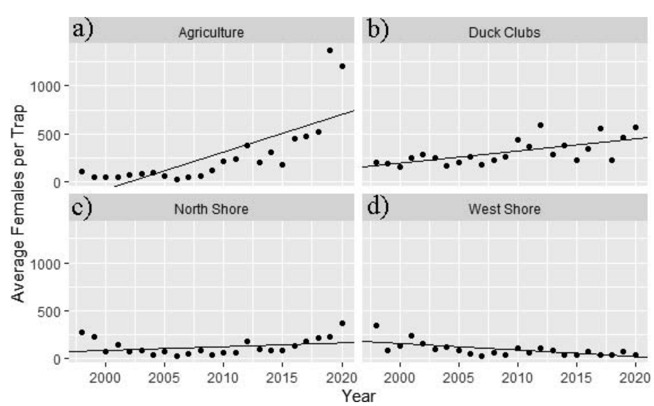


Figure 2.—Average monthly *Cx. tarsalis* abundance per trap night at (a) Agriculture, (b) Duck Clubs, (c) North Shore, and (d) West Shore, with linear regression lines.

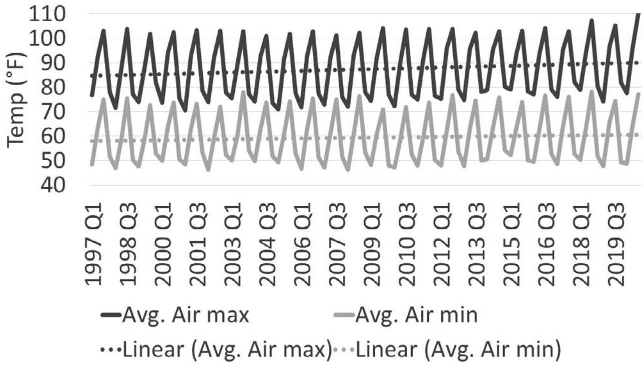


Figure 3.—Quarterly average maximum and minimum air temperatures at Oasis, CA.

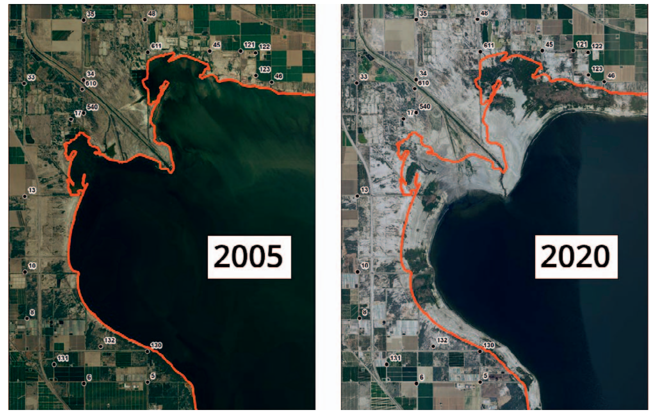


Figure 5.—Comparison of Salton Sea aerial images from 2005 and 2020. The red line traces the 2005 shoreline.

decline from 1997-2004, then to increase after 2010. At West Shore (Fig. 2d), there was a decrease in abundance over time ($F=14.1$, $p<0.01$, slope $=-6.96$, $R^2=0.40$). Because mosquitoes are poikilothermic, rising air temperatures may lead to faster mosquito development and reproduction rates. Examination of the local mean daytime air temperatures (Fig. 3) showed a general increase, which may have contributed to the changes in *Cx. tarsalis* abundance at Agriculture, Duck Clubs, and North Shore (Bachelet et al. 2016). The increase in *Cx. tarsalis* abundance at Agriculture and Duck Clubs, and the apparent increase at North Shore in recent years also may have been attributable to the declining Salton Sea water surface elevation (Fig. 4), caused by evaporation exceeding the inflow of water. With the decline in the water levels, the irrigation channels no longer reach the sea, which has led to water being released onto the dry shoreline, creating large pools of mosquito habitats, particularly around the Agriculture and Duck Clubs areas (Fig. 5). Other factors of consideration included changes in land-use, human population growth, and seasonal precipitation. Including these in future analyses may improve forecasts of virus transmission risk and an evaluation of the District's integrated vector management program.

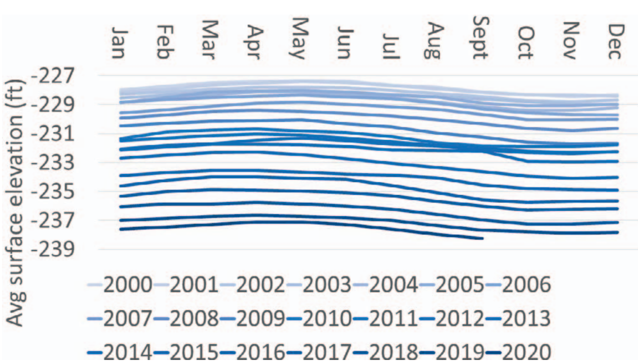


Figure 4.—Monthly average Salton Sea water surface elevation in feet below sea level.

Acknowledgements

Thanks to Samantha Przeklasa from CA Dept. of Fish and Wildlife for providing us the Riverside County base maps from 2005 and 2020. A big thank you to the District Surveillance and Quality Control Staff present and past without whom this program would not exist.

References

Bachelet, D., K. Ferschweiler, T. Sheehan, and J. Strittholt. 2016. Climate change effects on southern California deserts. Journal of Arid Environments. 127: 17–29.

California Department of Water Resources. **2021**. California Irrigation Management Information System (CIMIS). (https://cimis.water.ca.gov/Default.aspx).

R Core Team. **2020**. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reisen, W. K., and H. D. Lothrop. **1999**. Effects of sampling design on the estimation of adult mosquito abundance. JAMCA. 15: 105–114.

RStudio Team. **2020**. RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA.

U.S. Geological Survey. **2021**. Surface-Water Monthly Statistics for California. (https://nwis.waterdata.usgs.gov/ca/nwis/monthly/?search_site_no=10254005&agency_cd=USGS&referred_module=sw&format=sites_selection_links).

An evaluation of the California mosquito-borne virus surveillance and response plan relative to human West Nile virus disease risk

Mary E. Danforth[1], Christopher M. Barker[2], Emma Lonstrup[2], Robert E. Snyder[1], Vicki L. Kramer[1]

[1]California Department of Public Health, Vector-Borne Disease Section, Sacramento CA
[2]University of California, Davis, Davis Arbovirus Research and Training, Davis CA

Corresponding author: Mary E. Danforth, Mary.Danforth@cdph.ca.gov

The California Mosquito-Borne Virus Surveillance and Response Plan is used by local vector control and public health agencies in California to monitor and evaluate West Nile virus (WNV) activity and prompt responses to reduce the burden of WNV disease. The plan includes a formula to estimate the response level and associated human risk of infection based on the abundance and WNV infection rates in *Culex tarsalis* and the *Culex pipiens* complex mosquitoes as well as the numbers of dead birds, seroconversions in sentinel chickens, and ambient air temperature. In most other vector control programs in the US, the vector index is exclusively used to estimate the risk of human WNV transmission. We aggregated the number of reported human cases by mosquito control agency and county (whichever spatial scale was smaller) by three-week periods from 2009 through 2018 and compared these to the calculated response level and the vector index during the prior two weeks using negative binomial regression. The total response levels based on *Cx. tarsalis* and *Cx. pipiens* complex were statistically significantly associated with increased human cases and had a better model fit than the vector index. The current Response Plan is effective at estimating the risk of WNV transmission to humans in California.

Characterizing areas with increased burden of West Nile virus disease in California, 2009-2018

Mary E. Danforth[1], Marc Fischer[2], Robert E. Snyder[1], Nicole P. Lindsey[2], Stacey W. Martin[2], Vicki L. Kramer[1]

[1]California Department of Public Health, Vector-Borne Disease Section, Sacramento CA
[2]Centers for Disease Control and Prevention, Division of Vector-Borne Diseases, Fort Collins CO

Corresponding author: Mary E. Danforth, Mary.Danforth@cdph.ca.gov

---

West Nile virus disease, caused by a mosquito-borne flavivirus, can lead to neurological symptoms and has no treatment or vaccine. From 2009-2018, California reported more human cases than any other state in the US. Our study sought to identify smaller geographic areas within high burden counties that accounted for disproportionately large numbers of human cases from 2009 to 2018. Eleven of those areas, consisting of groups of high burden ZIP codes, were identified in nine counties, all within Southern California and California's Central Valley. These high burden ZIP codes accounted for 2.3% of California's area and 5.8% of the state's population, but 44.3% of cases reported within that time period and had an average incidence 2.5 times the incidence for the state as a whole. These areas should be considered for enhanced mosquito control and targeted public education, currently the only ways to lower the disease burden.

Expanding mosquito surveillance methods to accommodate seasonal fluctuations in native species and to monitor for invasive *Aedes* species

Melissa Doyle*, Jacqueline Cordova, Aviva Goldmann

San Gabriel Valley Mosquito and Vector Control District, 1145 North Azusa Canyon Rd, West Covina, CA 91790

*Corresponding author: mdoyle@sgvmosquito.org

Understanding seasonal fluctuations in mosquito populations is essential for providing effective control. Several insect traps target mosquitoes at specific points in their lifecycle. Traditionally, light traps baited with carbon dioxide placed in static locations monitor fluctuations in adult host-seeking populations. These data inform adult mosquito control decisions providing clear information regarding the efficacy of a treatment. In the San Gabriel Valley, California, light pollution and weather conditions often reduce the success of light traps baited with carbon dioxide forcing vector control agencies to rely on other traps such as the Reiter-Cummings CDC gravid trap to monitor the WNV vector *Culex quinquefasciatus* and the Biogent's Sentinnel 2 (BG) trap to monitor *Aedes* populations. Modifications to the lures of Reiter-Cummings CDC gravid traps and refining the placement methods for oviposition traps can enhance these methods in detecting and monitoring infestations of *Aedes* mosquitoes.

Dirofilaria immitis prevalence in Southern California's invasive *Aedes* and native *Culiseta* species

Zaina Chaban[1], Susanne Kluh[2], Tara Thiemann[1]*

[1]University of the Pacific, 3601 Pacific Ave., Stockton, CA 95211
[2]Greater Los Angeles Vector Control District, 12545 Florence Ave., Santa Fe Springs, CA 90670

*Corresponding author: tthiemann@pacific.edu

Introduction: Dog heartworm caused by the filaria, *Dirofilaria immitis,* is a mosquito-borne disease causing over 100,000 cases in dogs each year in the United States (CAPC 2021). Since 2013, dog heartworm disease cases have increased by approximately 21% in the United States as reported by the American Heartworm Society (AHS, 2019), and in Los Angeles County (County of Los Angeles Public Health, 2019). Increased disease reports are commonly attributed to an increase in the local vector population, and invasive mosquitoes belonging to the genus *Aedes* were introduced around this time The purpose of our research was to determine the prevalence of *Dirofilaria immitis*, the parasite that causes dog heartworm, in invasive *Aedes aegypti* and *Aedes albopictus* as well as in native *Culiseta incidens*.

Methods: The Greater Los Angeles County Vector Control District (GLACVCD) collected and pooled mosquitoes separated by species and location collected. *Aedes* pools were sent to the University of California at Davis where whole mosquitoes were homogenized for viral testing prior to testing for *D. immitis*. *Culiseta incidens* mosquitoes were sent directly to the Thiemann laboratory at the University of the Pacific, where only heads and thoraces were tested to target infective L3 larvae. All samples were tested with a *D. immitis*-specific primer: 5s-sp (U.S. Patent No.: 6,268,153 Bl, Huang et al. 2013). PCR products were visualized on an agarose gel, and all positives were confirmed by DNA sequencing.

Results and Discussion: *Dirofilaria immitis* was detected in all three mosquito species tested. Of the 403 pools tested, 14 *D. immitis* positive pools were detected across 12 cities. *Aedes aegypti* was sampled most frequently (n=4017 collected) with 8 positive pools, *Ae. albopictus* was least sampled (n=233) with 1 positive pool, and 5 *Cs. incidens* (n=1740) pools positive for *D. immitis* were detected. All *Ae. aegypti* positive pools were found between August-October of 2018 although more mosquitoes were collected in 2019. All *Cs. incidens* pools were collected between April and June of 2019, with all infected pools detected in June of 2019. Frequent *D. immitis* detections in wild caught *Ae. aegypti* and *Cs. incidens* within a relatively short period of time and in a small geographic region implies local *D. immitis* transmission and the potential for both species to serve as vectors in Los Angeles County. Further research should determine their role as a vector with respect to abundance and geographical distribution.

Additionally, this study demonstrated that different genera, species size and potentially different pool sizes may impact the detection of *D. immitis*. Dilutions of DNA template and modifications to PCR protocols should be considered based on species and overall DNA concentration present.

Acknowledgements

Thank you to The Greater Los Angeles County Vector Control District for collecting and sending the samples required to complete this project and for their support.

Reference List

AHS (American Heartworm Society). 2019. AHS Survey Finds Increase in Heartworm Cases. https://www.heartwormsociety.org/veterinary-resources/veterinary-education/ahs-board-speaks-out/368-ahs-survey-finds-increasein-heartworm-cases. Sept. 26, 2019.

CAPC (Companion Animal Parasite Council). 2021. Parasite Prevalence Maps. https://capcvet.org/maps#/2017/all-year/heartworm-canine/dog/united-states. May 1, 2021.

County of Los Angeles Public Health. 2019. Heartworm Disease in Los Angeles County. http://publichealth.lacounty.gov/vet/heartworm.htm. March 20, 2019.

Huang, S., D. J. Smith, G. Molaei, T. G. Andreadis, S. E. Larsen, and E. F. Lucchesi. 2013. Prevalence of *Dirofilaria immitis* (Spirurida: Onchocercidae) infection in *Aedes, Culex,* and *Culiseta* mosquitoes from north San Joaquin Valley, CA. J Med Entomol. 50: 1315-1323.

Public outreach in the era of Covid-19

Luz Maria Robles

Sacramento-Yolo Mosquito and Vector Control District, 8631 Bond Rd., Elk Grove, CA 95624

Corresponding author: lrobles@fightthebite.net

Introduction

In 2020, the novel Covid-19 virus drastically changed public outreach efforts and District operations. It resulted in constant change, adjustments and ongoing challenges as everyone quickly adapted to the many recommendations set forth by health and government officials. This presentation provides an overview of the changes that had to be implemented due to Covid-19 and how the Sacramento-Yolo Mosquito and Vector Control District (District) moved forward with public outreach and education efforts despite the pandemic.

Methods

There were many expectations for 2020: it was a new year, a new decade and many exciting projects were planned to be completed. Specifically for our District, 2020 was going to be a year where we would continue with extensive outreach efforts on invasive mosquitoes, because they had been detected the previous year. In addition, we were excited about the expansion of our school program to include interactive assemblies to engage children, and we had new community events planned to continue our education efforts around mosquitoes, West Nile virus and invasive mosquitoes. However, all of our plans quickly came to a halt with the introduction of Covid-19. Specifically in March 2020, life dramatically changed as a result of a shelter in place mandate issued by California Governor, Gavin Newsom. Despite the health recommendations and a "new normal", we had to continue with our public outreach efforts. While our District had to close its doors to the public, we produced video detailing service modifications and District operation changes for the public around the services being offered. We also re-affirmed our commitment to continue protecting the public health during a time of stress and high uncertainty. The video was posted on our website and disseminated on social media. Other strategies to continue our outreach and education efforts during the pandemic included Zoom media interviews and community presentations to service organizations such as rotary clubs and neighborhood associations. District updates to local elected officials during city council meetings were also done via Zoom and by submitting written reports. A key part of our outreach efforts included enhancing our social media efforts as a way to relay information to the public. Social media became even more important as invasive mosquitoes were detected in areas of Sacramento and Yolo counties. We partnered with local media outlets to launch a digital and print advertising campaign along with utilizing other advertising elements such as radio/television spots, digital ads, and billboards to disseminate our messages.

Because all in person community events were cancelled due to the pandemic, our District field technicians took on the enhanced role of public educators. They became star ambassadors and public outreach experts. Every time they conducted a home service inspection or had interaction with the public, they took extra time to educate and provide information. In addition, they also gave out District bags that contained repellent packets, brochures and other information that would normally be given out at community events or presentations. Because our office and members of the public were not able to pick up repellent wipes, our staff delivered boxes of wipes to senior centers, parks/recreation departments, and agencies that serve the homeless.

Results and Discussion

The implementation of new public outreach strategies was useful in ensuring residents continued to receive mosquito prevention information amid the Covid-19 pandemic. Although the outreach efforts looked different and had to be adjusted to comply with the coronavirus public health restrictions of social distancing and wearing a mask, they were successful and carried out in the best way possible. Some of the challenges that the District faced included finding ways to connect with the public because traditional techniques (events, presentations, etc.) were not available. While social media was a tremendous help to disseminate information, we realized that not everyone utilizes social media platforms, therefore our reach was limited. Lastly, even though District staff did a tremendous job with public outreach and it allowed for great contact into the community, it added another element to their daily workload.

Conclusion

Despite having to significantly change our District operations due to Covid-19, we were able to adapt public outreach strategies to successfully reach residents and continue educating about the importance of mosquito prevention. At the same time we were able to uphold our commitment to protect the public health and welfare of residents in Sacramento and Yolo counties. Moving forward the District will continue implementing similar outreach efforts while still abiding by all Covid-19 public health recommendations. Use of social media, Zoom presentations and advertising/media campaigns will continue to be used to reach the public until traditional outreach opportunities resume.

Acknowledgements

The Sacramento-Yolo Mosquito and Vector Control District staff for their assistance in helping to ensure public outreach continued despite Covid-19.

Enhancing emergency preparedness: Activating PIO/ communication groups to help spread the mosquito control message

Mary-Joy Coburn*, **Anais Medina Diaz, Caroline Gongora, Diana Gutierrez, Helen Kuan**

Greater Los Angeles County Vector Control District 12545 Florence Ave. Santa Fe Springs, CA 90670

*Corresponding author: mjcoburn@glacvcd.org

Introduction

Establishing relationships and collaboration among communicators from agencies is important for protecting public health and promoting community awareness. In addition to collaborating with other vector control districts in the state, the Greater Los Angeles County Vector Control District (GLACVCD/District) formed a county-wide public information officials/communications group to establish relationships and coordination with local government agencies. These joint efforts can provide outcomes for partner agencies that may not have been accomplished individually, because services and programs can be coordinated and resources can be pooled. These outcomes can include enhancing advocacy and resource development, creating more recognition and visibility for vector control, and granting additional opportunities for new projects. Our paper will discuss the process of forming and activating a local communications group, and how to leverage these relationships to increase public awareness about mosquito control.

Greater Los Angeles County Vector Control District is one of the five Vector Control Districts in Los Angeles County and provides service for six million residents. It is comprised of up of 88 Cities plus Unincorporated Areas, with over ten million residents. The County alone is comprised of 34 Departments, plus related agencies and approximately 200 Committees and Commissions. In total, there are over 500 political Districts and Sanitation Boards that have varying responsibilities and constituencies within the county.

Methods

GLACVCD formed the Greater Los Angeles County Public Information Officials Group (LAPIO) because there was a need for collaboration among the District and the over 500 other public agencies in LA County. Forming the group required considerable coordination, but it also allowed ample opportunities to engage with other leaders, collaborate with other agencies, and establish relationships that did not formerly exist (Figure 1).

Establishing relationships and collaboration among communicators from other agencies is important for protecting public health and promoting community awareness. Forming a local Public Information Officials Group consists of the following:

#1. Contact the California Association of Public Information Officials, or a similar organization that may already have a comprehensive list of Communicators in the area. The list will need to be thoroughly reviewed, narrowed, and updated.

#2. Form a Planning Committee to assist with collaboration such as meeting planning (determining agenda and topics, identifying speakers, coordinating locations and refreshments, etc.), and advertising the event.

#3. Identify qualified speakers to keep the group engaged, interested, and beneficial to attendees. In addition, the Committee will need to plan for a keynote or panel speaker(s), whether or not to have a live question and answer forum, or to have breakout rooms for networking opportunities and discussions (Figure 2).

Figure 1.—Meeting Invitation

Figure 2.—Meeting flyer with names of speakers

#4. Conduct surveys and polls to gather feed-back and help improve future meetings.
#5. Send a recap video highlighting the main points from the meeting, spotlighting an agency, and sharing activities and events from other agencies.

Results and Discussion

When people think of a government agency, mosquito control doesn't always top the list. The challenge is how to make vector control known not just to the public, but to other public agencies so that they can help share the mosquito control message, or include the District in times of crises. As FEMA and the FCC states, "while we don't have control over when or where the next disaster will strike, we do have control over what we do to prepare."

As part of emergency preparedness, it is crucial to maintain a list of emergency phone numbers of all the various agencies in the County. It's also helpful to have the names and direct lines to agency representatives and to share our name on their lines of communication. The goal is to introduce vector control to the various agencies in the county so that they become familiar with our agency and our services, BEFORE times of crises.

With multiple opportunities to highlight activities and events for all agencies, vector control has been featured as a panelist in various relevant discussions, highlighted on the eNewsletter distributed to the membership, and has shared information and video previews of upcoming events and campaigns such as California Mosquito Week, Mosquito Moment, as well as the upcoming summer campaign and the Mosquito Watch Program.

Having acquired an extensive list of the various public agencies and contact persons, we have helped our Operations crew in finding proper contacts for their projects. For example, we were able to assist our Underground Storm Drain supervisor find the correct Public Works Division to assist with unearthing maintenance hole covers.

The first official LAPIO meeting held in the Fall of 2020 had 71 attendees and focused on emergency preparedness. GLACVCD was introduced as the group founder with an opportunity to share information about vector control and the five vector control agencies in Los Angeles County. There have been two additional quarterly meetings and attendance has steadily included 50-70 communicators. With a global pandemic, meetings have been conducted virtually with topics on digital trends and social media best practices.

Conclusion

The LAPIO Group was formed with the hope to create more recognition and visibility for vector control that would lead to additional opportunities for new projects. We have made connections and presentations from referrals from the membership. We have high hopes for this group, and luckily many of the members are interested in keeping the momentum going. We will be meeting quarterly and Vector Control will continue to share campaigns and relevant information. We are hopeful that joint efforts can provide outcomes for partner agencies that may not have been accomplished individually, as services and programs can be coordinated and resources can be pooled. The LAPIO Group shows that there are opportunities for partnerships between vector control and other agencies into the future.

A neighborhood approach to mosquito control: Mosquito Watch

Caroline Gongora*, Mary-Joy Coburn, Diana Gutierrez, Helen Kuan, Liliana Moreno, Anais Medina Diaz

Greater Los Angeles County Vector Control District
12545 Florence Ave.
Santa Fe Springs, CA 90670

*Corresponding author: cgongora@glacvcd.org

Abstract

With nearly six million residents and established invasive *Aedes* populations spreading to new areas each year, it has been increasingly challenging for inspectors in the Greater Los Angeles County Vector Control District (GLACVCD) to conduct individual property inspections during the mosquito season. As a collaborative effort between the Operations, Surveillance, and Community Affairs departments, Mosquito Watch, a neighborhood mosquito control program, was launched as part of the year 2020 'Tip, Toss, Take Action' summer campaign. The current presentation will discuss how a neighborhood approach to mosquito control can help alleviate the need for the inspection of individual properties in the same neighborhood. The Mosquito Watch program worked with cities and partnered with homeowner associations and neighborhood watch groups across the County to provide guidance in mosquito reduction and disease prevention. Tool kits, informational packets, and social media kits were provided to mobilize neighborhoods to conduct their own backyard inspections. In addition, another tool kit with resources was provided to cities and local organizations to help increase mosquito control awareness to their respective residents. A campaign website and tool kits with readily available materials facilitated the sharing of GLACVCD messaging to cities and local partners. As a result of this collaborative effort, Mosquito Watch helped alleviate the need for inspectors to conduct door-to-door outreach and property inspections in neighborhoods with high *Aedes* biting pressure.

INTRODUCTION

The Greater Los Angeles County Vector Control District (GLACVCD/District) is a public health agency and the largest of the five vector control districts serving Los Angeles County. Since 1952, GLACVCD has been protecting the Greater LA area from the world's deadliest animal- mosquitoes. These tiny, yet potentially deadly mosquitoes have become increasingly challenging to control since the introduction of invasive *Aedes* mosquitoes in 2011. The *Aedes* mosquitoes not only pose a threat to public health, but they are changing the way of life for millions of Los Angeles residents with their aggressive day-time biting behavior. As a result, GLACVCD has experienced a high influx of service requests (SRs) from residents across its service area requesting individual/neighborhood property inspections.

With nearly six million residents and established invasive *Aedes* populations spreading to new areas each year, it was challenging for GLACVCD Operations staff to conduct door-to-door outreach and individual property inspections during the mosquito season. Mosquito Watch launched as part of the year 2020 'Tip, Toss, Take Action' summer campaign with a neighborhood approach to mosquito control and with the goal for residents, cities, and community organizations to take action against mosquitoes and to prevent the spread of mosquito-borne diseases in their own home and community.

METHODS

After receiving countless SRs and questions from the public at community events, presentations, and social media, it was determined that residents needed simple, long-term mosquito solutions. Therefore, a resource website, www.TipTossTakeAction.org, was created with digital resources and tools for residents and cities/agencies. The website acted as a landing page where the District directed individuals to find all the resources to conduct their own property inspection. In addition, it included a list of ways cities/agencies could partner with the District. The resources and tools featured on the website included (Figure 1):

- **Tip, Toss, Take Action pledge:** residents, organizations, and public officials make a commitment to take action against mosquitoes
- **Mosquito Watch:** a mosquito control program for neighborhoods

Figure 1.—The resources and tools that were part of the year 2020 'Tip, Toss, Take Action' summer campaign's website. The website was a landing page where residents and cities/agencies could find mosquito solutions and learn how they could partner with GLACVCD.

- **Advocate tool kit:** included general multi-lingual information regarding mosquitoes, mosquito-borne diseases, insect repellent, and breeding sources
- **City/Agency tool kit:** included a list of tools that cities/agencies could use to partner with GLACVCD to raise awareness about mosquitoes and mosquito control. Some of the tools included were virtual presentations, a social media tool kit, and the Take Action public service announcement (PSA) videos
- **Take Action PSAs:** videos to promote the summer campaign and Mosquito Watch
- **Mosquito News:** latest mosquito news

Along with the summer campaign, 'Mosquito Watch' was also launched. The program consisted of four steps: Take the Pledge, Advocate, Mobilizer, and Community Champion, with associated tasks to move forward to the next step (Figure 2). The first step was to take a pledge to take action against mosquitoes. Participants were asked to fill out an online form with their name, email address, and zip code. After taking the pledge on our website, participants became Advocates. As Advocates, they used the Advocate tool kit to conduct their own property inspection. They became Mobilizers once they conducted their property inspection. As Mobilizers, they were encouraged to educate and mobilize their neighbors to also conduct inspections on their respective properties. Participants needed to submit a photo/video of them conducting their home inspection to become a Mobilizer and share MW packets/MW social media posts to their neighbors to become a Community Champion. In the last step, participants scheduled a presentation with a Community Liaison and invited their neighbors to learn how they could partner with GLACVCD to reduce mosquitoes as a community. Mailchimp and Google sheets were used to track the pledges and participants.

RESULTS & DISCUSSION

Despite the COVID-19 pandemic, residents, cities, local organizations, agencies, and local officials engaged with

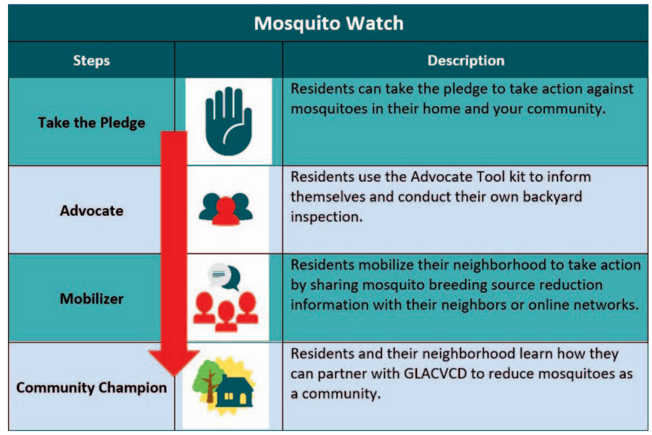


Figure 2.—Mosquito Watch is a mosquito control program for neighborhoods. The program consists of the Take the Pledge, Advocate, Mobilizer, and Community Champion steps. Participants moved forward in the program by completing activities specific to each step.

the summer campaign and Mosquito Watch in various ways. The resource website had a total of 11,567 views between June and December 2020 and the tool kit pages had over 800 views. In addition, 303 residents and public officials across LA County pledged to take action against mosquitoes (Figure 3). This suggests that people did engage with our website and resources.

Social media was used to promote the campaign and engage with residents and stakeholders. Our content was shared 221 times by cities, public officials, and organizations on their social media platforms, websites, newsletters, etc. The readily available online materials allowed cities and organizations to promote awareness and encourage their constituents to protect their family from mosquito-borne diseases. In addition, GLACVCD partnered with cities and organizations to preview the summer campaign and Mosquito Watch PSAs at virtual community events and drive-in movie series. At one drive-in movie series, it was estimated that 480 people saw the PSAs.

Despite the 303 pledges and promoting Mosquito Watch on social media, the program had zero participants advance to the other steps. This may have been a result of the inability to promote the program at in-person community events and presentations due to the COVID-19 pandemic. It was later discovered that many individuals interested in Mosquito Watch were not very tech-savvy and did not use social media. Although Mosquito Watch was a resource for residents, it became an effective tool for the Operations department to address the influx of SRs during the peak of the mosquito season. By referring residents who were requesting an inspection of their entire neighborhood/apartment complex/Homeowner's Association (HOA) to Mosquito Watch, the Operations department did not have to conduct door-to-door outreach and property inspections to over 400 properties in high biting pressure areas. Through Mosquito Watch, 444 MW packets with general mosquito information were delivered to residents and 5 virtual presentations with over 50 attendees were scheduled

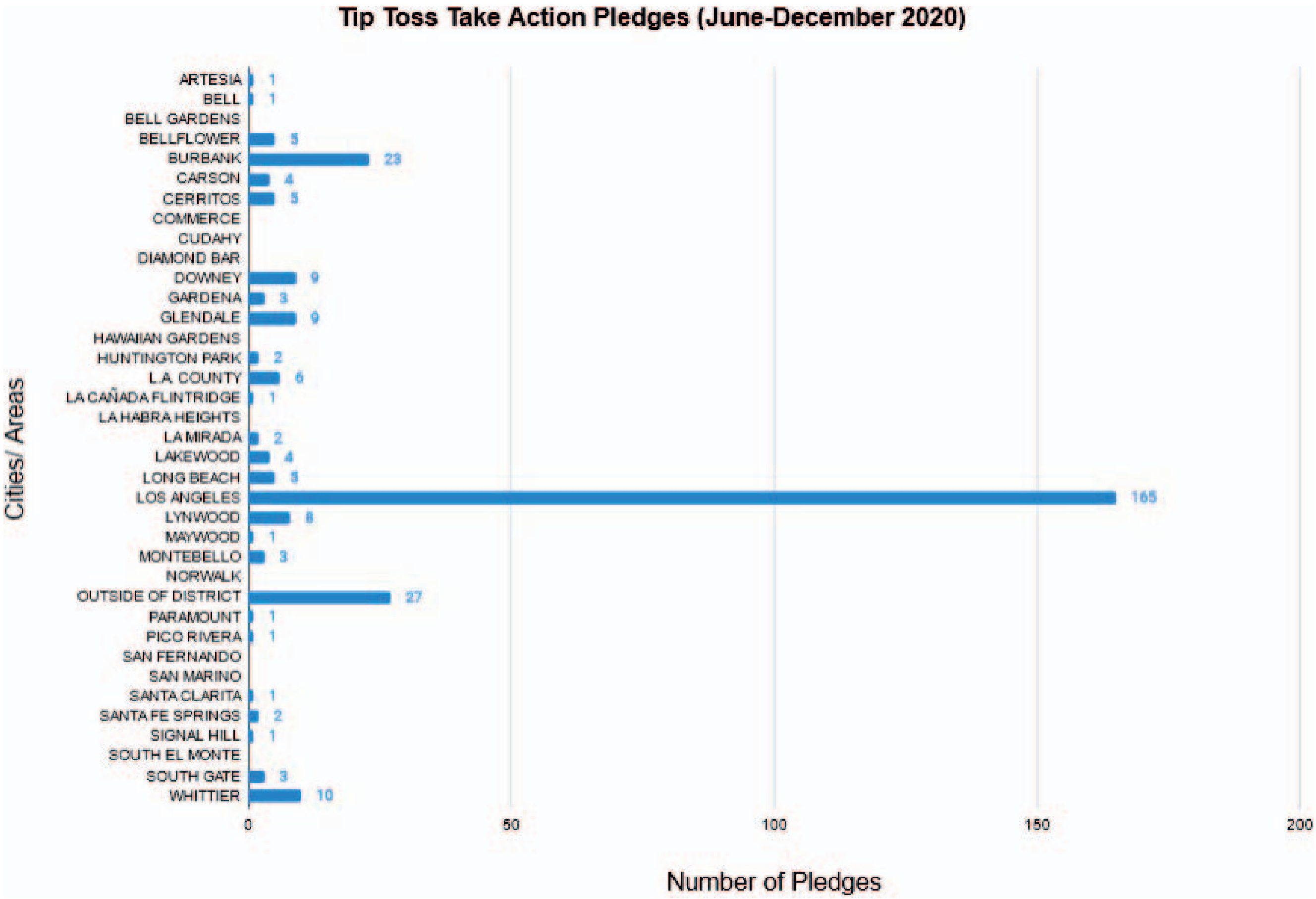


Figure 3.—The number of pledges by cities/areas between June and December 2020. Individuals within and outside of the District's service area pledged to take action against mosquitoes. Despite the COVID-19 pandemic and the limitations it imposed on community outreach activities, a total of 303 individuals made the commitment to take an active role in reducing mosquito breeding sources in and around their home.

to replace property inspections. The packets made it easier and safer to inform and distribute information to many residents and the virtual presentations addressed the questions and concerns of residents in real time. In addition, it allowed inspectors to focus on maintaining and treating public mosquito breeding sources.

CONCLUSION

With the continued spread of the *Aedes* mosquitoes and increased service requests from the public every year, it is imperative to provide residents with resources and tools to take an active role in reducing the threat of mosquitoes and mosquito-borne diseases both in their home and neighborhood. Mosquito Watch helped to alleviate the need for Operations staff to conduct door-to-door outreach and property inspections by providing resources such as a tool kit, packets and virtual presentations for residents and their neighbors. Moving forward, Mosquito Watch will continue to evolve and explore ways to engage and mobilize residents, and Districts in a similar situation should consider creating an outreach program with a neighborhood approach to shift the responsibility of controlling mosquitoes on private properties to residents.

ACKNOWLEDGEMENTS

A special thank you to GLACVCD General Manager Truc Dever and Community Affairs Director Mary-Joy Coburn for the opportunity to develop and launch Mosquito Watch. The campaign and Mosquito Watch would not have been possible if it was not for the collaborative work and support of Anais Medina Diaz, Diana Gutierrez, Helen Kuan, Liliana Moreno, and GLACVCD's Operations and Scientific-Technical Services departments.

Adapting a new education strategy in the age of COVID-19: MQA (Mosquito Questions Answered)

Francis Fernando*, Gregory Mercado, and Mary-Joy Coburn

Greater Los Angeles County Vector Control District
12545 Florence Ave.
Santa Fe Springs, CA 90670

*Corresponding author: ffernando@glacvcd.org

Abstract

The emergence of COVID-19 forced educators to abruptly change how they plan and implement curricula. For years, the education program at the Greater Los Angeles County Vector Control District (GLACVCD) has been predicated on an immersive interactive experience using the district's mobile education unit, the Mosquito SWAT Lab. Our presentation will discuss one approach to accommodate distance learning using a multimedia experience in a new video series called Mosquito Questions Answered (MQA). By deriving key components of Mosquito SWAT Lab's curriculum and mosquito questions submitted by students and members of the public, MQA seeks to provide concise information produced in a fun and easy to understand manner. Each MQA episode was designed to include language, comedy, and popular media trends that appropriately resonated with elementary and middle school student populations. MQA has increased social media activity and interaction, diversified the content used in current virtual programs, and provided a sustainable approach not only restricted to distance learning but to provide extracurricular content that may be used outside of the academic school year.

BACKGROUND

On Thursday, March 19, 2020 the Greater Los Angeles County Vector Control District began operating under the "Safe at Home" order in response to the novel COVID-19 pandemic. This caused the education program to effectively cease all in-person direct education; placing the Mosquito SWAT Lab out of commission indefinitely. Prior to the order, the education program was reaching over 150 students (approximately 3 schools) per week totaling 1,384 students between January and March 2020. The typical program focuses on a highly interactive and hands-on approach to mosquito education split between the SWAT lab and classroom settings.

As schools abruptly shifted and experimented with distance learning, the education program converted the lesson plan to be deliverable online; however, it took two months for schools to look for guest presenters and reserve the virtual program. A Household Pulse Survey found that 93% of students were engaged in some form of distance learning and among these students 80% shifted to the use of online resources (as opposed to materials sent from schools) (McElrath 2020). The prevalence of students engaging in distanced learning was widespread within our district's boundaries. An EdSource poll was administered following the initial incorporation of distance learning and reported that approximately 75% of parents believed distance learning was worse than in-person learning and approximately 55% thought the past Spring Semester of distance learning was not effective. Parents thought the top two challenges were the difficulty in sustaining a child's interest and their child not being motivated for online schooling (McElrath 2020). Similarly, our education team's observations with the initial distance learning experience included: students requiring an adjustment period for this new form of learning (unfamiliarity with audio/video mute, fragmented focus, speaker-audience dynamics), school planning (oversized virtual rooms), and the need for more interactive elements in the virtual presentation (assessed from 5 virtual presentations reaching 132 students during the Spring Semester). Following the conclusion of the 2019-2020 academic year, the education team analyzed the feedback from both in-person and virtual presentation and deliberated on ways to find a new approach to education during the pandemic.

