PROCEEDINGS AND PAPERS

of the

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

January 28 – 31, 2018
Held at the Marriott Monterey, California

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Published October 2018

Mosquito and Vector Control Association of California

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Submitted Papers

Posters

Surveillance for Mosquito-borne Encephalitis Virus Activity in California, 2017

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ABSTRACT In 2017, 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 47 out of 58 counties in California, and St. Louis encephalitis virus (SLEV) activity was reported from 15 counties. A total of 600 human WNV infections were reported, and enzootic WNV activity was detected among horses, dead birds, mosquitoes, and sentinel chickens. Four human cases of SLEV disease were identified, and SLEV positive chickens and/or mosquitoes were detected in 14 counties, including five counties in California that had not detected SLEV activity in several decades.

INTRODUCTION

The California Arbovirus Surveillance program is a cooperative effort of the California Department of Public Health (CDPH), the University of California Davis Arbovirus Research and Training laboratory (DART), 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 2017, the surveillance program included the following:

(1) Diagnostic testing of specimens from human patients exhibiting symptoms compatible with WNV infection.
(2) Monitoring mosquito abundance and testing mosquitoes for the presence of St. Louis encephalitis virus (SLEV), WNV, western equine encephalomyelitis virus (WEEV), and other arboviruses as appropriate.
(3) Serological monitoring of sentinel chickens for SLEV, WEEV, and WNV 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.
(7) Data management and reporting of non-human data through the CalSurv Gateway, the California arbovirus surveillance system.

West Nile virus activity was reported from 47 (81%) out of 58 counties in California (Table 1), and SLEV activity was reported from 15 (26%) counties (Table 2).

HUMAN DISEASE SURVEILLANCE

Serological diagnosis of human infection with WNV and other arboviruses was performed at the CDPH Viral and Rickettsial Disease Laboratory (VRDL), nine local county public health laboratories, and more than 75 commercial laboratories. Local laboratories tested for WNV using an IgM enzyme immunoassay (EIA) and/or an IgM or IgG immunofluorescence assay (IFA). Specimens with inconclusive results were forwarded to the CDPH-VRDL for further testing with a plaque reduction neutralization test (PRNT). Additional WNV infections were identified through nucleic acid test screening performed by blood donation centers.

In 2017, a total of 553 symptomatic and 47 asymptomatic infections with WNV were identified, a 24.2% increase in the number of infections compared to 2016 (Table 3). Of the 553 symptomatic cases, 401 (73%) were classified as West Nile neuroinvasive disease (e.g. encephalitis, meningitis, acute flaccid paralysis) and 152 (27%) were classified as non-neuroinvasive disease. Case-patients were residents of 27 counties and 358 (65%) were male. Incidence was highest (21.5 cases per 100,000 persons) in Inyo County, although Los Angeles County reported the highest number of cases (Figure 1, Table 3,). The median age of those with WNND was 62 years (range, 6 to 96 years), and among cases with non-neuroinvasive disease the median age was 51 years (range, 4 to 93 years). The median age of the 44 WNV-associated fatalities was 76 years (range, 50 to 96 years). Dates of symptom onset for

Table 1 Infections with West Nile virus in California, 2017. Includes asymptomatic infections detected through blood bank screening. NT = None tested

County	Humans	Horses	Dead Birds	Mosquito Pools	Sentinel Chickens
Alameda	1	0	2	0	0
Alpine	0	0	NT	NT	NT
Amador	0	0	NT	NT	NT
Butte	4	0	5	47	31
Calaveras	0	0	NT	NT	1
Colusa	0	0	1	NT	0
Contra Costa	4	1	19	9	7
Del Norte	0	0	NT	NT	NT
El Dorado	0	0	2	NT	NT
Fresno	14	1	5	167	NT
Glenn	0	2	2	12	1
Humboldt	1	0	1	NT	NT
Imperial	3	0	NT	0	NT
Inyo	4	0	NT	9	NT
Kern	32	2	3	152	NT
Kings	5	0	1	79	NT
Lake	0	0	5	17	2
Lassen	0	3	NT	NT	NT
Los Angeles	292	1	101	582	145
Madera	2	0	1	62	NT
Marin	0	0	1	0	NT
Mariposa	0	0	NT	NT	NT
Mendocino	0	0	NT	NT	NT
Merced	11	0	1	40	19
Modoc	0	0	NT	NT	NT
Mono	0	0	NT	NT	NT
Monterey	0	0	1	NT	NT
Napa	0	0	0	0	NT
Nevada	0	0	1	NT	0
Orange	39	0	57	280	NT
Placer	2	0	4	59	5
Plumas	0	1	NT	NT	NT
Riverside	35	4	41	196	NT
Sacramento	7	1	86	153	2
San Benito	0	0	0	0	1
San Bernardino	68	0	45	295	36
San Diego	2	1	43	9	NT
San Francisco	1	0	1	0	NT
San Joaquin	14	2	7	242	NT
San Luis Obispo	0	0	6	0	NT
San Mateo	0	0	1	0	0
Santa Barbara	0	0	1	1	4
Santa Clara	0	0	15	0	0
Santa Cruz	0	0	1	0	0
Shasta	1	0	2	0	4
Sierra	0	0	NT	NT	NT
Siskiyou	0	0	NT	NT	NT
Solano	1	0	4	9	11
Sonoma	0	0	5	1	NT
Stanislaus	31	0	6	196	NT
Sutter	3	0	3	16	22
Tehama	2	1	NT	NT	4
Trinity	0	0	0	NT	NT
Tulare	13	0	8	630	NT
Tuolumne	0	1	0	NT	NT
Ventura	1	0	2	3	0
Yolo	6	0	14	87	2
Yuba	1	0	6	18	8
State Totals	**600**	**21**	**510**	**3,371**	**305**

all reported cases ranged from March 16 to December 24, with the peak occurring during week 35 (August 27 – September 2).

Four symptomatic cases of SLEV infection were identified in 2017. Two cases (50%) were classified as neuroinvasive disease, and no fatalities were reported. Case-patients were residents of four counties (Table 2) and three (75%) were male. The median age was 61 years and dates of symptom onset ranged from July 24 to October 3.

Table 2 Infections with St. Louis encephalitis virus in humans, mosquito pools, and sentinel chickens, by county, California, 2017. NT = None tested

County	Humans	Mosquito Pools[1]	Sentinel Chickens
Butte	1	1	0
Fresno	0	63	NT
Imperial	0	3	NT
Kern	1	18	NT
Kings	0	21	NT
Los Angeles	0	1	2
Madera	0	10	NT
Merced	0	2	1
Placer	0	1	0
Riverside	0	23	NT
San Bernardino	0	2	6
Stanislaus	1	27	NT
Tulare	0	6	NT
Ventura	1	0	0
Yuba	0	1	0
Totals	**4**	**179**	**9**

[1]Positive mosquito pools included *Culex tarsalis* (111 pools), *Cx. quinquefasciatus* (60 pools), *Cx. pipiens* (7 pools), and *Cx. stigmatosoma* (1 pool)

MOSQUITO SURVEILLANCE

In 2017, mosquito testing was performed at DART and 13 local mosquito and vector control agencies. A total of 1,259,491 mosquitoes (46,193 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 were also tested for chikungunya, dengue, and Zika viruses at DART by a separate RT-qPCR.

West Nile virus was detected in 3,371 mosquito pools from 27 counties (Tables 1 and 4), and SLEV was detected in 179 mosquito pools from 14 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 2.7; the MIR was highest (6.9) in Tulare County. During the peak transmission period (July – September) the statewide MIR in *Culex* mosquitoes was as high as 5.0 and 11 counties reported MIRs greater than 5.0, the epidemic threshold value (Figure 2) (California Department of Public Health).

West Nile virus was identified from six *Culex* species (*Cx. erythrothorax*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. stigmatosoma*, *Cx. tarsalis,* and *Cx. thriambus)* and three other

Table 3 Reported West Nile virus human cases by county of residence, and year, California, 2008-2017.