METHODOLOGY

To address virtual learning, Mosquito Questions Answered (MQA) was created, an educational video series that seeks to answer student and community questions regarding mosquitoes (a highly anticipated part of normal program) and translate them into short and fun videos. Each episode included five components: (1) A template MQA introduction, (2) an introduction to a submitted mosquito question, (3) response, (4) debrief, (5) and template outro. Each episode amounted to a total run time between 1-3 minutes in length. The roles of MQA host and scientist

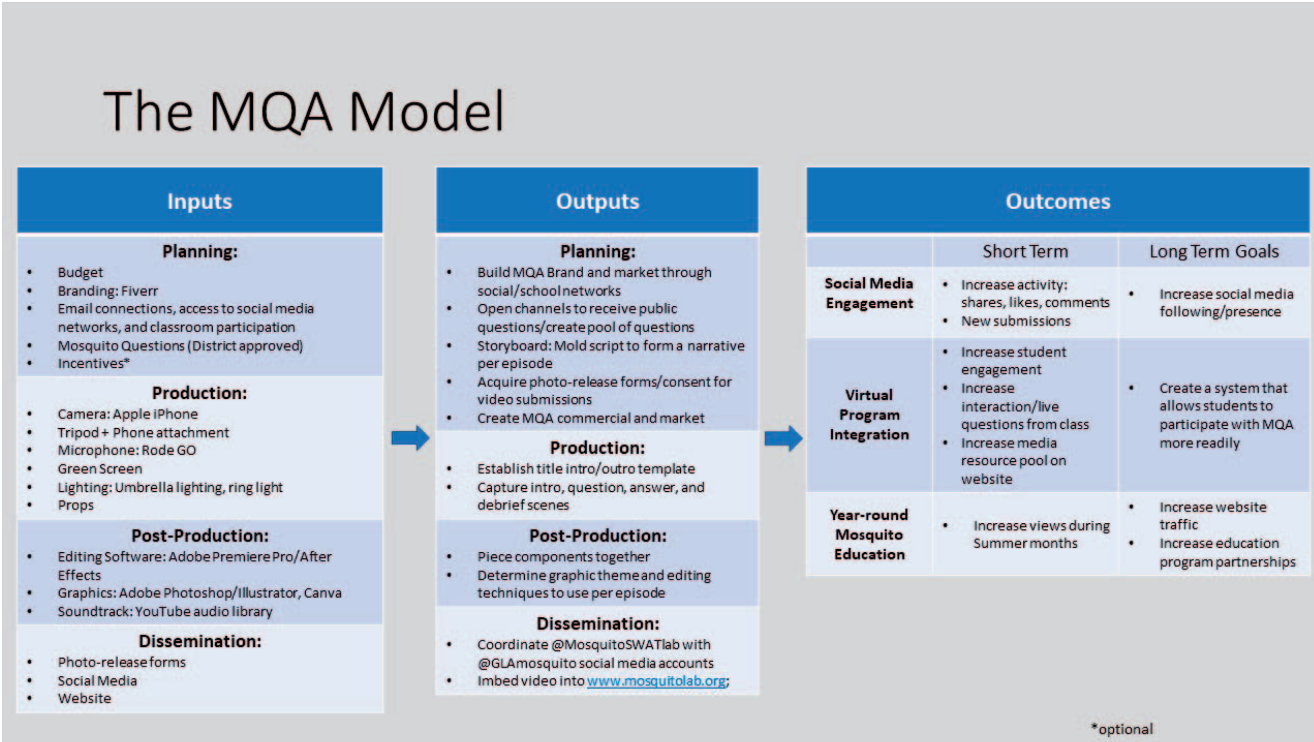


Figure 1.—Logic model for Mosquito Questions Answered (MQA) production and dissemination.

(answerer) alternated with each subsequent episode. Each episode was self-recorded in front of a greenscreen using a smartphone, tripod, and umbrella lights (Figure 1). Postproduction was conducted on Adobe Premiere Pro using accompanying elements through Canva.com. Final videos were disseminated on the @MosquitoSWATlab Instagram and Twitter accounts beginning April 2020 with the final episode airing in August 2020.

All episodes also were housed on the Education Program website (www.mosquitolab.org) for instant accessibility. Episode 8, "Why do mosquitoes bite me and not my brother?", is the most common question educators receive during Pre-COVID-19 in-person programming and distanced learning. It was then incorporated during the virtual program by showing the video prior to the live question and answer component (Figure 2).

RESULTS

MQA episodes received an increasing number of impressions with each subsequent month; with a peak in August reaching over 2,500 total impressions. Interactions remained relatively consistent among MQA posts; however, in comparison to the general cumulative content on the @MosquitoSWATlab account, more interactions were seen during months in which MQA were posted (Figures 3–5). MQA was well received when incorporated into the virtual

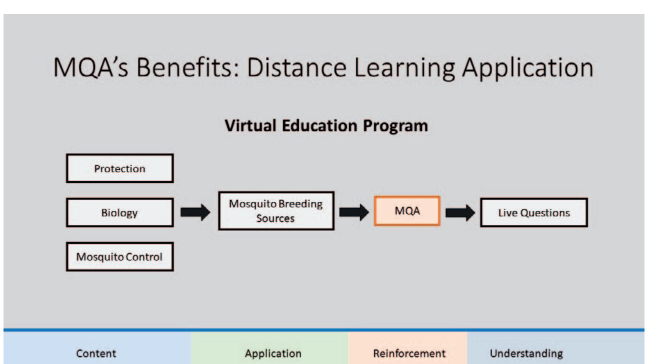


Figure 2.—Distance learning application of Mosquito Questions Answered (MQA) as a reinforcement tool.

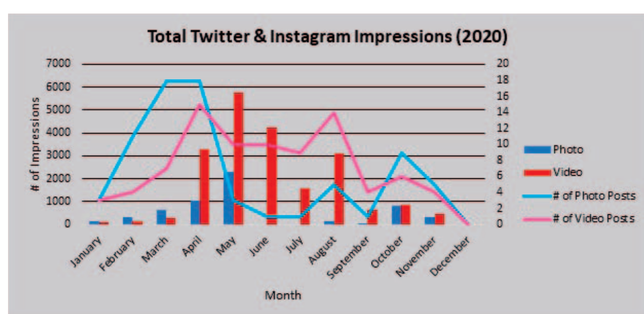


Figure 3.—Social media impressions (views and number of times content was displayed on a user's feed) comparing photos and videos.

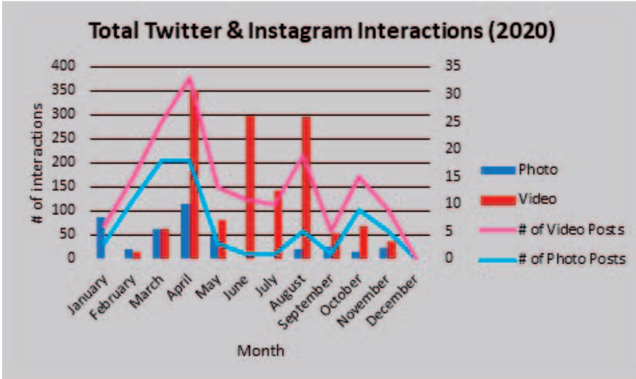


Figure 4.—Social media interactions (likes, comments, shares) comparing photos and videos.

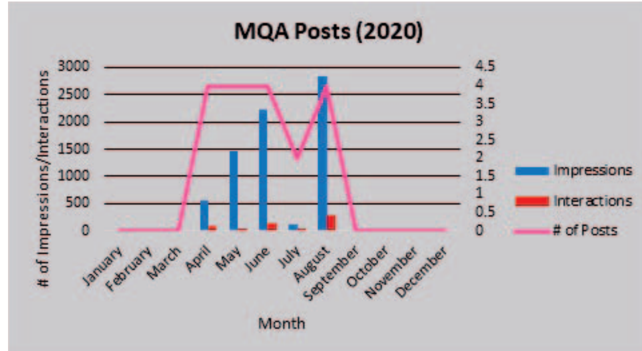


Figure 5.—Mosquito Questions Answered (MQA) posts' impressions and interactions in 2020.

education program as smiles and inaudible laughs were observed.

DISCUSSION

MQA became the Education Program's top posts for 2020. When assessing the increase in impressions as newer episodes were created and published, it is important to note that newer video editing techniques and styles were added and developed with more familiarity with the software and opportunities to build off previous episodes. Both students and teachers were observed to enjoy the strategically placed humor throughout Episode 8 when shared during virtual presentations. MQA was incorporated permanently into the design of the virtual program to address core learning principles outlined in Bloom's Taxonomy of learning objectives to aid learning and comprehension (Adams 2015). These elements included slapstick humor, sound effects, and simple animations that help illustrate key points. MQA can be replicated using only a portion of the resources listed under "inputs" in Figure 1. The biggest obstacles for MQA fall under the production side of each episode which consists of the use and set up of lighting, camera equipment, and green screen fixtures. Working from home during the pandemic exacerbated these issues due to confined living/workspaces as well as coordinating with others to produce videos that were suitable for post-production. Including post-production, producing episodes was exceptionally time consuming and restricted to the editing software that is available. Another limitation for MQA was receiving submitted questions. Although student questions regarding mosquitoes could be taken from previous classroom visits, MQA was designed to receive questions via social media submission which was dependent on social media following and the platform used; receiving video submissions was uncommon.

CONCLUSION

The creation of MQA was a successful opportunity to enter the distance learning realm by taking key elements of the in-person program (live questions, entertainment, and unique mosquito curriculum) and forming short videos to help educate the Education Program's social media following and stakeholders. Incorporation of MQA in the virtual program provides another opportunity to reinforce major topics discussed in the presentation while transforming the presentation into a multimedia experience. MQA season 1 has set the stage for subsequent seasons and allowed for continuing education beyond the school year with an easily accessible pool of education videos on the GLACVCD's education website.

ACKNOWLWEDGEMENTS

A special thank you to the Community Affairs Director, Mary-Joy Coburn for the opportunity to create a new virtual education campaign. MQA would not have been possible without all the students and schools that have participated in our education program and shared their inquisitive mosquito questions in 2020.

REFERENCES CITED

Adams, N. E. 2015. Bloom's taxonomy of cognitive learning objectives. J. Med. Library Assoc. 103: 152-153.

EdSource. 2020. Attitudes on key California education issues: Key findings from a statewide survey conducted August 29-September 7, 2020. Retrieved from https://edsource.org/2020/california-voters-grapple-with-education-in-age-of-pandemic-an-edsource-poll/640970

McElrath, K. 2020, August 11. Nearly 93% of households with school-age children report some form of distanced learning during COVID-19. Retrieved from https://www.census.gov/library/stories/2020/08/schooling-during-the-covid-19-pandemic.html

Assessing tick risk at schools in San Mateo County: Leveraging surveillance to improve tick safety education

Angie Nakano*, Tara Roth, Tina Sebay, Theresa Shelton, Arielle Crews

San Mateo County Mosquito and Vector Control District, Burlingame, CA 94010

*Corresponding author: anakano@smcmvcd.org

Introduction

The conventional wisdom in the San Francisco Bay Area has been that most tick exposures occur during outdoor activities outside the home at recreational areas such as parks or trails. However, the risk of encountering ticks at schools has not been well studied. The San Mateo County Mosquito and Vector Control District (District) assessed a number of school campuses in San Mateo County to ascertain the degree to which students and staff were at risk of tick exposure while at school.

Figure 1.—District staff members Arielle Crews and Tara Roth conduct sampling for ticks at a school campus in Pacifica, California.

Methods

Schools were initially chosen for consideration by proximity to areas of known tick habitat and other "green space" as viewed on online aerial maps. District staff drove by the outside of each campus to further determine their suitability for ticks. Administrators for selected schools were emailed and 15 schools were eventually visited in December 2019 and January 2020. Each visit consisted of contact with on-site office or maintenance staff, tick flagging of fields and other vegetation, and a visual inspection of the grounds (Figure 1). All *Ixodes pacificus* were tested for *Borrelia burgdorferi* and *B. miyamotoi*, and all *Dermacentor occidentalis* were tested for tularemia, using in-house PCR.

Figure 2.—Schools were given reports containing specific advice on areas needing vegetation management, as well as examples of ideal landscape attributes that minimize tick risk.

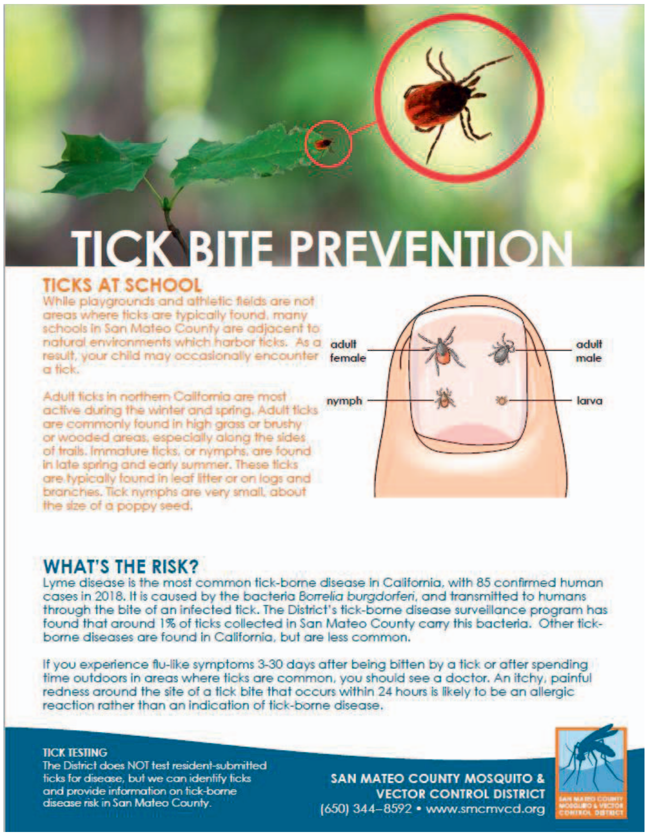


Figure 3.—Front of the tick bite prevention flyer given to schools for distribution to parents. Flyers were made available to participating schools in English and Spanish.

Results and Discussion

A total of 226 ticks were collected from 10 of the 15 schools. Five schools had no ticks, and one school had over 100 ticks collected. Most ticks found were *Ixodes pacificus*, but a small number of *I. spinipalpus*, *Dermacentor occidentalis*, and *D. albipictus* were also collected. Schools located adjacent to open space or heavily wooded areas were most likely to have positive tick collections. Ticks were rarely found on mowed athletic fields, except in some edge areas that were adjacent to natural brush or forest environments. All ticks collected and tested for pathogens were negative.

A personalized report was delivered to each school, including observations, findings, and recommendations for specific landscape modifications and tick safety messaging (Figures 2 and 3). Feedback from school administrators was positive and enthusiastic, and five schools commissioned special landscape work to enact the District's vegetation management recommendations (Figure 4). District staff returned to three of the schools in February 2020 after landscape maintenance work had been completed and re-sampled for ticks and observed a combined reduction of 85% of ticks collected.

Conclusions

This pilot study confirmed that schools located near open space areas with tick habitat often harbored ticks on campus. Conducting surveillance activities at schools offered opportunities for public education of staff and administrators. The surveillance data collected through campus visits allowed for improved and refined tick safety outreach information, which in turn resulted in a measurable reduction in tick risk at some schools.

Acknowledgements

We would like to thank the school administrators we worked with in San Mateo County for participating in this study. In particular, we would like to acknowledge Heather Olsen, Josie Peterson, Jorge Machado, Tim Molak, and Lou Roselli for their effort and commitment to reduce tick risk at their schools.

Figure 4.—Examples of in-field exercise stations before and after a Pacifica school performed vegetation management and landscape maintenance to reduce tick risk.

Maintaining school outreach momentum while navigating COVID-19 distance learning in Placer County

Meagan Luevano*

Placer Mosquito and Vector Control District, 2021 Opportunity Drive, Roseville, CA 95678

*Corresponding author: meaganl@placermosquito.org

Placer Mosquito and Vector Control District responded to the 2020 COVID-19 pandemic by adapting our mosquito control and outreach programs to keep staff safe. For public outreach programs, this required that the District to adapt all digital platforms for communication with the public. Although the District has existing online media such as social media, email newsletters and an actively updated website to reach the public, our successful school outreach program was reliant on in-person assemblies for over six years. To continue to provide meaningful outreach to elementary school aged students, we took our existing school assembly script and transformed it into a recorded virtual video assembly experience with enhanced graphics, quick transitions and even some 'slap-stick' comedy to engage students. The virtual assembly was quickly filmed, edited and tested on students to gauge reactions and assess engagement levels. The virtual assembly is currently being disseminated to Placer County schools and we are tracking its success.

Pandemic Pandemonium: How one southern California District confronted a world-wide pandemic by taking action with streamlined strategies resulting in minimal operational interference

Rick Howard*

Orange County Mosquito and Vector Control District, 13001 Garden Grove Blvd, Garden Grove, CA 92843

*Corresponding author: rhoward@ocvcd.org

The Orange County Mosquito and Vector Control District took the unprecedented action to declare a Local Vector Control Emergency on March 16, 2020 in response to the World Health Organization and Centers for Disease Control determination that the Coronavirus epidemic had been upgraded to a World Wide Pandemic. Three days later, and on March 19, 2020 California Governor Gavin Newsome Declared a State of Emergency and issued stay-at-home orders. By relying on an emergency operations model, the District assembled a COVID-19 response team representing a wide cross section of District employees. The Board of Trustees were engaged in an advisory role, and the District continued to perform its core services with limited interruption. The District's emergency COVID-19 Response team focused on three key components during this unprecedented time: 1) clear communications to the residents on the essential functions of OCMVCD, 2) developing and implementing Covid-19 safety protocols, and 3) transparent communication with district staff. Public engagement was seamless, and the District's Communications staff played a critical role in providing a calming message to the public during these stressful and unprecedented times. Safety programs were drafted and implemented, and guidelines that were at the forefront of the pandemic were shared with other agencies throughout California and the United States.

It’s not business as usual: Administration of a vector control district during the COVID-19 crisis

Truc Dever*

Greater Los Angeles County Vector Control District, Santa Fe Springs, CA 90670

*Corresponding author: tdever@glacvcd.org

Introduction

Maintaining daily operations during the initial days of the COVID crisis at the Greater Los Angeles County Vector Control District [GRLA] required up-to-date information, regular staff communication, and a lot of flexibility. Administrative staff and managers worked tirelessly to address emergency declarations, implement changes to state and federal paid family and sick leave laws, and develop new safety and sanitation guidelines. As the crisis wore on, the District turned its attention to workers’ compensation reporting, budget reserves, coordination of staff workspace, pre-shift COVID screening, employee communication and telecommuting policies. This presentation will highlight some of the many considerations for continued district operations and discuss lessons learned as agencies brace for the long-game.

Methods

The GRLA collaborated with neighboring vector control agencies to share resources and best practices and engaged legal counsel early into the pandemic. Management staff met weekly to discuss staff and resident needs and develop new protocols for continued mosquito control services while ensuring the safety of staff and the public. A weekly meeting of the Executive Officers of the Board of Trustees helped guide policies and district activities.

Results and Discussion

The presentation addresses management’s role in navigating this public health emergency and the many challenges of continuing operations during a statewide lockdown.

Conclusion

The District was successful in carrying out its mission to protect public health through the high mosquito season by taking proactive steps to stay ahead of changing safety guidelines and restrictions and anticipating potential obstacles to daily operations.

Greater Los Angeles County VCD's operational response to COVID-19

Wesley D Collins*

Greater Los Angeles County Vector Control District, Santa Fe Springs, CA 90670

*Corresponding author: wcollins@glacvcd.org

Introduction

Greater Los Angeles Vector Control District GLACVCD) runs a comprehensive Integrated Vector Management (IVM) program across 1000 sq mi of Los Angeles County to protect approximately six million residents from vector-borne diseases. For such a large undertaking pre-season planning and preparation is an important part of a successful mosquito control season. This involves strategic planning, staff training and seasonal recruitments, as well as a host of equipment related projects. All these activities usually involve in-person meetings, group collaboration and 'all-hands' meetings. On March 19, 2020 California Governor Newsom issued the nation's first statewide "Stay-at Home Order" due to the outbreak of COVID-19, presenting GLACVCD management and staff with a host of organizational challenges, because March is typically when we prepare for the coming mosquito season. After determining that, as a public health agency, we must be considered essential, we asked ourselves the following questions: How are we going to meet our obligation to protect the public from mosquito borne viruses while complying with National, State and County wide guidelines aimed to protect everyone from the pandemic? How can we accomplish our goals and objectives while keeping staff safe during a global outbreak of COVID-19 that may last into the foreseeable future?

Discussion

As a result of many meetings over the span of two weeks, during which all staff was allowed to work on various assignments from the safety of their home, GLACVCD management put together a comprehensive 'return to work plan', that was both in compliance with CDC and California Department of Public health guidelines and ensured to the best of our ability that staff would be and feel safe as they had to return to fulfill mission critical tasks. These measures allowed our district to move forward with our pre-season planning, so we would eventually be able to safely employ 41 seasonal vector control technicians without jeopardizing our 49 fulltime staff's safety.

As a result, we closed district facilities to the general public and enacted a strict face covering mandate, pre-shift temperature measurements and COVID symptom screening. We created daily sanitization protocols and schedules for the locker rooms, restrooms, work vehicles, workspaces, and other high contact areas. We also automated restroom equipment to prevent cross contamination. This was done by installing touchless devices such as soap dispensers, faucets, urinal flush valves and toilet flush kits.

Social distancing was enhanced by adding more workspace. This was accomplished by renting mobile office units and since most administrative staff were working from home, we were able utilize their office spaces for our field staff. We even transformed conference rooms to office spaces to facilitate safe staff distribution. We staggered work shifts to create additional space in the locker rooms as well as throughout the facility and to ensure only small groups of staff were in close contact, in case of COVID exposure and possible need for subsequent quarantine. There were five different work shifts in total and staff was not allowed to linger inside the office after changing into their uniforms or when returning from the field in the afternoon. We rented additional work vehicles to separate staff who would typically ride together due to their assignment. No one was allowed to ride in a vehicle together.

Communications was a huge part of our successful operation. Our staff have district issued cell phones and 4G tablets. The cell phones allowed for direct, group and text communications, whereas the 4G tablets allowed for field data capture, online training, email, direct, and group communications. Various Web communication platforms allowed us to 'go virtual' since in person contact was not allowed. All training was done virtually via zoom, with staff able to log into their assigned training on their district issued 4G tablets for tailgate meetings, all hands staff meetings and annual pesticide training. As more information became available and guidelines changed, so did our return-to-work plan that required impromptu or emergency meetings that again were all virtual. The staff could always be informed and kept up to date without physically being in the building. These virtual platforms also made it possible to interview and hire our seasonal staff.

Due to the pandemic restrictions we also had to change many of our practices out in the field. To ensure staff safety and make residents feel comfortable with our presence on their properties, we would only conduct in-person inspections after having made contact via phone to set the 'no-contact' parameters for our visit. When we had to contact residents, who had not reached out to us, we left door hangers asking for call-back, so we could make the appropriate arrangements.

Conclusion

Despite all the challenges due to the pandemic, we were able to accomplish our goals. We did all the necessary pre-season planning and training, and we were able to bring on all the seasonal staff needed. Our field expectations were fulfilled, and our objectives were met. We actually accomplished more in 2020 than in any typical year, although it was done differently with the help of modern technology. We believe our newly developed guidelines were effective because we have not documented any work-place related COVID-19 transmission amongst the staff at our agency.

Leveraging technology to quickly respond to an emerging invasive mosquito outbreak

Peter Bonkrude*

Shasta Mosquito and Vector Control District, Anderson, CA 96007

*Corresponding author: pbonkrude@shastamosquito.org

Introduction

In 2020, the Shasta Mosquito and Vector Control District (District) identified the first *Aedes aegypt*i and *Aedes albopictus* specimens in Shasta County. The locations of the collections quickly expanded and required the rapid response by District staff to effectively reduce the spread and potentially eradicate the invasive mosquitoes. To mobilize resources efficiently and effectively, the District leveraged their current data collection system, VeeMac (Figure 1), to better allocate resources, track progress and quantify the impact of surveillance and control. Using the adaptive nature of the system the District was able to "on the fly" build a program module distinct from their existing collection efforts without any additional developer involvement or cost.

Methods

In March of 2020, the District began the migration and use of a new VeeMac field data collection system. On 14 Aug 2020 District staff located an adult female *Ae. aegypti* in a routine trap. Two days later after confirmation of the species, we added several new trap types to our regular trapping routes and organized door-to-door inspections of the areas adjacent to the adult find (Figure 3). In the VeeMac system, we first created a separate "collection" or data type to capture and report out information generated during the invasive *Aedes* surveillance efforts (Figure 2). This allowed us to categorize all inspections, treatments, and workforce management specific to invasive *Aedes*. It also allowed us to visually filter information specific to invasive *Aedes* efforts and easily report to our stakeholders. In addition to the new data collection module, we began

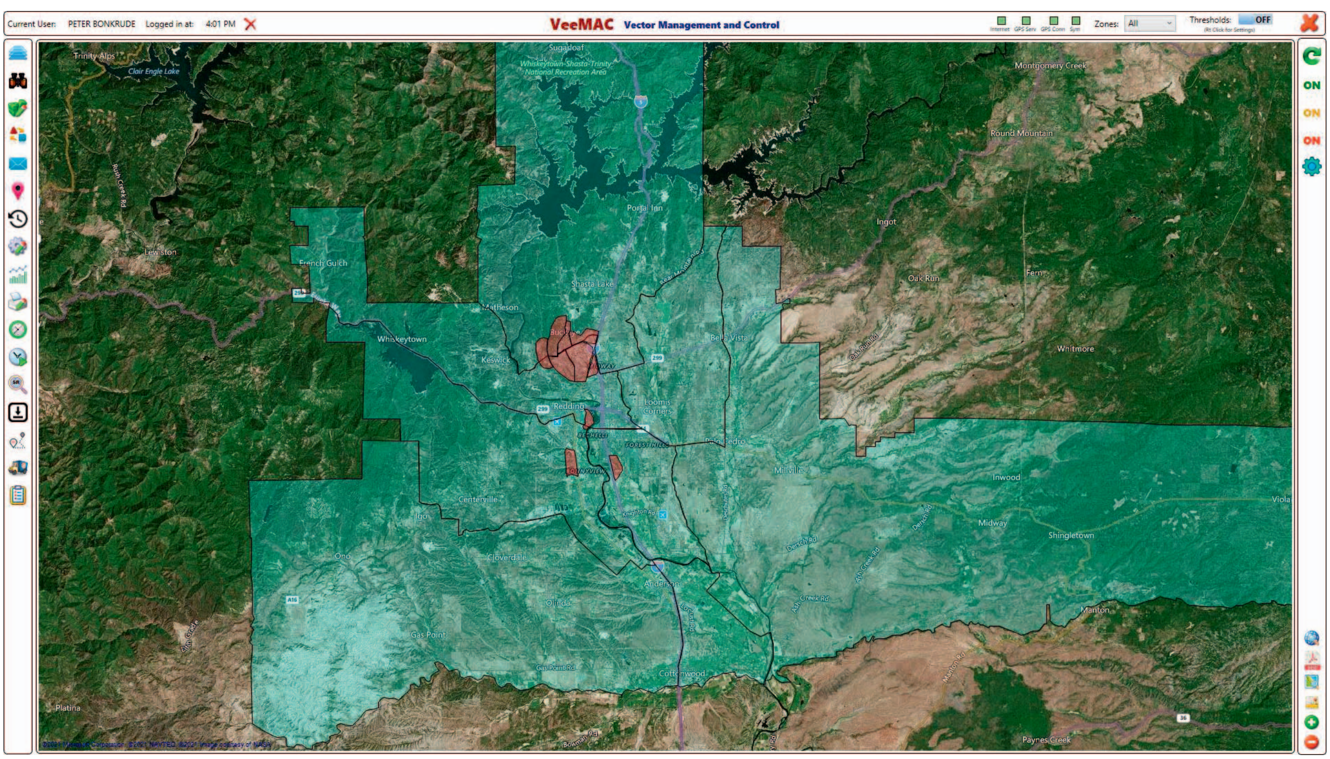


Figure 1.—VeeMac Dashboard showing new Aedes Operational Zones

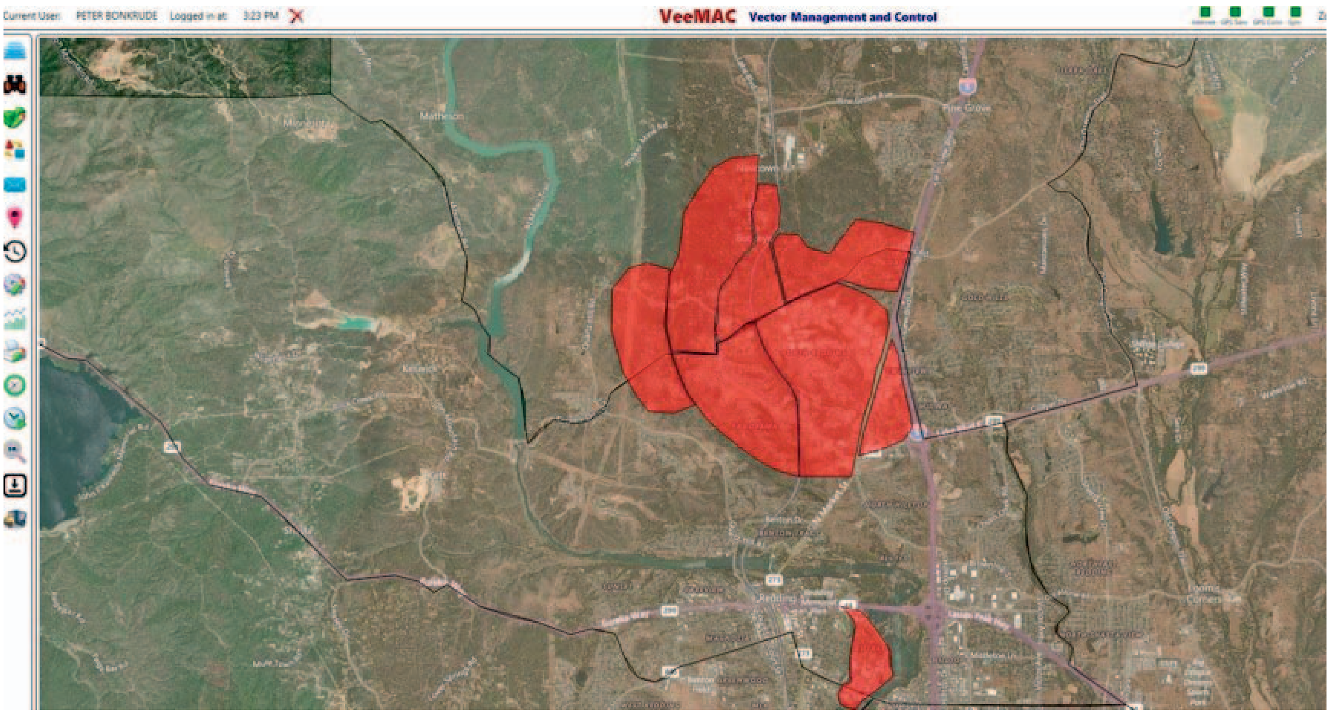


Figure 2.—Invasive *Aedes* Operational Zones

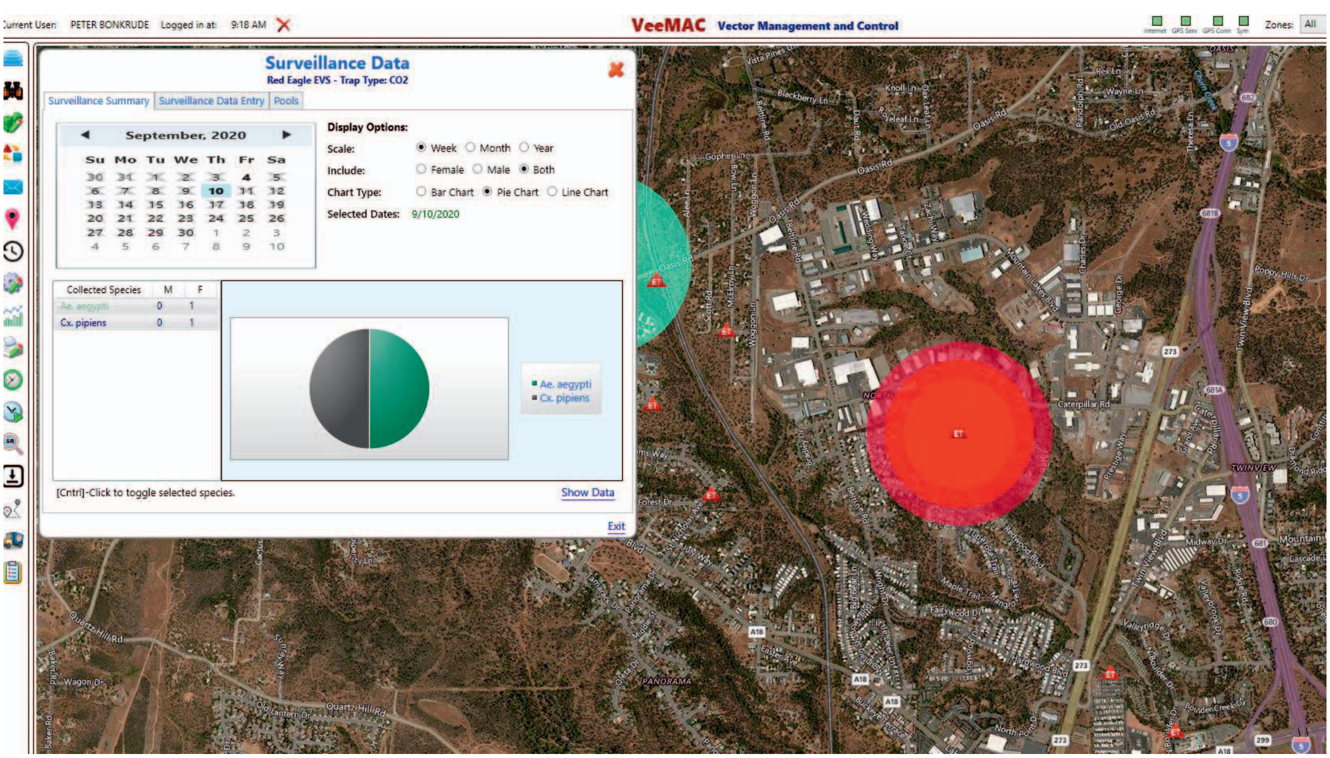


Figure 3.—Invasive *Aedes* Surveillance

collecting contact information from our door-to-door inspections and adding them as customers in VeeMac, so that we could easily contact those residents when the season began in 2021.

Results and Discussion

By leveraging our new data collection system, we were able to quickly respond to each new invasive *Aedes* find and seamlessly communicate that information across all Departments. District staff integrated new trap types, new control technologies and progress tracking into our operational response without additional programming.

Acknowledgements

Thank you to the Staff and Board of Trustees of the Shasta Mosquito and Vector Control District

References Cited

VeeMac: Vector Management Software, www.veemac.com

Decentralize your district today

Robert Ferdan*, Ryan Clausnitzer

Alameda County Mosquito Abatement District, Hayward, CA 94545

*Corresponding author: robert@mosquitoes.org

Introduction

Mosquito and vector control districts have faced many challenges in the last few years. Fires, COVID-19, and other natural and anthropogenic disasters could force a district to close its doors and operate in a decentralized environment. Adopting remote work technologies allowed districts to become flexible if the need arises. The Alameda County Mosquito Abatement District first decentralized several years ago during a remodeling project. Offices and employees had to move during construction, but as the project scope grew, it became apparent that all staff would have to vacate our building and work remotely.

Methods

Construction started early in 2019 and lasted 3 months. During that time we decentralized our District because our main building would be inaccessible. Each department in the District was asked to evaluate their readiness to work remotely. The compiled information was used to find solutions to issues that would produce a remote workforce. District needs were grouped into the following departments: Administration, Operations, Laboratory, Facility and Fleet. To produce a mobile and remote workforce, each facet of our business model was migrated to the cloud (e.g., computing, communication, and access functions). Jive, our cloud-based telephone system, allowed us to route calls from the District office to home offices or cell phones. Virtual meetings were already being used at the District, but we needed to scale up technologies for holding meetings from any location. Zoom was deployed to give staff, management and the Board of Trustees the ability to attend meetings and communicate from the field or their home office. Leading Edge, a geospatial database provider, enabled us to work remotely in the field using tablets that were connected by cellular technology. Field staff returned to the District only when they needed to replenish supplies. All data entry was performed in the field. District documents were converted to digital files in Office 365 and SharePoint, a cloud-based file repository. Employees now had access to District documents from any computer or tablet from any location that had cellular data. Remote timekeeping was mandatory. OnePoint, our human resources (HR) solution, allowed employees to clock in and out, along with all other HR functions, using their cell phone or the tablets remotely. All systems worked as planned and construction lasted three months with most office and field work completed remotely.

Results and Discussion

On March 19, 2020– almost a year after our construction project, California Governor Gavin Newsom ordered the closing all nonessential businesses due to the COVID-19 pandemic. That morning, our General Manager gave the order to begin remote work once again. District systems were already in place to work in a decentralized capacity facilitating our response to this lockdown. Our District was able to rapidly adapt and continued serving the public without interruption.

Conclusion

As we move into a post COVID-19 lockdown world, many companies have already decentralized and may never go back to the old way of doing business. The world has changed. Some aspects of the way the mosquito and vector control industry do business will never be the same either. We learned the value of decentralizing through a construction project, but it changed the way our District will do business for many years to come. I leave you with this question: “Are there ways to decentralize your district today to be ready for whatever comes next?”.

Acknowledgements

Eric Haas-Stapleton, Erika Castillo, and Erick Gaona assisted with decentralization and abstract preparation.

Are early adopters of Unmanned Aircraft Systems (UAS) transforming vector control agencies across the United States?

Bill Reynolds*, Piper Kimball

Leading Edge Aerial Technologies, Inc. 1990 Country Club Dr, Port Orange Fl 32128

*Corresponding author: breynolds@leateam.com

The use of unmanned aircraft systems (UAS) has grown exponentially in the last 15 months and transformed mosquito control operations across the United States. This presentation focused on agencies that have incorporated UAS for aerial surveillance, imagery, and applications. Specific solutions technology resolved included challenges surrounding the inspections of habitats, documentation of environmental events, and aerial applications to control immature and adult mosquito populations. Participants learned about the latest advancements in UAS technologies and future capabilities.

Enhanced routing methods for truck mounted applications in urban environments

Ruben Rosas*

Sacramento-Yolo Mosquito and Vector Control District, Elk Grove, CA 95624

*Corresponding author: rrosas@fightthebite.net

Introduction

The Sacramento-Yolo Mosquito and Vector Control District (District) has been using truck mounted low volume larvicide applications (LVL) to target cryptic sources in urban areas with high *Culex pipiens* populations to prevent the spread of West Nile virus. Due to the recent detection of *Aedes aegypti*, mapping and routing for effective LVL have become a critical component of successful urban larval control.

Methods

By utilizing multiple software platforms, the District can provide visual and voice guided navigation for pesticide applications in urban environments. Pesticide routes are created using Google MyMaps and exported to ArcGIS Desktop for post processing. After post processing, a navigation program called inRoute is installed on an iPad. The field technician then proceeds to use the iPad for navigation to conduct the pesticide treatment.

Results and Discussion

By converting paper maps to digital files and using an iPad for navigation, pesticide routes are now organized by folders and are ready for navigation at any moment. By utilizing waypoints and storing multiple routes on the cloud, technicians can receive turn by turn directions for any assigned route allowing staff to complete the mission appropriately and safely (Fig. 1).

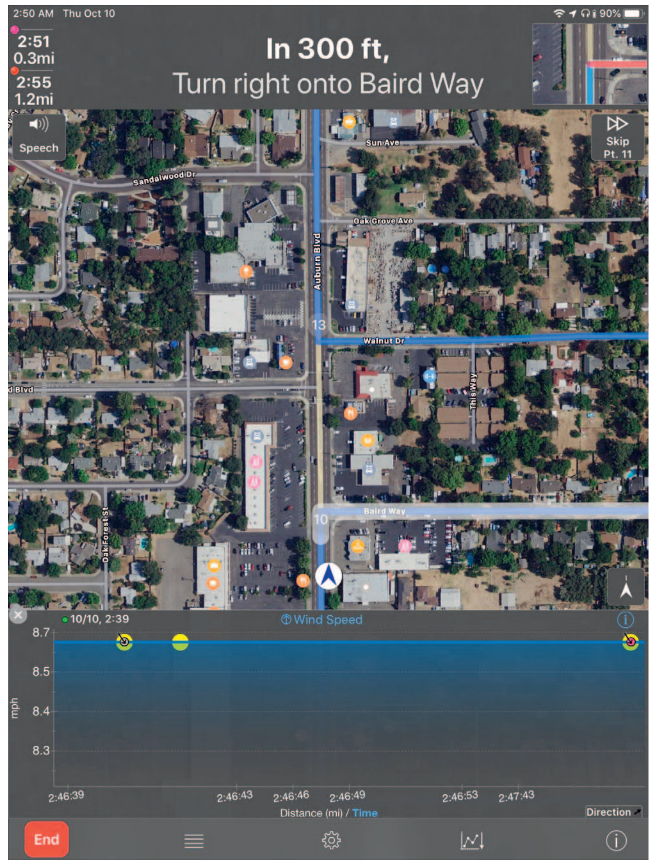


Figure 1.—Screen shot of the routing app during a pesticide application.

Conclusion

Pesticide route navigation has become a valuable tool for the District. By streamlining the workflow, the District can create and prepare routes for navigation easily and effectively. By utilizing ArcGIS, Google Maps, Google Drive and an IOS app called inRoute, the District has developed a systematic approach for seamless navigation in the field.

Software References

ESRI GIS Mapping Software: https://www.esri.com/en-us/home
Google Workspace: https://workspace.google.com
inRoute Navigation: http://carobapps.com/products/inroute

Interactive maps for West Nile virus risk

Shawn Ranck*, Jody K. Simpson, and Christopher M. Barker

Davis Arbovirus Research and Training (DART) Laboratory, Department of Pathology, Microbiology, and Immunology, University of California, Davis, CA 95616

*Corresponding author: sranck@ucdavis.edu

Introduction

The California Mosquito-Borne Virus Surveillance and Response Plan (CMVSRP) provides a basis for integrated vector management in California by outlining a framework to assess risk for West Nile virus (WNV) transmission to humans based on surveillance data and recommending public-health interventions as risk escalates. For many years, the CalSurv Gateway has generated weekly maps of WNV risk based on the CMVSRP risk assessment. These maps were generated from the latest surveillance data and delivered as PDF files to Gateway users' email addresses each week with links to view the files.

Methods

Recently, we have developed a new set of maps for WNV risk that replaced the previous static PDF files. The new maps are interactive and show graphs of changes over time in addition to the maps showing spatial patterns of risk. Users can download or share views of the maps, graphs, and underlying risk estimates for use in board reports or presentations.

Results and Discussion

Here we provide an overview of the capabilities of the new WNV risk maps and demonstrate important features that may be of interest to vector control agencies. The WNV risk maps are located at https://maps.calsurv.org/risk and are available to anyone with CalSurv login credentials. As with other CalSurv maps, the default page view includes a tutorial card which explains some of the features and contains a link to the methodology behind the visualization.

Many of the previous interactive characteristics are included in these new maps, such as species and date selection, mouse over capabilities, and geospatially informative charts. Interaction marks the primary benefit over the previous CMVSRP solution. Mousing over any point on the map creates a popup window that describing the underlying data at those coordinates. Clicking any point creates a bar chart describing filtered pieces of those data in greater detail as well as a time series for those data in the present year. Drawing a polygon on the map summarizes included locations and provides the same visualizations for summary data within that shape. From a desktop computer, any of the map, the chart, the time series, and a table containing underlying data for the present year and location can be downloaded at any time by clicking on the Share icon above the controls, then clicking the PDF Download button.

Conclusion

These materials have improved access to WNV risk assessment and response. Any questions about CalSurv maps may be addressed to help@calsurv.org.

Acknowledgements

We thank our partners including the state of California, California Department of Public Health, and the Mosquito and Vector Control Association of California for the long-standing support that makes the CalSurv websites possible. We also acknowledge funding support from the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement U01CK000516).

Long-term efficacy of Sumilarv 0.5G in inundated underground utility vaults to prevent adult mosquito emergence

Ryan L Amick*, Tanya Posey, Harold Morales, Susanne Kluh

Greater Los Angeles County Vector Control District, Santa Fe Springs, CA 90670

*Corresponding author: ramick@glacvcd.org

Introduction

Utility vaults are a necessity for utility companies such as cable providers and telephone companies to operate and maintain their lines underground. Vaults with high voltage owned by electrical companies were excluded from this study due to limited access and for safety reasons. The vaults are made of concreate boxes with gravel bottoms to allow for water to percolate into the ground. After some time, the gavel becomes clogged with sediment and becomes inundated with rain and irrigation water. Cooler unground temperatures, protection from wind and sun, as well as a constant source of water creates a problematic mosquito source.

Greater Los Angeles County Vector Control District (District) provides service for six million residents over a thousand square miles. In 2019, we had 118 utility vault sites requiring treatment, many of which had multiple vaults at each site, and resulted in 896 inspections and treatments. Almost daily, more of these vault locations were identified by District technicians and without regular maintenance by the utility companies, many new sources are created each year. It is the responsibility of the utility owning these vaults to keep them from breeding mosquitoes. However, due to the large number of vaults and the costs involved for the utility companies to bring them all into compliance, the District has to treat them to prevent mosquito emergence. Traditional larvicides last only 14-21 days. Sumilarv 0.5G, a sand granule coated with the insect growth regulator pyriproxyfen, has been advertised to prevent adult mosquito emergence for up to 150 days in catch basins. In the current study, we determined if it could work in a similar fashion within inundated utility vaults.

Methods

Ten g of Sumilarv 0.5G were added to water-soluble packets (WSP). In June and August 2020, five inundated utility vaults in the greater Los Angeles area with a history of breeding mosquitoes were treated with one ten g WSP and after seven days sampled for pupae at seven-day intervals. We planned to collect 20 pupae per vault per visit, but low numbers of immature mosquitoes led to late-stage larvae being collected as well. On many occasions, very few to no immatures were available for collection. Samples were brought back in source water to the laboratory and observed for adult emergence for seven days. In late fall, samples were collected and observed on a bi-weekly basis.

Results and Discussion

Low numbers of immature mosquitoes in the vaults made it difficult to collect a full sample of 20 pupae and larvae. Dead larvae and pupae were observed in vaults when samples were taken. Many pupae and larvae died in the emergence jars. Although a few individuals emerged early on in the observation period, overall emergence remained below 10%. Between days 98 and 231 no emergence was observed. The label states that Sumilarv 0.5G "affects reproductive viability of exposed mosquito eggs and adult female mosquitoes." It is possible that the pyriproxyfen effect on fertility and fecundity led to the low abundance of mosquitoes observed in the vaults.

On occasion, in the hot summer months, adults were observed resting in the vaults; however, trapping with an unbaited CO_2 trap yielded only two adult females. The lack of males, low number of females, and dead immature mosquitoes provided evidence that the inhibition of emergence seen in our samples in the laboratory was also happening in the vaults.

Guidelines for Sumilarv 0.5G from MKG led us to expect 150 days of control. Of the five vaults in the study, two vaults had no immature mosquitoes until 175 days post-treatment. Those vaults were erroneously retreated and subsequently removed from the study. The vault observed for the longest time period had more immature mosquitoes, but few adults emerged. Monitoring was conducted for 231 days before a cold snap killed most mosquitoes in all the vaults. Observation and treatment of two of the vaults started later in the season, but after 182 days all of the collected immatures began to emerge. Of the vaults that did have emergence, emergence was no greater than 10%. We elected not to use any controls in this study of Sumilarv, because we found it unethical to let mosquitoes emerge from known sources, given the risk of West Nile virus transmission. Consequently, we couldn't compare our results to an untreated control and calculate a percentage of inhibition of adult emergence. A study to take place in 2021 will use control vaults that are treated with a larvicidal oil after immatures are collected.

Conclusion

Although we have promising results, further investigation of this product is needed for the District to definitively conclude Sumilarv 0.5G is working as reputed by the manufacturer. Like most insecticides, resistance is also a concern for pyriproxyfen. Close monitoring of the product's inhibition of emergence needs to be conducted and the material should be used in rotation with other larvicides to reduce the chance of resistance.

Efficacy of Sumilarv 0.5G in unmaintained swimming pools

Faiza Haider*, Susanne Kluh, Ryan Amick, Tanya Posey

Greater Los Angeles County Vector Control District, Santa Fe Springs, CA 90670

*Corresponding author: shaider@glacvcd.org

Introduction

With traditional larvicides, unmaintained swimming pools can be kept mosquito free for 14-21 days after treatment. According to MGK, the manufacturer of Sumilarv 0.5G, a pyriproxyfen containing sand granule, Sumilarv 0.5G, can inhibit adult mosquito emergence for 28-35 days in water retention ponds. We conducted the current study to establish if similar results could be achieved in unmaintained swimming pools within the Greater Los Angeles County Vector Control District (District). With numerous unmaintained swimming pools, a great amount of time, effort, and resources are expended treating and inspecting these pools, and if a more effective, longer lasting option were available, those resources could be redirected to serve the residents of the District more efficiently.

Methods

We selected six pools in our district for this study: two were full, two half full, and two quarter full. Pretreatment larval collections were made by dipping each pool to gauge larval activity. We then administered Sumilarv 0.5G 10 gram water-soluble packets that were made in-house. Post-treatment, twenty-five pupae and/or fourth instar larvae (if pupae were not abundant in the pool) were collected on a weekly basis. Each sample was placed in a pint sized glass jar that was labeled with a corresponding date and address, then fitted with netting, and monitored to record adult emergence.

Results

Results varied among pools. We did not observe adult emergence in one of the full pools until day 70, whereas the other full pool had 86% emergence on day 21. In our half-full pools, we had 100% control until days 49 and 70. One of the quarter-full pools had a markedly high concentration of organic matter which may have impacted the efficacy of the pyriproxyfen and contributed to the 100% emergence observed at 28 days post treatment, warranting a re-treatment at day 35. The second quarter full pool dried up at day 50, but prior to that, we consistently observed 15% or less emergence.

Conclusion

We found that Sumilarv 0.5G did not surpass other products on the market that are currently in use District-wide. We plan to repeat this study with some changes in protocol during the coming season. We would like to include some field control sites to compare larval population density and emergence success. These control pools will be far enough away from our test sites to ensure no cross contamination due to autodissemination of the pyriproxyfen. A pupicide will be applied once emergence is observed in the control pools. The study design will move away from quarter full pools and only utilize full pools to ensure the swimming pools do not dry by mid-season.

Acknowledgements

The authors would like to thank the Greater Los Angeles County Vector Control's Operations department for their collaboration on this study.

An overview of the Chironomid Midge Management Program at Greater Los Angeles County Vector Control District

Rande M. Gallant*

Greater Los Angeles Vector Control District, Santa Fe Springs, CA 90670

*Corresponding author: rgallant@glacvcd.org

Abstract

The Greater Los Angeles County Vector Control District (GLACVCD) Chironomid Midge Management Program relies upon a system of surveillance and inter agency collaboration to maintain non-biting midge populations below nuisance levels. Although GLACVCD does not usually perform control measures, it advises local water management entities on control options such as water rotation and dry-down periods, as well as pesticide application options to maintain acceptable midge abundance levels. The Chironomid Midge Management Program employs a multi-faceted surveillance approach to determine midge population levels, both directly at the source as well as in affected communities, to allow for rapid identification of problem areas and to provide informed abatement strategy recommendations to responsible agencies. The current paper will discuss the use of Chironomid Midge surveillance equipment and strategies as they apply to a number of managed sites within the district service area.

INTRODUCTION

Among the objectives consistent with its mission, the Greater Los Angeles County Vector Control District (GLACVCD) engages in the control of mosquitoes, black flies, and chironomid midges. Although nonbiting midge populations do not pose a disease risk to residents like mosquitoes, severe midge blooms can create intense nuisance situations, lead to health issues due to individuals' allergic reactions to insect scales, and cause economic loss to local business such as automotive paint shops, meat packing plants or bakeries (Ali 1991). Although many of the district's activities involving mosquitoes and black flies include the use of pesticides, the district generally approaches midge control with the intent to assist third-party agencies in their control efforts on the properties they own. As such, the district acts in an advisory capacity initiated by a developing local crisis, typically brought to our attention by residents within close proximity of a source such as a spreading basin. , The intent of the GLACVCD program is to act preemptively by monitoring midge activity at both the source as well as in adjacent neighborhoods, so that control efforts may be enacted prior to a negative response from the community to high midge abundance. Monitoring results are used to provide feedback to outside agency responders to direct control measures, assess results, and advise further action.

MATERIALS

To collect the most meaningful data, various trap designs and strategies were initially tested during the 2014 season. Thereafter routine chironomid monitoring began in 2015, and continues to date with the exception of the winter months during which time all equipment receives maintenance and necessary repairs. Four sampling techniques are generally used, each with a distinct sampling purpose.

1) New Jersey Light Traps. These have been placed in five locations where midge activity has presented a chronic nuisance issue (Fig. 1).

Access to a 110 volt electric power outlet is required to run these traps. Therefore, they can only be installed at a facility with an outdoor power socket that is still in reasonable proximity to the presumed chironomid breeding source. The traps are equipped with a photocell that regulates operation to nighttime hours. Each trap has been fitted with a 60 watt black lightbulb operating in a range between 350 nm to 400 nm. Chironomids have demonstrated a positive phototaxis to this light range (Kokkinn and Williams 1989), but it is not noticeable to people nearby. The traps are positioned 2 to 3 m above the ground, depending upon the placement of the outdoor power source. Midges are attracted by the light, sucked in by a fan and blown into a collecting kill jar containing a dichlorvos pest strip. Trap content is generally collected once a week. For samples greater than 500 individuals approximately 10% of the collection is removed and counted. This portion as well as the whole collection are weighed and the weight of the subsample is used to extrapolate the total collection count. Any non-midge material in the collection is considered 'matrix' and is weighed along with sample. The resulting counts are tracked in a spreadsheet.

2) Floating Emergence Traps. These are of a pyramidal design, with a PVC frame (Fig. 2).

Figure 1.—New Jersey Light Trap.

The base is $1m^2$ and remains afloat via foam "pool noodles." The legs are 30 inches long and support a receptacle at the top for a plastic collection jar. Each side of the pyramid is covered in a panel of fine mesh (1mm) insect netting that attaches to the frame with hook and loop strips. The entire device is collapsible and can be placed in a nylon tote bag for transport and storage. The collection jar

Figure 2.—Floating Emergence trap.

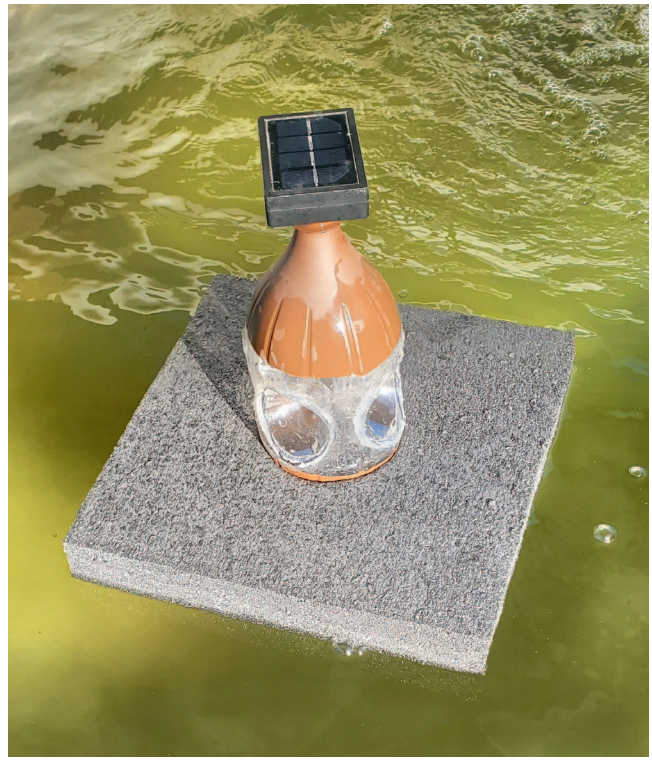


Figure 3.—Portable Gravid/Light Trap.

contains a vegetable glycerin solution in which the insects are trapped upon passing through a tube inserted through the bottom of the jar. The jars are generally swapped out once per week, the contents taken back to the laboratory and transferred into a PVC half pipe for counting.

3) The Portable Gravid/Light Trap (PGLT) This trap consists of a floating lantern produced with a 2L soda bottle fitted with solar batteries and an LED light that illuminates the trap throughout the nighttime (Fig. 3).

Four funnels within the sides of the bottle allow entry to the trap, where the insects come into contact with a 50% vegetable glycerin solution. The solution can be transferred to a tray for study. Gravid female midges tend to lay egg masses in the solution, which can then be identified with a dichotomous key (Morrow et al. 1968). The PGLT was conceived after observations were made in which midge flies collected in emergence traps differed from those collected in the nearby New Jersey Light Traps. This trap may detect midge flight activity where other traps cannot be placed. On the spreading basins, there is the added benefit of sampling species that tend to arise from different depths than those beneath the emergence trap position.

4) The Ghost Shrimp Pump. This is a plunger and tube PVC device which collects a sediment core sample 2 inches in diameter (Fig. 4).

This allows for a consistent sample draw, which can then be examined in a tray for larval chironomids. This

Figure 4.—Ghost Shrimp Pump.

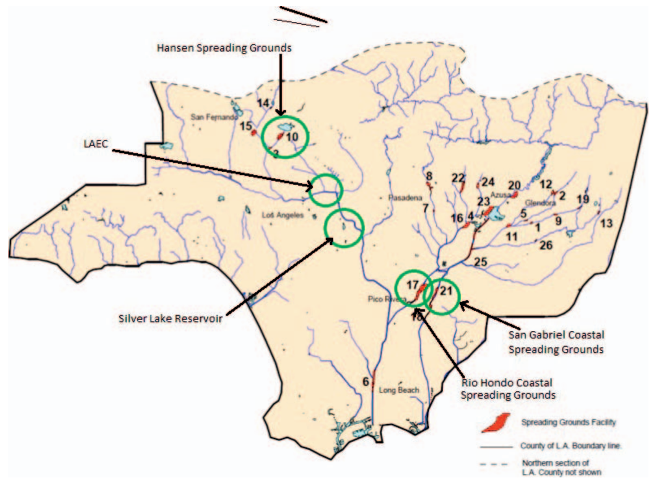


Figure 5.—Los Angeles County map showing GLACVCD chironomid midge monitoring sites. (Modified from https://dpw.lacounty.gov/wrd/SpreadingGround/SpreadingGroundMap.pdf. Date Accessed: April 1, 2021.

instrument can be used to determine sources of midge fly activity as well as for pre-and post-treatment evaluations.

METHODS

Five sites with chronic chironomid midge nuisance issues were monitored weekly (Fig. 5).

Three were under the jurisdiction of the County of Los Angeles Department of Public Works (LADPW): Rio Hondo Coastal Spreading Grounds, San Gabriel Coastal Spreading Grounds in Pico Rivera, and Hansen Spreading Grounds in the Eastern San Fernando Valley. LADPW uses the spreading grounds as percolation basins to recharge groundwater with storm runoff. These basins are periodically flooded and then allowed to dry. Water levels may fluctuate by several feet, but the shallow depth of the basins dictate that conditions are generally littoral. Midge problems occur when the basins retain water for extended periods during warm weather. Control efforts commence with midge abundance monitoring conducted by GLACVCD Scientific and Technical Services (Sci-Tech) staff, with the results relayed to one of the Public Works Principal Civil Engineering Technicians. The latter have the authority to decide about water movement within the spreading grounds, and upon GLACVCD advice, will usually transfer water into dry basins to allow the dry-down of the problematic basins to prevent further emergence and reduce midge nuisance.

Another problematic midge site is the Los Angeles Equestrian Center (LAEC) in Burbank adjacent to the north bank of the Los Angeles River and bordered to the east by the Burbank Western Channel. Sediment deposited by slow moving portions of the river and channel create habitat for chironomid midges.. Once the problem the midges caused for riders and horses was brought to GLACVCD's attention, a number of strategies were considered, including the use of the pesticide VectoBacl2AS® [active ingredient: *Bacillus thuringiensis var. israelensis* (B.t.i.)], because this product is already in use in the Los Angeles River for the control of black flies (*Simulium*). However, the high concentration needed midge larval control made this impractical. Ultimately it was determined that the best approach would be to identify all larval bearing sediment and remove it on a routine maintenance schedule.

The final site is the Silver Lake Reservoir complex which contains both Silver Lake and Ivanhoe reservoirs, maintained by the LADWP. The reservoirs have been decommissioned, but are maintained as community water features. Consequently water persists year round and midge larvae thrive in the accumulating sediment. Due to the permanent nature of the waterbodies, water rotation and dry-down periods have not been considered as control options and water surface agitation features are currently not part of the design. Larvicide driven control options will be costly and labor intensive, so it remains to be seen which approach LADWP choses once resident complaints rise to a level that demands action.

For surveillance, NJLTs are deployed throughout the warm months at all sites, while the floating emergence traps are only used at Rio Hondo Coastal Spreading Grounds and Hansen Spreading Grounds. PGLTs are deployed as needed to study unexpected midge fly occurrences. Collections are retrieved weekly, when possible. Data collected from the field includes: date, collector, site, water condition, and other observations including the presence of natural chironomid predators, such as Cliff Swallows (Graber et al. 1972). Water condition describes whether the basin is dry, filling, fully wet or partially ponded. Back in the lab, data recorded includes the count of all midge flies in each trap, the number of trap-nights, and the notable presence of other insects such as mosquitoes, caddis flies, and moths. Due to the extreme complexity of chironomid taxonomy, and because it is not essential for the success of this control program, collections are not identified to species at this time, but rather categorized and recorded by size as small, medium, or large. A "large" individual constitutes anything$>$6.4mm,"medium" 3.2 – 6.4 mm and small$<$3.2

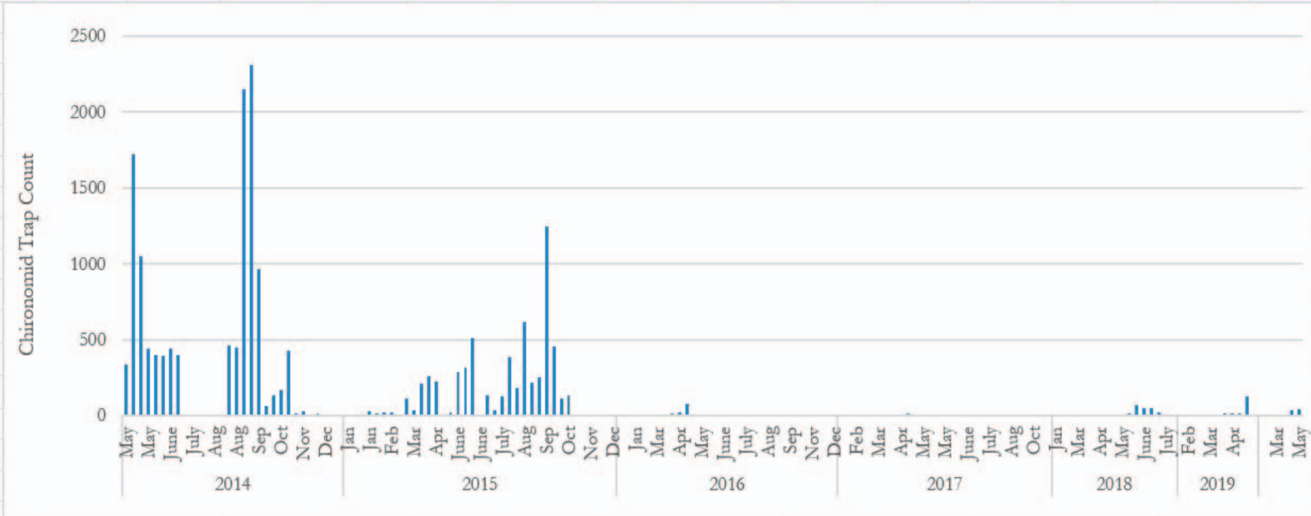


Figure 6.—Chironomid midge trap counts at Los Angeles Equestrian Center. During the 2015 season, spikes in abundance guided continuing efforts to seek out and remove sources of larval bearing sediment. By November 2015, all productive sources were identified.

mm. The purpose for this characterization is to determine whether there is a bias toward any specific size in relation to midge nuisance calls.

RESULTS

Both NJLTs and Floating Emergence Traps were good indicators of elevated chironomid midge activity, however, differences in appearance of the midges collected between each indicate that it cannot be assumed that those individuals collected in NJLTs originated at the site of the corresponding emergence traps. These differences in collection could be the result of a multitude of factors such as differences amongst species in phototaxis, wind speed and direction, changes in water depth, as well as distance between the NJLT and the respective water source. These discrepancies called for the creation of the PGLTs, which demonstrated their usefulness during the first week of August 2020 when one of them collected over 2000 chironomids while the emergence trap several feet away caught none.

The coastal spreading grounds at Rio Hondo and San Gabriel River are in close proximity to residential, commercial, and industrial properties. As such, it is critical that some form of chironomid control be maintained to minimize the nuisance and economic impact at these locations. Since the inception of the current program, chironomid midge abundance at these basins has been maintained below nuisance levels as determined by the lack of resident complaints without the use of pesticides. LADPW is consulted whenever midge counts begin to increase in a wet basin, and the water is shunted to a different basin allowing the first to dry down. In the early part of the season Cliff Swallow (*Petrochelidon pyrrhonota*) presence has proved to be a good indicator of heavy midge activity over a basin, and upon advice from GLACVCD staff, personnel at LADPW now remove the water from a basin when a number of these birds are observed feeding above the surface. Swallow presence peaks during the month of May (iNaturalist 2021); however, after which their usefulness as an indicator of midge activity decreases.

At LAEC constant communication between Sci-Tech staff and the vector control specialists allowed for the identification and removal of all significant chironomid larval sources after the 2015 season (Fig. 6).

Since that time, midge activity at the facility has been maintained at very low levels through continuous sediment management.

The Silver Lake Reservoir Complex remains problematic due to the inability of LADWP to shift water out of the reservoir basins. LADWP staff have stated that in the past, they have resorted to barrier treatments as necessary, and generally rely on community feedback as a trigger to take action.

DISCUSSION

Since the implementation of the program, it has been apparent, that each site must be treated in its own unique way. Each has its own set of characteristics and any attempt to standardize the program as a whole was met with futility. The key to successful midge emergence management at these facilities has been to become familiar with each site and seek consistency in respect to monitoring and treatment. Differing conditions include factors such as water movement and depth, prevailing wind speed and direction, the distance between a chironomid larval source and the closest outdoor power supply available for the New Jersey light trap, human disturbance, and disruptive wildlife.

Challenges to both aquatic traps include the establishment of webs of Long-jawed Orb Weaver spiders (*Tetragnatha sp.*) over the openings, and chewed tether lines by Coyotes (*Canis latrans*). The coyote problem was solved by applying ghost pepper sauce to the tether lines early in the season. No easy solution to the spider web issue has been found aside from frequent and thorough cleaning and removal of spiders and webbing, especially during the latter half of the season when they are most abundant. Other issues involve tearing of the emergence trap netting by turtles and water fowl (observed), which led to the development of independent replaceable side panels so that only one side of the trap required replacement if torn.

NJLTs are a good indicator of the midge nuisance level experienced by residents in nearby communities, but the source cannot be assumed without direct verification, especially in respect to spreading basins as they are typically associated with a nearby river and related drainage systems. They are sturdy traps and receive little damage from exposure to the elements, however, debris should be removed from the trap and the light bulbs tested with each visit. Although chironomids are attracted to the ultraviolet end of the light spectrum, it is useful for the bulb frequency to include some visible light to be able to determine if the lightbulb has burned out when observing it in daylight.

Monitoring midge abundance will continue in the future, just as communication with third party property managers continues to be critical in maintaining midge abundance at pre-nuisance levels. Trap counts will be regressed against variables such as time of year, temperature, time since inundation of a basin, and moon phase, in an effort to seek predictive modeling to aid in preemptive maintenance. Observations will continue in regards to natural predators in hopes of discovering other indicators of midge activity to fill in the gap vacated by the Cliff Swallows in the latter half of the season.

The effort to correlate chironomid midge nuisance levels by species size has not yet been realized due to insufficient data at this time. Since the outset of this program, the number of midge complaints has decreased, thereby denying us the opportunity to come to any conclusions regarding trap counts. Likewise, the Ghost Shrimp Pump has not been put to much use due to a lack of pre- and post-treatment evaluation necessity.

The PGLT trap is new and its potential may not yet be fully realized. It performs well over water, however, it is hoped that it will be just as effective on land. Studies show that horizontally polarized light produces a much stronger attraction to midges, presumably mimicking a turbid, nutrient rich water source for oivposition (Lerner et al. 2008). Incorporating a polarizing filter into the PGLT might provide the same draw as would a water source, even when the trap is on dry land.

ACKNOWLEDGEMENTS

My sincere gratitude to Susanne Kluh, Steve Vetrone, and Paul O'Connor for their direction and guidance throughout the development of this program and paper, and to Faiza Haider, Randy Hannie, Jessica Pena and all other GLACVCD personnel who have assisted me in the preparation and installation of field equipment necessary for this work.

REFERENCES CITED

ALI, A. 1991. Perspectives on management of pestiferous Chironomidae (Diptera), an emerging global problem. J. Am. Mosq. Control Assoc. 7: 260-281.

(LADPW) County of Los Angeles Department of Public Works. https://dpw.lacounty.gov/wrd/SpreadingGround/SpreadingGroundMap.pdf. Date Accessed: April 1, 2021.

Graber, R. R., J. W. Graber, and E. L. Kirk. 1972. Illinois Birds: Hirundinidae**.** Biological Notes No. 80. Illinois Natural History Survey. State of Illinois Department of Registration and Education. Urbana, Il. Natural History Survey Division.

Horvath, G. 2014. Polarized Light and Polarization Vision in Animal Sciences. Springer. (pg. 126).

iNaturalist. https://www.inaturalist.org/taxa/11858-Petrochelidon-pyrrhonota. Date Accessed: April 1, 2021.

Kokkinn, M. J., and W. D. Williams. 1989. An experimental study of phototactic responses of *Tanytarsus barbitarsis* Freeman (Diptera: Chironomidae). Australian J. Marine Freshwater Res. 40: 693–702.

Lerner, A., N. Meltser, N. Sapir, C. Erlick, N. Shashar, M. Broza. 2008. Reflected polarization guides Chironomid females to oviposition sites. J. Exp. Biol. 211: 3536–3543

Morrow. J. A., J. L. Bath and L. D. Anderson. 1968. Descriptions and key to egg masses of some aquatic midges in southern California (Diptera: Chironomidae). Calif. Vector Views l5:99-108.

PacVec's assessment of regional training needs related to vector-borne diseases

Celia Chen[1]*, William Walton[2], and Christopher M. Barker[1]

[1]University of California, Davis, CA, 95616
[2]University of California, Riverside, CA, 92521

*Corresponding author: celchen@ucdavis.edu

Introduction

The Pacific Southwest Center of Excellence in Vector-Borne Diseases (PacVec) aims to increase the capacity of the southwestern United States and Pacific Islands to respond to vector-borne disease threats. Training is a key pillar of PacVec's programs, and the center plans to develop a short-course training curriculum for vector control and public health professionals in the region. To identify the most pressing needs, PacVec conducted a needs assessment in late 2020 to understand the perceived gaps in training relating to vector-borne disease and public health throughout the region.

Methods

The needs assessment was developed and distributed to vector control and public health professionals working in state, county, and local vector control and public health agencies in the Pacific Southwest (including California, Arizona, Hawaii, Nevada, Utah, and US-Affiliated Pacific Islands) using the online survey tool Qualtrics. There were 28 questions in the needs assessment question bank: 10 were optional, 14 were required, and 4 questions were presented to participants depending on how they answered the required questions. The needs assessment listed six modules containing vector-borne disease training topics for participants to rank the relevance of each topic using the Likert scale. It also assessed participants' training delivery preferences and potential module attendance. The needs assessment was open for participation and advertised widely for four weeks including a 1-week extension. Advertisement consisted of social media postings (Twitter and Facebook), direct email announcements to PacVec subscribers, and inclusion in PacVec, MVCAC, and other associations' periodic newsletters.

Results and Discussion

The resulting sample size was 94 participants, with 55 who answered on behalf of their agency or a group within their agency and 39 who answered for themselves. Most participation came from California (n=31) and Arizona (n=29), followed by Nevada (n=10), US-Affiliated Pacific Islands (n=10), Utah (n=8), other (n=4), and Hawaii (n=2). Participants who stated "other" are from Navajo Nation, Wyoming, Maryland, and air force worldwide. Most participants identified themselves as agency managers (n=30) or senior staff (n=30), followed by other (n=10), field staff (n=9), researchers (n=7), laboratory staff (n=4), and students (n=4). Participants who identified as "other" were epidemiologists, presidents of boards of trustees, public information or technological officers, or a small number who did not specify their position.

Of the 94 participants, 86 participants completed the entire section regarding the relevance of module topics. Topics deemed most relevant for training from each module were as follows:

1. Mosquito-borne diseases of the Pacific Southwest
2. Host Biology (vertebrate host dispersal, life cycles, behavior, etc., as relevant to transmission of vector-borne pathogens)
3. Epidemiology of Vector-Borne Diseases (major regional diseases and emerging threats)
4. Mosquito Control Practices (e.g., IMM/IVM, adulticiding, larviciding, source reduction, biological control, defining thresholds)
5. Collection and Processing of Vectors (review of trap types, sampling methods, handling specimens, and applying techniques in multiple settings)
6. Vector-Borne Disease Communication Approaches (best practices and approaches to communicating with the public, press, and policy makers on vector-borne disease topics)

Of the 86 participants who ranked the relevance of module topics, 82 reported their likelihood of attending each training module. Most indicated their attendance to be extremely likely across all six modules. Most participants (n=43) indicated that they are extremely likely to attend module 1, followed by module 2 (n=41), module 3 (n=40), module 4 (n=40), module 6 (n=38), and module 5 (n=33). In regards to training delivery, participants indicated that a web-based, series format – a training on a single weekday in each of a series of weeks – would be ideal. In terms of time preference, results reflect that most participants preferred earlier times, with the top choice being 8-11 AM Pacific Time (n=38).

Conclusions

Information gained from the needs assessment will be used to develop a PacVec short-course training curriculum to be initiated in 2021 to meet the needs of vector control and public health agencies and to expand the potential recruitment pool for the vector control workforce. Based on the needs assessment, conversations with agencies, and the COVID-19 pandemic, PacVec plans to host the training as a web-based series of 2-3 hour modules.

Acknowledgements

We thank the members of the Mosquito and Vector Control Association of California and other partner agencies and associations within the Pacific Southwest who responded to this needs assessment and help us to realize the collaborative goal of making our region more resilient against threats from vector-borne diseases. This study was enabled by financial support from the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement U01CK000516).