County	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2017 incidence per 100,000 person-years	Ten-year incidence per 100,000 person-years
Alameda	1	0	1	0	2	0	1	0	0	1	0.06	0.04
Alpine	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Amador	0	0	0	1	0	0	0	0	1	0	0.00	0.52
Butte	6	2	1	3	10	24	24	53	21	4	1.77	6.54
Calaveras	1	0	0	0	0	0	0	0	0	0	0.00	0.22
Colusa	1	0	0	0	3	2	3	1	2	0	0.00	5.44
Contra Costa	4	5	4	3	4	5	5	1	4	4	0.35	0.34
Del Norte	0	0	0	0	0	0	0	0	0	0	0.00	0.00
El Dorado	1	1	0	1	0	1	0	0	1	0	0.00	0.27
Fresno	3	13	23	9	24	8	43	8	14	13	1.31	1.59
Glenn	1	0	2	1	7	9	10	19	6	0	0.00	19.14
Humboldt	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Imperial	0	0	0	0	1	0	1	1	0	3	1.59	0.32
Inyo	0	0	0	0	0	0	0	0	0	4	21.48	2.15
Kern	2	18	15	18	25	25	11	11	17	30	3.35	1.92
Kings	2	3	1	1	3	1	4	0	8	5	3.34	1.87
Lake	0	0	0	0	1	0	1	2	1	0	0.00	0.77
Lassen	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Los Angeles	156	20	4	58	163	151	253	286	151	277	2.70	1.48
Madera	0	1	7	2	3	3	3	4	6	2	1.28	1.98
Marin	0	0	0	0	0	2	0	1	0	0	0.00	0.11
Mariposa	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Mendocino	0	0	0	0	0	0	1	2	0	0	0.00	0.34
Merced	1	4	1	1	13	0	1	1	0	10	3.64	1.17
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	1	0	0	0	1	0	0.00	0.07
Napa	0	0	0	0	0	1	0	0	0	0	0.00	0.07
Nevada	0	0	0	0	0	0	0	2	0	0	0.00	0.20
Orange	71	4	1	10	42	10	263	92	32	33	1.03	1.75
Placer	6	0	3	1	12	6	7	0	7	0	0.00	1.10
Plumas	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Riverside	62	3	0	7	19	35	14	127	11	32	1.34	1.30
Sacramento	13	0	12	4	29	11	10	4	25	6	0.40	0.75
San Benito	0	0	0	0	0	0	0	0	0	0	0.00	0.00
San Bernardino	36	2	5	4	33	13	21	54	8	57	2.64	1.08
San Diego	35	4	0	0	1	0	11	42	20	2	0.06	0.35
San Francisco	0	0	1	0	1	1	0	0	0	1	0.11	0.05
San Joaquin	12	10	6	5	13	8	9	2	13	14	1.87	1.23
San Luis Obispo	0	0	0	0	0	0	0	0	0	0	0.00	0.00
San Mateo	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Santa Barbara	1	0	0	1	0	1	0	0	0	0	0.00	0.07
Santa Clara	1	0	0	1	0	2	10	8	1	0	0.00	0.12
Santa Cruz	0	0	0	1	0	0	0	0	0	0	0.00	0.04
Shasta	1	0	0	0	1	1	2	3	1	1	0.56	0.56
Sierra	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Siskiyou	0	0	0	0	0	0	0	1	0	0	0.00	0.22
Solano	1	0	0	0	2	1	5	1	4	1	0.23	0.34
Sonoma	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Stanislaus	17	14	12	11	26	17	33	13	26	28	5.11	3.59
Sutter	0	0	0	0	8	10	8	2	12	3	3.09	4.44
Tehama	4	0	0	1	4	5	4	5	5	2	3.13	4.69
Trinity	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Tulare	5	4	12	11	7	5	21	13	10	12	2.54	2.12
Tuolumne	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Ventura	0	0	0	0	7	2	1	6	7	1	0.12	0.28
Yolo	1	2	0	0	10	6	15	8	16	6	2.74	2.92
Yuba	0	1	0	3	4	13	6	10	11	1	1.34	6.57
Total WNV Cases	**445**	**112**	**111**	**158**	**479**	**379**	**801**	**783**	**442**	**553**	**1.40**	**1.08**
Asymptomatic Infections	53	17	20	18	48	54	91	77	41	47		
Total WNV infections	**498**	**129**	**131**	**176**	**527**	**433**	**892**	**860**	**483**	**600**	**1.52**	**1.20**

Table 4 Results of mosquito and sentinel chicken testing for West Nile virus, California, 2017.

County	No. mosquitoes tested	No. mosquito pools tested	WNV + pools	No. flocks	No. chickens	No. WNV positive flocks	WNV + sera
Alameda	5,985	480	0	2	10	0	0
Butte	20,732	442	47	7	42	7	31
Calaveras	0			1	10	1	1
Colusa	0			1	10	0	0
Contra Costa	16,496	552	9	5	50	2	7
Fresno	43,286	1,387	167	0			
Glenn	2,254	47	12	1	7	1	1
Imperial	7,307	671	0	0			
Inyo	4,403	94	9	0			
Kern	29,626	725	152	0			
Kings	14,894	452	79	0			
Lake	21,775	749	17	2	12	1	2
Los Angeles	118,172	4,302	582	48	318	36	145
Madera	16,309	446	62	0			
Marin	1,363	100	0	0			
Merced	9,200	376	40	8	48	6	19
Napa	3,887	139	0	0			
Nevada	0			4	24	0	0
Orange	156,448	5,750	280	0			
Placer	37,284	2,359	59	2	12	1	5
Riverside	187,677	5,716	196	0			
Sacramento	93,209	4,908	153	2	12	1	2
San Benito	216	6	0	1	10	1	1
San Bernardino	72,451	3,896	295	9	72	6	36
San Diego	10,818	691	9	0			
San Francisco	324	20	0	0			
San Joaquin	121,632	2,929	242	0			
San Luis Obispo	1,796	47	0	0			
San Mateo	6	4	0	3	30	0	0
Santa Barbara	6,396	174	1	5	50	1	4
Santa Clara	1,421	134	0	8	56	0	0
Santa Cruz	2,166	206	0	2	20	0	0
Shasta	15,628	544	0	7	54	1	4
Solano	10,715	302	9	3	36	2	11
Sonoma	12,954	535	1	0			
Stanislaus	54,460	1,400	196	0			
Sutter	9,127	255	16	6	42	4	22
Tehama	0			3	30	1	4
Tulare	91,203	3,080	630	0			
Ventura	2,847	60	3	5	54	0	0
Yolo	48,362	2,022	87	2	12	1	2
Yuba	6,662	193	18	2	14	2	8
Total	**1,259,491**	**46,193**	**3,371**	**139**	**1,035**	**75**	**305**

species (*Aedes aegypti, Culiseta incidens,* and *Cs. inornata*) (Table 5); positive pools were collected from March 22 – November 28, with the peak occurring during week 31 (July 30 – August 5). St. Louis encephalitis virus was identified from four *Culex* species (*Cx. pipiens, Cx. quinquefasciatus, Cx. stigmatosoma,* and *Cx. tarsalis)*; positive pools were collected from June 9 – October 12.

CHICKEN SEROSURVEILLANCE

In 2017, 29 local mosquito and vector control agencies in 25 counties maintained 139 sentinel chicken flocks (Table 4). Blood samples were collected from chickens every other week and tested for IgG antibodies to WNV, SLEV, and WEEV by an EIA at the CDPH Vector-Borne Disease Section Laboratory. Presumptive positive samples were confirmed by IFA or western blot. Samples with inconclusive results were tested by PRNT at the VRDL.

A total of 305 seroconversions to WNV were detected among 75 flocks in 18 counties, and 9 SLEV seroconversions were detected among 4 flocks in 3 counties (Tables 1, 2, and 4). Seroconversions to WNV occurred from June 15 – November 14, with the peak occurring during week 32 (August 6 – August 12). The first and last SLEV seroconversions occurred July 14 and September 22, respectively.

DEAD BIRD SURVEILLANCE

In 2017, the WNV Dead Bird Hotline and website received 7,745 dead bird reports from the public in 55 counties (Table 6). Oral swabs or tissue samples from dead bird carcasses were tested at DART or at one of 13 local agencies by RT-qPCR. Of the 2,058 carcasses deemed suitable for testing, WNV was detected in 510 (25%) carcasses from 39 counties (Figure 3, Tables 1 and 6). Thirty-five species tested positive for WNV: 68% were American crows, 12% were California scrub-jays, 5% were other corvids, and 15% were non-corvid species. Positive birds were detected from January 5 – December 29, with the peak occurring during week 37 (September 10 – September 16).

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 2017, West Nile virus infection was detected in 21 horses from 13 counties (Table 1). Eight (38%) of the horses died or were euthanized as a result of their infection.

DISCUSSION

West Nile Virus In 2017, 553 WNV human disease cases were reported from 27 counties, which was an increase of approximately 24.2 percent compared to the number of cases reported in 2016, and was the third highest number of cases reported within the last 10 years (Table 2). In addition, 44 fatalities were reported, which

Table 5 Mosquitoes tested for West Nile virus, California, 2017.

***Culex* species**	**No. Pools**	**No. mosquitoes**	**WNV +**	**MIR**
Cx. erraticus	1	6	0	0.0
Cx. erythrothorax	1,483	55,486	6	0.1
Cx. pipiens	7,905	165,399	287	1.7
Cx. quinquefasciatus	17,455	481,221	1,935	4.0
Cx. stigmatosoma	1,175	13,612	53	3.9
Cx. tarsalis	15,593	511,349	1,085	2.1
Cx. thriambus	139	752	1	1.3
All *Culex*	**43,751**	**1,227,825**	**3,367**	**2.7**

***Anopheles* species**	**Pools**	**No. mosquitoes**	**WNV +**	**MIR**
An. franciscanus	15	136	0	0.0
An. freeborni	3	8	0	0.0
An. hermsi	39	352	0	0.0
All *Anopheles*	**57**	**496**	**0**	**0.0**

***Aedes* species**	**Pools**	**No. mosquitoes**	**WNV +**	**MIR**
Ae. aegypti	1569	12,301	2	0.2
Ae. albopictus	90	774	0	0.0
Ae. dorsalis	1	3	0	0.0
Ae. melanimon	1	2	0	0.0
Ae. nigromaculis	2	58	0	0.0
Ae. notoscriptus	1	1	0	0.0
Ae. squamiger	8	108	0	0.0
Ae. taeniorhynchus	1	50	0	0.0
Ae. vexans	41	1,923	0	0.0
Ae. washinoi	18	828	0	0.0
All *Aedes*	**1,732**	**16,048**	**2**	**0.1**

Other species	**Pools**	**No. mosquitoes**	**WNV +**	**MIR**
Culiseta incidens	361	6,848	1	0.1
Culiseta inornata	107	656	1	1.5
Culiseta particeps	26	403	0	0.0
Unknown	159	7,215	0	0.0
All other	**653**	**15,122**	**2**	**0.1**

was the second highest number of fatal WNV cases ever reported in California (www.westnile.ca.gov). The annual incidence rate in California was 1.4 cases per 100,000 persons. Although incidence rates were highest in more rural and less populous counties, 50% of all reported cases were residents of Los Angeles County (Figure 1, Table 3). The proportion of WNND cases among all reported cases in California was 73%, which suggests that several thousand non-neuroinvasive cases also occurred, as these cases are less likely to be diagnosed, laboratory confirmed, and reported (Centers for Disease Control and Prevention, 2010).