Relating mosquito collection effort to the reliability of West Nile virus risk estimates

Karen M. Holcomb[1,2]* and Christopher M. Barker[1,2]

[1]Davis Arbovirus Research and Training Laboratory, Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616
[2]Pacific Southwest Center of Excellence in Vector-Borne Diseases, University of California, Davis, CA 95616

*Corresponding author: kmholcomb@ucdavis.edu

Introduction

Integrated vector management to prevent human infections with West Nile virus (WNV) is guided by entomological indices derived from surveillance (Gubler et al. 2000, Barker 2019). Due to time and resource constraints, these indices often are calculated using inadequate data, with little consideration given to the uncertainty around the estimated value. Understanding the associations between surveillance effort and reliability of calculated entomological indices of arbovirus activity is essential for optimizing allocation of available surveillance resources to guide control. However, there are no specific guidelines on the trapping effort (i.e., total collection events or mosquitoes tested) necessary to achieve a desired level of certainty in these estimated indices. In this study, we considered the effects of sample size on the estimated WNV infection rates in mosquitoes necessary to achieve certainty about the risk levels defined in the California Mosquito-Borne Virus Surveillance and Response Plan (CDPH et al. 2020) under a range of real-world scenarios.

Methods

We simulated typical mosquito collection and testing by a surveillance program across a range of background infection prevalence (0.5-20 infected females per 1,000 tested) to describe the relationship between trapping effort (number of trap collections and total numbers of mosquitoes tested) and the accuracy of the resulting risk estimate, using the risk levels defined in the California Mosquito-Borne Virus Surveillance and Response Plan (CDPH et al. 2020). We also determined statistical measures of diagnostic performance, including WNV detection probability, sensitivity (probability that the simulated risk estimate is high enough), and specificity (probability that the simulated risk estimate is not too high), based on the level of trapping effort and background infection prevalence. We then estimated the accuracy, detection probability, and underestimation resulting from low, medium, and high intensities of collection effort under contrasting seasonal patterns of typical mosquito and WNV activity in the Sacramento and Coachella Valleys to assess risk estimation performance under the range of field conditions present in California.

Results and Discussion

We found that across all simulated infection prevalence, risk estimates often underestimated the true level of risk with a large proportion of underestimation resulting from a failure to detect WNV, especially at low levels of collection effort. Overestimation of WNV risk was less common than underestimation. The probability of detecting WNV rapidly increased with increasing collection effort, but a larger number of collections were required to achieve accuracy of risk estimates at each level of effort. Similarly, sensitivity increased as collection effort increased, reflecting the increase in precision with increased data. In contrast, specificity remained high across levels of collection effort, incorporating the relationship of accuracy and underestimation with risk estimation.

Simulations of realistic patterns of mosquito and viral dynamics in the Sacramento and Coachella Valleys indicated that accuracy of risk estimates is particularly low during periods of low vector abundance, even during periods of high infection prevalence. To achieve a consistent level of reliability, greater collection effort was required during the early-season periods of low infection prevalence as compared to the middle of the season when infection prevalence is typically elevated.

Conclusions

Underestimation of the WNV risk is more likely than overestimation, especially during periods of low abundance or infection prevalence. Therefore, adaptive sampling (i.e., increased collection effort in periods with low abundance or infection prevalence) is needed to improve the reliability of risk estimates during the early and late margins of the season. A future direction is the development of an online app for vector control and public health to assess the relationship between collection effort and reliability of risk estimates. Quantifying the uncertainty in risk estimates derived from surveillance will guide effective vector control strategies to prevent viral amplification, thereby protecting human health.

Acknowledgements

We would like to thank all the Davis homeowners who hosted a mosquito trap during the summer of 2019; the data from that study was the inspiration for this study. We acknowledge funding support from the Pacific Southwest Center of Excellence in Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516). The project was also supported by the National Center for Advancing Translational Sciences, National Institutes of Health, through grant number UL1 TR001860 and linked award TL1 TR001861. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

References Cited

Barker, C. M. 2019. Models and surveillance systems to detect and predict West Nile virus outbreaks. J. Med. Entomol. 56: 1508-1515.

(CDPH) California Department of Public Health, Mosquito and Vector Control Association of California, and University of California. **2020**. California mosquito-borne virus surveillance and response plan. https://westnile.ca.gov/download.php?download_id=4502

Gubler, D. J., G. L. Campbell, R. Nasci, N. Komar, L. Petersen, and J. T. Roehrig. **2000**. West Nile virus in the United States: guidelines for detection, prevention, and control. Viral Immunol. 13: 469–475.

The development of a spatially resolved ensemble forecast model of West Nile virus transmission in the Coachella Valley, CA

Matthew J. Ward[1]*, Meytar Sorek-Hamer[1], Jennifer Henke[2], Krishna Vemuri[3], Nicholas DeFelice[3]

[1]Universities Space Research Association (USRA) at NASA Ames Research Center, Moffett Field, CA 94043.
[2]Coachella Valley Mosquito & Vector Control District, Indio, CA 92201.
[3]Environmental Medicine and Public Health, Icahn School of Medicine at Mount Sinai, NY, NY 10029.

*Corresponding author: mjwa88@gmail.com

Introduction

West Nile virus (WNV) is the leading cause of domestically acquired arboviral disease in the continental United States and exhibits considerable inter-annual and geographical variation in transmission (Davis et al. 2008). Although transmission of WNV exhibits a pronounced sensitivity to a complex seasonal ecology, our ability to predict in real-time the timing, duration and magnitude of local WNV outbreaks remains limited (DeFelice et al. 2019). As a consequence, effective allocation of public health resources is challenging and often reactive, a circumstance highlighting the need for additional environmetal variables to help define fine spatial scale real-time estimates of local transmission. Here, we report on our ongoing development of a spatially refined model that uses ECOsystem Spaceborne Thermal Radiometer Experiment on Space Station (ECOSTRESS) data to capture the variability in micro-climates across the Coachella Valley, CA and incorporates it into a graphical model describing local WNV transmission dynamics.

Methods

Mosquito pool infection data was obtained for the years 2006 though 2020 from the Coachella Valley Mosquito and Vector Control District (CVMVC). This data was used to calculate the maximum likelihood estimates (MLE) of the WNV infection rate at the annual time step and different remote sensing platform scales. Model testing is currently underway using remote sensing variables including temperature, precipitation, specific humidity and evapotranspiration from NLDAS, HRRR and ECOSTRESS platforms (Table 1). *Culex* species are currently aggregated but we plan to disaggregate between *Cx. quinquefasciatus* and *Cx. tarsalis* for further model testing. Statistical analysis is being preformed using R version 4.0.3 (R Core Team 2018).

Results

The MLE indicates variability among years and geo-spatial areas across the 15 years under analysis (Figures 1, 2), while also demonstrating an average peak infection rate in the Coachella Valley between weeks 26 - 38 each year (Figure 3). This is in contrast with mosquito abundance data where we observe distinct bimodal peaks on either side of the peak in infection rates (Figure 3). Additionally, mapping the evapotranspiration (ET) measurements from ECOSTRESS shows distinct seasonality as well as consistent hydrology indicative of agricultural irrigation that may provide sources for mosquito production and WNV amplification during the hot and dry summers of the

Table 1.—Climate and hydrological variables, their resolutions and platforms.

Variable	Platform	Sensor/Model	Spatial Resolution	Temporal Resolution
Mosquito abundance	CVMVC surveillance	Trap data	~ 81 traps per week	Weekly (~ 45.2 weeks)
WNV incidence (mosquitoes)	2006 - 2020	Pooled Testing		
Soil moisture	Radar	HRRR	3 km x 3 km	Hourly
Atmospheric ambient temperature				
Precipitation				
Specific humidity				
Land surface temperature	Terra & Aqua (MODIS)	MOD21_L2 (Terra)	1 km x 1 km	Daily
		MYD21_L2 (Aqua)		
Evapotranspiration	ISS	ECOSTRESS	70 m x 70 m	~4 days
Evaporative stress index				

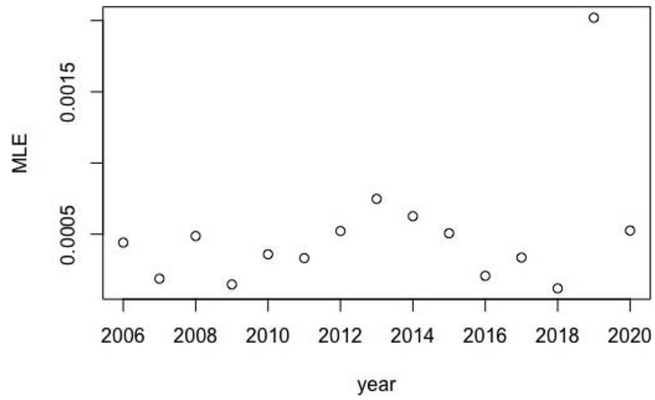


Figure 1.—WNV mosquito infection rates (MLE) for each year of available data from the Coachella Valley, CA.

Coachella Valley (Figure 4). This is made apparent by the darker blue areas that indicate consistently higher ET throughout the year while the ET in the rest of the valley fluctuates.

ECOSTRESS's near real-time space-based remote sensing observations provide a new set of highly resolved fine-scale environmental observations that may be used to further inform existing and future WNV forecast models. The inclusion of ECOSTRESS' high spatial resolution (70 m^2) and highest re-visit frequency (1-5 days) thermal infrared data in the state space model should provide structure and allow us to capture tipping points within micro-ecosystems that explain the observed biology. The model-inference system can then be used to better understand the spatial variability of amplification and the relationship between zoonotic amplification and potential human spillover events in near real-time. This work is an initial step in the development of a statistically rigorous framework for spatially resolved indicators of WNV risk.

Conclusions

WNV exhibits considerable inter-annual and geographical variation making the effective allocation of

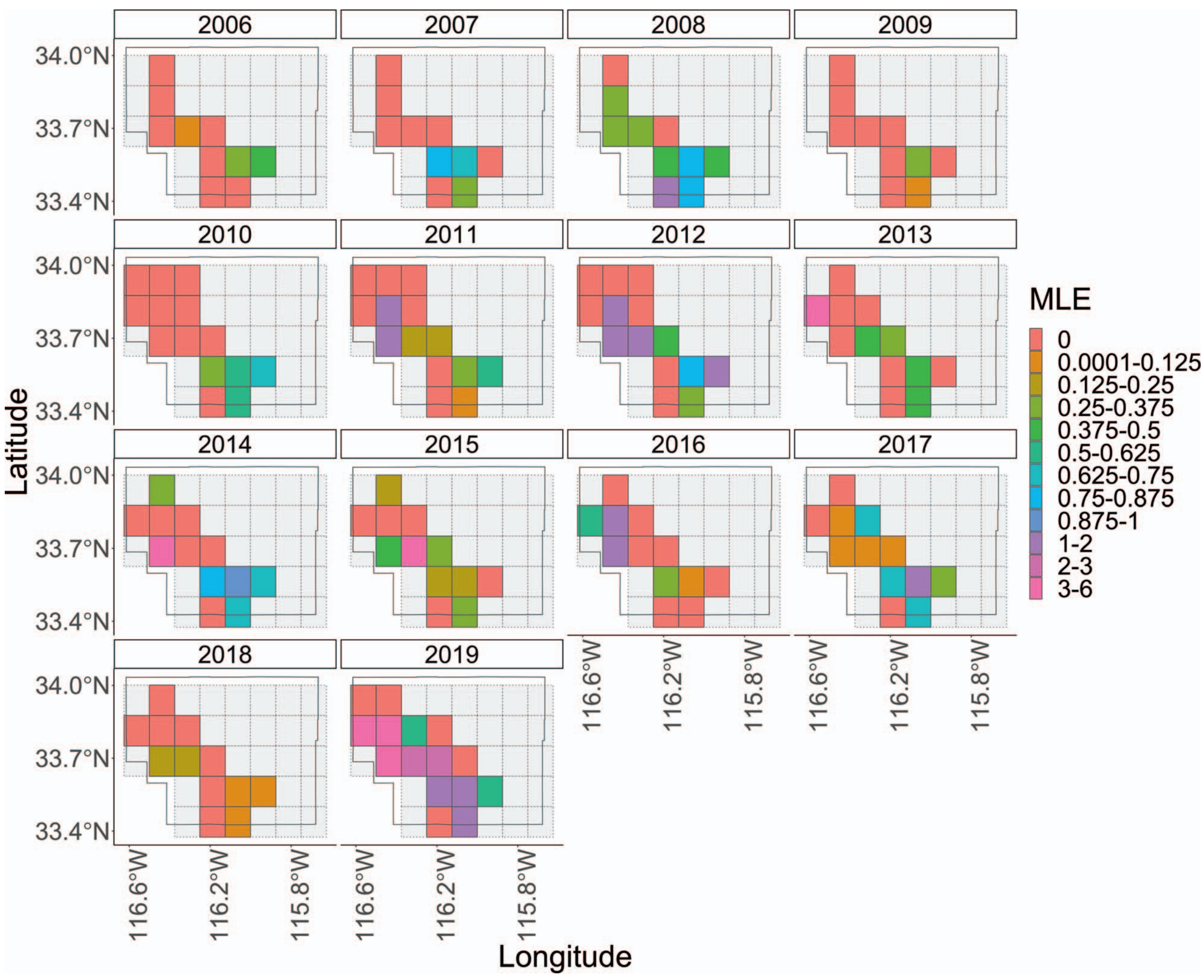


Figure 2.—Historical annual mosquito infection rates (MLE) stratified by NLDAS grid across the Coachella Valley, CA.

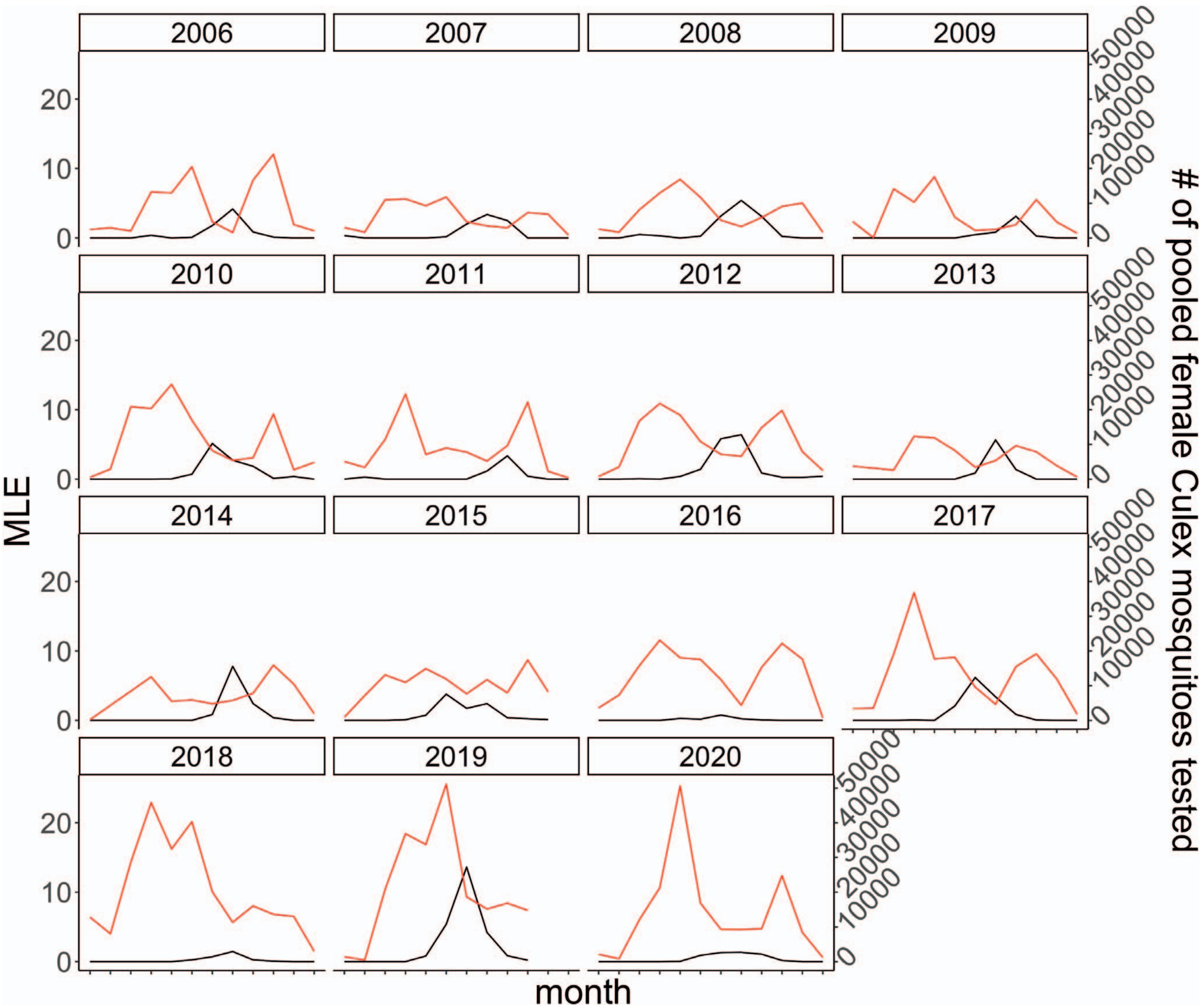


Figure 3.—Monthly infections per 1,000 of *Culex* mosquitoes tested (MLE) (black) and the total number of female *Culex* mosquitoes tested (red) in the Coachella Valley, CA for the years 2006 - 2020. *Cx. quinquefasciatus* and *Cx. tarsalis* are combined.

public health resources challenging, highlighting the need for accurate forecasts of WNV transmission. Additionally, mosquito control interventions are traditionally informed by trap-level monitoring. By integrating trap-based surveillance with fine-scale remote sensing, we hope to provide better spatial resolution for more informed control efforts. Remote sensing products such as ECOSTRESS, which provide near real-time fine scale observations of potential mosquito sources and WNV amplification sites hold the potential to provide the information needed to facilitate highly targeted and effective mosquito control interventions. Such decision support tools would help stakeholders target control of infectious mosquito populations and activate public health interventions in a more timely and economical fashion.

Acknowledgements

We would like to thank the CVMVC district staff for the use of their data, field work and operational research funding (141316E1-CFDC-4B63-9418-DEDB9AE8BBDB). Additoinally, we would like to acknowledge our funding support for this project including the Pacific Southwest CoE Training Grant Program (1U01CK000516) funded by the Centers for Disease Control and Prevention (CDC). Contents are solely the responsibility of the authors and do not necessarily represent the official views of the CDC or the Department of Health and Human Services. The CDC had no role in the design of the study, the collection, analysis, and interpretation of data, or in writing the manuscript. Additional funding was provided by NASA Health and Air Quality Applied Science Program, NASA Grant ECOSTRESS18-0046 and

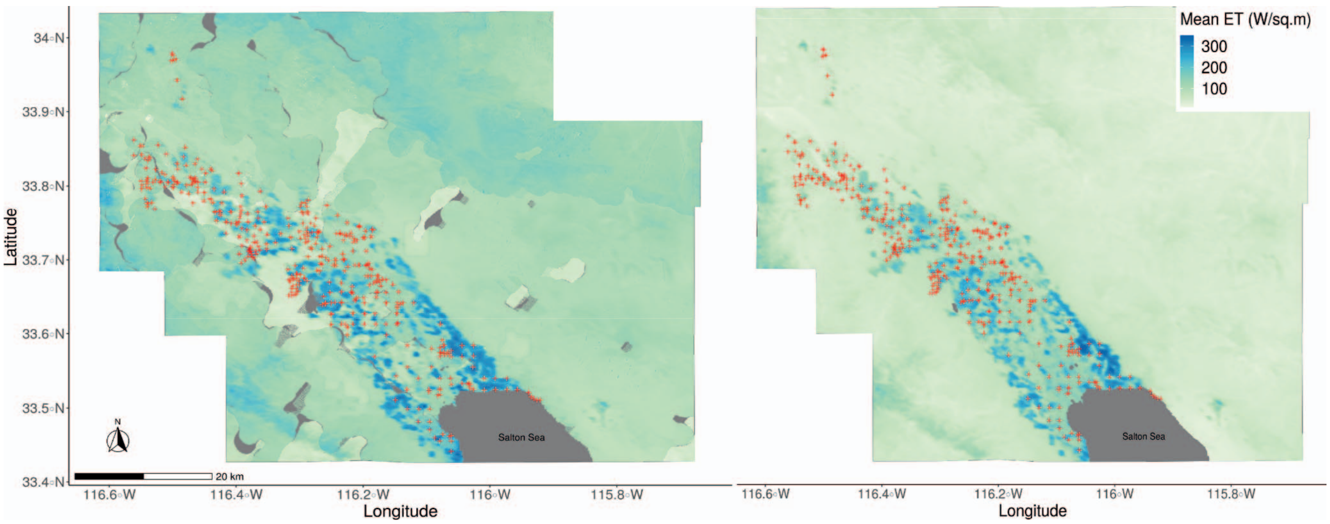


Figure 4.—Mean ET (W/m^2) as measured by ECOSTRESS in the Coachella Valley, CA during the early season (March - May) (left panel) and late season (June - Aug) (right panel) with trap locations (red x) for 2019.

NASA/USRA's Environmental Analytics Group. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

Davis, L. E., J. D. Beckham, and K. L. Tyler. 2008. North American encephalitic arboviruses. Neurol Clin 26: 727-757, ix.

DeFelice, N. B., R. Birger, N. DeFelice, A. Gagner, S. R. Campbell, C. Romano, M. Santoriello, J. Henke, J. Wittie, B. Cole, C. Kaiser, and J. Shaman. 2019. Modeling and Surveillance of Reporting Delays of Mosquitoes and Humans Infected With West Nile Virus and Associations With Accuracy of West Nile Virus Forecasts. JAMA Netw Open 2: e193175.

R Core Team. 2018. R: A Language and Environment for Statistical Computing computer program, version By Team, R. C., Vienna, Austria.

Assessing changes in the greenness of neglected swimming pools in Fresno, California, through a time series of satellite images

Matteo Marcantonio*, Christopher M Barker

Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, California 95616

*Corresponding author: marcantoniomatteo@gmail.com

Introduction

Synanthropic mosquito species exploit available habitats such as neglected swimming pools, whose presence has been associated with the above-average incidence of disease in humans caused by West Nile virus in urbanized areas (Reisen et al. 2008). Swimming pools that lack effective filtration systems, circulation pumps, or sanitation during the warmer parts of the year can rapidly become highly suitable for mosquito oviposition and larval development. The role played by neglected swimming pools as mosquito habitat may be more important where private swimming pool density is high and the availability of other suitable habitats is limited, such as in urbanized areas of California's Central Valley, where precipitation is limited in summer (Pkdata 2013).

Due to the hazards they pose, neglected swimming pools are important surveillance targets for mosquito control agencies. In the recent years, remotely sensed imagery has been coupled with ground inspections to locate suitable habitats for mosquitoes in urbanized areas, such as neglected swimming pools (Kim et al. 2011, McFeeters 2013, Thompson et al. 2013). However, due to the infrequent or null revisiting times of orbiting satellites or other air-borne devices, on-board instruments acquire imagery only at a singular time point "on demand" or at revisit cycles that are too infrequent for reliable detection of changes in swimming pool condition. This has limited the utility of remote sensing for mosquito control, with past studies lacking temporal resolution and the ability for documenting longitudinal changes in swimming pool conditions during the mosquito season. More recently, the temporal resolution of satellite imagery has become finer due to the development of smaller satellites coordinated in constellations that have the ability to image the entire Earth at weekly or daily frequency, while retaining a high spatial resolution.

Methods

We used a sub-weekly time series of satellite observations from the PlanetScope satellite constellation (Planet, California, USA) to derive the Normalized Difference Vegetation Index (NDVI) and: 1) determined whether this dataset could be reliably used to separate neglected pools from clear pools and 2) assessed whether inspections of neglected swimming pools by mosquito control agencies resulted in a change in pool greenness and suitability as mosquito oviposition habitats in Fresno, California.

The Consolidated Mosquito Abatement District provided us with a dataset containing the geo-location of swimming pools inspected by the district between 2013 and 2018 with detailed information on the status of each swimming pool. From this dataset we selected 30 pools which were reported green/dark for at least 3 consecutive checks in 2018 and 30 pools which were reported clear (i.e. clean and blue) during the same year (Figure 1). Afterwards, we obtained 95 cloud free multi-spectral raster tiles from the PlanetScope dataset which were used to derive NDVI values for the 60 swimming pools from the 14 March to 1 October 2018, thus covering the warmest part of the year for Fresno.

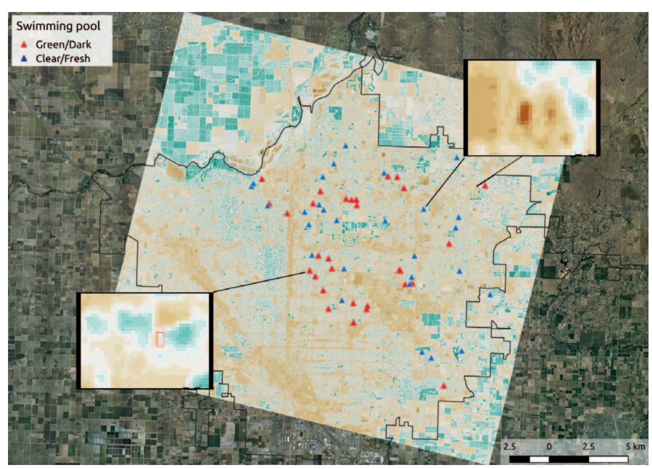


Figure 1.—Locations of clear (blue triangles) and dark/green (red triangles) swimming pools. In the background there is a true color satellite image (from GeoEye IKONOS) of the study area, whereas the overlapped square layer represents NDVI (derived from PlanetScope ortho-scenes) values on the 29th of May 2018. The two insets report examples of the NDVI signature of a dark/green (bottom-left) and a clear (top-right) swimming pool (in red).

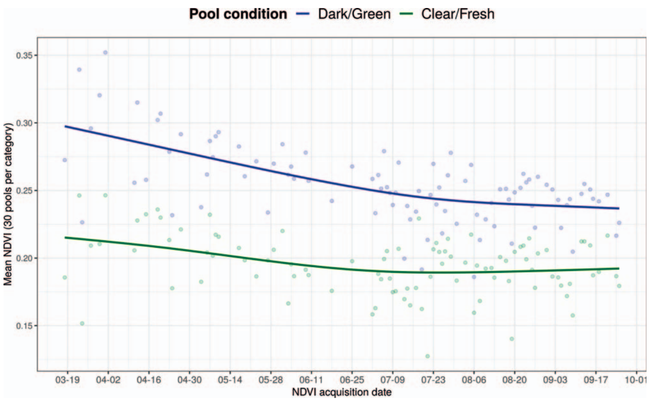


Figure 2.—Average daily NDVI values (dots) and smoothed trends (lines) for the sets of 30 dark/green (blue) and clear/fresh (green) swimming pools from March to October 2018.

Results

On average the NDVI of green pools was 0.07 (GLM 95% CI 0.03-0.11) higher than clean pools. This confirmed that the PlanetScope imagery may be used to separate neglected and clean swimming pools. Moreover, we found that the NDVI of green pools decreased during the year for green pools, but did not change for clear pools (Figure 2). This could be due to many factors, such as chemical treatments of the neglected pools by owners as the season progressed.

Subsequently, to find whether inspections carried out by the district would trigger a decrease in NDVI in neglected pools, we used a linear mixed model (LMM) in which NDVI values for swimming pools were the response variable and covariates were inspections lagged by 1, 2, or 3 dates (corresponding to NDVI acquisition dates) as well as the average temperature of the NDVI acquisition date. The pool IDs and weeks of the year were modeled as hierarchical levels (i.e., random effects). Results showed that the effect of inspections by the district was not associated with a significant change in the greenness of the pools until the second lagged date, or 9 days (on average) after an inspection, when a decrease in NDVI was found (LMM IR: -0.012, -0.02). Temperature also was negatively associated with NDVI (LMM IR: -0.017,-0.013) which may be due to restored pool conditions with warmer temperature during the year regardless of inspections by the district (Figure 3).

Conclusions

The NDVI derived from PlanetScope dataset may be a valuable index to quantify differences in greenness between neglected and clear pools, and therefore could classify pool status over time. Inspections by the mosquito control

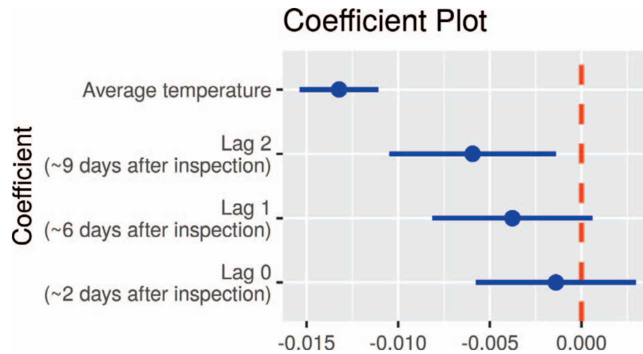


Figure 3.—Forest plot showing Linear Mixed Model coefficient values for the four regression parameters. Blue dots and lines represent model estimates and 95% CI, whereas the vertical red line indicates 0.

district were associated with a decrease in the greenness of neglected pools several days after the inspections. This latter finding could be explained by pool owners taking some time to improve conditions after an inspection.

As future directions, the high temporal frequency and daily update of Planet data could be exploited to assess the status of swimming pools throughout the year and may be used to follow green-to-clear or clear to green transitions for individual swimming pools. Moreover, other indices derived from multispectral data, such as the Normalized Difference Water Index (Gao 1996) or the utilization of single bands, should be explored as they may provide more accurate results.

Reference list

Gao, B. 1996. NDWI—A normalized difference water index for remote sensing of vegetation liquid water from space. Remote Sensing of Environment. 58: 257–266.

Kim, M., J. B. Holt, R. J. Eisen, K. Padgett, W. K. Reisen, and J. B. Croft. **2011**. Detection of Swimming pools by Geographic Object-based Image Analysis to support West Nile virus control efforts. Photogrammetric Engineering & Remote Sensing. 77: 1169–1179.

McFeeters, S. K. 2013. Using the Normalized Difference Water Index (NDWI) within a geographic information system to detect swimming pools for mosquito abatement: a practical approach. Remote Sensing. 5: 3544–3561.

Pkdata. **2013**. U.S. Residential Swimming Pool Market Report.

Reisen, W. K., R. M. Takahashi, B. D. Carroll, and R. Quiring. **2008**. Delinquent mortgages, neglected swimming pools, and West Nile virus, California. Emerg Infect Dis. 14: 1747–1749.

Thompson, D. R., M. de la Torre Juárez, C. M. Barker, J. Holeman, S. Lundeen, S. Mulligan, T. H. Painter, E. Podest, F. C. Seidel, and E. Ustinov. **2013**. Airborne imaging spectroscopy to monitor urban mosquito microhabitats. Remote Sensing of Environment. 137: 226–233.

Vector competence of Californian mosquitoes for chikungunya virus

Kasen K. Riemersma, Ana L. Ramírez and Lark L. Coffey*

Department of Pathology, Microbiology and Immunology, University of California, Davis, CA 95616

*Corresponding author: lcoffey@ucdavis.edu

Introduction

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus transmitted by *Aedes aegypti* which has circulated in the Americas since 2013 causing debilitating joint and muscle pain. Although no local transmission has been observed in California, from 2015 to 2020, there were more than 250 travel-associated CHIKV cases in Los Angeles alone (CDC 2021; CDPH 2021). Here we evaluated whether *Ae. aegypti* mosquitoes from Los Angeles are competent vectors for CHIKV (Riemersma and Coffey 2019).

Methods

We orally exposed colonized *Ae. aegypti* from Los Angeles to artificial bloodmeals containing CHIKV generated from an infectious DNA clone of IOL CHIKV outbreak strain 06–049 (Coffey and Vignuzzi 2011) using a membrane feeder apparatus in two replicate experiments. On days 5 and 12 post-feeding (dpf), we collected mosquito bodies, legs and wings (L/W) and saliva to determine infection, dissemination, and transmission rates, respectively, and viral titers in mosquito tissues using qRT-PCR (Lanciotti et al 2008). Additionally, saliva samples that were positive for CHIKV RNA were titrated by plaque assay to determine the quantity of infectious CHIKV expectorated (Baer and Kehn-Hall 2014).

Results and Discussion

At 5 dpf, infection, dissemination, and transmission rates were 36%, 26%, and 11%, respectively. At 12 dpf, infection, dissemination, and transmission rates were 80%, 64%, and 41%, respectively, which were significantly higher compared to 5 dpf. While mean CHIKV RNA titers in bodies, L/W and saliva were similar at both time points, higher levels of infectious CHIKV were expectorated at 5 dpf compared to 12 dpf (1.3 $\log_{10}$ plaque forming units [PFU] vs. 0.4 $\log_{10}$ PFU). Additionally, the percentage of RNA positive saliva samples containing infectious CHIKV as determined by plaque assay was higher at 5 dpf than 12 dpf (85% vs. 22%). This temporal decline in transmission of infectious CHIKV may be explained by antiviral mechanisms in the salivary glands, although this needs further evaluation.

Conclusions

Overall, our results indicated that *Ae. aegypti* mosquitoes from Los Angeles are competent laboratory vectors for CHIKV. Altogether, the evidence of laboratory vector competence for CHIKV, endemicity of *Ae. aegypti* and importation of infected travelers support a high risk of local CHIKV transmission in Los Angeles and possibly other areas in California.

Acknowledgements

Funding support was provided by a National Institutes of Health Ruth L. Kirschstein National Research Service Award (T32 OD O11147 to K.K.R.) and R01 AI125902 to L.L.C. A.L.R. is supported by PacVec.

References Cited

Baer, A. and K. Kehn-Hall. 2014. Viral concentration determination through plaque assays: using traditional and novel overlay systems. J. Vis. Exp. 93: e52065

(CDC) Centers for Disease Control and Prevention. 2021. CDC ArboNet. Available: https://wwwn.cdc.gov/arbonet/maps/ADB_Diseases_Map/index.html. Accessed 30 April 2021.

(CDPH) California Department of Public Health. 2021. CDPH update on number of chikungunya infections in California April 1, 2021. Available: https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/Chikungunya.aspx. Accessed 30 April 2021.

Coffey, L.L. and M. Vignuzzi. 2011. Host alternation of chikungunya virus increases fitness while restricting population diversity and adaptability to novel selective pressures. J. Virol. 85: 1025–1035

Lanciotti, R.S., O.L. Kosoy, J.J. Laven, J.O. Velez, A. J. Lambert, A.J. Johnson, S.M. Stanfield and M.R. Duffy. 2008. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerging Infect. Dis. 14: 1232–1239

Riemersma, K.K. and L.L. Coffey. 2019. Chikungunya virus populations experience diversity-dependent attenuation and purifying intra-vector selection in Californian *Aedes aegypti* mosquitoes. PLoS Negl. Trop. Dis. 13: e0007853

Proteomic analysis of the peritrophic matrix from *Aedes aegypti* fourth instar larvae

Sajleen Phagura[1,2]*, Sarjeet Gill[1,2]

[1]Environmental Toxicology Graduate Program, [2]Department of Molecular, Cell and Systems Biology, University of California, Riverside, CA 92521

*Corresponding author: sphag001@ucr.edu

Aedes aegypti is the principal vector of arboviruses (dengue virus, yellow fever virus, chikungunya virus, Zika virus and many more) throughout the tropics and subtropics globally. The best way to control virus transmission is to control the vector population. Larvicides need to cross the peritrophic matrix (PM), an acellular chitinous barrier that envelopes all ingested material, to reach the midgut cells. The PM could be modified to make the mosquito more susceptible to larvicides. Before that can happen, there is a need to understand the structural composition of the larval PM. We report for the first time, the complete PM proteome of the *Ae. aegypti* larvae. Mass spectrometric analyses identified 167 proteins, of which 23 were chitin-binding peritrophic matrix proteins. We additionally report 2 putative chitin-binding proteins identified by cysteine residues pattern and presence of glycosylation. This extensive list of peritrophic membrane proteins can be investigated further to develop control methods for the *Ae. aegypti* larvae.

Diel activity patterns of *Aedes aegypti* in suburban Madera County

Sarah T. Abusaa[1,2]*, Trinidad Reyes[3], Matteo Marcantonio[1,2], Christopher M. Barker[1,2]

[1]Davis Arbovirus Research and Training (DART) Laboratory, Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616
[2]Pacific Southwest Center of Excellence in Vector-borne Diseases, University of California Davis, Davis, CA 95616
[3]Madera County Mosquito and Vector Control District, Madera, CA 93637

*Corresponding author: stabusaa@ucdavis.edu

Introduction

Aedes aegypti were first reported in Madera County, California, in 2013 and have been targeted by surveillance and control programs since that time due to their status as pests and vectors of significant human pathogens such as dengue and Zika viruses (California Department of Public Health, 2019). Host seeking and feeding behaviors of *Ae. aegypti* have been studied at length in both laboratory and field settings, and findings from these studies have demonstrated great variability in diel activity patterns across different environments. Host availability (varying with urbanization and land use), photosensory signals (varying with ambient light created by sunlight patterns or artificial light sources), and abiotic environmental features (temperature and humidity) have been shown to be related to the timing and length of observable peaks in activity (Chadee and Martinez 2000; Focks et al. 1993; Jones, 1981; Scott et al. 2000; Smith et al. 2018; Trpis et al. 1973). Due to the recency of the *Ae. aegypti* introduction to California and the contrast between the Central Valley Mediterranean climate compared to the typical tropical and subtropical habitat at the center of origin, there is a need to understand their behavior in this hot, arid climate. Identifying the timing of host-seeking throughout the day will facilitate understanding the potential overlap between mosquito and human activity periods that result in biting exposure and provide specific timing for vector control and to inform community education projects. The aims of our study were to identify periods of greatest host-seeking activity within the day based on time-segregated trap counts of female *Ae. aegypti* and to describe the relationships between counts of adult females and the microclimates at each sampling location and time period.

Methods

Collection bottle rotator traps (John W. Hock Company, Gainesville, FL, USA) were set weekly from September 2 to October 15, 2020 at five residential sites in the city of Madera, California. Traps were baited with CO_2 (dry ice) and BG-Lure^{TM} cartridges (Biogents USA, Moorefield, WV, USA). Temperature and humidity sensors were placed adjacent to each trap. The traps were programmed for seven intervals, with collection coverage from 12:00 PM to roughly 9:30 AM the following day. Mosquitoes were identified by sex and species. *Aedes aegypti* were counted and hourly rates of *Ae. aegypti* female collection were calculated for each site. Activity curves based on the hourly rates were plotted, and peaks were related to timing of sunrise and sunset, as well as within-day temperature and humidity trends.

Results and Discussion

Host seeking activity peaked during crepuscular periods, i.e. the time surrounding sunrise and sunset. All sites showed activity peaks corresponding to these times, though the intensity of the peaks varied, and three sites showed greater peaks in the morning compared to evening, whereas the remaining two showed greater peaks in the evening compared to the morning. All sites had similar distributions of temperature and relative humidity, with crepuscular periods characterized by lower temperatures and higher relative humidity. Additional baseline differences in activity, such as magnitude of activity peaks, may be attributable to unmeasured factors such as the presence of nearby larval sources or refugia, which we will take into account in our future models by setting a random effect for collection site.

Conclusions

Our preliminary findings support the hypothesis that in drier, hotter climates, *Ae. aegypti* seek hosts primarily during crepuscular periods rather than hotter midday hours, as is common for the tropical and subtropical climates in which they are endemic. Future analysis will focus on model fitting to quantify the seasonal effects of sunlight, temperature, and humidity on host seeking activity as well as relating activity patterns of male *Ae. aegypti* to females to elucidate the timing of mating behaviors.

Acknowledgements

We thank Manager Alex Scalzo, Manager Teresa Hamilton, and the staff of Madera County Mosquito and

Vector Control District for their collaboration. We acknowledge financial support from the Pacific Southwest Center of Excellence in Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516).

References Cited

Biogents USA. (n.d.). BG-lure - Biogents USA. https://us.biogents.com/artificial-human-skin-scent/bg-lure/. Retrieved March 24, 2021.

(CDPH) California Department of Public Health. 2020. Guidance for surveillance of and response to invasive Aedes mosquitoes and dengue, chikungunya, and Zika in California. https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/InvasiveAedesSurveillanceandResponseinCA.pdf. Retrieved March 24, 2021.

Chadee, D. D., and R. Martinez. 2000. Landing periodicity of *Aedes aegypti* with implications for dengue transmission in Trinidad, West Indies. J Vector Ecol. 25: 158–163.

Focks, D. A., D. G. Haile, E. Daniels, and G.A. Mount. 1993. Dynamic life table model for *Aedes aegypti* (Diptera: Culicidae): Simulation and validation. J Med Entomol. 30: 1018–1028.

John W. Hock Company. (n.d.). Collection Bottle Rotator - John W. Hock Company. https://www.johnwhock.com/products/programmable-collection/collection-bottle-rotator/. Retrieved March 24, 2021.

Jones, M. D. R. 1981. The programming of circadian flight-activity in relation to mating and the gonotrophic cycle in the mosquito, *Aedes aegypti*. Physiol Entomol. 6: 307–313.

Scott, T. W., P.H. Amerasinghe, A.C. Morrison, L. H. Lorenz, G. G. Clark, D. Strickman, and J. D. Edman. 2000. Longitudinal Studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: Blood Feeding Frequency. J Med Entomol. 37: 89–101.

Smith, M., D. Dixon, C. Bibbs, D. Autry, and R. De Xue. 2018. Diel patterns of *Aedes aegypti* (Diptera: Culicidae) after resurgence in St. Augustine, Florida as collected by a mechanical rotator trap. J Vector Ecol. 43: 201–204.

Trpis, M., G. A. H. McClelland, J. D. Gillett, C. Teesdale, and T. R. Rao. 1973. Diel periodicity in the landing of *Aedes aegypti* on man. Bull Wld Hlth Org. 48: 623–629.

Characteristics of resistance to Cry11Aa of *Bacillus thuringiensis subsp. israelensis* in *Aedes aegypti*

Haonan Zhang*, Sarjeet Gill

Molecular, Cell and Systems Biology, University of California, Riverside, CA

*Corresponding author: haonan.zhang@ucr.edu

Bacillus thuringiensis subsp. israelensis (Bti) expressing Cry and Cyt toxins provides a promising strategy for the biological control of mosquitoes, but the evolution of resistance is a primary threat for the long-term efficacy of Bti. Previously we have developed an *Aedes aegypti* strain that shows 124-fold resistance to Cry11Aa crude toxin. To further elucidate the resistance mechanism to Cry11Aa toxin, we used purified Cry11Aa toxin to continually select this strain for 16 additonal generations and obtained a resistance fixed strain Gill-R. Compared to the susceptible strain Gill-S, the Gill-R showed more than 1000-fold resistance to Cry11Aa crude toxin, 217-fold resistance to Cry11Aa purified toxin, 19-fold and 15-fold cross resistance to Cry11Ba and Cry4Ba toxins, respectively, but no resistance to Cry4Aa and CytAa toxins. Resistance to Cry11Aa was completely suppressed by combining CytAa in a 1:3 ratio with Cry11Aa. Genetic analyses indicated that Cry11Aa resistance in Gill-R was autosomal, incompletely recessive, and monogenic. Binding assays showed that there is no significant difference in Cry11Aa toxin binding to brush border membrane vesicles (BBMVs) between the Gill-R and Gill-S. RNA-seq-data revealed a total of 596 up-regulated and 561 down-regulated genes in the larvae midgut of Gill-R compared to Gill-S. A range of 2-4 fold changes were observed in previously reported genes associated with Cry toxin resistance (ABC transporters, tetraspanin, aminopeptidase, alkaline phosphatase and cadherin), but no target-site mutations in the sequences of these genes were detected. Gene Ontology (GO) analysis of differentially expressed genes (DEGs) with statistical significance showed that up-regulated DEGs were significantly enriched into the molecular function category involved in DNA-binding transcription factor activity. Collectively, these results provide fundamental information for further research of the mechanisms of potential *Bti* resistance in mosquitoes.

Pyrethroid residues in California urban catch basin water

Nathan D. Sy[1]*, Sarah S. Wheeler[2], Mir Bear-Johnson[3], Robert F. Cummings[4], Eric Haas-Stapleton[5], Jennifer Henke[6], Susanne Kluh[7], Tianyun Su[8], Trinidad Reyes[9], Marcia Reed[2], Katherine K. Brisco[10], Jay Gan[1]

[1]Department of Environmental Sciences, University of California, Riverside, CA 92521
[2]Sacramento-Yolo Mosquito and Vector Control District, Elk Grove, CA 95624
[3]Delta Vector Control District, Visalia, CA 93291
[4]Orange County Mosquito and Vector Control District, Garden Grove, CA 92843
[5]Alameda County Mosquito Abatement District, Hayward, CA 94545
[6]Coachella Valley Mosquito and Vector Control District, Indio, CA 92201
[7]Greater Los Angeles County Vector Control District, Santa Fe Springs, CA 90670
[8]West Valley Mosquito and Vector Control District, Ontario, CA 91761
[9]Madera County Mosquito & Vector Control District, Madera, CA 93637
[10]Mosquito Control Research Laboratory, Kearney Agricultural Center, Department of Entomology and Nematology, UC Davis, Parlier, CA 93648

*Corresponding author: nsy001@ucr.edu

With many cases of pyrethroid resistance having been documented in California of mosquitoes, assessing environmental contributors is crucial for limiting the prevalence of resistance. In urban settings, pyrethroid residues retained on surfaces such as concrete can become a persistent source of low-dose exposure. Urban runoff can reach larval habitats such as those in stormwater conveyance systems. Previous work observed that pyrethroids were ubiquitous in samples of catch basin media. The current project seeks to expand the scope of the previous screening. Street-level residential catchments were sampled for water in 8 areas across California. After filtration, both water and suspended solids were extracted for pyrethroid residue analysis on GC-MS/MS. Partial results of this project were discussed as was planned work for the evaluation of certain sources of pyrethroid exposure to mosquitoes.

Acknowledgements

Funding was provided by the Pacific Southwest Center of Excellence in Vector-Borne Diseases, CDPH Award A18-0612-S001.

Pyrethroid resistance in *Culex tarsalis* in Northern California

Tara C. Thiemann[1]*, Sumiko De La Vega[1,2], Bonnie Ryan[1]

[1]University of the Pacific, Stockton, CA 95211
[2]San Joaquin County Mosquito & Vector Control District, Stockton, CA 95206

*Corresponding author: tthiemann@pacific.edu

Introduction

Culex tarsalis is one of the most abundant vectors of encephalitis viruses in California. Pyrethroid insecticides play a significant role in the reduction of mosquito populations and thus the transmission of vector-borne pathogens, but the effectiveness of vector control is threatened by increasing insecticide resistance. The goal of the current study was to determine the prevalence of pyrethroid resistance and characterize resistance mechanisms in several *Cx. tarsalis* populations.

Methods

Bottle bioassays with permethrin and permethrin plus piperonyl butoxide (PBO) were conducted for 17 populations across Lake, Placer, San Joaquin, Sacramento, and Yolo Counties during the summer 2018. Mosquitoes collected concurrently from the same populations also were tested for levels of detoxifying enzymes (Brogdon 1984) and for the presence of the target-site mutation known as knockdown resistance (*kdr*) (modified from Hughes 2017).

Results and Discussion

Mortality in bottle bioassays ranged from 8.6 to 96.2% after 2 hours of exposure to permethrin. In all but one population, mortality rose to over 90% when mosquitoes were exposed simultaneously to both permethrin and PBO, suggesting that oxidases play a role in resistance. Results from enzyme assays also supported this idea, as oxidase levels were higher in some field populations than in the susceptible *Cx. tarsalis* colonies (both KNWR and BFS) used for comparison. The levels of glutathione S-transferases (GSTs) and acetylcholinesterase also showed a positive correlation with resistance at the population level. Additionally, the *kdr* mutations leucine (L) → phenylalanine (F) and leucine (L) → serine (S) were prevalent in all wild populations tested. In fact, the susceptible leucine allele was the least common allele detected in this study.

Conclusions

Data analysis is ongoing, but it appears that both *kdr* mutations and increased enzyme levels are contributing to *Cx. tarsalis* pyrethroid resistance in these study areas.

Acknowledgements

We would like to thank our vector control collaborators at Lake County Vector District, Placer Mosquito and Vector Control District, Sacramento-Yolo Mosquito and Vector Control, and San Joaquin County Mosquito & Vector Control District. These Districts providing funding as well as critical mosquito collections and bottle bioassays. This project also received funding support from the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516).

References Cited

Brogdon, W.G. 1984. Mosquito protein microassay. I. Protein determinations from small portions of single-mosquito homogenates. Comp Biochem Physiol 79:457–459.

Hughes, B.D. 2017. Monitoring insecticide resistance mechanisms in *Culex tarsalis* from Sutter County, California. University of the Pacific, Thesis.

Community ecology in California is driving genetic variation and infectivity of the Lyme disease etiological agent

Marie Lilly, Arielle Crews, Liliana Cerna, Andrea Swei*

San Francisco State University, San Francisco 94136

*Corresponding author: aswei@sfsu.edu

Introduction

Lyme disease is the most common vector-borne illness in North America (CDC 2018). In the U.S., Lyme disease is caused by the transmission of the bacteria *Borrelia burgdorferi* (*Bb*) from *Ixodes spp*. ticks to vertebrate hosts, termed as reservoirs (Johnson et al. 1984). The cycling of *Bb* between ticks and vertebrates creates a heterogeneous environment for the bacteria (Swei et al. 2015). Borrelia use outer surface proteins to adapt to these variable environmental conditions in ticks and reservoir hosts. One outer surface protein, C or ospC, is highly expressed when *Bb* enters the reservoir host and is believed to function in evasion of the host immune system (Barbour et al. 2010, Brisson et al. 2004). With a transmission cycle relying on multiple host species, host community diversity is hypothesized to drive the maintenance of diverse ospC genotypes (Brisson et al. 2004, Qiu et al. 2014). Different *Bb* ospC genotypes also are associated differentially with human disease and symptoms—some strains are "human invasive" (HIS), meaning they can infect humans, whereas others are not (non-HIS) (Brisson et al. 2004, Vuong et al. 2014). Therefore, the ecological drivers of ospC diversity have important public health implications. Nevertheless, there is poor understanding of the distribution and factors responsible for maintaining ospC genotypes, especially in the western United States (Barbour et al. 2010, MacDonald et al. 2017). The aim of our research was to characterize the distribution and host community composition associations of HIS *Bb* ospC genotypes in the western United States.

Methods

We employed field epidemiological surveys of *Bb* presence and prevalence in tick (N=1,355) and mammal hosts (N=1,105), coupled with generalized linear mixed-effect models (GLMM) to test the relationship between host community composition and pathogen genotype diversity in Northern California (Swei et al. 2012). Data were collected across 14 field sites in the San Francisco Bay Area during the peak larval and nymphal questing period from 2006-2010 and from 2016-2019 for a total of 9 years of data.

Results and Discussion

We detected 12 ospC genotypes in nymphal *Ixodes pacificus* populations across our field sites in the Bay Area, three of which were human invasive strains. Genotype Hb was the most commonly detected and is unique to the West Coast system (Fig. 1) (Fedorova et al. 2014). Our GLMM analyses found rodent species richness to be a significant predictor of ospC genotypic richness ($p=0.015$), indicating that rodent host community composition is an important driver of pathogen genotype composition (Fig. 2).

Conclusion

The risk of Lyme disease has increased markedly on the East Coast and in the northern Midwest regions of the United States (CDC 2018). Although not as prevalent on the West Coast, Lyme disease is still the most devastating vector-borne illness in California (Swei et al. 2015, Macdonald et al. 2017). The West Coast Lyme ecology is different from that of other parts of the United States and provides an opportunity to understand how *Bb* cycles in this unique ecological landscape. Beyond the implications for human Lyme disease risk, our study elucidates how community composition plays a role in driving genetic variation of *Bb* on the West Coast, an area currently lacking detailed investigation of the types and drivers of Bb genotype diversity.

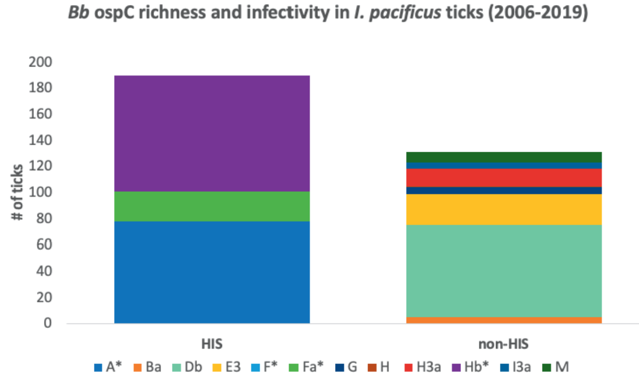


Figure 1.—*Borrelia burgdorferi (Bb)* human invasive (HIS) and non-human invasive (non-HIS) outer surface protein C genotypic richness in *Ixodes pacificus* collected during 2006-2010 and 2016-2019.

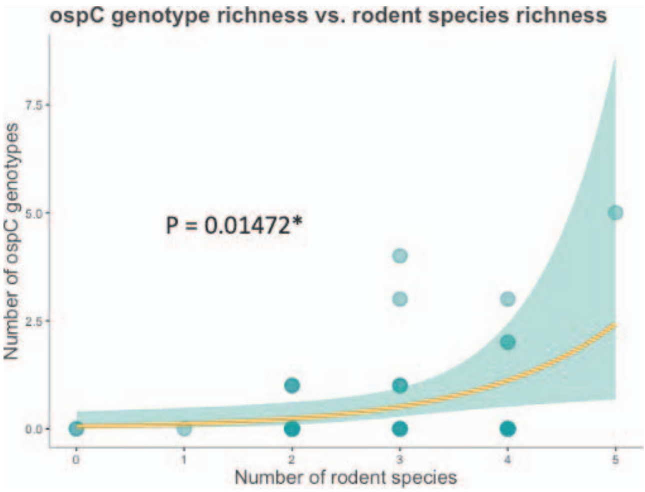


Figure 2.—*Borrelia burgdorferi (Bb)* outer surface protein C genotypic richness plotted as a function of rodent species richness (2016-2019).

Acknowledgements

We acknowledge Jordan Salomon, Samantha Sambado, Kacie Ring, Ceili Peng, Grace Shaw, Vincent Mai, Jacoby Clark, Nghia Tran, Monika Koczela-Stillman, Laura Hughes, and Adrienne Almarinez for their help with sample collection and processing. This research was conducted on Native lands of: Ramaytush, Ohlone, Chochenya and Wappo. We thank the City of Belmont, California State Parks, East Bay Municipal Water District, Marin Open Space Trust, San Mateo County Parks, Sonoma Regional Parks, Midpeninsula Regional Open Space and the Town of Los Gatos for access to conduct research in their parks. This project was made possible through funding from the NSF, CDC, and PacVec.

References Cited

Barbour, A. G., and Travinsky, B. 2010. Evolution and distribution of the ospC gene, a transferable serotype determinant of *Borrelia burgdorferi*. MBio, 1: e00153-10.

Brisson, D., and Dykhuizen, D. E. 2004. ospC diversity in *Borrelia burgdorferi*: different hosts are different niches. Genetics, 168: 713-722.

CDC. 2018. Reported Cases of Lyme Disease — United States, 2018. https://www.cdc.gov/lyme/datasurveillance/maps-recent.html

Fedorova, N., Kleinjan, J. E., James, D., Hui, L. T., Peeters, H., and Lane, R. S. 2014. Remarkable diversity of tick or mammalian-associated Borreliae in the metropolitan San Francisco Bay Area, California. Ticks and tick-borne dis. 5: 951-961.

Johnson, R. C., Schmid, G. P., Hyde, F. W., Steigerwalt, A. G., and Brenner, D. J. 1984. *Borrelia burgdorferi sp. nov*.: etiologic agent of Lyme disease. Int. J. System. Evol. Microbiol. 34: 496-497.

MacDonald, A. J., Hyon, D. W., Brewington, J. B., O'Connor, K. E., Swei, A., and Briggs, C. J. 2017. Lyme disease risk in southern California: abiotic and environmental drivers of *Ixodes pacificus* (Acari: Ixodidae) density and infection prevalence with *Borrelia burgdorferi*. Parasites & vectors, 10: 7.

Qiu, W. G., and Martin, C. L. 2014. Evolutionary genomics of *Borrelia burgdorferi sensu lato*: findings, hypotheses, and the rise of hybrids. Inf. Genetics Evolution 27: 576 593.

Swei, A., Briggs, C. J., Lane, R. S., and Ostfeld, R. S. 2012. Impacts of an introduced forest pathogen on the risk of Lyme disease in California. Vector-Borne and Zoonotic Diseases, 12: 623-632.

Swei, A., Bowie, V. C., amd Bowie, R. C. 2015. Comparative genetic diversity of Lyme disease bacteria in Northern Californian ticks and their vertebrate hosts. Ticks and tick-borne Dis. 6: 414-423.

Vuong, H. B., Canham, C. D., Fonseca, D. M., Brisson, D., Morin, P. J., Smouse, P. E., and

Ostfeld, R. S. 2014. Occurrence and transmission efficiencies of *Borrelia burgdorferi* ospC types in avian and mammalian wildlife. Inf., Genetics and Evolution 27: 594-600

Effects of short-term weather on the timing of mosquito host-seeking activity and implications for integrated vector management

Pascale C. Stiles[1]*, Gurmanpreet Kaur[1], Mary Sorensen[2], Mario Boisvert[2], Jake Hartle[2], Marcia Reed[3], Sarah Wheeler[3], and Christopher M. Barker[1]

[1]University of California, Davis, DART Lab, Davis, CA 95616
[2]Placer Mosquito and Vector Control District, Roseville, CA 95678
[3]Sacramento-Yolo Mosquito and Vector Control District, Elk Grove, CA 95624

*Corresponding author: pcstiles@ucdavis.edu

Introduction

Information derived from entomological surveillance for arboviruses, such as West Nile virus (WNV), aids local vector control agencies in decision-making to enact adult mosquito control to quickly reduce mosquito populations in periods of epidemic risk (California Department of Public Health 2020). Understanding the factors that influence the beginning of mosquito host-seeking activity in a night is critical for ensuring that target vector populations are impacted by these control applications. Our study aims to relate daily weather conditions to the start of host-seeking activity.

Methods

We collected data over a two-month period consisting of 15-minute counts of mosquito catches, temperature, wind speed, and relative humidity from ten automated Biogents Counter traps (Pruszynski 2016) in Placer, Sacramento, and Yolo Counties. The study was conducted during the seasonal peak of *Culex tarsalis* activity near irrigated rice fields during the summer of 2019 in collaboration with Placer and Sacramento-Yolo Mosquito and Vector Control Districts. We used the R package "suncalc" to compute daily sunset times at each site (Thieurmel and Elmarhraoui 2019). We approximated 15-minute counts of *Cx. tarsalis* by multiplying the BG-Counter numbers, which reflect total mosquitoes captured, by the hourly proportion *Cx. tarsalis* observed in paired CO_2-baited traps with collection bottle rotators (Hock 2015) and estimated the time at which host-seeking activity commenced as the earliest 15-minute period in a night where a nonzero count of *Cx. tarsalis* was observed. Finally, we related weather conditions at a range of times in the afternoon to the timing of host-seeking activity onset relative to sunset time using linear regression models that adjusted for baseline differences among the ten trap sites using the "lme4″ package in R (Bates et al. 2015).

Results and Discussion

Overall, we observed 427 trap-nights between July 3 and September 5 where the traps 1) collected at least 50 *Cx. tarsalis* females and 2) did not experience a malfunction that prevented the observation of all 96 15-minute periods in 24 hours. The observed timing of mosquito activity onset ranged from 42.57 minutes before to 58.97 minutes after sunset (SD: 11.36 min). Wind speed and temperature at 8:00 PM resulted in the best model for the onset of host-seeking activity relative to sunset time. From the regression model, onset time varied by site and ranged from 10.62 to 27.00 minutes after sunset in average weather conditions. This confirms findings from other studies that *Culex* host-seeking activity begins shortly after sunset (Bailey et al. 1965, Reisen et al. 1997, Godsey et al 2010). We found that increases in both wind speed and temperature at 8:00 PM independently resulted in a delay of activity onset, with wind speed having a stronger effect than temperature. Other studies examining the influence of weather on the timing of mosquito host-seeking activity have not found such associations, but these studies were typically conducted on windless evenings and did not account for the change in the photoperiod in the outcome (Reisen et al. 1997, Veronesi et al. 2012, Montarsi et al. 2015). We have demonstrated that meteorological factors have independent effects on the start of mosquito host-seeking activity after adjusting for time of sunset.

Conclusions

Taken together, predicting host-seeking periods based on the effects of weather on the onset of host-seeking activity will help vector control agencies target the window of time during which a single mosquito control application could have maximum impact. Agencies should expect to enact adulticide applications later in the night following windier- and/or warmer-than-average evenings.

Acknowledgements

The authors acknowledge Tom Moore of Placer MVCD and Elizabeth Stovall and Kylie Letamendi of Sacramento-Yolo MVCD for showing us the trap sites and for conducting routine trap maintenance during the course of the study period, Mirsha Torres of Placer MVCD for aiding in trap collections and species identification, and Placer, Sacramento, and Yolo County homeowners for permitting us to collect from traps on their properties.

References

Bailey, S. F., D. A. Eliason, and B. L. Hoffmann. 1965. Flight and dispersal of the mosquito *Culex tarsalis* Coquillett in the Sacramento Valley of California. Hilgardia. 37: 73–113.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. J Stat Softw. 67: 1–48.

California Department of Public Health, Mosquito & Vector Control Association of California, and University of California. 2020. California Mosquito-Borne Virus Surveillance and Response Plan. Sacramento.

Godsey, M. S., K. Burkhalter, M. Delorey, and H. M. Savage. 2010. Seasonality and time of host-seeking activity of *Culex tarsalis* and floodwater *Aedes* in northern Colorado, 2006-2007. J. Am. Mosq. Control Assoc. 26: 148–159.

Hock, J. W. 2015. Collection Bottle Rotator. John W. Hock Co. (https://johnwhock.com/products/programmable-collection/collection-bottle-rotator/).

Montarsi, F., L. Mazzon, S. Cazzin, S. Ciocchetta, and G. Capelli. 2015. Seasonal and daily activity patterns of mosquito (Diptera: Culicidae) vectors of pathogens in Northeastern Italy. J. Med. Entomol. 52: 56–62.

Pruszynski, C. 2016. The BG-Counter: A new surveillance trap that remotely measures mosquito density in real-time. Wing Beats. 27: 13–18.

Reisen, W. K., H. D. Lothrop, and R. P. Meyer. 1997. Time of host-seeking by *Culex tarsalis* (Diptera: Culicidae) in California. J. Med. Entomol. 34: 430-437.

Thieurmel, B., and A. Elmarhraoui. 2019. suncalc: Compute Sun Position, Sunlight Phases, Moon Position and Lunar Phase.

Jeep modifications for source reduction and mosquito control in Los Angeles County flood control channels.

Mark A. Daniel*

Greater Los Angeles County Vector Control District, Santa Fe Springs, CA 90670

*Corresponding author: mdaniel@glacvcd.org

Introduction

Los Angeles County Flood Control District has approximately 500 miles of open flood control channels. A majority of these open flood control channels have concrete bottoms and steep or vertical sides. The Los Angeles County Flood Control District usually clears these channels semi-annually, once before winter rains and again after the rains have stopped. During the summer, non-storm water flows lead to sediment accumulation and algal growth, as well as the deposition of refuse. These conditions provide the perfect habitat to produce mosquitoes and midges. The use of Jeeps has been a critical part of accessing and treating flood control channels since the early days after the Districts formation.

Methods

Modifications were done in-house by Operations and Fleet Maintenance staff. Most of the work was done during the winter when mosquito production was low. Modifications were the result of suggestions from the operators who need their vehicles to be self-contained and practical.

Discussion

Modifications to the Jeeps have evolved over the years to fit the District's vector control needs. Initially, the Jeeps carried personnel and equipment into the channels to apply pesticides. Modifications have evolved to include, on board pesticide tanks, bumper mounted spray heads, protective cab enclosure for operator safety, solid filled tires, bumper mounted wenches and a lightweight plow blade for source reduction. The plow blade is especially useful for clearing algal mats, sediment and accumulated debris. The use of the plow blade has reduced the quantity of pesticide used in the channels to mitigate the larval mosquitoes and midges.

Conclusion

The Greater Los Angeles County Vector Control District developed new methods to access and treat the flood control channels, district, to address mosquito and midge production that creates a nuisance and threatens public health.

Acknowledgements

Thanks to all our staff who have contributed their time and effort to creating an efficient and effective tool for our District and our resident's safety: Jovino Martinez (current operator Sylmar), Tom Veloz (current operator/Jeep fabrication Santa Fe Springs), Frank Ochoa (former operator Sylmar), Steve Hillyer (retired operator Santa Fe Springs), Victor Perez (design and Jeep fabrication), Tom Griep (fabrication), and Luis Guerrero (fabrication)

Conducting physical control at residential mosquito sites

Ryan Thorndike*

San Mateo County Mosquito and Vector Control, Burlingame, CA, 94010

*Corresponding author: rthorndike@smcmvcd.org

Introduction

The removal of standing water using physical control techniques on residential properties lowers the risk of mosquito production and, by extension, mosquito-borne pathogen transmission risk. Various tools and equipment are needed to obtain this goal for the short or long term. A sampling of physical control methods and materials currently used in residential sites by the San Mateo Mosquito and Vector Control District (SMCMVCD) are described herein.

Methods

Physical control at residential sites may include: dumping and draining containers, removing containers, removing water from landscape features such as ponds and fountains, drilling holes and breaking-up landscape features. The use of buckets, brooms, sumps or submersible pumps, drills and sledgehammers can be used to drain or alter landscape features to prevent standing water. It is recommended to carry out this process in early summer when rain is no longer expected and thereby keep water features dry for as long as possible.

Example 1: Early-season clean-out of ponds (temporary abatement methods):
A technician can often remove standing water from shallow in-ground ponds (Figure 1) by first using a bucket to remove as much water as possible, followed by a push-broom to sweep out water and debris from the bottom surface of the pond. For narrow or deep ponds, it may be necessary to use a hand or sump pump to drain the pond (Figure 2). If the property owner does not have an accessible power outlet, use of a sump pump may require the technician to bring a generator and/or an extension cord to power the pump.

Example 2: Permanent methods of decommissioning ponds
Technicians can offer more permanent abatement solutions for residents who do not intend to keep water in a pond or other vessel in the future. Drain holes in the bottom of ponds can be achieved using an appropriate-sized impact drill (Figure 3, 4). It is recommended for the technician to bring a generator to run the impact drill, because this type of equipment can cause power surges if plugged into a residential power source. Proper personal protective equipment should always be used when using an impact drill, including leather gloves and eye and ear protection (Figure 4).

Another option for permanent abatement of unwanted water-holding vessels is to break up the feature with a sledgehammer (Figure 5). As with any physical control method involving heavy tools, leather gloves, eye and

Figure 1.—In-ground pond holding water in a residential yard.

Figure 2.—Use of a sump pump and hose to drain a deep pond. Bucket with screened ports serves to prevent debris from clogging the pump.

Figure 3.—Two commonly used types of impact drills for pond drainage: shown are a Bosch 11245EVS with a 1-1/8" drill bit and a Dewalt D25231 with a 1" drill bit.

ear protection, and reinforced work boots are recommended.

The SMCMVCD requires a waiver to be signed by residents to consent to any permanent alterations of their water features.

Figure 4.—Technician using an impact drill to provide drainage to ensure the pond will no longer hold water.

Figure 5.—Birdbath broken into pieces using sledgehammer

Conclusion

Incorporating physical control methods can result in permanent or semi-permanent mosquito abatement in a variety of residential source types. After draining water utilizing a bucket, broom or submersible pump after the winter rains, water features will often remain dry throughout the summer. After drilling holes in ornamental ponds and gaining resident participation in keeping the holes free of leaf litter and debris, water was unable to accumulate in about half of all ponds drilled by SMCMVCD staff. The demolition of bird baths and other unwanted yard vessels involves considerable physical effort, but results in the complete elimination of perennial mosquito breeding sources.

Benefits to mosquito and vector control districts from conducting physical control at residential mosquito sites include: limiting pesticide use, isaving pesticide costs, saving time doing inspections and re-inspections, reducing mosquito populations, and lowering the risk of mosquito borne pathogen transmission

Acknowledgments

Thank you to Angie Nakano for assistance with abstract preparation, Tara Roth for Power Point suggestions, Casey Stevenson for suggestions on presentation content and Sean Jones for equipment specifications.

Tools and techniques in rodent surveillance

Tara Roth*

San Mateo County Mosquito and Vector Control, Burlingame, CA 94010

*Corresponding author: troth@smcmvcd.org

Many techniques exist for rodent surveillance and control (examples have been provided in the attached Supplement: Tools and Techniques in Rodent Surveillance); however, not every technique will work for every situation. The reason for this is that commensal rats (*Rattus* spp.) are neophobic (afraid of the new and unknown) and capable of intercommunication and social learning. Although rats are curious by nature, they will avoid traps, baits, or monitors if they perceive them to be a threat – either through negative interactions themselves or through watching harm come to their nest mates.

In general, it is recommended that a successful rodent control program employ multiple techniques for surveillance before control efforts commence. Control methods should be chosen based on the distribution of the target species and the presence or absence of non-targets such as pets, wild rodents, species of concern, mesocarnivores, or other predators. This is especially important in sensitive habitats and the failure to adequately protect non-target species from rodent control measures can lead to penalties from regulatory agencies and negative perceptions from the public. Before embarking on any control program, first contact local authorities and agencies (e.g. Environmental Health, Code Enforcement, Housing, County Agriculture, Fish and Wildlife etc.) to establish guidelines, responsibilities and working protocols. Laws may change, so maintain a working relationship with local authorities to stay up-to-date on current legislation.

While baiting and trapping are important in reducing the population, they tend to have a temporary effect on rodent populations. Exclusion work, improvements to sanitation, and updates to equipment are necessary for control work to have any long-term effect. Baiting and trapping programs should be employed to enhance exclusion work and should never be utilized on their own.

Acknowledgements

I would like to thank Brian Weber and Casey Stevenson for assisting me in designing this talk and Angie Nakano for providing feedback and edits on both the talk and the written supplemental.

Underground storm drain spray wand components

Yessenia L. Curiel*

Greater Los Angeles County Vector Control District, Santa Fe Springs, CA 90670

*Corresponding author: ycuriel@glacvcd.org

Introduction

The Underground Storm Drain System (USDS) in Los Angeles is a complex network of drains, catch basins and manhole chambers. These systems catch runoff from rainstorms as well as from irrigation and other residential runoff. Greater Los Angeles County Vector Control District (GLACVCD) has over 3,000 miles of underground storm drains within its boundaries. The USDS program was initiated in the Spring of 2002, in anticipation of the impending arrival of West Nile virus, to treat approximately 33,000 maintenance hole covers within the GLACVCD boundaries in the attempt to lower *Culex quinquefasciatus* abundance. Currently, a total of 15 staff members, 8 teams of 2 and 1 lead, are dedicated to the program of inspecting approximately 62,000 maintenance covers on a yearly basis. The USDS is an important part of GLACVCD's Integrated Vector Management (IVM) program, and its treatments and inspections are crucial in protecting the public from vector borne diseases as well as nuisance biting.

Discussion

The originally pristine USD water conveyance system below most of Los Angeles county has been modified since its inception: earthquakes, reconstruction and debris accumulation have let to water ponding rather than flowing in many places. Warmth, humidity and lack of maintenance make for ideal mosquito conditions and GLACVCD has documented the mass emergence of both *Culex* and *Aedes* mosquitoes in the past. Therefore, the right equipment is necessary to make efficacious treatments.

The USDS program is mainly based on larviciding and this is accomplished by turning a liquid formulation into a coarse mist that is injected injecting said mist into the system through the opening in the manhole cover lid and letting air currents distribute the material along the horizontal line, with droplets deposited during the process. These applications initially were made using a short-stemmed Hydro-Blast Cleaning Tool and siphon gun sprayer made by Amflo. Using this early version of the tool required constant kneeling or bending, was not ergonomic, and left applicators with poor traffic visibility. As the program moved into its daily routine, a better solution was needed.

Modifications were made by taking our short-stemmed Hydro-Blast Cleaning tool and siphon gun sprayer, disassembling it to only use the venturi valve, and attaching it to a 58-inch steel tube. Other components were added to enhance sprayer function (Figure 1). A 3-way valve was added to turn that larvicide application on and off. We added clear tubing to transfer the larvicide from the 3-way valve to the venturi valve and added a coiled hose, with one end connected to the 3-way valve and the other end connected to the pesticide tank via quick disconnect. The upper section of the assembled wand therefore includes a quick disconnect, 3-way valve, on/off valve, a clear hose and a coiled hose. The 3-way valve which is connected to the clear hose via an elbow barbed fitting is then connected to the venturi valve in the lower section of the wand. A quick disconnect to the on/off valve allows air from the compressor mounted in our trucks, to flow through the steel tube and disperse the larvicide from the venturi valve. The coiled hose connects to the 3-way valve which allows larvicide to travel from the tanks into the wand.

The lower section of the wand includes a venturi valve, brass coupler, clear tubing and steel tip to protect the venturi valve. The venturi valve from our hydro-blast cleaning tool is connected to the end of the steel tube via a brass coupler and a steel tip is connected to end of the venturi valve to protect the venturi valve. The steel tip also facilitates our treatments by fitting into the access hole of the manhole cover. The clear tubing, connected to the 3-way valve in the upper section of the wand, runs along the 58-inch steel tub and is connected to the venturi valve via an elbow barbed fitting which carries larvicide from the pesticide tank in our trucks.