Non-human WNV activity was reported from 46 counties in 2017 (Table 1). Although ecological surveillance data documented WNV activity throughout the year, most detections occurred from June through October, with peak activity in August. Enzootic activity was elevated in several counties in southern California and the Central Valley, areas which subsequently reported a higher incidence or number of human cases. Notably, Los Angeles County reported the highest MIR of WNV in mosquitoes during the summer months, at 11.3 (Figure 2).

St. Louis Encephalitis Virus Prior to the introduction of WNV into California in 2003, SLEV was routinely detected in mosquitoes and sentinel chickens, but seemingly disappeared thereafter and was not detected again until 2015. In 2015, SLEV re-emerged in the Coachella Valley in Riverside County, and in 2016 the virus spread to nine additional counties. In 2017, SLEV activity was reported from 15 counties located in southern California and the Central Valley. Notably, SLEV was detected for the first time in more than 40 years in five northern California counties: Butte, Merced, Placer, Stanislaus, and Yuba. Outreach to local health departments was conducted in areas with environmental detections of SLEV and medical providers were encouraged to include SLEV testing for suspect WNV cases. This resulted in the identification of four human SLEV disease cases from four counties (Butte, Kern, Stanislaus, and Ventura); none were fatal. Enzootic SLEV activity was detected in 14 counties via mosquito and sentinel chicken testing.

CONCLUSIONS

In 2017, there was an increase in human WNV disease cases compared to 2016, although almost three-quarters of 2017 cases were reported from southern California counties, primarily Los Angeles County. St. Louis encephalitis virus continued to expand into additional counties, including areas without reported activity for over 40 years. Environmental detections of SLEV and WNV documented the presence of both viruses in the same areas, indicating that the two similar flaviviruses can co-exist in the same ecological niche. Environmental detections of both viruses preceded the occurrence and rise in human cases, highlighting the value of environmental surveillance to direct mosquito control efforts and decrease the risk of transmission of mosquito-borne arboviruses 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 Hotline; the local public health laboratories which tested samples; the many physicians and veterinarians who submitted specimens from clinical cases, and the valuable contributions of the staff of MVCAC, DART (especially Sandra Garcia), and the CDFA Animal Health Branch. From CDPH, we thank the VRDL (especially Shoruq Alhajmohammad, Giorgio Cosentino, Jill Hacker, Maria Liu, Ruth Lopez, Meghana Madala, Oliver Oyler, Leo Oceguera, Peter Patiris, Chris Preas, Maria Salas, and Diana Singh), the Veterinary Public Health Section (especially Curtis Fritz), the Infectious Diseases Branch (especially Claudia Erickson), and VBDS (especially Ervic Aquino, Mary Joyce Pakingan, Robert Payne, Aidan Ward, and the WNV Hotline staff).

This study was supported by the Epidemiology and Laboratory Capacity for Infectious Diseases Cooperative Agreement number 6 NU50CK000410-04-01 from the US Centers for Disease Control and Prevention.

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Table 6 Dead birds reported, tested, and positive for West Nile virus, California 2017.

County	Reported	Tested	Positive	(%)
Alameda	276	39	2	(5.1)
Alpine	1	0		
Amador	11	0		
Butte	98	24	5	(20.8)
Calaveras	8	0		
Colusa	10	2	1	(50.0)
Contra Costa	695	44	19	(44.2)
Del Norte	0			
El Dorado	72	18	2	(11.1)
Fresno	168	11	5	(45.5)
Glenn	6	2	2	(100)
Humboldt	12	2	1	(50.0)
Imperial	3	0		
Inyo	12	0		
Kern	76	3	3	(100)
Kings	28	3	1	(33.3)
Lake	40	25	5	(20.0)
Lassen	0			
Los Angeles	889	160	101	(63.1)
Madera	27	3	1	(33.3)
Marin	64	9	1	(11.1)
Mariposa	4	0		
Mendocino	11	0		
Merced	64	7	1	(14.3)
Modoc	0			
Mono	2	0		
Monterey	34	4	1	(25.0)
Napa	39	5	0	(0)
Nevada	25	8	1	(12.5)
Orange	674	477	57	(11.9)
Placer	250	123	4	(3.3)
Plumas	1	0		
Riverside	289	68	41	(60.3)
Sacramento	945	358	86	(24.0)
San Benito	9	1	0	(0)
San Bernardino	258	59	45	(76.3)
San Diego	246	109	43	(39.4)
San Francisco	55	11	1	(9.1)
San Joaquin	233	40	7	(17.5)
San Luis Obispo	59	13	6	(46.2)
San Mateo	404	94	1	(1.1)
Santa Barbara	37	6	1	(16.7)
Santa Clara	515	107	15	(14.0)
Santa Cruz	80	18	1	(5.6)
Shasta	47	3	2	(66.7)
Sierra	1	0		
Siskiyou	1	0		
Solano	104	21	4	(19.0)
Sonoma	130	26	5	(19.2)
Stanislaus	217	22	6	(27.3)
Sutter	58	22	3	(13.6)
Tehama	9	0		
Trinity	1	1	0	(0)
Tulare	82	16	8	(50.0)
Tuolumne	7	1	0	(0)
Ventura	119	16	2	(12.5)
Yolo	195	61	14	(23.0)
Yuba	44	16	6	(37.5)
Totals	**7,745**	**2,058**	**510**	**(24.8)**

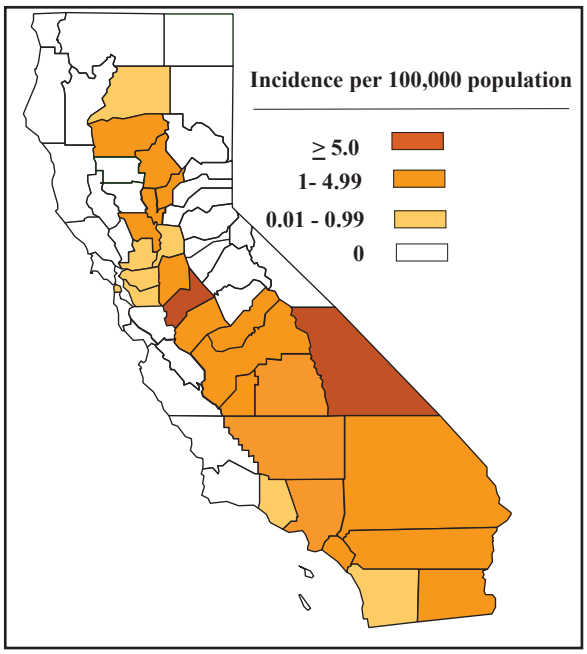

Figure 1 Incidence of human cases of West Nile virus in California, 2017.

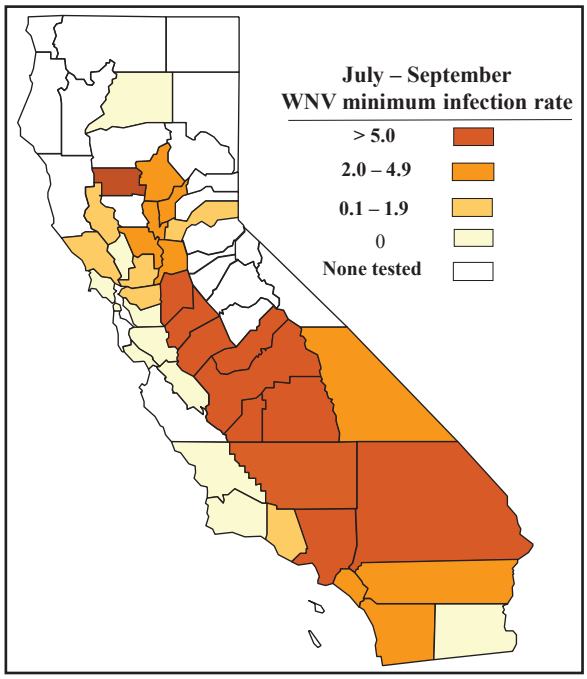

Figure 2 West Nile virus minimum infection rate in *Culex* mosquitoes, by county, California, July – September, 2017. Minimum infection rate is defined as the minimum number of infected female mosquitoes per 1,000 tested.