For operation, the technician opens the on/off valve on the upper section of the wand to allow air at 100psi to travel down the steel tube. The technician then opens the 3-way valve to draw larvicide from the pesticide tanks into the coiled hose to the 3-way valve down the clear hose and into the venturi valve, where it is mixed with the air and dispersed into the underground storm drain. This creates droplets between 80 - 120 microns in diameter. Once the desired amount of larvicide is dispersed, the technician turns off the 3-way valve and maintains the air on/off valve open for a few seconds more to allow the larvicide to be pushed down the chimney of the drain and into the tube where the air in the system and the droplet size created, allow the material to drift approximately 0.10 miles, treating the water along the way. The underground storm

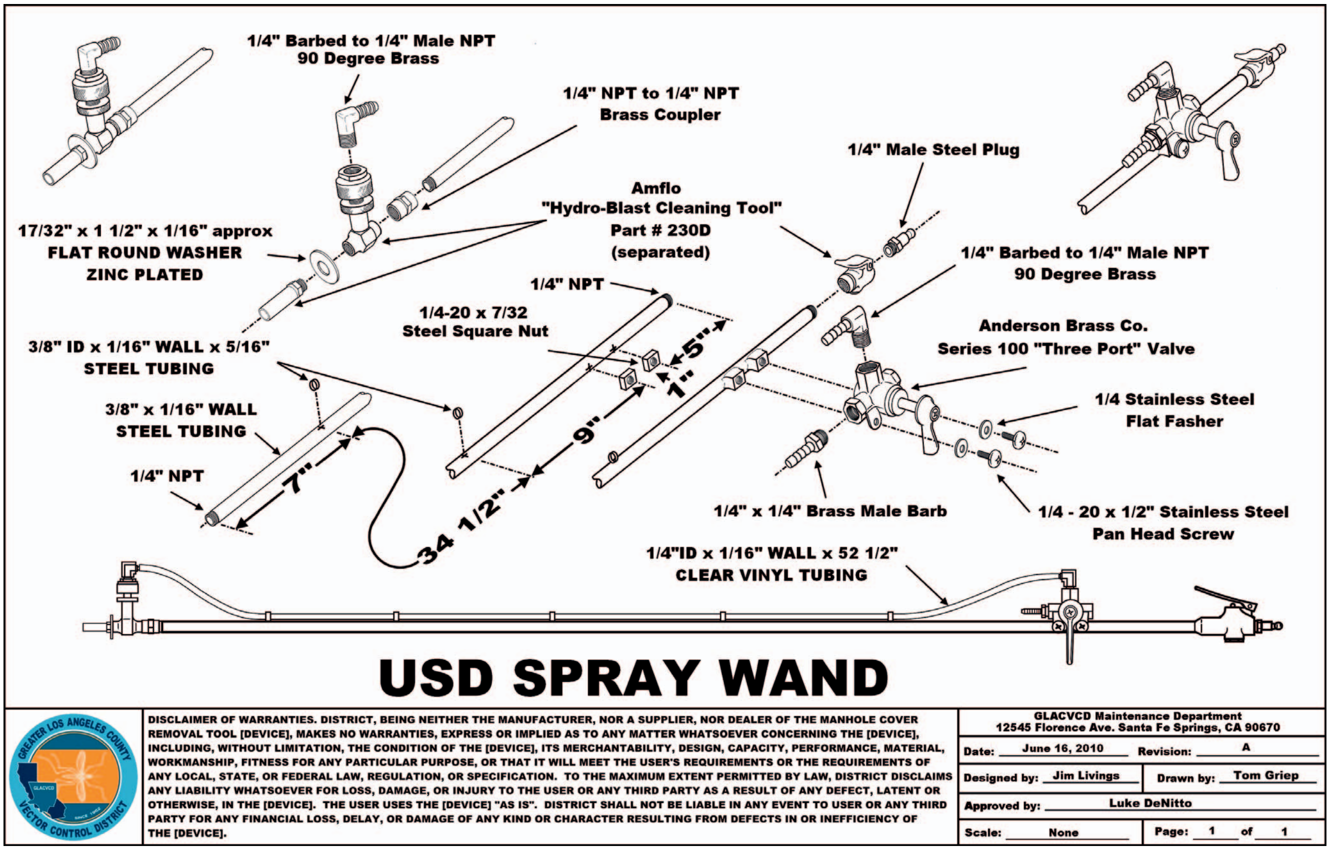


Figure 1.—The Underground Storm Drain (USD) wand for infection of larvicides. See text for a description and use of the components shown.

drains are treated with a 12.8 oz mix of VectoBac 12AS and VectoLex WDG. Individual treatments contain 0.4 oz of VectoBac 12AS and 0.8 oz of VectoLex WDG which effectively treats 0.10 miles. Our wands are calibrated to ensure that the approximate amount of larvicide is dispersed by using a stop watch which is attached on the upper section of the wand.

Conclusion

The challenges that we presently face is that the brass venturi valve made by Amflo has been discontinued, but there is a cheaper alternative made by NAPA which is made out of aluminum. However, anecdotal information indicates that the aluminum venturi tends to clog easily compared to the brass valve from Amflo and we currently are working with our sci-tech department to determine if we can change the calibration of the aluminum venturi valves and still be with in droplet size range to make effective treatments. This wand set-up has been functioning for more than 15 years with minor modifications, but we are always open for new ideas from our staff to improve our equipment.

Acknowledgements

Special thanks to Susanne Kluh and Steve Vetrone for providing information relevant to the calibration of the wands and special thanks to Tom Griep for providing schematics to assemble these spray wands.

Overview of unmanned aircraft system operation in the Northern Salinas Mosquito Abatement District

Ken Klemme*

Northern Salinas Valley Mosquito Abatement District, PO Box 10053, American Canyon, CA 94503

*Corresponding author: Ken@montereycountymosquito.com

Introduction

Unmanned Aircraft System (UAS) operations have become popular among vector control districts. In the current presentation, the utility and use of UAS equipment at the Northern Salinas Valley Mosquito Abatement District are described to assist those Districts deciding on whether UAS equipment will be useful in their operations.

Methods

We created a Best Management Practices approach to flying based on use and observation in addition to the normal pre-flight checklist.

Results and Discussion

Over time and through experience we have learned how to expedite a safer launch and return of the equipment. Constructing a portable shelter and having pamphlets ready for the public have been a great help. Safety is our number one concern for the public and the pilots. Adding informative pamphlets that are readily available away from the landing zone, allows the pilots to keep their attention on the flight, while satisfying public observers with information. We also clearly mark the landing and takeoff sites, so that everyone is aware of where the UAS should land. We also have employed the use of walkie talkies to increase distance between spotters and pilots to keep 'better eyes' on the UAS.

It is important to understand the different features on the equipment before going to the field. Again safety is the number one concern and we cannot stress enough how important it is to follow all the pre-flight check lists. Precautionary measures include adding Locktites to all bolts that are to remain attached, because these may become loose during flight and make for a precarious landing. The built in "Return to home" feature uses the most direct route to come back to the starting position, which in one instance was straight through a tree which ended in the total loss of the UAS. Flying at altitudes above any obstructions is very important to keep in mind. The UAS must be treated as if it were a vehicle with the capabilities of injuring people, property and wildlife. Overall despite the total loss of one UAS vehicle. we continue to learn and are available as a resource for those interested in the use of UAS.

Conclusions

In the video, we shared some tips, advice and pitfalls concerning UAS applications that may help a District make to decide if UAS technology will be useful. UAS technology should be taken seriously especially because of the potential damage to property and persons. We have learned from previous mistakes and continue to apply that knowledge to future flights. UAS use in environmentally sensitive areas has been extremely beneficial, because of the low environmental impact, thereby improving interactions with land stewards from different foundations.

Acknowledgements

Thanks to Paul Palomo for recording, editing and help scripting our video.

Inside look at the $1,000,000 Mosquitofish rearing facility at Delta Vector Control District

Mark Nakata*, Crystal Grippin, Mir Bear-Johnson, Mustapha Debboun

Delta Vector Control District, Visalia 93291

*Corresponding author: mnakata@deltavcd.com

Mosquitofish, *Gambusia affinis*, were historically collected at Delta Vector Control District by setting traps in nearby ponds and reservoirs. This method proved to be time-consuming and yielded a minimal amount of mosquitofish that were often infected with parasites and bacteria. In 2018, the District decided to improve and expand its mosquitofish program by building a new indoor mosquitofish rearing facility and updating the outdoor holding facility. In 2019, architectural designs and drawings were drafted, city plan checks were completed, and construction permits were obtained. In 2020, the construction began in March and was completed by October (Fig. 1). The indoor rearing facility is fully optimized for mosquitofish with tank and filter equipment supplied from Gambusia Solutions, custom built mosquitofish feeders, tunable white LED lighting, and dehumidifiers to control humidity (Fig. 2). Future projects include equipment modifications to the tanks and fish feeder system, building custom Arduino based sensors with a variety of testing probes, and biological experiments to test mosquitofish development. Delta Vector Control District strives to acquire the latest technological advances to provide District residents with healthy mosquitofish year around.

Figure 1.—Delta Vector Control District Laboratory and Mosquitofish Rearing Facility.

Figure 2.—(A) Indoor mosquitofish rearing facility (B) Semi-covered outdoor mosquitofish holding facility.

SIT IRL: Developments for the *Aedes aegypti* sterile insect technique (SIT) program in Lee County, FL

Rachel Morreale*

Lee County Mosquito Control, Lehigh Acres, FL

*Corresponding author: morreale@lcmcd.org

Lee County Mosquito Control District (LCMCD) has been developing a sterile insect release program to control *Aedes aegypti* since 2017. In-house experimentation has led to substantial improvements in irradiation, blood feeding, and rearing methods. Improvements in membrane blood feeding have allowed for the change from live vertebrates to artificial hosts with comparable egg production. Additionally, LCMCD obtained several pieces of equipment that are essential for scaling up our mass rearing operations. These advances in technology facilitate the processes of larval rearing and of sex separation. Background population surveillance using BG Sentinel traps began at the pilot study site on Captiva Island in June 2017 and has now surpassed the two year goal proposed by the International Atomic Energy Agency. This consistent monitoring has provided an abundance of information regarding the population dynamics of *Ae. aegypti* over time. Baseline sterility is also being assessed in eggs collected from oviposition cup monitoring. Several mark-release-recapture (MRR) studies have been performed to assist in population estimates, dispersal of released males, and duration of released sterile male survival through marked male recaptures. Operational releases of 50,000 sterile male *Ae. aegypti* twice per week began on June 10, 2020 (total = 100,000 males per week). Ongoing surveillance by adult trapping and oviposition cups have provided insights into the impact that these releases have had on the population.

Wide open thinking on wide-area applications

Derek Drews*

Clarke, 675 Sidwell Court, St. Charles, IL 60174

*Corresponding author: ddrews20@gmail.com

Applying larvicides through air blast equipment is an emerging application method for public health that borrows practices from both the traditional adulticiding and larviciding approaches. Having gained notoriety by controlling container-breeding species during the Zika outbreak in Miami-Dade County, Florida, air blast applications have gained traction with abatement programs across the country looking for more efficiency and flexibility in larval control. This session examines the many facets of wide-area applications, highlights lessons learned, and identifies best practices gleaned from field trials and operational work with air blast equipment.

A new perpetual capture carbon dioxide trap for year-round adult mosquito surveillance

Sarah.S. Wheeler*, Ben Weisenburg, Kylie Letamendi, Marcia Reed

Sacramento-Yolo Mosquito and Vector Control District, 8631 Bond Rd, Elk Grove, CA 95624

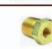
*Corresponding author: S. Wheeler, swheeler@fightthebite.net.

Introduction

The Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) conducts year-round adult mosquito abundance surveillance. Traps are serviced weekly and provide baseline mosquito abundance metrics. Previously abundance traps have included New Jersey light traps (NJLT; BioQuip Products, Rancho Dominguez, CA), AC-powered Gravid Mosquito Traps (BioQuip Products, Rancho Dominguez, CA), and Mosquito Magnet Commercial Pro traps (MMT; Safer Brand; Lititz, Penn). The SYMVCD began using MMT in 2000, with the majority of traps purchased before 2005. By 2017, the MMTs had been in constant operation for ten or more years and could no longer be economically repaired. Therefore, the search for a suitable replacement was conducted. After investigating commercial options the SYMVCD decided to design and build a simple AC powered trap that used compressed CO_2 as a lure. Because abundance traps are only serviced weekly, the CO2 release needed to be limited to dusk through dawn and the trap had to withstand all weather conditions.

The new trap design was compared to MMT in 2017 and NJLT in 2019. The newly designed trap has been deployed and incrementally improved each year since 2017 and is currently referred to as the Locker Trap (LCKR). In 2020 the LCKR was fully integrated into the SYMVCD's surveillance program, and has now replaced both MMT and NJLT at year-round surveillance sites. The current paper describes the performance, design, and construction of the LCKR trap.

Table 1.—Components used in the construction of Locker Traps.

Major components	Manufacturer	Item #	Image
Single gauge CO2 regulator	Taprite	T741	
Footlocker	Sterilite	18429001	
Photoswitch	Taxnele	AS-10-12 10A	
1/4" NPT to #10-32 Female Reducer	Clippard	4CQF-PKG	
#10-32 Male with Captivated O-Ring to Barb, 1/8"	Clippard	11792-4-PKG	
Tubing	Clippard	URH1-0804-CLT-050	
.006" Precision Noryl Orifice Restrictor with 1/8" Barbs	SMC Pneumatics	F-2815-060-B85-NORYL	
Flex-A-Spout Downspout Extension	Amerimax Home Products	85019	
6V Fan (FFB0912VH)	Delta Electronics	FFB0912VH	
ASCO Solenoid 411L1112HVS	ASCO	411L1112HVS	
12 V 2.5A AC DC power supply adapter	Zuext	JCY-1225	
Other supplies			
Plywood for floor of trap 2" X 4" lumbar for trap footing Wood screws Red and black wire Fuse Switch Catch bag			

Methods

The LCKR was designed for ease of construction and repair by District personnel. The components of the LCKR are enclosed inside a plastic foot locker that both protects the components and the collected mosquitoes from weather, and shields the trap components from view (see Table 1 for a parts list). The trap (Fig. 1) is designed to use compressed liquid carbon dioxide (CO_2) as an attractant for host-seeking mosquitoes and uses a fan to draw mosquitoes into a catch bag. The CO_2 flows from the tank, through the regulator, and the flow is controlled using a 0.006" inline restrictor orifice and a regulator. Gas is released by a solenoid that is controlled by a photo switch that opens the solenoid valve under low light conditions when target mosquitoes are active. A flashing LED was wired on the same circuit as the solenoid (Fig. 2) so that technicians could easily visualize when CO_2 was flowing. The fan is mounted at the end of the catch tube (flexible downspout) and pulls mosquitoes into the catch bag without passing them through the fan. Catch bags were sewn by district personnel using 0.9 oz Noseeum mesh (Ripstop by the roll, Durham NC) and were designed to fit within the catch tube and not interfere with the fan; catch bags are installed from the outside of the trap (Fig. 1a).

Locker trap collection rates were compared to both MMT and NJLT. Traps were run concurrently at the same

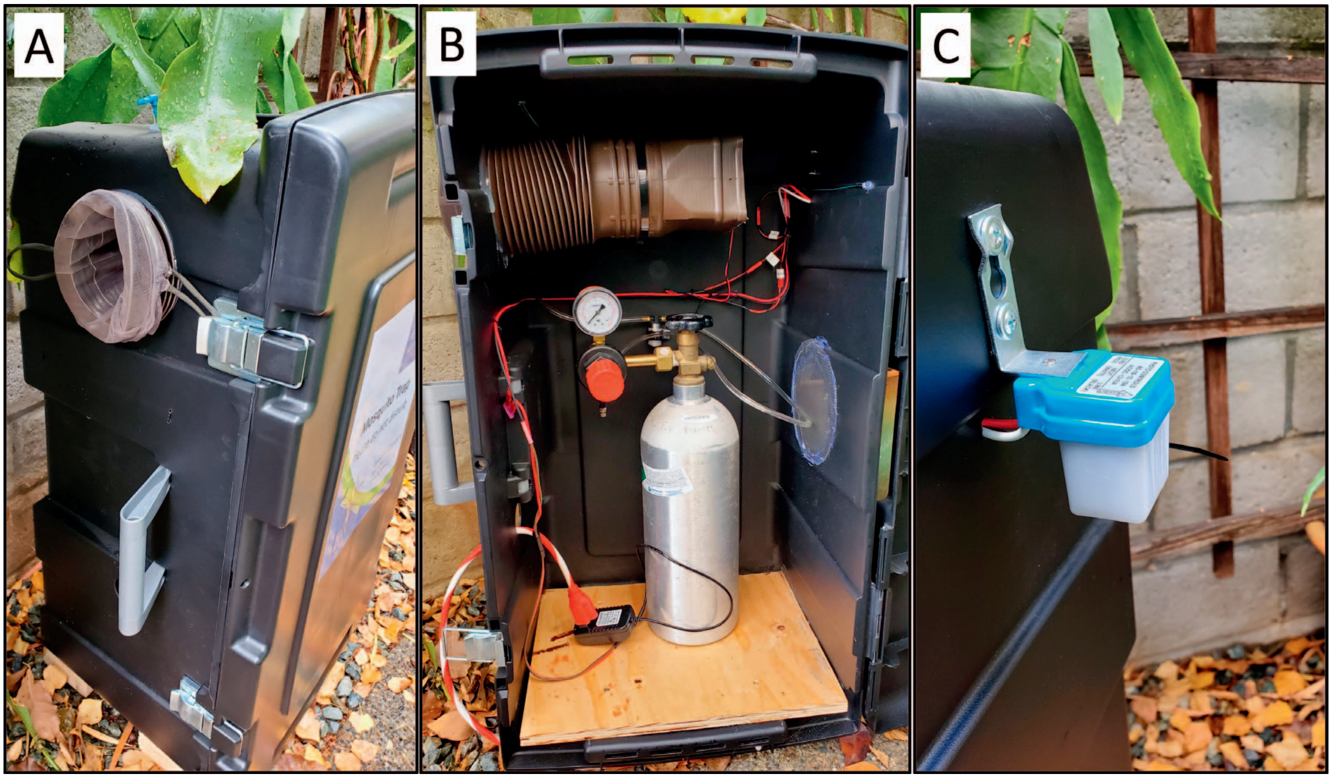


Figure 1.—Locker Trap A) outside view, shown with a collection bag mounted on the capture tube; B) inside view with a 5lb CO2 tank attached; C) photo switch, installed on the outside, opposite the capture tube.

location 150m apart. Each trap was set to run for one-week intervals, the mosquitoes collected were identified to species and sex, and then counted. The LCKR was compared to MMT at four different locations in 2017 (Fig. 3a) and compared to NJLT at two different locations in 2019 (Fig. 3b). The duration of the comparisons varied by location, but LCKR traps were rotated among available MMT locations to allow for spatial variation, whereas LCKR and NJLT comparisons took place at the same two locations from January to September in 2019.

The total number of female mosquitoes collected over a seven-day period were compared by trap type. When traps were operated for more or less than seven days, the mean number of females per trap night were calculated, multiplied by seven then rounded to the nearest integer. Data were processed in this way to preserve the Poisson distribution of the count data. To model the number of female mosquitoes collected as a function of the covariates, a Poisson generalized linear model was fit with trap location, week of collection, and trap type as covariates. This method was also used assess the species diversity between the compared trap types, models were fit that predicted the total number of species collected over a seven-day period (female mosquitoes only) by trap location, week of collection, and trap type. All models were run in R (R Core Team 2021).

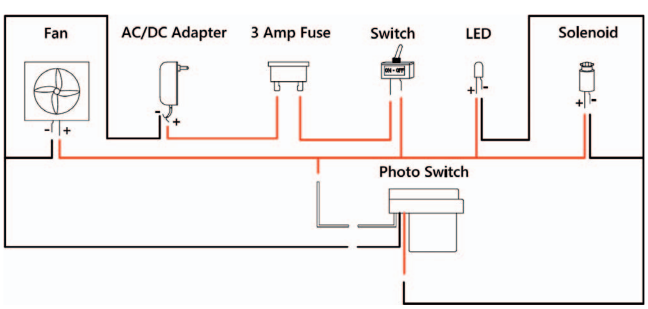


Figure 2.—Locker Trap component wiring diagram.

Results and Discussion

The design of the LCKR has steadily improved over the last five years. Repeated deployment of the trap has allowed for selection of components that meet trapping needs with minimal trap failures. By necessity LCKR CO_2 flow rates varied between 250 mL/min and 400 ml/min. Flow rate was dependent on night length. April through September the traps ran at the higher flow rate and a single 10 lb tank was sufficient for two weeks of trapping. During October - March when night length was longer the lower flow rate was used and the 10 lb tank was changed on a weekly basis.

The comparison of MMT to LCKR was conducted before LCKR traps had been fully developed and at the end of the service life for MMT. Therefore, it was not clear how the current LCKR would compare to a recent MMT model. These comparisons were conducted to better understand how surveillance data would change if/when LCKRs were

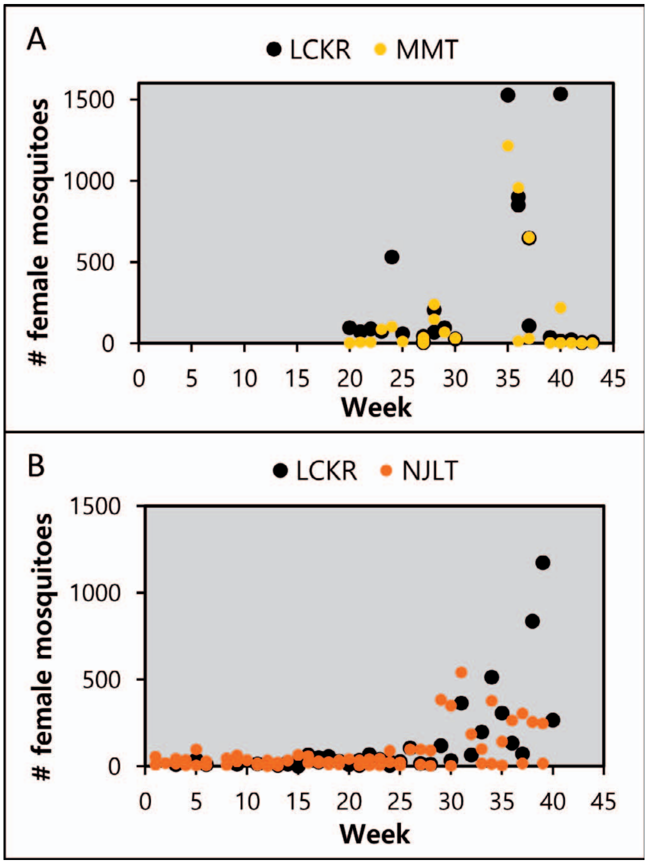


Figure 3.—Aggregation of all abundance counts of female mosquitoes (all species) collected by trap type over a seven-day period, plotted by week of collection; A) Locker traps (LKCR) and Mosquito Magnet Pro traps (MMT); B) LKCR and New Jersey light traps (NJLT)

adopted. To assess trap performance, the model coefficients for each trap comparison were evaluated. Comparison of LCKR to MMT indicated that for every 10 female mosquitoes collected in a LCKR trap, the MMT collected 6.1 ($P < 0.001$). LCKRs and NJLTs collected similar numbers of mosquitoes; i.e., for every 10 female mosquitoes collected in a LCKR, there were 9.6 collected in the NJLT. Overall, the number of female mosquito species collected weekly for the comparison of LCKR (mean $\pm$ SD $= 3.1 \pm 1.0$ species) to MMT (mean $\pm$ SD $= 2.7 \pm 1.2$ species) and LCKR (mean $\pm$ SD $= 2.9 \pm 1.4$ species) to NJLT (mean $\pm$ SD $= 3.2 \pm 1.7$ species) were similar and not predicted by trap type ($P > 0.05$). These data indicated that a shift to LCKR for abundance surveillance would have minimal impact on female mosquito abundance data. However, LCKR do not collect male mosquitoes, and males are readily collected by NJLT. Therefore, switching from NJLT to LCKR meant a loss of male mosquito abundance data.

Conclusions

We anticipate that as LCKR traps are used and serviced, incremental improvements will continue. The next area for optimization will be assessment of different fan speeds. The current fan rotates at 3700 rpm. This fan speed was selected to maximize catch, but leads to desiccation and damage of the catch over the collection week. Future work will evaluate fans with the same form factor, but lower fan speeds, to determine the impact on catch rate and specimen condition. Overall, the LCKR has fulfilled our District's need for a sturdy, easy to construct and repair, and effective trap for year-round surveillance. The LCKR has now become an integral part the SYMVCD surveillance program.

Acknowledgements

We thank the Sacramento-Yolo Laboratory Technicians for their assistance with construction, setting, servicing, and feedback on the Locker Trap during development and implementation.

References

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Pay to play: Targeting notifications to specific neighborhoods for wide area larvicide treatments for combatting the invasive *Aedes*

Tammy Gordon*

Coachella Valley Mosquito and Vector Control District, Indio, 92201

*Corresponding author: tgordon@cvmvcd.org

Abstract

In the Coachella Valley of Southern California, nighttime larvicide applications were scheduled to combat the spread of the invasive *Aedes aegypti* in the summer of 2020. Six applications were scheduled over the course of eight weeks using both aerial and truck mounted methods. Starting with a good strategy and targeted goals, we explored a case study in public notification to over 26,000 residents across two cities in the Coachella Valley. Although traditional media plays a part in awareness and education, the evolution of social media – specifically geo-targeted ads – have revolutionized the opportunities to reach and collect data from users. But challenges such as Facebook's ever-changing algorithm, time needed to participate on a multitude of new platforms, and barriers of entry to applications like Nextdoor, inhibit the opportunities to catch the attention of residents. We explored challenges, best practices, and information gleaned from follow up surveys in our case study.

Introduction

Detection of the invasive mosquito species *Aedes aegypti* has been steadily increasing throughout the Coachella Valley of California since it was first detected in May 2016. This mosquito species does not occur naturally in Southern California but research and experience has shown once introduced, it thrives in Valley neighborhoods. This is particularly daunting because as this mosquito becomes established, the risk of mosquito-borne diseases they can transmit such as chikungunya, dengue, and Zika increases.

In the fall of 2019, the District Call Center noticed an increase in service requests, specifically in the La Quinta Cove area of the Coachella Valley. At the time, there was one trap located within the Cove and the abundance of trapped invasive *Aedes* adults did not support the increase in service requests. Subsequently, additional traps were set and a team of technicians dubbed "The Hot Spots Team" spent one day per week for six weeks going door-to-door and conducting residential inspections.

In 2020, the Coachella Valley Mosquito and Vector Control District's Integrated Vector Management (IVM) program staff identified the La Quinta Cove and one additional neighborhood in the city of Palm Desert as "hot spots" for *Ae. aegypti* activity. Although each area contains roughly the same number of households, the demographics differed in a variety of ways.

Staff created a wide area larvicide treatment plan to lessen the abundance of *Ae. aegypti* in neighborhoods over the summer, with one application every weekend (day depending on the zone) for four consecutive weekends. Two additional applications would follow every other weekend for a total of six applications per zone, over an eight week period from July 18-September 5.

One of the most influential factors was the availability of District equipment to access the residential areas. In Palm Desert, many of the subdivisions within the application area were gated communities which would require additional staff resources to gain access to each community. This made area wide larvicide treatments by helicopter the most logical option. The La Quinta Cove application area was mostly an ungated community built in a grid like system which made this location ideal for truck mounted area wide larvicide treatments. We estimated about 26,000 residents live within these two application zones.

The current paper evaluated the public notification tools that the District used for these area wide treatments. Additionally, we explored the public's perception of the efficacy of treatments by analyzing results of a follow-up survey advertised to the public living within the treatment zones.

Developing goals of the project

A common technique used to create goals is the SMART goals process. This method helped define objectives within the project. The goals included 1) to geo-target neighborhoods within the application routes, 2) track reach/impressions, click throughs, webpage views, and complaint calls, 3) following the last application, a public opinion survey was promoted within one month of the last application, and 5) the project should be reasonable for one person to add to their workload.

The District used a variety of outreach methods to notify residents in targeted neighborhoods. Traditional notification methods included City Council presentations, posting of notification signs throughout the neighborhoods, and weekly emails through the District's notification ListServ.

City Council presentations: Maintaining good relationships with elected officials and staff is a basic outreach approach, because different officials will have different levels of interest in vector control. As in most cases, interest tends to spike when an elected official is being effected by an invasive *Aedes* infestation, such as was the Mayor of La Quinta. The Mayor requested a City Council presentation beyond the informational press release.

Sign placement: Where the notification signs are placed is at the discretion of a Lead Vector Control Technician who studies the area's access points and installs temporary signs at strategic locations and high trafficked intersections.

Email ListServ: By promoting our "Sign up for our notification emails" in 2019, our ListServ jumped from around 200 to nearly 2,400 users by the time we sent notifications for the area wide larvicide treatments. Although helpful, notifications were not nearly as targeted as other tools. One benefit from the weekly email was the ability for quick updates which generated six media articles or interviews.

The application routes contained only about 5% of the total full-time residential population in the valley, or about 26,000 full time residents. Therefore, a traditional advertising campaign would be costly and irrelevant to a lot of people. So by targeting only the neighborhoods within the application zone, we saved resources for the District and remained relevant for residents that would have the most to benefit from the notifications.

After the series of treatments, the metrics we analyzed included: tracking reach (unique users) or impressions (number of times the post was shown), click throughs, web page views, and complaint calls to measure our notification's effectiveness.

Lastly, we followed-up the series of applications in each treatment zone with a survey measuring the residents' perception of efficacy.

Advertising campaigns

To provide additional notification and transparency to residents, the District purchased a series of advertisements to notify residents within the application routes. This strategy is known as geo-targeting. It provided the best value for advertising campaigns by showing the advertisements only to users that are within the designated geospatial area. Social media advertising is a cost effective way to notify residents. Online platforms used to geo-target the campaign included Facebook, Instagram, Twitter, online banner ads, and Nextdoor.

Facebook ads: For now, Facebook still holds value when it comes to high return on marketing investment. Two geo-targeted Facebook ads ran 2-3 days before each of the first four weekend applications for a total cost of $406.21. These ads were linked to custom webpages that showed maps, dates, times, and frequently asked questions for applications. In total, the advertising reports indicated that more than 18,000 people viewed the paid-for posts which told us that 72% of the total estimated population viewed the notification ad on Facebook/Instagram.

In the La Quinta Cove, there are an estimated 5,000 households in the application zone. The Facebook ad report showed that 8,430 unique users saw the ad. Of those, 338 clicked the link directing them to the custom webpage for their neighborhood averaging $0.58 per link click.

In the Palm Desert aerial application zone, there are an estimated 5,000 households in the application zone with 10,395 unique users seeing the ad. Of those, 893 clicked the link directing them to the custom webpage for their neighborhood averaging $0.23 per link click.

Alpha Media ad results: Two geo-targeted ads were purchased through Alpha Media to place digital ads on radio station websites. These ads ran three days before the applications for the first two weeks of scheduled treatments for a total cost of $480. The campaign report indicated that the banner ads received 44,649 impressions, meaning the advertisement was placed and seen 44,649 times. The report also indicated that the ads only received 95 clicks. However, we were able to gain the insight that in La Quinta, 66% of users viewing ads were on a mobile device. The 76% of those that clicked on the ad from a mobile device was greater than the community of Palm Desert in which 58% of users viewed ads on a mobile device and 66% of those clicked on the ad.

Nextdoor: In 2020, the District engaged in a paid pilot program with the Nextdoor platform. The cost to use the tool was based on residents that signed up on the Nextdoor platform, or "members." At the time of the contract, members on Nextdoor residing in the Coachella Valley for more than six-months of the year, or Reachable Nextdoor Members (RNM), totaled 78,809 and the unit cost per member was $.0069 per month for a total of $543.78 per month. As of December 2020, the number of members has risen to 114,000, nearly 33% of estimated households in the Coachella Valley. As a cost saving measure in the coming year, the contract will be seasonal and only paid for between April-November which is generally considered the District's mosquito-borne virus season. In that timeframe, posts on Nextdoor yielded almost 15,000 impressions from residents that resided near a virus detection area or within an area wide mosquito treatment area.

Nextdoor uses a verified residential address to create neighborhoods based on the concept that neighbors talk to neighbors. A problem with these designated neighborhoods is that they don't always coincide with subdivisions which sometimes make notifications problematic. However, by using a paid version of this platform, the Nextdoor engineers built neighborhoods using our route maps indicating that each post only went to people within that application zone.

Starting on July 13, we posted to each of the Area wide larvicide treatment neighborhoods. According to Nextdoor, the La Quinta Cove neighborhood had 4,494 Nextdoor members and the Palm Desert application zone had 1,672 members. Because Nextdoor is relatively new to the District, we tried posting at different times, dates, and used different subject lines to better reach residents. For example in Palm Desert, posting a few days before an application *and* a follow up reminder post the day of treatment with the subject line "Helicopter Mosquito Control Treatments Tonight (2am-7am)" was more engaging and resulted in fewer follow up posts from residents.

Website: Another measuring tool we used was web traffic. We pointed all online hyperlinks to custom web pages for each application zone and tracked page visits. These webpages restated dates, times, and map area. Additionally, we included detailed information that an interested resident might want such as product used, how it works, and what to do with pets during the application. Total page views between July and September was 2,563: 1,795 views on the Palm Desert application page and 768 on the La Quinta Cove application page.

Call Center: The Call Center received about 15 phone calls from residents regarding the area wide larvicide treatments over the first six weeks of applications. Of those, thirteen calls were questions regarding pets, pools, etc. The two other complaint calls focused around pesticide abhorrence. Although we categorize these as the only two complaints, it is noteworthy that both of these individuals called multiple times and required considerable staff time and legal guidance. One complainant went so far as to use their vehicle to block the treatment trucks which required police escort to complete the applications.

Postcards: Postcards were not produced for these applications as the total cost to produce and mail one postcard, did not justify the return on investment which is difficult to calculate with mail notifications. Another limitation is the program the United States Postal Service uses to deliver these notices. The Every Door Direct Mailer (EDDM) can be delivered to every address within the postal route without prejudice. But, similar to the limitation we saw in the Nextdoor platform, the application route did not coincide with the postal route. For example, the estimated households in the Palm Desert route was 5,000, but with overlapping postal routes, to deliver a postcard to every address within the zone, the EDDM program would deliver to 6,509 addresses. We estimated that a postcard sent to households within the application areas before each application would have cost the District around $16,800.

Outreach Survey

Essential to any outreach program is measuring efficacy. In this case, the efficacy was based on the residents' perception of the efficacy of mosquito control. After the completion of the series of treatments, an online survey was promoted. The goals of the survey were to find 1) is there an understanding of invasive *Ae. aegypti,* 2) do residents think mosquitoes are an issue in their community, 3) were residents notified of the treatments and where did they hear about them, and 4) did the treatments work.

Paid advertising: In an attempt to gain more participants in the survey, we paid for ads via Facebook and Twitter using the geo-targeting method discussed above. Facebook ads totaling $434.84 received a reach of 8,734 and 271 link clicks. On Twitter, we opened the survey three weeks after the completion of the last larvicide application and promoted for 10 days and gained 31,346 impressions, for $360.57.

In general, surveys have low response rates and experiencing a 1% response rate is common, especially for the length of time the survey was open. With our estimated 26,000 residents living within the application zones, we then were anticipating roughly 260 respondents. It is important to note, however, that the residential population is estimated to include *all* residents, including children. We would not expect a child to respond to a survey, so closing the survey with 194 responses was predictable and, although low, acceptable.

Survey questions and responses

The 10-question survey was offered in both English and Spanish. Overall, 99.4% responded in English, 53% indicated they lived within the Palm Desert application zone, and 47% indicated residency in La Quinta. Questions included:

1) ***Did you know that an invasive mosquito (Aedes aegypti) has been detected in your neighborhood? Even though no viruses have yet been transmitted in California, this mosquito may carry viruses (such as dengue, yellow fever, Zika, and chikungunya) that can cause illness and even death in people.*** 77.5% Yes, 22.5% No.
2) ***How concerned are you about the presence of mosquitoes in your community?*** Respondents rated their level of concern using a five point Likert scale (1=Extremely, 2=Greatly, 3=Somewhat, 4=Hardly, 5=Not at all). 50% of respondents were extremely concerned, whereas 2% were not concerned at all. The weighted average rating score was 1.8, indicating the overwhelming majority of respondents was extremely or greatly concerned about mosquitoes in their neighborhood.
3) ***The District completed mosquito control applications in your neighborhood this summer. Were you notified about these mosquito control applications?*** Although some respondents answered that they were not in the Coachella Valley during the series of treatments, 66% of survey respondents disclosed that they did know that treatments were taking place, 25% were not notified, and 6% were unsure.
4) ***If you knew about the applications, how did you learn about them?*** Of those who did hear about the control treatments, the top responses for where they heard about them included: Nextdoor, social media ads, signs posted in neighborhood and their City's social media posts, respectively.

5) ***Have you or your family been bitten by mosquitoes while in your neighborhood?*** 73% Yes, 24% No, 3% Unsure.
6) ***Have you noticed a change in the number of mosquitoes in your neighborhood since the applications?*** Discouragingly, a noticeable decrease in abundance by respondents was not certain: 20% did not know if they noticed a difference, whereas 30% believed they were seeing the same amount or more in their neighborhood. However, 50% of respondents did perceive a noticeable decrease.
7) ***Additional questions.*** While we had their attention, we collected some information concerning general behaviors and attitudes. We asked if respondents know that mosquitoes only needed a tablespoon of water to develop, what steps do they take to reduce sources in their yard, what personal protection do they use, and if they had additional comments or questions. The responses varied among the answers we supplied, but by allowing respondents to add their own responses, we were able to glean some of the misinformation that our residents believe in and use to protect themselves against mosquitoes, including essential oils and planting mosquito fighting vegetation.

Many of the respondents commented at the end of the survey for closing remarks. Beyond reading comments, we used content analysis to categorize responses for further study and pull out common themes. By a three-to-one margin, positive comments overwhelmingly were greater than negative comments ("thank you for spraying" vs "fearmongering and unnecessary"). Not surprisingly, the majority (55%) of comments in the survey were neither positive nor negative, and simply stated the respondents distaste relating to mosquitoes and mosquito bites. Some common themes gleaned from comments included: neighborhood signs were too small, the helicopter was too loud, and requests to repeat/expand treatment areas.

The information and insights gleaned from the responses were invaluable in measuring the efficacy of the outreach tools we used. Information from the survey will be used to improve our education efforts and our notification processes in the coming season.

Conclusion

The key to a good campaign strategy is to create goals. The SMART goal exercise is easy to use and helps create measurable goals that are attainable. After identifying hot spot geography, we then estimated households and residential population. In doing so, we could then design a geo-targeted ad campaign to notify residents of the application period. We tracked our ads using reach/impression numbers, webpage views, and complaint calls.

Although some of the takeaways from this case study support outreach ideas and tactics already in use, by following up the notification process with a survey, we created a baseline of information to improve on. This included misinformation and general questions residents have before an application to help guide our webpage text.

Social media advertising is cost effective and contains better reports than traditional advertising campaigns. It is difficult to estimate the number of people who read a postcard sent to their home, but social media reports offer numerical data points on not only how many times the ad was seen (impressions), but also the number of unique users exposed to the ad (reach), and the number of people who clicked on the additional information link (click throughs).

Surveys get little engagement but yield invaluable insight. Without a public opinion survey, we could only draw assumptions as to what our population understands about mosquito control. By asking specific questions, we can identify what topics need more development for public education. Debunking mosquito control myths and offering additional information regarding pets have been added to the social media outreach campaigns for 2021.

Lastly, do not discount the importance of traditional notification and partnerships with stakeholders. Posting physical signs throughout treatment areas is vital for targeted audiences that could be outdoors during the application time. The survey indicated that many respondents not only saw the signs, but requested additional signs be placed for easier access to read the notifications. City staff from both cities were actively engaged in sharing our information on a variety of their information channels. City Council presentations, though time consuming, are an effective way of gaining additional attention, particularly from the community's influencers who then share the notifications.