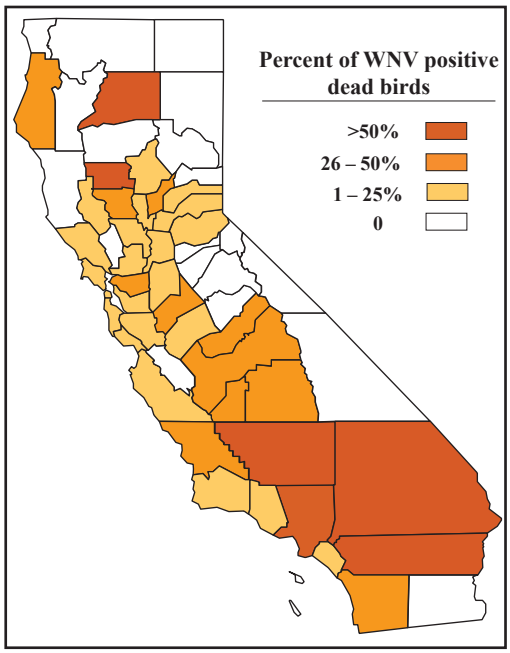

Figure 3 West Nile virus infection prevalence in dead birds, by county, California, 2017.

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.

This year, there were three applicants for the Reeves Award. First place was awarded to Mary Beth Danforth for her paper "The impacts of cycling temperature on West Nile virus transmission in California's Central Valley." Second place was awarded to Carrie De Jesus for her paper "Timed observations of precopulatory interactions between *Aedes aegypti* and *Aedes albopictus*." The manuscripts or summaries for all Reeves Award candidates appear on the pages following.

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-resistant 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	----------	----------

House Fly (*Musca domestica* L.) Attraction to Insect Honeydew*

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This manuscript is a summary of Hung et al. (2015) published previously in PLOS ONE

INTRODUCTION

Flies magnify the risk of food-borne disease by contacting animal wasteand then transporting pathogens to places where the risk of human disease is greatly amplified (Lysyk and Axtell 1986, Wang, Chang, et al. 2011). Flies also may play a key role in the distribution of human pathogens from domestic livestock to human food crops (Talley et al. 2009), but it is unknown whether flies are attracted to human food crops or simply encounter them randomly during undirected movement through the environment. In 2006, an outbreak of *Escherichia coli* O157:H7 associated with bagged spinach affected over 200 individuals in 26 US states (CDC 2006). Subsequent studies in the region where the contaminated spinach originated resulted in the capture of filth flies carrying this pathogen in spinach and lettuce field crops adjacent to a cattle pasture (Talley et al. 2009). Within a field of leafy greens, filth flies were more abundant where honeydew-producing insects were present (Talley et al. 2009). The authors have observed house flies feeding on honeydew, a carbohydrate-rich excretion produced by phloem-feeding insects (Downes and Dahlem 1987), presumably to obtain the carbohydrates needed to sustain flight or other physiological functions (Stanfield and Hunter 2009). Some insects orient toward honeydew odors as an indicator of food or oviposition sites (Wang, Johnson, et al. 2011, Leroy et al. 2012); however, honeydew sugars are not volatile or odorous. Thus, we hypothesized that house flies detect and orient toward honeydew by following odor plumes associated with honeydew, and that fungal digestion of honeydew sugars may produce such odors.

METHODS

House flies were collected from a southern California dairy facility and reared as a colony in an insectary. Citrus mealybugs (*Planococcus citri* Risso) feeding on butternut squash and pea aphids (*Acyrthosiphon pisum* Harris) feeding on faba bean plants were also reared in the insectary. Other honeydew-producing insects, the honeydew, and the corresponding plant on which they feed were collected from the field and brought back to the laboratory as test stimuli for use in the bioassays, and for collecting specimens of the fungi associated with the honeydew. These collections included whiteflies on orange and grapefruit leaves; citrus mealybugs and cottony cushion scales on mandarin leaves; lerp psyllid on red ironbark eucalyptus leaves; lerp psyllids on red river gum eucalyptus leaves; and aphids on crepe myrtle leaves. Eight bioassay cages were placed in a room maintained at 25 ± 2°C, 40% relative humidity, and 12L:12D photoperiod. Each cage contained two glass beakers to hold test materials. For each bioassay period, the treatment (insect honeydew on the plant cutting) and control (plant cutting only or empty beaker) were randomly assigned a position within the test cages. White sticky cards were placed on top of the beakers to capture flies that landed near the beaker. Groups of 50 female flies (3-5 d old) were starved for 40-44 hr before being released into each cage. The numbers of flies on each sticky card were recorded 24 hr later. We examined house fly responses to fungal odors by isolating and culturing the fungi associated with the honeydew collected from the field and the laboratory. The fungi were identified to genus or species by morphology and color. The two most frequent species collected among the attractive samples, *Aureobasidium pullulans* and *Cladosporium cladosporioides*, were grown on a potato dextrose broth (PDB) medium and used in the cage bioassay to assess house fly responses to the fungi. The control for these bioassays was the PDB.

RESULTS

House flies were attracted to the mealybug-infested squash but not the uninfested squash or a decaying squash. Flies were attracted to aphid-infested faba bean plants, combined samples of whitefly-infested orange and grapefruit leaves, and to citrus mealybug and cottony cushion scale-infested mandarin orange

leaves. In contrast, flies were not attracted to lerp psyllid-infested foliage of red ironbark eucalyptus. Wetting the psyllid honeydew on red ironbark eucalyptus foliage followed by incubation for 24 hr under humid conditions did not increase fly attraction. Flies were also not attracted to aphid-infested crepe myrtle or to lerp psyllid-infested red river gum eucalyptus leaves. House flies were attracted to odors from *A. pullulans* cultured in PDB but not to odors from *C. cladosporioides* in PDB, relative to sterile PDB.

DISCUSSION

House flies were attracted to odors associated with honeydew produced by a range of honeydew-producing insects. The honeydew that collected more flies than their respective controls tended to be sticky to the touch and showed noticeable mold growth. House flies may not be attracted to honeydew produced by the psyllids on eucalyptus, because the odors of eucalyptus may interfere with the flies' detection of the honeydew or the quality of the honeydew was not wet or odorous enough to attract the flies. Aphids on crepe myrtle did have a sticky honeydew residue and noticeable mold growth; however the level of aphid infestation was relatively low, which may explain the lack of house fly attraction to this substrate.

Two fungi (*A. pullulans* and *C. cladosporioides*) were repeatedly isolated from field-collected plant materials contaminated with honeydew that were attractive to house flies in laboratory bioassays. Odors from *A. pullulans* have been shown to increase the number, particularly of Diptera, and diversity of insects captured in an agricultural field (Davis and Landolt 2013). Because of the ubiquity of this fungus as a colonizer of insect honeydews, odors from *A. pullulans* may therefore be general insect attractants (Davis and Landolt 2013) and odors resulting from metabolism of honeydew by *A. pullulans* might signal the availability of a carbohydrate-rich food source. The results from this study support our hypothesis that honeydew production by insects infesting food crops may contribute to attraction of house flies to those crops, particularly when crops are adjacent to animal rearing facilities or other sites that produce large numbers of house flies. Thus, managing honeydew-producing insects on food crops may reduce house fly visitation, and consequently the risk of crop contamination with food-borne pathogens.

ACKNOWLEDGEMENTS

Research funding was provided by the USDA NIFSI #2009-51110-05856. We thank the UC Cooperative Extension Farm Advisors, commercial farm managers, and local gardeners who assisted with honeydew collection. Dr. Greg Walker provided the pea aphid colony and Dr. Mark Hoddle provided the citrus mealybug colony. Ryan Puckett and ShuaiFei Chen assisted with enumerating and identifying fungi from honeydew samples.

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Prevalence and seasonality of fleas associated with California ground squirrels and the potential risk of tularemia in an outdoor non-human primate research facility[1, 2]

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INTRODUCTION

In 2010 there was an epizootic of tularemia at the California National Primate Research Center (CNPRC) in Yolo County, California that resulted in 20 confirmed and suspect clinical cases in rhesus macaques (*Macaca mulatta*) as well as a 53% seroprevalence in the southern outside-housed colony (Sammak et al. 2012). Although ticks are an important biological vector for *Francisella tularensis* (the agent of tularemia), the spatial distribution of cases as well as the results of multiple wildlife surveys conducted in the region surrounding the CNPRC were not supportive of tick transmission (Sammak et al. 2012, Roth et al. 2017). California ground squirrels (*Otospermophilus beecheyi*) were highly prevalent at the site and have been directly implicated in the amplification and spread of *F. tularensis* to non-human primates in the past. They also serve as hosts of several species of flea that will willingly feed on humans (such as *Oropsylla montana*) and that are capable of mechanically transmitting a variety of pathogens such as plague and *Bartonalla washoensis*. While the ground squirrels are heavily managed at the site via live trap and removal, and infestation of macaques with fleas is rarely noted, it is unclear how effective these control methods are at preventing transmission.

We studied ectoparasite burdens in California ground squirrel burrow systems at the CNPRC to provide data on ground squirrel-associated ectoparasite density and distribution. This information may help characterize the risk of disease transmission from ground squirrels to outdoor housed animals and allow facilities to develop informed management strategies for the prevention of vector-borne disease transmission.

METHODS

We sampled fleas from ground squirrel burrows and collected host-seeking fleas from transect lines within the CNPRC. We also considered a number of risk factors that may impact flea populations within burrows including whether the burrow appeared to be in recent active use by a rodent, whether the swab could be inserted 0.5 m or more into the burrow (impacting the surface area the swab could come into contact with), whether the burrow was located under a permanent structure such as a tree or building, whether the burrow had been filled in with soil or gravel during the previous sampling period by pest control staff, and the number of burrow system entrances. We performed Mann-Whitney U tests to determine whether mean numbers of fleas per swab were different between paired risk factors.

RESULTS AND DISCUSSION

From March 1 to October 31, 2015 we recorded 52 California ground squirrel burrow systems and collected 560 fleas from the CNPRC grounds. The largest number of fleas (n = 184) was collected in October. The most common species were *Hoplopsyllus anomalus* (n=331), *Oropsylla montana* (n=158), *Echidnophaga gallinacea* (n= 60), and *Ctenocephalides felis* (n= 11), all of which have been reported to parasitize humans. It is not unreasonable to suppose these species may also parasitize non-human primates as well. In general, burrow systems with six or more entrances had higher loads of fleas per swab than burrow systems with less than six entrances (p=0.006) which may be due to larger numbers of ground squirrel hosts living within larger burrow systems. The

[1]*Summary of a previously published paper: Roth, T.M., Sammak, R., and Foley, J.E. 2017. Prevalence and seasonality of fleas associated with California ground squirrels and the potential risk of tularemia in an outdoor non-human primate research facility. Journal of Medical Entomology 55: 452-458*

[2]*Presented at the Annual MVCAC Conference in 2018 under the title "Investigating an Outbreak of Tularemia in the California Central Valley"*

average number of fleas recovered per swab was significantly greater from burrows which had been open the previous sampling period (1.33) compared to burrows that had been filled with gravel or soil (1.05, p=0.014) which is likely due to the activity of the hosts. Free, non-host associated fleas collected from transects included 12 *H. anomalus*, 9 *C. felis*, 6 *O. montana* and 1 *E. gallinacea* which indicated that these flea species do not exclusively remain in the burrows of their rodent hosts. Despite the abundance of host-seeking fleas at the site, we collected only one *H. anomalus* from a rhesus macaque after 650 person-hours of searching.

For non-human primates, outdoor housing encourages natural behavior and lowers stress, but may also expose the animals to wildlife-associated pathogens which can destabilize social structure, affect animal welfare, and affect research outcomes. Control of ground-dwelling pests via toxicants and burrow destruction are not viable methods at research centers as the primates themselves may become exposed or the burrows may be inaccessible due to their location under immovable structures. Our results suggest that the management of ground squirrels by live trapping and removal does not significantly reduce the potential for ectoparasite spill-over, and that flea control measures should be given a high priority. Although we were unable to recover fleas from the non-human primates, it is unlikely the fleas cannot access the non-human primates or are uninterested in feeding on them. It is possible fleas are rarely recovered during routine veterinary inspections because the non-human primates are anesthetized before examination and fleas often leave anesthetized hosts due to a dropping body temperature. It is also very likely that ectoparasites are regularly removed by the non-human primates themselves via social grooming. Many of the 2010 clinical cases presented with symptoms consistent with the consumption of infected materials (oropharyngeal tularemia) which could have been related to consuming fleas infected with *F. tularensis*.

CONCLUSION

In conclusion, ectoparasite control must be performed concurrently with rodent control in order to reduce the risk of pathogen spillover. Previous studies have demonstrated that bait laced with the neonicotinoid insecticide imidacloprid had a nearly 100% efficacy at reducing flea abundance on ground squirrels for at least 29 days without evidence of mammalian toxicity (Borchert et al. 2009). We recommend pre-baiting with insecticides before conducting mammalian pest removal as a safe and effective option for ectoparasite control at facilities with a risk of wildlife associated diseases.

ACKNOWLEDGEMENTS

We thank Brett Farnham and Jaleh Janatpour for their information regarding animal control methods and the veterinary and the animal care staff at the CNPRC for their efforts in recovering fleas from the NHP's during routine examinations and hospital visits. We also thank Jeffrey Roberts and Christopher Barker for their feedback on this text and the Center for Vector-Borne Diseases as a source of funding this project.

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Understanding the Importance of Social Media

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ABSTRACT We live in a digital world where virtually any piece of information we're looking for is at our fingertips using our smartphones! Social media plays a critical role in how we obtain information and how we communicate with others. Use of social media as a marketing technique is no longer optional, and it has grown to become a vital and integral part of every modern successful business. As people spend more time on their mobile devices and less time on other forms of media such as watching television or listening to the radio, use of social media to disseminate information becomes even more critical.

Since 2010, the Sacramento-Yolo Mosquito and Vector Control District has been utilizing social media platforms to educate, communicate and interact with the public. These avenues of communication have also proven to be a useful platform to disseminate information on District activities and services. This presentation focuses on the importance of understanding the use of social media by discussing the various social media platforms, reviewing the benefits of a social media strategy, providing an overview of what makes good social media content and evaluating the effectiveness of social media posts in reaching your audience. This presentation also offers recommendations for Districts who are looking to set up or expand their social media outreach efforts.

The Red Zone: Using a Hazard Communication Model to Convey Risk, Bolster Partnerships, and Increase Public Awareness of Mosquito-Borne Disease Threats in Nine High Risk Cities in Orange County

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ABSTRACT After 14 years of county-wide West Nile virus activity (699 human infections, 28 deaths) and the recent rapid expansion of invasive *Aedes*, the Orange County Mosquito and Vector Control District (OCMVCD) has identified nine of the county's 34 cities with the highest risk for mosquito-borne disease. In response, OCMVCD launched the High Risk Area 9 (HRA9) Initiative in the spring of 2017 to mitigate the disease risk by: 1) enhancing communication through increased city and community engagement; 2) elevating risk awareness outreach in the most impacted areas of these cities; 3) increasing mosquito source reduction efforts; and 4) streamlining the implementation of response plans which guide the escalation of mosquito suppression activities. This presentation discussed OCMVCD's development and roll-out of the HRA9 Initiative which engaged city administrators and staff in a collaborative effort to improve the integration of interagency resources to maximize protection for the citizens and visitors of Orange County.

From Fire Ants to Mosquitoes: An Analysis of the Door to Door (D2D) Mosquito Education, Inspection, and Control Program, 2017

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ABSTRACT This presentation is an analysis of the new workflow process utilized by the Orange County Mosquito and Vector Control District's (OCMVCD) Door-to-Door (D2D) mosquito education, inspection, and control program. In June, 2017, OCMVCD began notifying neighborhoods 2-5 days in advance of D2D mosquito control activities in areas with high West Nile virus and invasive *Aedes* activity. OCMVCD modeled this process on its successful Red Imported Fire Ant (RIFA, *Solenopsis invicta)* control program to reduce RIFA populations in targeted neighborhoods. In 2015 and 2016, D2D backyard inspections were conducted at approximately 40% of parcels in targeted areas. In 2017, utilizing the notification protocol prior to inspection, 50% of backyards in targeted neighborhoods received inspections. Due to high levels of nuisance mosquitoes in Santa Ana, D2D teams notified and inspected three residential neighborhoods on three consecutive Saturdays during peak mosquito season. OCMVCD staff were able to inspect and educate residents of >70% of parcels in the targeted areas during Saturday. Notification of parcels in targeted areas prior to D2D mosquito control efforts engages the public so that there is increased acceptance of OCMVCD D2D teams accessing backyards. Increasing access to backyards helps in the effort to reduce populations of biting mosquitoes in high risk areas of Orange County.

Partnering For a New Experience in Education

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ABSTRACT Managing mosquitoes in our changing landscape requires an updated approach. No longer can Districts just rely on laboratory and operations staff to find and suppress *Culex* populations. With the establishment of invasive *Aedes* in our dense urban landscapes, and limited suite of tools for their control, public engagement and participation is critical. To succeed, outreach approaches - whether they are media campaigns or outreach programs - must stimulate the senses, elicit an emotional response, and inspire action.

When the Greater Los Angeles County Vector Control District's beloved mobile outreach vehicle, the VecMobile (aka: BugBus), finally reached its 'end of life', the District took a hard look at the program. While not cheap, the program is consistently in high demand by teachers, anticipated by younger students, and regularly requested by cities. Its possible closure led students and teachers to rally the Board to keep the program alive.

Justifying and paying for an expense of this magnitude challenges even the largest of districts, but through creativity, perseverance, and out-of-the-box thinking, the District was able to purchase and outfit an exciting, high-tech outreach vehicle that accomplishes many goals. Programmatic elements are designed to align with California Science Standards and incorporate STEAM (science, technology, engineering, art, & math) elements. Donated microscopes allow immersive hands-on study of the mosquito, inspiring and motivating our next generation; giant scanning electron microscopy (SEM) images, a 65" touch screen interface, and an anatomically correct 3D interactive provide that 'wowfactor'; and an edgy new look and catchy rap video provide the hook needed to leave a lasting impression on students and adults alike.

Fundraising and unique partnerships provide viable pathways to improve the outreach experience. Through perseverance, these tools have allowed this District to continue providing free outreach programs on the new Mosquito SWAT Lab for many years to come.