These processes will be repeated with some adjustments in the 2021 pre-scheduled area wide larvicide application series in an attempt to increase the number of residents notified as well as include additional frequently asked questions for those residents.

Acknowledgements

I would like to thank my District cohorts, without their extraordinary efforts and dedication this work would not be possible. Thank you to the IVM team that spent countless hours strategizing and talking through points of concern and addressing them always with protecting public health as the most influential driving factor. Also thank you to Jennifer Henke for her willingness to share her knowledge and expertise with me at all times. And lastly, thank you to Jeremy Wittie for taking the time to encourage my ideas and giving constructive feedback to develop projects and programs to the best of our abilities.

“*Aedes* juice” as an effective oviposition attractant for ovicups

Javier Valdivias*, Jesse Erandio, Crystal Grippin, Mark Nakata, Mir Bear-Johnson, and Mustapha Debboun

Delta Mosquito and Vector Control District, Visalia, CA 93291

*Corresponding author: jvaldivias@deltavcd.com

INTRODUCTION

Delta Mosquito and Vector Control District (DMVCD) has baited passive traps such as ovicups and autocidal gravid ovicup (AGO) traps with either an alfalfa-based infusion or tap water. The alfalfa-based infusion primarily attracts gravid *Culex* species, performing poorly with *Aedes aegypti*. The current study evaluated infusion mixtures to find a more effective substrate that specifically targets gravid *Ae. aegypti* to increase the effectiveness of ovicups for surveillance.

METHODS

Aedes larval samples were collected from man-made containers during property inspections. Each positive larval sample was filtered through a mesh screen to ensure all larvae and debris were removed and then stored in a 5-gallon jug at room temperature. Eight locations throughout the Visalia Public Cemetery were selected as appropriate trap sites at which three ovicups were set for a duration of four days during each of four weeks. The ovicups were set side by side (Fig. 1) and each contained one of the three tested attractants: “*Aedes* juice” (filtered larval source water), tap water, and standard gravid trap hay infusion (275 grams of Kruse’s Perfection Brand Rabbit food, 30 grams of NOW Brewer’s yeast and 50 gallons of tap water). After four days, the germination papers were removed and dried for two days before eggs were counted using a Nikon SMZ 800 microscope. The mean egg counts in cups with each of the three attractants were compared by two-way repeated measures ANOVA and post-hoc Tukey’s Test.

RESULTS AND DISCUSSION

A total of three ovicups were set in eight locations throughout the cemetery each week. These eight sites were sampled for four weeks; however, in the fourth week all three cups from one location were missing before retrieval. A total of 6,332 *Ae. aegypti* eggs were collected. The results from two-way repeated measures ANOVA indicated there was a significant difference among the mean egg counts in the three infusions ($F=18.22$; $df=2$; $P<0.001$). A post-hoc Tukey’s Test was used to compare 4-day mean total egg counts in the infusions during each week. Ovicups baited with “*Aedes* juice” had a significantly greater egg count than the alfalfa-based infusion on weeks 1 – 3 ($P<0.05$); however,on week 4, no significant difference was found ($P=0.12$). On week 1, no significant difference was found between ovicups baited with “*Aedes* juice” and tap water ($P=0.21$). On weeks 2-4, ovicups baited with “*Aedes* juice” had a significantly greater mean egg count than tap water ($P<0.05$). On weeks 1-4, no significant difference was found between mean egg counts in tap water and the alfalfa-based infusion (Fig. 2).

CONCLUSION

Water from sources that previously contained *Ae. aegypti* larvae were more attractive to gravid females than both the alfalfa-based infusion and tap water. Utilizing ‘*Aedes* juice’ as an oviposition attractant to bait passive traps, such as AGOs and Biogents Gravid *Aedes* Traps (BG-GAT), may lead to effective suppression methods in the form of egg sinks for areas where source reduction inspections are completed.

Figure 1.—Example of the multi-choice behavioral bioassay arrangement of ovicups in the field.

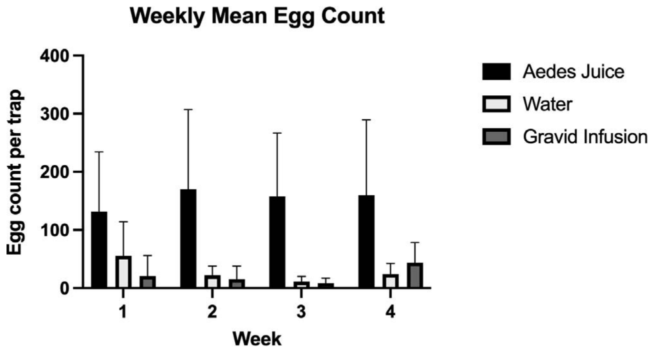


Figure 2.—Comparison of the 4-day mean egg count per trap for *Aedes* juice, tap water, and alfalfa-based infusion for the duration of the 4 weeks.

ACKNOWLEDGEMENTS

We thank the Visalia Public Cemetery personnel for their support throughout this study.

How much product do we need? Using different rates for *Aedes* control

Jennifer A. Henke*, Gabriela Perezchica-Harvey, Gerald Chuzel, Melissa Snelling, Arturo Gutierrez, Kim Y. Hung, Roberta Dieckmann, Greg Alvarado, Olde Avalos, Chris Cavanaugh, Michael Martinez, Edward Prendez, and Tammy Gordon

Coachella Valley Mosquito and Vector Control District, Indio, CA

*Corresponding author: jhenke@cvmvcd.org

Aedes aegypti mosquitoes were detected in the Coachella Valley in May 2016. Since then the Coachella Valley Mosquito and Vector Control District has conducted several missions of large area larviciding for the control of mosquitoes on residential properties. Mosquito collections in La Quinta Cove in 2019 indicated that there was a robust population. Truck larviciding applications were made weekly at 0.5 per acre in the northern two blocks and 0.35 lbs per acre in the southern two blocks during weeks 29-32, and then again on week 34 and 36 (July through September 2020) for a total of 6 applications over 8 weeks. The first four applications were made using the same A1 Super Duty over two days with one route treated on Saturday and the other on Sunday. The final two applications were made using 2 pieces of equipment on the same day. Mosquito collections were made using 8 BG-Sentinel traps baited with dry ice and BG lure set in each block, with comparisons made to trap collections in other cities where no large area applications were made, although routine control efforts through service requests and treatments to the stormwater system continued. When comparing the mosquito collections in La Quinta with similarly sized communities where large area applications did not occur, the numbers of female *Ae. aegypti* collected per trap fell and remained low until 6 weeks following the final application.

Fighting a phantom: Response to initial detection of *Aedes aegypti* in Yuba City

Erik Blosser*, Merv Hunt, Joe Songer, Tim Houser, Bill Terbush, and Stephen Abshier

Sutter-Yuba Mosquito and Vector Control District, Yuba City, CA 95991

*Corresponding author: eblosser@sutter-yubamvcd.org

INTRODUCTION

During the summer and fall of 2020 at least six cities located in the Sacramento Valley reported first-time detections of invasive *Aedes aegypti* (CDPH 2021), greatly expanding the known species range in this region. In Yuba City, the Sutter-Yuba MVCD first detected *Ae. aegypti* in a CDC light trap collected on August 14, 2020 and proceeded with efforts to determine the scale of the infestation and simultaneously treat areas with known detections.

METHODS

Published flight ranges of *Ae. aegypti* (Marcantonio et al. 2019) were used as a guide for initial surveillance efforts using BG sentinel traps and ovicups that were baited with tap water and checked weekly. Simultaneously, wide area ULV deltamethrin applications and Vectobac water-dispersible granule (WDG) wide-area larvicide spray (WALS) treatments were performed over an approximately 200 acre spray block surrounding the detection point. Following repeated detections at two sites, properties within a 100 meter radius received front and back yard surveillance utilizing ovicups as well as biweekly inspections and treatments by handheld fogging with deltamethrin. Focal areas were switched to treatment with In2Care traps once egg detections ceased.

RESULTS AND DISCUSSION

Follow-up surveillance trapping resulted in ten detections of *Ae. aegypti,* with nine of these coming from ovicups. Ovicups were more useful than BG Sentinels due to the ability to deploy large numbers for extended periods of time with little risk of theft. All detections were located at two "hotspots", each consisting of two neighboring houses. Each hotspot was surrounded by adjacent houses which were consistently negative for eggs and larvae. Property inspections discovered two potential sources, larvae in a corrugated gutter downspout extension and a suspect leaf-clogged gutter, both of which were fed by condensation from roof air conditioning units and both of which were likely inaccessible to WALS treatments.

CONCLUSIONS

Limited detections of *Ae. aegypti* in Yuba City during 2020 seemed to indicate a fairly small-scale infestation. However, the easily missed, small-sized detection areas, difficulty in discovering larval habitats, and limited large-scale surveillance made it difficult to ascertain the true extent of the infestation. Continued surveillance of known hotspots along with a larger area survey for infestations will help answer questions about overwintering success and the scale of infestation in the coming year.

ACKNOWLEDGEMENTS

Zach Samay, Alyssa Aguirre, Richard Herrington, Shane Loyd and Darrell Jew contributed to these efforts.

REFERENCES CITED

CDPH (California Department of Public Health). 2021. *Aedes aegypti* and *Aedes albopictus* mosquitoes in California by county/city. https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/AedesDistributionMap.pdf. Accessed 3-24-21.

Marcantonio, M., T. Reyes, and C.M. Barker. 2019. Quantifying *Aedes aegypti* dispersal in space and time: a modeling approach. Ecosphere, 10(12):e02977.

The WALS to the cemetery: A look at efficacy under field conditions

Crystal Grippin*, Javier Valdivias, Jesse Erandio, Paul Harlien, Tim Christian, Bryan Ruiz, Mir Bear-Johnson, Mustapha Debboun

Delta Mosquito and Vector Control District, Visalia, CA 93291

*Corresponding author: clgrippin@deltavcd.com

INTRODUCTION

Vases, plant trays, and other man-made containers placed at gravesites place cemeteries at high risk for *Aedes aegypti* (L.) colonization (Vezzani et al. 2002, Wilke et al. 2020). *Aedes aegypti* were first discovered at the Visalia Public Cemetery in 2019, with a 5% container index (CI, percent of water-holding containers positive for larvae or pupae). Despite extensive physical control efforts and public education, the cemetery continued to produce *Ae. aegypti* the following year with the CI reaching 40% by the first week of August 2020. This study evaluated the use of truck-mounted Wide Area Larviciding (WALs) to control *Ae. aegypti* under field conditions.

METHODS

VectoBac WDG was applied in the cemetery once a week for four weeks using a truck-mounted A1 Super Duty fogger (Valent BioSciences 2019). Larval abundance was monitored using a randomized container survey conducted weekly before each WALs application and for four weeks following the last application. Female *Ae. aegypti* were monitored with five BG Sentinel mosquito traps placed weekly, before each WALS application and for five weeks following the last application.

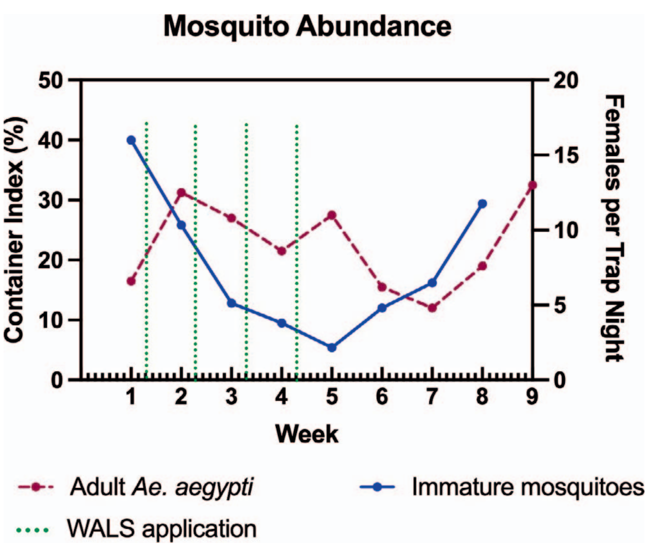


Figure 1.—Female *Ae. aegypti* abundance and the container index during and after truck-mounted wide area larviciding in the cemetery.

RESULTS AND DISCUSSION

The CI rapidly decreased from 40.0% before the first WALs application to 5.4% by the fifth week following the four applications (Figure 1). The CI slowly recovered to near original levels by the eighth week. Changes in adult *Ae. aegypti* abundance appeared to lag about two weeks behind changes in the CI (Figure 1). In week seven, adult abundance decreased to 4.8 female *Ae. aegypti* per trap night. Female *Ae. aegypti* abundance increased during week 5, when a source outside the cemetery was found and then treated.

CONCLUSION

Truck-mounted WALs applications were effective in reducing the CI within the cemetery under field conditions. However, this reduction only continued for about a week after the last application, with changes in adult abundance following about two weeks after changes in the CI. Products with a longer residual efficacy may be more effective in maintaining control under field conditions. Physical control is the best solution, but there is not enough commitment by individuals within the community to make this a reality within the cemetery.

REFERENCES CITED

Valent BioSciences. 2019. Container mosquito vector control: Vehicle mounted WALS of VectoBac WDG bacterial larvicide, standard operating procedure for the USA version 1.6.

Vezzani, D., and N. Schweigmann. 2002. Suitability of containers from different sources as breeding sites of *Aedes aegypti* (L.) in a cemetery of Buenos Aires City, Argentina. Mem. Inst. Oswaldo Cruz. 97: 789–792.

Wilke, A. B. B., C. Vasquez, A. Carvajal, M. Moreno, Y. Diaz, T. Belledent, L. Gibson, W. D. Petrie, D. O. Fuller, and J. C. Beier. 2020. Cemeteries in Miami-Dade County, Florida are important areas to be targeted in mosquito management and control efforts. PLoS One. 15.

WALS®: Treatments using an A1 Super Duty Sprayer in three habitats of Orange County, California

Timothy Morgan*, Sokanary Sun, Xiaoming Wang, Amber Semrow, and Robert Cummings

Orange County Mosquito and Vector Control District, Garden Grove, CA 92843

*Corresponding author: tmorgan@ocvector.org

Abstract

In 2020, the Orange County Mosquito and Vector Control District (OCMVCD) conducted efficacy studies for three A1 Super Duty Sprayer, VectoBac® water dispersible granule (WDG) applications. Treatments were evaluated against pyrethroid susceptible laboratory colony *Culex quinquefasciatus* Say larvae using mortality assays by placing plastic cups at different distances downwind from the drive path to collect larvicide droplets at a freshwater marsh, cemetery, and residential neighborhood. Twenty-four-hour mortality was assessed to estimate the efficacy of each treatment. Post spray adult *Aedes aegypti* (L.) abundance was evaluated for an ultra-low volume (ULV) adulticide and wide area larvicide combination treatment in the residential neighborhood. Overall, the 24 h *Cx. quinquefasciatus* larval mortalities in the marsh, cemetery, and residential neighborhood were 95.6%, 99.8%, 82.9%, respectively. The A1 Super Duty Sprayer was highly effective at delivering larvicide to the target areas and will continue to be evaluated by OCMVCD.

INTRODUCTION

Aedes aegypti (L.) is a notorious, globally invasive mosquito. It was discovered in 2013 in California and for the first time in Orange County, inside a home in the city of Anaheim, during 2015 (Metzger et al. 2017). Within five years, all but two of the county's 34 cities were inhabited by this species. The rapid spread of *Ae. aegypti* and the simultaneous detection of two other invasive species, *Ae. albopictus* (Skuse) and *Ae. notoscriptus* (Skuse), leave many residents distressed and the Orange County Mosquito and Vector Control District (OCMVCD) working to develop control strategies against these container breeding mosquitoes. Although currently absent, autochthonous transmission of chikungunya, dengue, and Zika viruses is now possible when county residents infected abroad return home with one of these viruses to invasive *Aedes* infested communities. These conditions warrant the establishment of targeted invasive *Aedes*-vectored disease surveillance and control programs (CDPH 2020), and the acquisition of efficient and effective pesticide application equipment necessary for area-wide control (Harris et al. 2020). OCMVCD has added a powerful truck-mounted spray blower to its control program for larvicide applications to help address increasing invasive *Aedes* mosquito activity and vector-borne disease risks. Three studies were performed to evaluate the utility of the spray blower in delivering water dispersible granular larvicide to artificial mosquito breeding targets, placed within three different habitats, in the urban matrix of Orange County: a freshwater marsh, a cemetery, and a residential neighborhood.

METHODS

In 2020, OCMVCD conducted three area-wide VectoBac® WDG (37.4% *Bacillus thuringiensis* subsp. *israelensis*, strain AM 65-52, Valent BioSciences, Libertyville, IL) applications (also known as a Wide Area Larvicide System, WALS®) using a truck-mounted A1 Super Duty Sprayer (A1-SD), (A1 Mist Sprayers, Ponca NE), equipped with a Micronair AU5000 Atomiser (Micron Group, Bromyard, U.K.). All three applications were evaluated against pyrethroid susceptible laboratory colony *Culex quinquefasciatus* Say 2nd and 3rd instar larvae using plastic cup bioassays. Control cups were set upwind and wind speeds ranged from 0.8 to 3.2 km/h (0 to 2 mph) during the applications. Cups were collected 30 minutes after each application.

Freshwater Marsh

On 12 February 2020, a cup bioassay was used to assess the efficacy and longevity of a daytime WALS® application at a freshwater marsh. Although the system is described to utilize VectoBac® WDG, we used a mixture, with an additional dispersible granule product for this application while targeting marsh mosquitoes, primarily *Cx. erythrothorax* Dyar. We applied an aqueous mixture of VectoBac® WDG at 395.4 g/ha (160 g/acre) and VectoLex® WDG (*Bacillus sphaericus* 2362, Serotype H5a5b, Strain ABTS 1743, Valent BioSciences, Libertyville, IL) at 1,285 g/ha (520 g/acre) to a 5.3 ha (13-acre) freshwater marsh located south of the Villa Park Dam, Orange County, CA. The spray plume volume median diameter was calculated at 134 microns using DropVision® Ag (Leading Edge, Fletcher, NC). Three passes were made along a single drive path at

Table 1.—ULV adulticide and WALS® larvicide treatment dates and pre/post treatment reduction of female *Aedes aegypti* abundance. Abundance reduction was calculated with Henderson-Tilton formula.

			Female *Aedes aegypti* Abundance (per trap night)	
Events	Treatment	Date	Treatment	Control
Pre-Treatment Surveillance	None	9/10/20	3	4
Treatment	WALS®/ULV	9/11/20	-	-
Treatment	ULV	9/15/20	-	-
Treatment	ULV	9/16/20	-	-
Treatment	WALS®/ULV	9/17/20	-	-
Post-Treatment Surveillance	None	9/30/20	1	5
Abundance Reduction (Pre/Post Treatment)			73.3%	

4.8 km/h (3 mph). Fourteen 500 ml polyethene cups were placed 20.7 to 115.2 m (68 to 378 ft.) downwind from the drive path either on the ground or floating on anchored polystyrene pads on the water surface. After the treatment, cups were returned to the laboratory, filled halfway with distilled water, and three larval cohorts of 25 *Cx. quinquefasciatus* were added to each cup 2 hours, 4 days, and 7 days after treatment. Mortality was assessed at 24 h and all larvae were removed prior to subsequent introductions. Laboratory conditions were as follows, 12:12-h light: dark cycle, 23°C ambient air, 21°C cup water, and 50-60% humidity and were the same for each evaluation. Larval *Cx. quinquefasciatus* was used as a substitution for the actual target species to assess delivery of the larvicide to the target area.

Cemetery

On 13 October 2020, the same basic larval cup assay was used to evaluate a daytime WALS® treatment of 495 g/ha (200 g/acre) VectoBac® WDG applied to a 42.1 ha (104 acres) cemetery complex in Santa Ana, CA. Sixty-one cups were placed on the ground in five linear transects ranging from 1.5 to 228.6 m (5 to 750 ft.) perpendicular to the drive path. A single pass was made at 14.5 km/h (9 mph). Twenty-five *Cx. quinquefasciatus* larvae were added to each cup and mortality evaluated after 24 hours.

Residential Neighborhood

In response to an imported dengue case, two nighttime WALS® applications were scheduled for a 30.4 ha (75 acres) single-family-home residential neighborhood in the city of La Habra, CA, on 10 September and 17 September 2020, using 495 g/ha (200 g/acre) rate of VectoBac® WDG. The first WALS® application was evaluated using a larval cup mortality assay with a single introduction of 25 *Cx. quinquefasciatus* to each cup. Four cups were placed on the ground, two (under cover and open) in both the front and back yards, at seven sites. Additionally, a truck-mounted cold fogger (Pro Mist Dura®, Clarke, St Charles, IL) delivered a single ULV application of DeltaGard® (1.5 g/ha, or 0.00134 lb. deltamethrin/acre, AI) (Bayer, Research Triangle Park, NC), immediately following the first WALS® treatment. The WALS® treatment area was located within the boundary of larger area, which later required area-wide control for West Nile virus (WNV) and received three additional consecutive nightly ULV treatments with DeltaGard® at the maximum label rate. The final ULV treatment occurred immediately after the second and final WALS® application (Table 1). The ULV spray droplet spectrum met the product label requirements ($8\mu \leq Dv0.5 \leq 30\mu$ and $Dv0.9 < 50\mu m$).

To evaluate the treatment efficacy of the WALS® applications, we averaged *Cx. quinquefasciatus* 24 h mortalities (total number larvae dead/total number introduced) for cups grouped together based on increasing distance from the upwind spray path. To evaluate the efficacy of the combination WALS® and ULV adulticide applications in the residential neighborhood treatment area, we compared *Ae. aegypti* abundance (number of adult females collected per trap night) from four BG-Sentinel® traps (Biogents AG, Regensburg Germany) baited with BG-Lure® and dry-ice (CO_2) to that of 11 routinely set traps in *Ae. aegypti* infested areas with no areawide control, using the Henderson-Tilton formula (Henderson and Tilton 1955). Adult mosquitoes were removed from the traps, killed with dry-ice, and brought to the laboratory where they were identified to species, sexed, enumerated, and the data recorded.

RESULTS

Cup distances from the spray path varied among the freshwater marsh, cemetery, and residential study sites, ranging from 20.7 - 115.2 m (68-378 ft.), 1.5 - 228.6 m (5-750 ft.), and 4.5 – 70.1 m (15-230 ft.), respectively. The overall 24-hour *Cx. quinquefasciatus* larval mortalities in the marsh, cemetery, and residential neighborhood were 95.6%, 99.8%, 82.9%, respectively. Control mortality was less than 3% in all studies, so no control corrections were used (WHO 2005).

Of the three *Cx. quinquefasciatus* cohorts introduced to cups at 2 hrs, 4 days, and 7 days after treatment in freshwater marsh, we observed greater than 90% *Cx. quinquefasciatus* larval mortality in the first cohort, 24 hours after introduction, at all distance categories (Fig. 1). Twenty-four hour mortality decreased for the second cohort compared to the first, but remained above 85% for all distance categories. Mortality in the 3rd cohort decreased with distance from the truck, with greater than 90% mortality observed in cups set within 30.5 m (100 feet) from the spray path, to no mortality in cups placed beyond

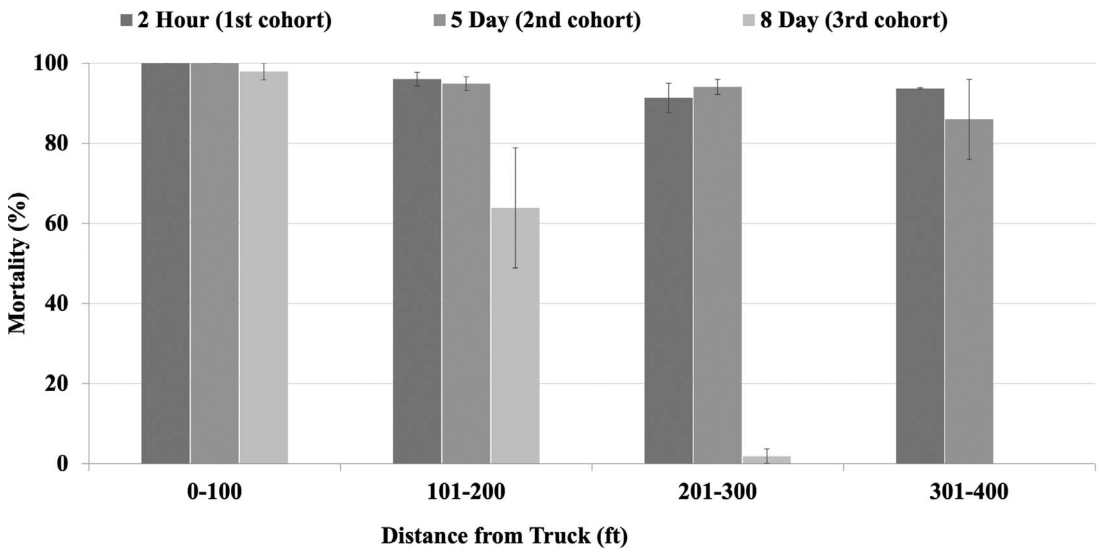


Figure 1.—Laboratory strain *Cx. quinquefasciatus* 24 h mortality by distance from truck application in larvicide cup bioassays at a freshwater marsh. Larval cohort introductions at: 2 hour, 4 days, and 7 days after treatment. Bars show standard error.

91.4 m (300 feet). (Fig. 1). We observed greater than 99% *Cx. quinquefasciatus* larval mortality at all distance categories in the cemetery trial 24-h post larval introduction (Fig. 2). In the residential neighborhood assessment, we found greater than 90% mortality in both the covered and uncovered cups, up to 30.5 m (100 feet) from the spray path (front yards), and a decreasing trend of mortality at greater distances, reaching a low of 45% at the furthest distance for cups under cover (backyards) (Fig. 3). High standard error values in the figure reflect small sample sizes for those distance categories. Two weeks after the last WALS® and ULV combination treatment, *Ae. aegypti* female abundance decreased 73.3% from pre-treatment abundance when compared to the control area (Table 1).

DISCUSSION

Overall, the results indicated that the truck-mounted A1 Super Duty Sprayer was very effective at delivering the larvicide to the intended targets in all three habitats, even in low wind conditions. Greater levels of mortality were observed in the less developed freshwater marsh and cemetery habitats compared to the more developed residential neighborhood. This may have been due, in part, to the vegetation profile within each habitat. The marsh drive path was on an elevated road (~ 15 m) above the marsh, which consisted primarily of 2 to 4 m emergent aquatic vegetation and upland shrubs. Trees were densely clumped in patches across the cemetery, whereas trees

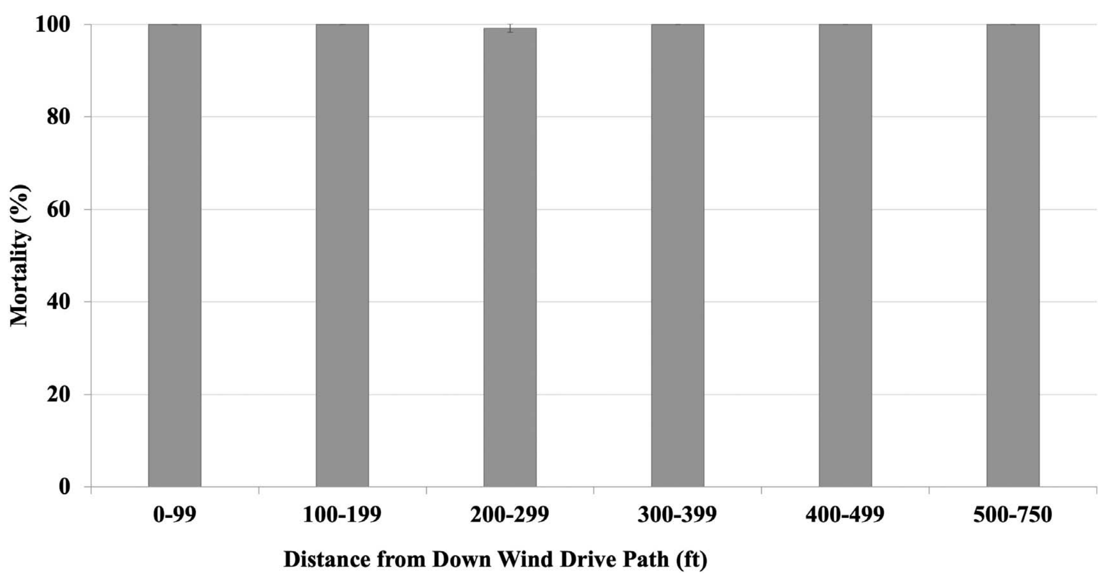


Figure 2.—Laboratory strain *Cx. quinquefasciatus* 24 h mortality in larvicide cup bioassays by distance downwind from the drive path at a *Bti* treated cemetery. Bars show standard error.

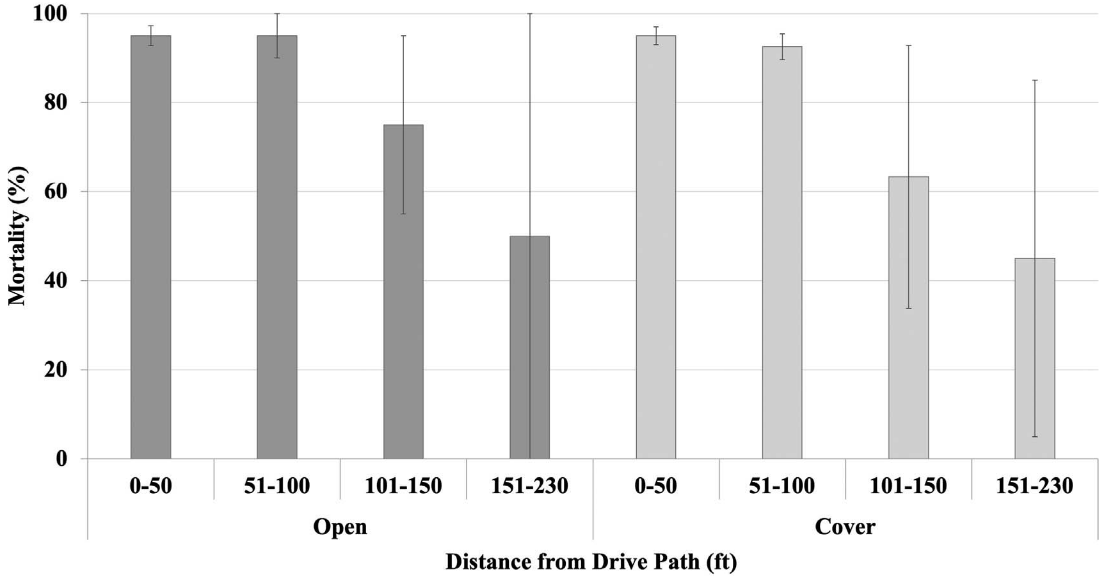


Figure 3.—Laboratory strain *Cx quinquefasciatus* 24 h mortality in a cup bioassay by distance from a *Bti* treated residential neighborhood. Cups were placed in front and backyards, under cover (light grey) and in the open (grey).

lined the drive path and backyards in the residential neighborhood. To minimize spray droplets captured by large trees in the cemetery and residential neighborhood directly adjacent to the spray path, we directed the spray head vertically during all applications. The high velocity spray output propelled the plume vertically (>30.5 m capacity) above the canopy through the unimpeded space above the road, where the low-speed wind could then disperse the descending droplets. Similar to aerial-based larvicide treatments of tree covered collection cups (Luas et al. 2005), the larvicide product from our vertically oriented ground treatment penetrated the foliage and effectively reached cups. The unexpected high mortality in the far distance cups (152.4 to 213.4 m, or 500 to 700 feet) in the cemetery, may have been due, in part, to the drive path configuration which was tangential to each downwind end of the transects. We hypothesized that back drift may have occurred if the plume expanded at its vertical apex and then settled back down on both sides of the drive path in the low wind conditions (0 to 0.8 km/h, or 0 to 0.5 mph), inflating the observed mortality in the furthest downwind cups. In the residential neighborhood treatment, larval mortality was lower in cups beyond 30.5 m (100 feet) from the spray path (backyards) than cups placed closer than 100 feet (front yards), regardless of placement under cover or in the open. The difference in mortality between the front and back yard positioned cups may not be due to an obstructive tree canopy, but from droplet obstruction by various impervious manmade structures found in the habitat (e.g., homes, garages, fences, patio covers etc.).

The combination of WALS® and ULV adulticiding in urban settings has been documented to reduce *Ae.* aegypti abundance (Wilke et al. 2021). Although we observed a promising 73.3% reduction in the adult female *Ae. aegypti* abundance after the larvicide and adulticide combination treatments, compared to the non-treated control area, the absolute change in the number of mosquitos per trap night of two adult females seems nominal. Ernst et al. (2017) found that autochthonous dengue transmission was occurring in Hermosillo, Mexico, with *Ae. aegypti* abundance averaging around two per trap night when using BG-sentinel traps for surveillance. This reported low vector density in a dengue endemic area indicates that similarly low *Ae. aegypti* abundance in Orange County may support local dengue transmission and warrants control measures around travel related cases. Although the absolute reduction in abundance may have been small, there may have been important impacts on the mosquito population age structure (Ernst et al. 2020) and other biological parameters that may reduce disease transmission risk.

OCMVCD will continue to utilize and evaluate WALS® applications using the A1-SD for larval control in both early and peak seasons. The impact of WALS® and ULV combined control on our local WNV vector, *Cx. quinquefasciatus*, invasive *Ae. aegypti* abundance, and mosquito-borne virus prevalence will also be examined.

ACKNOWLEDGEMENTS

We greatly appreciate OCMVCD operations and lab staff for assisting with the bioassay field work and the District Manager, Rick Howard, for supporting this work.

REFERENCES CITED

CDPH (California Department of Public Health). 2020. Guidance for surveillance and response to invasive *Aedes* mosquitoes and dengue, chikungunya, and Zika in California. https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/InvasiveAedesSurveillanceandResponseinCA.pdf. Accessed March 30, 2021.

Ernst, K. C., K. R. Walker, P. Reyes-Castro, T. K. Joy, A. L. Castro-Luque, R. E. Diaz-Caravantes, M. Gameros, S. Haenchen, M. H. Hayden, A. Monaghan, E. Jeffrey-Guttierez, Y. Carrière, and M. R. Riehle. 2017. *Aedes aegypti* (Diptera: Culicidae) longevity and differential emergence of dengue fever in two cities in Sonora, Mexico. J. Med. Entomol. 54: 204-211.

Harris, A. F., J. Sanchez Prats, N. Nazario Maldonado, C. Piovanetti Fiol, M. García Pérez, P. Ramírez-Vera, J. Miranda-Bermúdez, M. Ortiz, and P. DeChant. 2021. An evaluation of *Bacillus thuringiensis israelensis* (AM65-52) treatment for the control of *Aedes aegypti* using vehicle-mounted WALS® application in a densely populated urban area of Puerto Rico. Pest Manag. Sci. 77: 1981-1989.

Henderson, C. F. and E. W. Tilton. 1955. Tests with acaricides against the brow wheat mite, J. Econ. Entomol. 48: 157-161.

Metzger, M. E., M. Hardstone Yoshimizu, K. A. Padgett, R. Hu, and V. L. Kramer. 2017. Detection and establishment of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) mosquitoes in California, 2011-2015. J. Med. Entomol. 54: 533-543.

Porse, C. C., V. Kramer, M. H. Yoshimizu, M. Metzger, R. Hu, K. Padgett, and D. J. Vugia. 2015. Public health response to *Aedes aegypti* and *Ae. albopictus* mosquitoes invading California, USA. Emerg. Infect. Dis. 21: 1827-1839.

Lucas, K. J., P. Brake, S. Grant, L. Lake, R. B. Bales, R. R. Ryan, N. Philips, and P. Linn. 2020. Characterization and efficacy of VectoBac® WDG application targeting container-inhabiting mosquitoes using an unmanned aerial vehicle. J. Fla. Mosquito Vec. Cont. 67: 84-90.

WHO (World Health Organization). 2005. Guidelines for laboratory and field testing for mosquito larvicides. 39 pp.

Wilke, A. B. B., C. Vasquez, A. Carvajal, M. Ramirez, G. Cardenas, W. D. Petrie, and J. C. Beier. 2021. Effectiveness of adulticide and larvicide in controlling high densities of *Aedes aegypti* in urban environments. PLoS One. 16: 1-15.

Evaluation of Wide Area Larviciding Spray (WALS) efforts to control *Culex* and *Aedes* mosquitoes in Los Angeles County

Steven F. Vetrone and Susanne Kluh*

Greater Los Angeles County Vector Control District, 12545 Florence Ave., Santa Fe Springs, CA 91670

*Corresponding author: skluh@glacvcd.org

Abstract

The Greater Los Angeles County Vector Control District (GLACVCD) provides services to over one million residential and commercial properties located within approximately a 1000 square mile service area. A consequence of such a high density of properties is that they account for the largest proportion of mosquito breeding sources encountered by the GLACVCD. Performing property inspections to identify and abate open and cryptic larval habitats for *Culex* and *Aedes* mosquitoes requires a tremendous investment of both time and resources. This is of particular concern during times of high mosquito abundance and elevated virus transmission activity. Novel treatment approaches such as the Wide Area Larvicide Spray (WALS) technique potentially provide the ability to treat difficult to reach larval habitats by delivering the biorational larvicide, VectoBac WDG, over a broad geographic area. This article discusses the use of droplet characterization, larval bioassay, and adult abundance monitoring to evaluate the effectiveness of a truck mounted WALS application on adult *Culex* and *Aedes* abundance as well as West Nile virus (WNV) infection rates in populations of *Culex quinquefasciatus* in a 200 acre area in Studio City, CA.

Introduction

In the past decade, Los Angeles County has experienced the introduction and establishment of three invasive *Aedes* species: *Ae. albopictus*, *Ae. aegypti,* and *Ae. notoscriptus* (Metger et al., 2017). The presence of these new species has resulted in marked increases in biting pressure as well as the risk for outbreaks of diseases such as dengue, chikungunya or Zika (Olmos 2020). Unfortunately, characteristics common to all three of these new species such as their ability to lay eggs which are resistant to desiccation and exploit small and cryptic sources found in abundance on a large number of properties, synergistically makes these mosquitoes incredibly hard to control.