Don't drone on: Providing clear and concise communications for unmanned aerial systems programs

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INTRODUCTION

In 2016, the Placer Mosquito and Vector Control District (District) began to explore the utility of unmanned aircraft systems (UAS), otherwise known as drones, in mosquito and vector control. In January of 2017, the District's Board of Trustees passed a resolution recognizing the vector control benefits which resolved "that the Placer Mosquito and Vector Control District Board of Trustees hereby support the development of UAS technology for the purposes of protecting public health, and shall develop and implement policies and procedures to ensure the judicious and safe use of UAS technology in vector assessment and control operations". Since then, the District has purchased several UAS and has committed staff to development and implementation of UAS in field operations.

PROBLEM STATEMENT

As the Placer Mosquito and Vector Control District began the exploratory process of UAS technology integration into existing surveillance and control programs, District staff recognized the importance of external communications surrounding the use of UAS. As certain UAS products became readily available to public and private agencies, as well as the general public, it was expected that there would be concerns about what drone users should be allowed and not allowed to do. Privacy concerns over UAS use were also a part of the popular culture zeitgeist at the time.

APPROACH

District staff understood the importance of communicating with partner agencies and the public about the benefits of UAS technology for mosquito control to help assuage potential concerns over data collection and privacy. A communication strategy was developed to help address public concerns, as well as educate and recruit partner agencies to assist with any potential communications from members of the public around UAS activities .

RESULTS

The Public Affairs Manager, with guidance from the District's General Manager, then developed a UAS Communication Strategy that included the following components:

Backgrounder Sheet
MAC UAS Flight Notifications
Webpage
Social media
Media advocacy

CONCLUSION

Implementation of the UAS Communication Strategy is ongoing, and additional meetings with partner agencies are currently underway. The District has found that being proactive with UAS communications in anticipation of questions or concerns has been beneficial as far as being able to direct the narrative around UAS use with the media, fostering positive relationships with partner agencies, and gaining and maintaining public trust.

Using a Web-Based Survey Interface for Real-Time Data Collection for Invasive *Aedes* Mosquito Response

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INTRODUCTION

In April of 2017, San Mateo County Mosquito and Vector Control District (SMCMVCD) participated in Silver Dragon XI: Operation Zika, a full-scale county emergency preparedness exercise coordinated by San Mateo County Health System and the San Mateo County Sheriff's Office simulating the response to a mosquito-borne disease outbreak in San Mateo County. CERT (Community Emergency Response Team) members visited nearly 7,000 residences throughout San Mateo County and distributed reusable shopping bags filled with printed materials related to mosquitoes and mosquito-borne disease prevention to each household. Three local CERT teams also agreed to test a real-time mobile data collection component during the exercise. The purpose of the real-time mobile data collection component was to determine whether CERT volunteers were able to use smart phones to enter data into the survey interface, and whether the resulting data was accurate enough to be useful for planning mosquito surveillance and control activities in the event of an invasive *Aedes* mosquito detection in San Mateo County.

METHODS

Members of participating teams used smartphones or tablets to enter information on: 1) the number of containers they saw in the visible front yard area ("Record the number of containers or other items that may hold water."), 2) whether residents reported getting daytime mosquito bites at that address in the past 6 months ("Has any resident experienced daytime mosquito bites at this address in the past 6 months?"), and 3) whether residents reported purchasing new plants or outdoor items in the past 6 months ("Has resident introduced plants, pots, or other outdoor items to this address in the past 6 months?"). If no resident was available at the time of the visit, team members noted that and entered only the number of containers they observed. All CERT teams participating in the exercise were offered training by SMCMVCD staff; however, only one CERT team participating in the mobile data collection component attended training. Three team leaders from another CERT team attended a separate training. The third CERT team did not receive any in-person training. Participating teams were provided with a set of instructions for using the survey on a mobile device, including text instructions and screen captures. Participants were given the option of entering data on their own personal mobile device or using an iPad provided by the District. The data was collected using an online survey collection interface (SurveyMonkey). Approximately 8 weeks after the completion of Silver Dragon XI: Operation Zika, SMCMVCD staff visited 83 addresses from two neighborhoods surveyed by the CERT team that had received training and entered an estimate of the number of containers observed in the front yard of each residence.

RESULTS AND DISCUSSION

Most CERT team members had access to a personal mobile device and were willing to use it during the exercise. There were no reports of technical difficulties during the exercise. There were 714 survey submissions collected during the exercise, of which 41 were duplicate entries. Another 47 entries did not

Table 1 Responses to survey question 1: Record the number of containers or other items that may hold water

Estimated Containers	Responses	% Response
0	304	48.56%
1-5	236	37.70%
6-15	53	8.47%
15+	31	4.95%
No response	2	0.32%
Total	626	

Table 2 Responses to survey question 2: Has any resident experienced daytime mosquito bites at this address in the past 6 months?

Reports daytime mosquito bites?	Responses	% Response
Yes	10	1.60%
No	16	2.56%
Unsure	29	4.63%
Refused to answer	5	0.80%
Resident not available	551	88.02%
No Answer	15	2.40%
Total	626	

contain a complete, valid address. The remaining 626 entries were considered complete responses, of which 624 (99.68%) contained an answer to the question "Record the number of containers or other items that may hold water" (Table 1); 55 (8.79%) contained a resident-supplied answer to the question "Has any resident experienced daytime mosquito bites at this address in the past 6 months?" (Table 2); and 52 (8.33%) contained a resident-supplied answer to the question "Has resident introduced plants, pots, or other outdoor items to this address in the past 6 months?" (Table 3). Based on SMCMVCD staff's assessment of 83 properties in two neighborhoods previously visited by a CERT team during the exercise, 48.19% of CERT responses noted fewer containers than observed by SMCMVCD staff, 48.19% of responses indicated the same number of containers observed by SMCMVCD staff, and only 3.61% of responses indicated more containers than observed by SMCMVCD staff (Table 4). CERT teams were willing and able to use their own personal mobile devices to enter data into the survey interface. The vast majority of data entered was usable,

Table 3 Responses to survey question 3: Has resident introduced plants, pots, or other outdoor items to this address in the past 6 months?

Reports new outdoor items?	Responses	% Response
Yes	7	1.12%
No	17	2.72%
Unsure	28	80.00%
Refused to answer	16	2.56%
Resident not available	523	83.55%
No Answer	35	5.59%
Total	626	

Table 4 Comparison of SMCMVD and CERT-collected container data for 83 properties

Containers as Assessed by SMCMVCD	CERT Overestimate	CERT Accurate	CERT Underestimate
0	1	38	n/a
1-5	2	2	27
6-15	0	0	9
15+	n/a	0	4
Total	3	40	40

and a large amount of data was collected in a short period of time. It is likely that this technique would be useful in the event that the District needed to collect information or responses from many households quickly. During the exercise, the CERT teams made contact with only a small number of residents. This was likely both by choice – the CERT team leaders reported that the information bags were left on doorknobs in the interest of distributing as many as possible – and because the exercise was completed during work hours on a weekday when few residents were at home. In the event of a real CERT team deployment, particularly if resident contact was desired, it would be preferable for teams to work on weekends or evenings when more residents are home. CERT teams in San Mateo County are made up of mostly retired residents. One CERT team leader expressed concerns with team members' physical abilities, noting that they are not able to cover large areas quickly, particularly in neighborhoods with hills and slopes. However, it is likely that CERT deployments on weekends will attract a wider range of volunteers. Even with training, the CERT teams were not very effective at assessing the number of potential mosquito breeding sources present on a property. They tended to underestimated the number of visible containers, particularly at homes where the SMCMVCD staff observed a relatively small number of containers. This is not unexpected, as many potential breeding sources are familiar items, such as plant saucers, children's toys, and decorative items. Unaccustomed to looking for mosquito breeding sources, the CERT team members likely overlooked these items at otherwise well-kept properties.

CONCLUSIONS

Although CERT teams were not effective at assessing the number of potential mosquito breeding sources visible on properties, it would be feasible to deploy them for other purposes, such as administering questionnaires and surveys to residents, distributing information, or assessing the presence or absence of more obvious features.

Texts from the Pool - Streamlining Resident Response to SacYolo's Pool Program

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ABSTRACT As our aerial pool surveys have grown in size, the number of notices posted when residents are not home has increased, and with it, the amount of time it takes to manage phone calls and appointments to return to the property for an inspection. This past season, we implemented online appointment requests and text messaging as options to respond to notices. We will explain how this changed our program and streamlined response, what best practices we learned in the process, and what we will change looking forward to next season.

A tale of 3 competencies: Mosquito surveillance and control capabilities across the United States and its legislative implications

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ABSTRACT Mosquito-borne diseases are an ongoing public health concern in the United States. In 2016 alone, mosquitoes transmitted over 2,000 cases of endemic West Nile Virus (WNV) and 224 cases of emerging Zika virus (ZIKV) disease. Controlling the spread of vector-borne diseases is the responsibility of a variety of departments across the country, including local health departments, yet little data exists on these departments' level of preparedness.

The National Association of County and City Health Officials (NACCHO), in partnership with the Centers for Disease Control, developed and distributed an electronic survey to assess mosquito surveillance and control capacity among almost 2,000 local vector control programs nationwide. The responses were scored quantitatively by evidence-based activities essential to a competent vector control program. Using this criteria, the nearly 1,100 respondents (57% response rate) ranked 8% "Fully Capable," 4% "Competent," and 84% "Needs Improvement." Of the vector control programs ranked as "Needs Improvement," all of them lacked the capability or capacity to perform pesticide resistance testing and more than half lacked competency in performing routine mosquitio surveillance and species identification.

This assessment gathered previously unavailable baseline data on local mosquito surveillance and control in the United States. A majority of local departments reported significant gaps in programmatic activities, indicating significant susceptibility to mosquito-borne diseases caused by WNV and ZIKV. By identifying these gaps, this assessment enables local, state, and federal agencies and their partners to prioritize and develop targeted approaches, regulatory and legislative solutions for improving vector control programs. Local vector control programs need quality and ongoing staff training, enhanced partner engagement, and resources to support essential mosquito surveillance and control activities.

Modernizing a mosquito district through process discovery and technological innovations.

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ABSTRACT Alameda County Mosquito Abatement District (ACMAD) has been modernizing its information technology (IT) infrastructure, workflows, and analytical processes over the past 2 years. Our vision is to increase fiscal responsibility, efficiency, and transparency of the District to its employees, the Board of Trustees and the public. We analyzed the current business model and developed solutions to enhance daily operations. Internal systems, policies and processes were systematically inspected for relevance; although many had worked, they were wildly inefficient. ACMAD has changed its operational model from a paper-based cumbersome organization, to a cloud-based, open and distributive environment. We learned through modernization projects that many mosquito districts share similar issues that arise from being small to medium sized government entities. To increase fiscal responsibility, we renegotiated contracts by partnering with larger entities to reduce cost. Upgrading our IT infrastructure gave us the ability to move storage, human resources, mosquito fish management and geodatabase into the cloud. By reducing reliance for hardware on premise, there was less need for IT resources. By adding redundancies to connecting systems we have achieved 5 9's uptime (99.999%). Developing a substantially enhanced geo-database allowed us to empower our employees in the field and laboratory to work collaboratively rather than in siloed departments. Information now flows instantly among support staff, operations and the lab. Requests for service from the public are automatically assigned to technicians in the field with zero wait time. A technician can respond within 5 minutes after the office receives a call for service: that's faster than Amazon. The future will be dependent on the ability to share data internally, and to distribute it to partners worldwide. We are now looking at innovations in satellite imagery, virtualization, unmanned air systems and artificial intelligence as needed tools in the next evolution of the ever-changing landscape of mosquito control.

Susceptibility monitoring in mosquitoes to permethrin and biorational larvicides in California and Utah (2007-2016)

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INTRODUCTION

During 2007-2016, 152 bioassays were conducted on mosquito collections submitted by nine vector control agencies in California and Utah to West Valley MVCD for pesticide resistance detection. These agencies included Butte County MVCD, Moorpark VCP, Northwest MVCD, Orange County MVCD, Salt Lake City MAD, San Diego County VCP, San Joaquin County MVCD, Turlock MAD, and West Valley MVCD.

METHODS

The mosquito species submitted were *Aedes aegypti*, *Aedes nigromaculis*, *Ae. taeniorhynchus*, *Culex pipiens*, *Cx. quinquefasciatus*, and *Cx. erythrothorax*. Bottle bioassays were conducted for permethrin on 15 samples. Cup bioassays (Su and Cheng 2014a) were carried out for methoprene (Altosid® LL) on 46 samples, *B.t.i.* (VectoBac® WDG) on 35 samples, *Lysinibacillus sphaericus* (VectoLex® WDG) on 45 samples, a combination of *B.t.i.* and *L. sphaericus* on one sample, and spinosad (Natular® G30) on 10 samples. The susceptible colonies of *Cx. quinquefasciatus* from West Valley MVCD and of *Cx. pipiens* from San Mateo County MVCD were bioassayed concurrently with field collections for calculation of resistance ratios. Collections from untreated areas of *Ae. nigromaculis* were bioassayed concurrently with collections from treated areas for resistance ratio calculations.

RESULTS AND SUMMARY

Assay results were summarized in Table 1. Tolerance or low-to moderate level resistance to permethrin was observed in numerous samples of *Cx. pipiens* complex. Resistance to methoprene was detected in *Cx. quinquefasciatus* from Orange County MVCD and Northwest MVCD at low levels, and in *Aedes nigromaculis* from Turlock MAD at high level. Tolerance to *L. sphaericus* was revealed in *Cx. quinquefasciatus* from Northwest MVCD, whereas resistance to *L. sphaericus* was shown in *Cx. quinquefasciatus* from San Diego County VCP at low- to moderate-levels and in *Cx. pipiens* from Butte County MVCDA and Salt Lake City MAD, UT, at high levels. No tolerance or resistance was encountered in all samples tested against *B.t.i.* or spinosad. Pyrethroids have been extensively used to combat pestiferous arthropods of agricultural, urban and public health importance, various levels of resistance have been well documented (Zhu et al. 2016). Mosquito populations are exposed intentionally or unintentionally to the applications of pyrethroids, often leading to development of tolerance or resistance. Biorational mosquito larvicides based on microbial organisms (Su 2016a) and juvenile hormone analogs are important tools to control disease vectors and other nuisance species, their applications have been intensified recently, particularly since the invasion of West Nile virus. All the mosquito larvicides we depend on bear various levels of resistance risk (Su 2016b, Su et al. 2014b), more cases of resistance development are reported in field populations (Su 2016b, Su et al. 2018). It is strongly suggested to monitor the susceptibility of mosquito populations of concern to the products that are planned to use.

Table 1 Tolerance or resistance to permethrin, methoprene and Lysinibacillus sphaericus among mosquito samples submitted for bioassay from varioius agencies in California and Utah.

Pesticides	Species	Resistance ratios	Resistance ranks	Agencies
Permethrin	*Culex pipiens* and *Cx. quinquefasciatus*	5.2-23.5	Low to moderate	Butte County MVCD, Northwest MVCD, Orange County MVCD, West Valley MVCD
	Culex pipiens complex	22.8-47.7	Moderate	San Joaquin County MVCD
Methoprene	*Cx. quinquefasciatus*	2.1-3.5	Tolerance	Northwest MVCD
		5.6-18.1	Low	Northwest MVCD, Orange County MVCD
		21.0-32.5	Moderate	Orange County MVCD
	Ae. nigromaculis	27.3-1,914.5	Moderate to High	Turlock MAD
Lysinibacillus sphaericus	*Cx. quinquefasciatus*	2.2-3.4	Tolerance	Northwest MVCD
		6.2-53.1	Low to moderate	San Diego County VCP
	Cx. pipiens	687.4-22,878.6	High	Butte County MVCD
		20,780.0-23,926.9	High	Salt Lake City MAD

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Low Efficacy of a Methoprene Product: Reduced Target Susceptibility or Product Performance Issue?

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ABSTRACT The failure of methoprene field applications to control *Culex quinquefasciatus* larvae in Orange County, CA, has been noticed by field technicians for the past few years. In response to these field observations, the efficacy of sustained release methoprene (Altosid XR briquettes, 2.1% A.I.) was evaluated in semi-natural, mesocosm environments, using four 480-gallon capacity test tanks, for a duration of 150 days by the Orange County Mosquito and Vector Control District. Freshly-laid egg rafts from wild caught Orange County mosquitoes and a methoprene-susceptible strain of *Cx. quinquefasciatus* supplied by the West Valley Mosquito and Vector Control District (WVMVCD) were introduced to the four test tanks and two control tanks for a total of nine trials (4 replicates per test tank, 8 replicates per test cage; 2 replicates per control tank, 4 replicates per control cage). Wild type and susceptible mosquito larvae were kept separate using individual floating bio-assay cages. Inhibition of Emergence (IE) in pupae was recorded at the end of each trial. Laboratory larval bio-assays were also conducted by the WVMVCD on colonized wild type and susceptible *Cx. quinquefasciatus* to determine resistance ratios (RR) to methoprene. Results of the semi-field trials demonstrated low IE (<90%) and likely evidence of methoprene tolerance in the wild *Cx. quinquefasciatus*. Laboratory larval bioassay RR results paralleled these field observations. The Orange County field population with low susceptibility to methoprene was colonized and were subjected to laboratory selection at the WVMVCD for further studies.

Field evaluation of pyrethrin resistance in *Culex tarsalis* from a rice growing area

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ABSTRACT There are more than 33,000 acres of rice in the Sacramento-Yolo Mosquito and Vector Control District's service area. These rice fields serve as productive breeding sites for mosquitoes, including the important West Nile virus vector *Culex tarsalis*. Organic rice can create additional challenges due to the limited number of products labeled for use over organic crops. Previous bottle bioassay and microplate assessment of *Culex tarsalis* collected from rice growing areas have indicated that pyrethrin resistance is present. To better understand the impact of resistance on control efforts, field trials of Pyronyl Oil Concentrate 525 over conventional rice and Merus 2.0 over organic rice by aerial Ultra Low Volume were conducted. Sentinel cages and spinners were placed within each spray block to determine the efficacy of each application. Each sentinel cage location was comprised of two cages, one cage containing susceptible colony mosquitoes (*Culex tarsalis*, BFS) and the second contained wild *Culex tarsalis* collected near rice fields located at the Vic Fazio Yolo Wildlife Area. Twelve hours post-treatment, the cages were observed for mortality and both surviving and dead mosquitoes were evaluated by microplate assay to better understand the mechanisms contributing to pyrethrin resistance. The results of the field trials and microplate assay will be discussed.