In addition to the challenges posed by invasive *Aedes* mosquitoes, the warmer inland areas of Los Angeles County experience annual *Culex quinquefasciatus* driven outbreaks of West Nile virus (WNV) (Kwan et al., 2010). Despite population levels of *Cx. quinquefasciatus* being kept at low levels through an extensive larviciding program, small numbers of older females persist and can transmit the virus to residents. Ground based adulticiding efforts targeting those older infectious females have had limited success. This lack of adult control can be attributed, in large part, to urban heat islands common throughout the GLACVCD service area, where the heat that rises from the concrete surfaces for most of the night precludes the formation of effective treatment conditions including thermal inversions.

VectoBac WDG, when applied as a mist, has been shown to have the ability to disperse into cryptic *Aedes* backyard sources (Pruszynski et al., 2017; Garcia-Luna et al., 2019; Harris et al., 2021). *Bacillus thuringiensis israelensis* (Bti) is known to impact adult female survivorship in some mosquito species when ingested from a treated water source during oviposition (Klowden and Bulla, 1984). In the search for a treatment approach that can both reach cryptic *Aedes* sources in back yards and reduce WNV activity in those same neighborhoods, a truck mounted WALS application was conducted in approximately a 200 acre area of Studio City, a suburb north-west of downtown Los Angeles. The area was selected due to the presence of high *Aedes* abundance, elevated *Cx. quinquefasciatus* populations and above average WNV infection rates at the time of application.

Materials and Methods

In accordance with the WALS Standard Operating Procedure (SOP) provided by Valent BioSciences Corporation (Valent BioSciences 2019), a total of five vehicle mounted WALS treatments were conducted within the same 200-acre spray block in Studio City, California. Although the treatment protocol calls for six treatments, the sixth and final treatment was omitted due to inclement weather conditions. Among the five treatments conducted within the spray block, the four initial ones were carried out at seven-day intervals. The remaining fifth treatment was

Figure 1.—Location of sampling cup sites (Red), Kromekote cards (Blue), sampling cups/Kromekote cards (Green), and trap sites (Yellow) within treatment area.

conducted 14 days later. Each application was made using a truck mounted A1 Super Duty Mist Sprayer (Model SDX-100 R18; A1 Mist Sprayers, Ponca, NE.) fitted with a Micronair AU5000 atomizer (Micronair, Isle of Wight, UK). To increase the likelihood of producing droplet spectra within the targeted range (30 to 236μm), the Micronair atomizer was configured with a 20-mesh screen and a blade pitch set to a 35° angle. The VectoBac® WDG (Bti, Valent BioSciences LLC Libertyville, IL.) material was applied as an 18% solution (1.5 lbs/gal) at a rate of 8 oz/A.

The evaluation of treatment effectiveness involved the examination of four parameters: characterization of droplet spectra, larval bioassay of field exposed cups, differences in pre- and post-treatment abundance, and WNV infection rates in the *Culex* population. Given the WALS treatments were conducted during the peak of the COVID-19 lockdown in the Los Angeles area, recruitment of residential sampling sites was restricted due to social distancing guidelines enacted by GLACVCD, which included a "no-knock" policy to limit resident contact. As such, all surveillance site recruitment had to be conducted with the use of door hangers in hopes of resident participation. This, in turn, resulted in a limited number of sites to choose from that were not always in the ideal location.

Figure 2.—Example of bioassay cup locations at residential sampling sites. 'Open' cups shown in green and 'Covered' cups shown in red.

Droplet characterization of pesticide aerosols was conducted by placing Kromekote paper cards (3-inch x 5-inch) along four side streets perpendicular to the predetermined spray path (Fig. 1). Cards were secured to a CD jewel case to prevent movement and ensure proper orientation. Along these side streets, cards were spaced at 30-foot intervals for a total distance of 180 feet downwind of the spray pass. Sprayed cards were allowed to dry and analyzed using DropVision® AG software (Leading Edge, Fletcher, NC). The aggregate results for all card lines were averaged to estimate Volume Median Diameter (VMD) and droplet density.

Treatment coverage and downwind deposition of material was evaluated through larval bioassay of sampling cups exposed, on site, during treatment. A total of six cup-sampling sites were selected within the spray block. Due to the COVID-19 related social distancing guidelines described above, only four residential sampling sites were used (Fig. 1). Among these, two were located immediately downwind of the spray path whereas the remaining two were on the upwind side (i.e., farthest downwind in the spray swath). To compensate for the small number of sampling sites, additional cups were placed on two side streets perpendicular to the predetermined spray path as was done with the Kromekote papers. At each residential site, a total of six 200ml cups (70mm dia.; Mold Rite Plastics, Plattsburgh, NY) were distributed throughout the property (front yard, side yard, and backyard), with half of the cups exposed ("open") and the remainder placed under vegetation ("covered") (Fig. 2). Cups placed on side streets were evenly spaced along the entire width of the block using the previously described exposure sequence. A series of control cups were placed several blocks away, upwind of the treatment area. Kromekote card lines and sampling cups were deployed for the first three events of the five-treatment series.

Treated side street cups were collected no sooner than 30 minutes post application, whereas those placed around residential properties were retrieved the following morning. Cups were sealed with their respective lids and returned to the laboratory. Sealed cups were washed to remove all larvicidal material from the exterior surfaces of the cups to

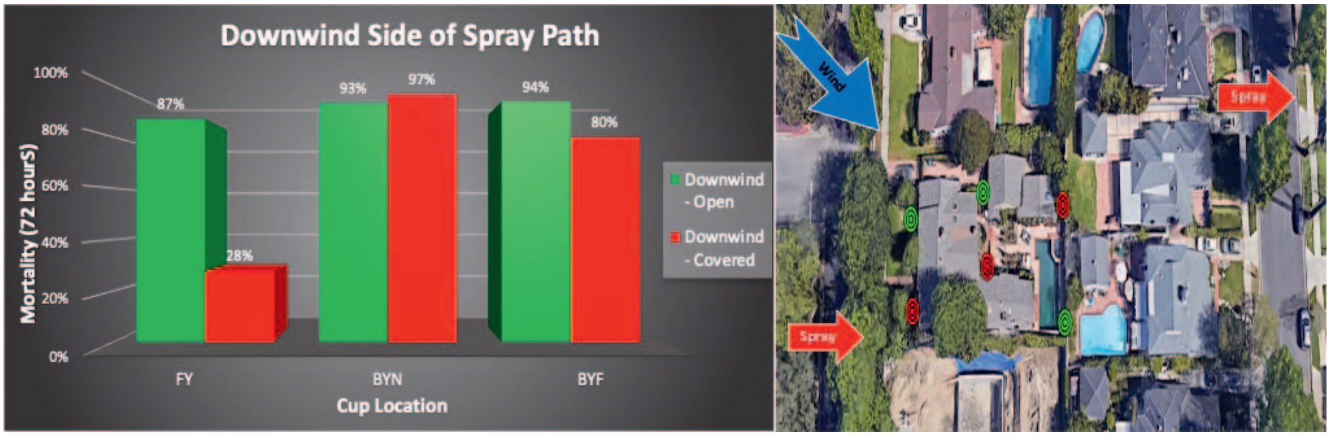


Figure 3.—Bioassay results for cups placed at properties immediately downwind of spray path. (Cup placement: FY = front yard, BYN = side yard, and BYF = backyard)

prevent cross-contamination. All sampling cups, including controls, were filled with 100ml of distilled water, to which ten 2nd and 3rd instar susceptible colony reared *Cx. quinquefasciatus* larvae (VCq4 colony) were added. Lastly, three drops of 6% beef liver powder suspension were added to each cup as a larval food source. Larval mortality was recorded every 24 hours over a 72-hour period.

The impact of treatments on the abundance of adult *Cx. quinquefasciatus* and *Ae. aegypti* was evaluated through weekly abundance trapping using a combination of Reiter gravid and BG Sentinel traps. Traps were set in the early afternoon and collected the following morning. Adult males and females were counted and identified to species. *Cx. quinquefasciatus* females were pooled and submitted for testing at the UC Davis Arbovirus Research and Training (DART) laboratory. Weekly pre-treatment trapping was initiated at eight sites (Fig. 1) two weeks before the first treatment and then during the night prior to each application, throughout the duration of scheduled treatments and for two more weeks after the final application. As with the bioassay cup sites, the adult trap location distribution throughout the treatment area was less than ideal due COVID-19 restrictions for resident contact, possibly leading to an undersampling of the northern half of the spray block.

Abundance and WNV infection rate data from bimonthly routine surveillance sampling in the surrounding cities and neighborhoods was used as untreated controls. Differences in *Aedes* and *Culex* abundance over the 10-week sampling period for both the treatment area and the untreated controls were assessed using least squares regression models in STATA 15 (StataCorp, College Station, TX)

Results and Discussion

The analysis of droplet spectra among sprayed Kromekote cards yielded an average VMD of 59.4μm (range: 26 to 210μm) across all replicates. While these values were within the recommended VMD range of 30 to 236μm, they were on the small end of the specified spectrum. However, given the low wind conditions on the mornings of the treatments (< 2 mph), we felt that the smaller droplet diameters would be advantageous by allowing droplets to remain suspended for a longer duration. We observed good droplet deposition with a mean density of 37.2 drops per cm^2 across all replicates. Taken together, the VMD and droplet density values indicated that the equipment configuration and calibration was sufficient to effectively deliver the larvicidal formulation over the treatment area.

Bioassay results demonstrated heterogeneous larval mortality consistent with cup position relative to the spray swath and exposure. Cups placed on properties immediately downwind of the spray path exhibited average larval mortality at 72 hrs of 87%, 90% and 94% among the 'open' cups positioned in the front, side and back yards, respectively. Similarly, the 'covered' cups showed larval mortality of 28%, 100% and 80% from front to back yards, respectively (Fig. 3). Conversely, cups placed on properties located upwind of the spray path, or more specifically, farthest downwind from the spray swath, demonstrated limited larval mortality. For these properties, larval mortality averaged less than 10% for both 'open' and 'covered' cups in backyards as well as 'covered' cups in the side yards. 'Open' cups placed in the side yards of these properties averaged 54% mortality. Larval mortality improved to 92% and 54% for the 'open' and 'covered' cups, respectively, placed in front yards (Fig. 4). The significant reduction in mortality which was observed in the backyard cups was likely due to a wind shadowing effect by natural and structural barriers (trees, hedges, fences, buildings, etc.) limiting the drift and deposition of the aerosol.

Lastly, the series of 4 cups placed on side streets exhibited good to moderate larval mortality across all downwind distances and exposure types. Among the 'open' cups, average larval mortalities of 92%, 68%, 62% and 98% were observed as distance increased from the spray path. A similar trend was seen among the covered cups at these locations, albeit with slightly reduced mortalities of

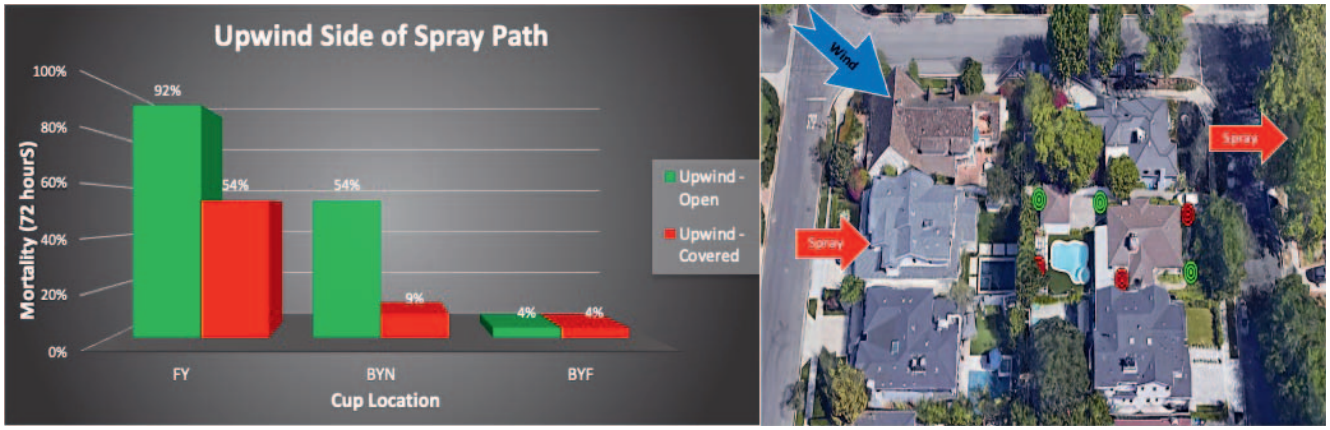


Figure 4.—Bioassay results for cups placed at properties upwind of spray path. (Cup placement: FY = front yard, BYN = side yard, and BYF = backyard)

80%, 57%, 50%, and 53%, respectively. As observed elsewhere, mortality increased in both open and covered cups at the end of the spray swath, indicating that these cups, despite being upwind, were impacted by mist generated from the spray vehicle on its pass down the previous street (Fig. 5).

During the ten weeks of adult surveillance, a significant reduction in both male ($m = -2.21$, $p < 0.01$) and female ($m = -2.88$, $p < 0.001$) *Ae. aegypti* abundance was observed within the treatment block. By comparison, the abundance of this species in surrounding cities and neighborhoods, while generally lower during the equivalent time period, remained unchanged ($p > 0.05$), demonstrating the effectiveness of the WALS treatment in reducing *Aedes* population abundance (Fig. 6). This reduction from an average of 30 females per trap-night to below five females per trap night (almost 90%), however, did not result in a noticeable relief in biting pressure according to resident perception. While vector abundance is not necessarily an accurate predictor of dengue virus transmission risk (Louis et al., 2014), a WALS treatment in conjunction with adulticide applications will certainly be a consideration in an *Aedes* transmitted virus outbreak.

Even though treatments also must have reduced *Cx. quinquefasciatus* emergence from shared backyard sources, abundance of both males and females of this species in the treatment area remained stable ($p > 0.05$) throughout the observation period (Fig. 7). In contrast, *Culex* numbers at surrounding neighborhood control sites slowly decreased towards the end of the season. This observation reaffirms the previous finding that backyard sources are not a major contributor to the overall *Cx. quinquefasciatus* abundance (Kluh 2020).

WNV infection rates in adult female *Cx. quinquefasciatus* collected from within the treatment block and the surrounding untreated control areas showed overall downward trends during the observation period, despite considerable fluctuation among sampling weeks. This trend may be explained by the fact that we were nearing the end of the mosquito and virus transmission season. However, although Maximum Likelihood Estimates (MLE) (Weidong et al. 2003) decreased by 50% in the

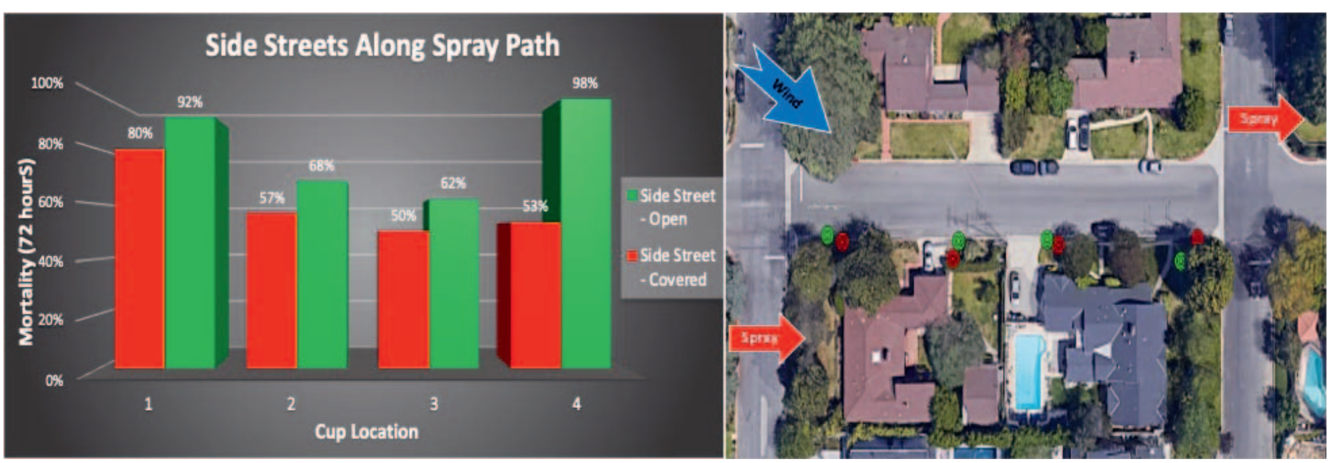


Figure 5.—Bioassay results for cups place along side streets (cups numbered sequentially proceeding downwind from the route of the spray vehicle)

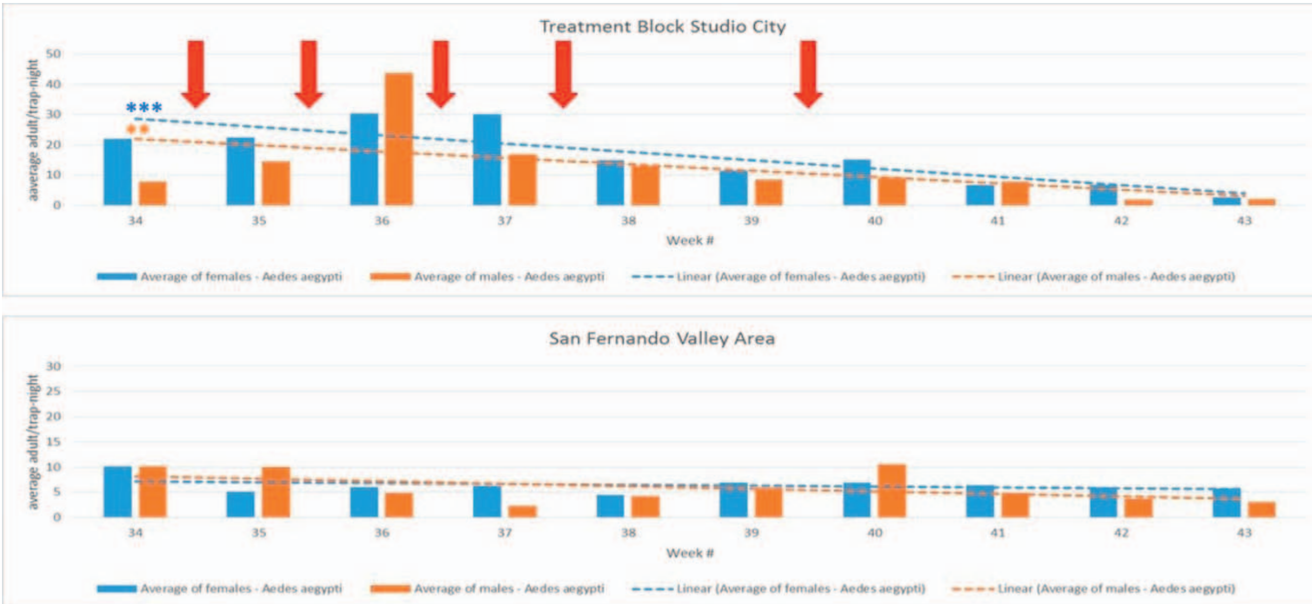


Figure 6.—*Aedes aegypti* adult abundance within the treatment area (top) and surrounding untreated control sites in San Fernando Valley communities (bottom). Red arrows indicate WALS treatment dates. Note: trend line slopes significant: ** $p<0.01$, *** $p<0.001$

treatment area, the MLE decreased by 80% in samples collected in the surrounding areas (Fig. 8). This indicated that the reduction in the infection rate in the treatment area could not be attributed to the treatments, because the infection rate concurrently decreased more in the surrounding untreated control sites. Considering the small area treated relative to *Cx. quinquefasciatus* potential flight range, it was thought to be unlikely to observe an effect of the Bti taken up from the water source during oviposition on the survivorship of adult females as measured by the infection rate.

Conclusions

Truck mounted WALS applications can be an effective treatment option for the multitude of mosquito sources in residential properties in densely populated Los Angeles County. The A1 Super Duty Mist Sprayer fitted with a Micronair AU5000 atomizer when used to apply VectoBac WDG as an 18% solution consistently produced the correct droplet size range and air velocity to effectively propel the material into front and backyards despite potential obstructions. The WALS treatment in Studio City achieved

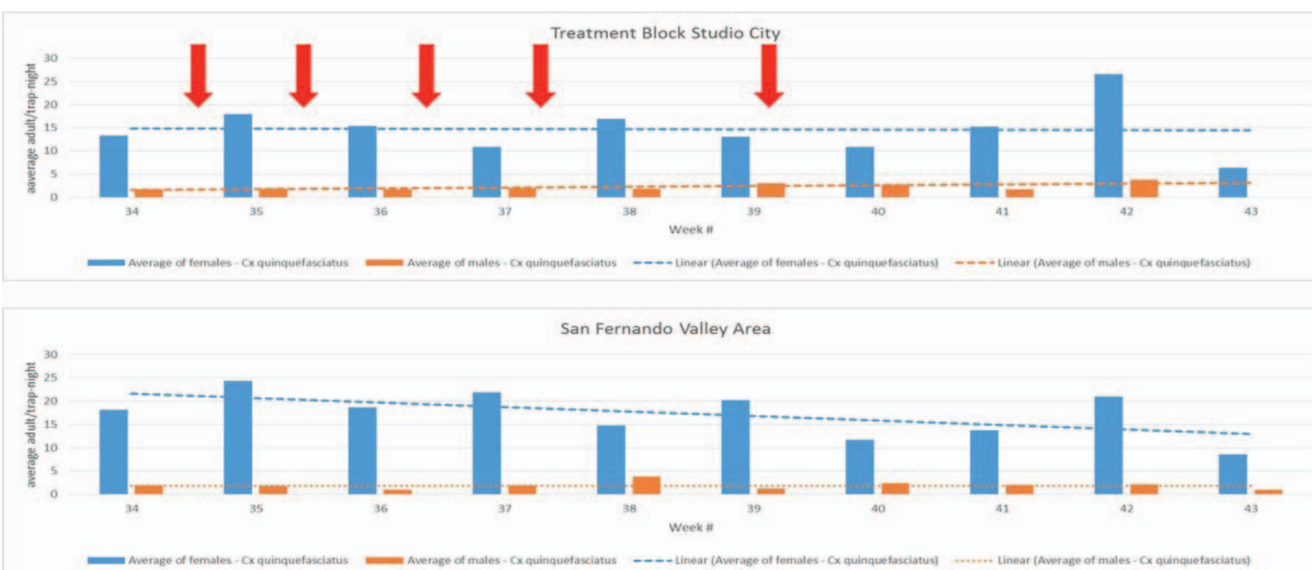


Figure 7.—*Culex quinquefasciatus* adult abundance within the treatment area (top) and surrounding untreated control sites in San Fernando Valley communities (bottom). Red arrows indicate WALS treatment dates.

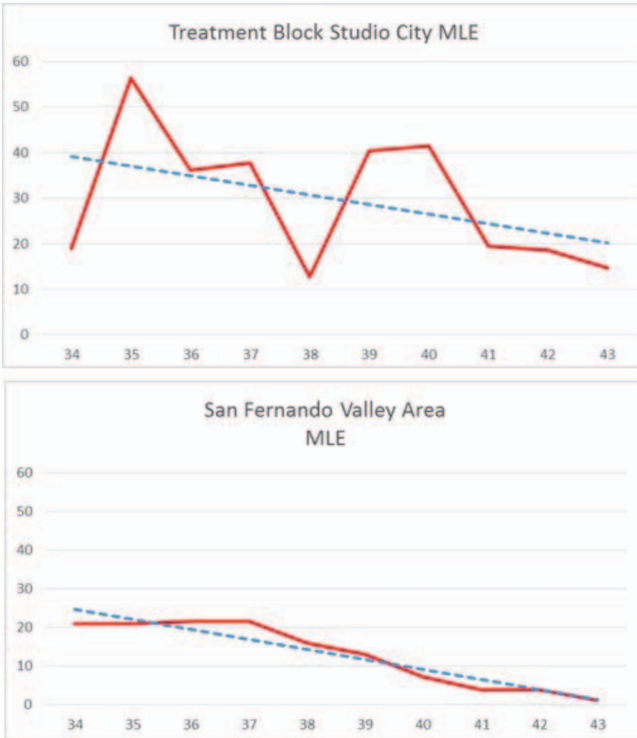


Figure 8.—Weekly WNV infection rate in *Culex quinquefasciatus* females collected within the treatment area (top) and surrounding untreated control sites in San Fernando Valley communities (bottom)

sufficient larval control in both open and cryptic front and backyard sources to result in close to a 90% reduction in adult *Aedes* trap counts. However, according to residents, this was not enough to alleviate the biting nuisance. At a cost of close to $25,000 for material alone to cover 200 acres (0.31 sq mi) of GLACVCD's 1000 square mile service area (0.03% of total), WALS was not a cost effective choice for nuisance control. It will, however, be a treatment option during a small dengue, chikungunya or Zika virus outbreak to reduce vector populations. In contrast, there was no measurable impact of the WALS treatment on either the abundance of *Cx. quinquefasciatus* or the WNV infection rate in the treatment area compared to surrounding untreated communities. Although this was probably due, at least partially, to the small size of the treated area given this species' flight range, it eliminates this approach as a possible small-scale ground based WNV control strategy.

References

Garcia-Luna, S.M., L.F. Chaves, J.G. Juarez, B.G. Bolling, A. Rodriguez, Y.E. Presas, J.P. Mutebi, S.C. Weaver, I.E. Badillo-Vargas, G.L. Hamer, W.A. Qualls, 2019. From surveillance to control: Evaluation of a larvicide intervention against *Aedes aegypti* in Brownsville, Texas. J Am Mosq Control Assoc. 35:233-237

Harris, A.F., J. Sanchez Prats, M.N. Nazario, F.C. Piovanetti, P.M. García, V.P. Ramírez, J.B. Miranda, M. Ortiz, P. DeChant, 2021. An evaluation of *Bacillus thuringiensis israelensis* (AM65-52) treatment for the control of *Aedes aegypti* using vehicle-mounted WALS® application in a densely populated urban area of Puerto Rico. Pest Management Sci. 77:1981-1989.

Klowden, M. & L. Bulla, 1984. Oral toxicity of *Bacillus thuringiensis subsp. israelensis* to adult mosquitoes. Appl. Environ. Microbiol. 48: 665-7.

Kluh, S. 2020. Impact of invasive *Aedes* species targeted backyard mosquito control measures on local *Culex quinquefasciatus* populations. Proc. Mosq. Vector Contr. Assoc. Calif. 88:141–145

Kwan J.L., S. Kluh, M.B. Madon, and W.K. Reisen, 2010. West Nile virus emergence and persistence in Los Angeles, California, 2003–2008. Am. J. Trop. Med. Hyg., 83: 400–412

Louis, V. R., R. Phalkey, O. Horstick, P. Ratanawong, A. Wilder-Smith, Y.Tozan, and P. Dambach. 2014. Modeling tools for dengue risk mapping – a systematic review. Intl. J. Health Geogr. 13: 50.

Metzger M.E., M. Hardstone Yoshimizu., K.A. Padgett, R. Hu, and V.L. Kramer. 2017. Detection and establishment of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) mosquitoes in California, 2011-2015. J Med Entomol. 54: 533-543.

Olmos M. 2020. The importance of inspecting and educating simultaneously when responding to *Aedes* and *Culex* service requests. Proc.Mosq.Vector Contr.Assoc.Calif.88: 146- 148.

Pruszynski, C.A., L.J. Hribar, R. Mickle, A.L. Leal, 2017. A large scale biorational approach using *Bacillus thuringiensis israeliensis* (Strain AM65-52) for managing *Aedes aegypti* populations to prevent dengue, chikungunya and Zika transmission. PLoS One. 15:12(2):e0170079.

Valent BioSciences. 2019. Container Mosquito Vector Control Vehicle Mounted WALS™ of VectoBac® WDG Bacterial Larvicide. Standard operating procedure (SOP) for the USA. http://www.valentbiosciences.com/publichealth/wp-content/uploads/sites/4/2017/02/VBac-WG-vehicle_SOP-2019_REV_V1.6.pdf

Weidong G., R. Lampman, R.J. Novak, 2003. Problems in estimating mosquito infection rates using the minimum infection rate. J Med. Entomol. 40: 595–596,

Two years of *Aedes aegypti*: Two counties, two different stories

Samer Elkashef*, Marcia Reed, Kevin Combo, Sarah Wheeler, Steve Ramos, Garth Ehrke, Debbie Dritz, Luz Maria Robles and Gary Goodman

Sacramento-Yolo Mosquito and Vector Control District, Elk Grove, CA 95624

*Corresponding author: selkashef@fightthebite.net

Introduction

In the late summer of 2020, the Sacramento-Yolo Mosquito and Vector Control District (District) detected *Aedes aegypti* for the first time in Yolo County, more specifically in the City of Winters. This infestation was characterized by having higher trap counts and more traps with positive detections of invasive *Aedes* than had been observed previously within the District's boundaries. To address this issue, the District activated its newly revised invasive mosquito response plan which included an increase in both seasonal and full-time staff dedicated to addressing invasive *Aedes* issues.

Methods

Outreach efforts included mailers sent directly to residents in the affected area as well as an article in the local newspaper. The District also initiated an active social media presence which included Facebook, Instagram and Neighborhood Nextdoor applications.

For surveillance, the District deployed BG Sentinel traps as a means of accessing the scope of the infestation, with one to two traps deployed per acre. Surveillance was augmented by field technicians doing door-to-door larval surveillance and physical control when possible.

Mosquito control treatments were done via truck mounted cold foggers for adulticides and mist blowers for larvicides. The products used in these applications were Deltagard (Bayer Environmental Science) and VectoBac WDG (Valent Biosciences), respectively.

Results and Discussion

In 2019, the detection of *Ae. aegypti* in Sacramento County triggered an intensive door-to-door campaign that included many backpack applications of both adulticides and larvicides directly to properties where the invasive mosquitoes were detected. Although effective at reducing the population to an undetectable level, these activities caused a strain on District resources. This triggered District staff to examine its processes and revise how the District addresses new detections. This led to a shift away from direct applications to residential properties to using truck mounted applications to treat areas of concern. This strategy was used during the summer of 2020 to address *Ae. aegypti* detection in the City of Winters in Yolo County.

Conclusions

The 2020 mosquito season presented a new challenge with the detection of invasive *Aedes* in Yolo County and the comprehensive response to the detection. The District's trapping efforts in 2021 will elucidate how effective this new strategy was and what steps need to be taken to improve the invasive response plan.

Acknowledgements

We would like to thank all of the staff at the Sacramento-Yolo Mosquito and Vector Control District for their efforts in tackling invasive *Aedes* issues. We would also like to thank our partners in the City of Winters for coordination of efforts.

A side-by-side evaluation of A1 SuperDuty Mist Blowers

Steven Ramos*, Samer Elkashef, Sarah S. Wheeler, and Marcia Reed

Sacramento-Yolo Mosquito and Vector Control District, Elk Grove, CA 95624

*Corresponding author: sramos@fightthebite.net

Introduction

The Sacramento-Yolo Mosquito and Vector Control District (District) simultaneously evaluated two A1 SuperDuty Mist Blowers (A1) both equipped with AU5000 Atomizers (Micron Group; Bromyard, Herefordshire, UK) to determine whether there were performance differences between units. The District utilizes two A1 machines, the first #92 was an unaltered unit with all factory components, whereas the second #3 was modified by the District with an electric pump as opposed to the original belt-driven water pump. No modifications were made to the electric fans which drive wind speed and atomize the output. The A1 has become the District's primary application tool for wide area larvicide sprays (WALS). The District's goal was to determine whether individual A1 units can be set up using universal calibration values, such as blade pitch, or if calibration values are specific to each machine.

Methods

The A1 units used for this trial were truck-mounted and tested concurrently using a vacant property in Elk Grove, California. Within 12 hours of the scheduled trial, a 12% solution of Vectobac WDG (Valent BioSciences, Libertyville, IL) and 2% Red #40 granular dye (Sensient Technologies, St. Louis, MO) were mixed using a venturi inductor. The mixture was split between the two A1 units, each calibrated to release 1.5 gal/min at 10mph for an application rate of 0.25lbs Vectobac WDG/acre. The atomizer blade pitch on each A1 was set to a starting point of 35°, then adjusted to attain a droplet spectrum that spanned a 300ft swath width.

Weather monitoring units (Kestrel Weather Instruments USA; Boothwyn, PA) were placed at 5 and 30 ft above ground level and were used to monitor wind speed and temperature. Applications were performed at or after sundown and were delayed until there was a thermal inversion of at least 0.5° F (i.e., temperature at 30 ft > 5 ft), and a wind speed greater than 2 mph. A 300 ft transect was set up downwind for each truck with tripods set every 25 ft. The two transects were set up so that the wind blew perpendicular to the spray path, and with 1,000 feet of open field between the transects to eliminate overspray. Each tripod was fit with a plastic cutting board (8.5 in x 6 in) with two 2.5 in diameter holes that secured the 6oz polycarbonate bioassay cups (Mold-Rite Plastic® USA; Chicago, IL) and a binder clip to secure a 3 in x 4 in card made from Kromekote cast-coated cardstock (CTI Paper USA; Sun Prairie, WI). Cups were used to conduct later laboratory larval bioassays and cards were used to collect droplet data. Both trucks started spraying at the same time, with spray coordination enabled by 3-way calling between the drivers and a technician that was monitoring the weather stations. After the transect was sprayed, the cups and cards were left in the field for 30 min and then collected. If a subsequent run was performed, the blade pitch was adjusted in 5° - 10° increments and fresh cups and cards were deployed. Typically, up to three blade pitches per machine were tested during a single evening.

The following day larval bioassays were conducted. Each cup was filled with 80 mL of distilled water, the lid was secured, and the cup was inverted five times to resuspend any product on the sides of the cup. Colony reared *Culex quinquefasciatus* (CQ1) were used for the assay, with 3rd instar larvae suspended in 20 ml of distilled water then poured into each bioassay cup. Larvae were provided with 0.075 g of ground TetraMin Tropical Flakes (Spectrum Brands Pet; Blacksburg, VA) as food. Larval mortality was counted at 6, 24, and 48 h post-introduction. Cards were visually inspected for the presence of droplets and then sent to Valent Biosciences for droplet analysis (data not shown). Target droplet size was 140 -160 μm and drops were readily visible by the unaided eye, so that cards could be quickly inspected to ensure droplet coverage.

Results and Discussion

When applications were made at 10 mph, the two A1 units required different blade pitches to produce 100% larval mortality throughout a 300ft swath width. A1 #92 had been used by the District since 2018 and had an optimal blade pitch of 45° (Figure 1a), whereasA1 #3 was purchased in 2019 and had an optimal blade pitch of 60° to 70° (Figure 1b). Although both units operated in a similar fashion and the engine speed of each unit was fixed, the blower speeds were variable. Variation in blower speed impacted the performance of the atomizers and led to shifts in droplet size. The higher blade pitch for A1 #3 was required to compensate for a higher blower speed. Running A1 #3 at a 35° blade pitch resulted in no visible droplets on cards and minimal mortality in larval bioassays. However, a 60° blade pitch resulted in visible droplets on cards throughout the transect and larval mortality in all bioassay cups. These data indicated that universal blade pitch settings were not possibleTo produce the proper droplet spectrum, each A1 sprayer should be individually calibrated to determine the optimal blade pitch required to deposit droplets across the target swath width. More work is needed to determine whether different weather conditions impact blade pitch calibrations, but variables such as wind speed, inversion and humidity seem to play a vital role in the application success.

Acknowledgements

We would like to thank the staff at Sacramento-Yolo Mosquito and Vector Control with their help in performing these trials, and Valent BioSciences for their continued support in these evaluations.

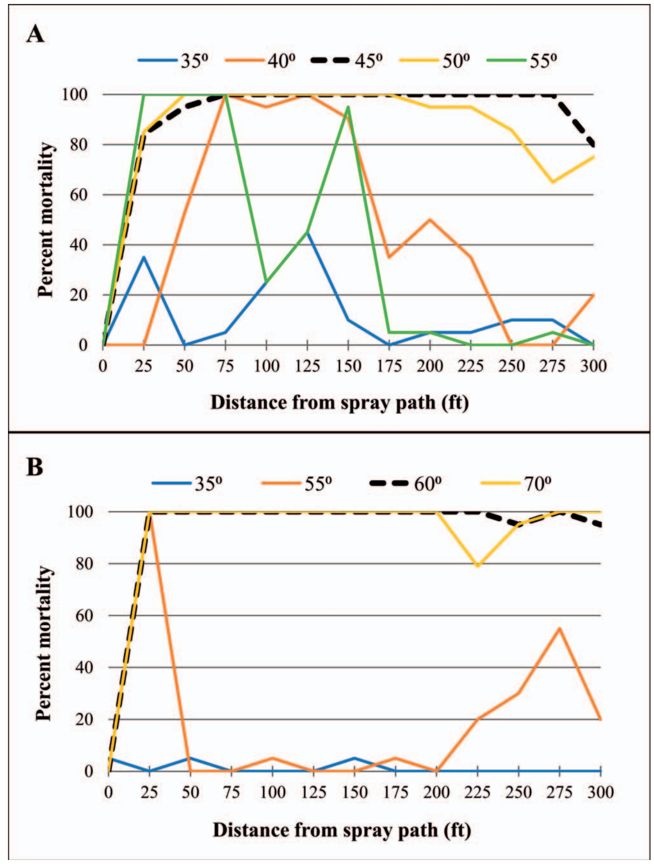


Figure 1.—Larval bioassay mortality across the spray transects at different blade pitch settings for two A1 SuperDuty Mist Blowers: A1 #92 (A) and A1 #3 (B). The dashed line indicates the optimized blade pitch setting for each unit.