Variability in the frequencies of genetic mutations associated with pyrethroid resistance in *Aedes aegypti* from central and southern California

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ABSTRACT The first breeding populations of *Aedes aegypti* were identified in California in 2013, and have since been detected in 13 counties in central and southern CA. Recent studies suggest two introductions likely occurred, with genetically distinct populations in central and southern CA. Given the recent threat of Zika virus transmission, it is imperative to understand if these populations harbor potential resistance to pyrethroids, the most commonly used class of adulticides in the state. In 2017, the California Department of Public Health, Vector-Borne Disease Section implemented an *Ae. aegypti* pesticide resistance screening program to look at two common knockdown resistance mutations on the sodium channel gene. These mutations prevent the insecticide from acting on the mosquito nervous system, thereby potentially conferring resistance to certain pyrethroids. Real-time polymerase chain reaction assays were used to analyze the V1016I and F1534C mutations for *Ae. aegypti*. Preliminary results from mosquitoes collected by ten mosquito and vector control agencies indicate that populations in the central and southern regions vary in the frequency of these two mutations. The central CA populations are displaying nearly fixed resistant mutations at both mutation sites, while the southern CA populations are more variable. Though the presence of these mutations does not guarantee control failure, it does indicate that these mosquitoes may be predisposed to surviving pyrethroid treatments. In southern CA, fixation of these resistant mutations in populations may be prevented by following best management practices for preventing resistance.

Heterogeneity in pyrethroid resistance in *Aedes aegypti* in Miami/Dade, Florida

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ABSTRACT The objective of this study was to determine the susceptibility of adult *Aedes aegypti* in Miami/Dade, Florida, to four mosquito adulticides: MosquitoMist 2, MosquitoMaster 412, Duet, and Merus. From 2016 to 2017, we collected *Ae. aegypti* eggs from thirteen neighborhoods in and around Miami/Dade. F1 females were tested by bottle bioassays using formulated product. Diagnostic time (DT) and diagnostic dose (DD) were calculated for each product by comparison with a susceptible *Ae. aegypti* strain. MosquitoMist 2 was as or more effective against field-collected mosquitoes compared to the colony strain. *Ae. aegypti* from Miami/Dade exhibited delayed time-to-mortality against Duet and Merus, which contain pyrethroids and pyrethrin, respectively. Despite the delay in time-to-mortality, 90-100% of *Ae. aegypti* from seven neighborhoods died within one hour. These results indicate local heterogeneity in *Ae. aegypti* to formulated pyrethroid products. Because the CDC bottle bioassay does not reflect field-applied rates, these results indicate the need for field testing with product at the label rate to determine field efficacy.

Resistance detection in *Culex tarsalis*: from conventional bioassay to molecular approach

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INTRODUCTION

Conventional bioassays are crucial for evaluating functional resistance in a mosquito population. However, standard bioassays do not give great insight into the mechanisms behind the resistance. Target-site mutations are a category of resistance mechanism in which DNA mutations lead to changes in protein structure. These changes confer resistance, typically by altering or preventing the binding of an insecticide. The two most common target-site mutations are (1) *kdr*, a mutation in the voltage-gated sodium channel that leads to pyrethroid resistance and (2) *ace-1*, a mutation in the gene encoding acetylcholinesterase that confers resistance to organophosphates (Martinez-Torres et al. 1998; Soderlund and Knipple 2003; Weill et al. 2004). Unlike conventional bioassay methodology, which can typically be used across multiple species, molecular testing requires the development of new primers or primer/probe sets to detect target-site mutations for each mosquito species. Here, we develop molecular diagnostics to detect *kdr* and *ace-1* in *Culex tarsalis*, an abundant mosquito in the Western United States that is an important vector of West Nile and other arboviruses.

METHODS

Degenerate primers were used to amplify the DNA regions surrounding *kdr* and *ace-1* in *Cx. tarsalis* from over 50 individual mosquitoes. From these sequences, a PCR using GC-rich primer tags (Germer and Higuchi 1999; Saavedra-Rodriguez et al. 2007) and SYBR green with melt curves was developed to distinguish three *kdr* alleles: leucine (wild-type; TTA), phenylalanine (TTT), and serine (TCA).

Wild-caught mosquitoes collected in Yuba County were separated into resistance levels for pyrethroids and organophosphates using a CDC bottle bioassay (Brogdon and McAllister 1998) with a modification that dead mosquitoes were removed from the bottle at various time points. DNA sequencing was used to look for *ace-1* mutations, and the newly developed SYBR green assay was used to characterize *kdr* in these mosquitoes.

RESULTS AND DISCUSSION

Nearly 90% of the tested mosquitoes displayed some resistance to pyrethroids. Preliminary molecular analysis revealed that *kdr* mutations (both leucine to phenylalanine and, to a lesser extent, leucine to serine) were prevalent. All mosquitoes resistant to pyrethroid insecticides in the bottle bioassay were homozygous for *kdr* mutations. Additionally, most individuals susceptible to pyrethroids had at least one *kdr* allele, suggesting that a single *kdr* mutation was not enough to confer resistance or that there may be additional factors involved. With regard to organophosphates, little resistance was detected in the bottle bioassays and, thus far, no *ace-1* mutations have been discovered in this *Cx. tarsalis* population.

CONCLUSIONS

This, to our knowledge, marks the first molecular testing for insecticide resistance markers in *Cx. tarsalis* and the first characterization of *kdr* mutations in this species. Further work is necessary to assess the prevalence of *kdr* and other resistance mechanisms in *Cx. tarsalis* across a broader geographic area.

ACKNOWLEDGEMENTS

We would like to thank the Sutter-Yuba and Sacramento-Yolo Mosquito & Vector Control Districts for help with collecting mosquitoes for this project. Funding was provided by the Mosquito Research Foundation.

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Detoxification Enzyme Levels in *Aedes aegypti* Associated with Pesticide Resistance

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ABSTRACT The detection of *Aedes aegypti* and travel associated Zika virus infected individuals in California makes mosquito control a vital part of preventing local Zika virus transmission. Studies have demonstrated that accumulated chemical sprays, especially using pyrethroids, lead to insecticide resistance, causing these control measures to become ineffective. A biochemical microplate assay is an effective way to monitor mosquito detoxification enzymes. These enzymes could either hydrolyze or oxidize toxins into non-toxic substrates and eliminate them. In this study, we collaborated with local mosquito and vector control agencies in California to test field-caught *Ae. aegypti*, monitoring for α and β-esterases, oxidase, glutathione-S-transferase, and insensitive acetylcholine esterase. This work provided evidence of metabolic resistance levels, and differentiated pyrethroid, organophosphate, and organochlorine resistance within our field caught *Ae. aegypti*. Coupled with our knockdown resistance assay, our results provided a detailed representation of the potential resistance profile of *Ae. aegypti* populations in California. This information can help local agencies design appropriate chemical control strategies.

Field evaluation of three residual adulticides using a leaf bioassay

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ABSTRACT Residual insecticides applied to foliage for the control of adult mosquitoes are a part of an integrated pest management program utilized by Sacramento-Yolo Mosquito and Vector Control District. The release of a newly formulated residual insecticide prompted a re-evaluation of the best methods for integrating these products into our program. Three products were evaluated for residual efficacy using a leaf bioassay: Suspend SC, Suspend Polyzone, and Lambda 9.7 CS. Products were applied in the field at mid- and maximum-label rates and then observed over time. Leaf bioassay results indicated that Suspend SC and Suspend Polyzone have similarly extended periods of residual activity at both mid- and maximum-label rates. Lambda 9.7 CS was evaluated to a lesser extent and had a comparably shorter duration of residual efficacy.

INTRODUCTION

Effective mosquito control requires a diversity of tools. In addition to larviciding and ultra-low volume spraying for adult mosquito control, the Sacramento-Yolo Mosquito and Vector Control (Sac-Yolo) also has utilized residual insecticides. Residual insecticides applied to foliage have been shown to have utility in multiple studies conducted in a variety of locations including the Southern California Desert (Britch et al. 2009), Florida (Cilek and Hallmon 2006, Cilek 2008), Kentucky (Trout et al. 2006, 2007) and Queensland Australia (Royal 2004). Previously, Sac-Yolo primarily utilized Suspend SC (Bayer Environmental Science, Research Triangle Park, NC) applied at a mid-label rate (0.75 fl oz/gal/sq ft) to structures and foliage near homes and in parks in response to elevated mosquito abundance or West Nile virus activity. The recent release of Suspend Polyzone (Polyzone; Bayer Environmental Science, Research Triangle Park, NC) has triggered a re-evaluation of the use of residual insecticides by Sac-Yolo. Residual insecticides have the added advantage of flexibility in application time, so that applications can be made when people are least likely to be utilizing park spaces, and can provide extended periods of control.

The active ingredient in both Suspend SC and Polyzone is the pyrethroid deltamethrin. The product label states that Suspend Polyzone has been formulated to contain a microscopic polymer film that protects the active ingredient from weather, precipitation, irrigation, and mechanical abrasion. The Suspend SC label does not state a period of residual activity, but allows for reapplication every 21 days. The Polyzone label states that the product may provide residual control for up to 90 days, but also allows for reapplication every 21 days as needed. Lambda 9.7 CS (Lambda; Central Life Sciences, Schaumburg, Il) is a third product that was evaluated to a lesser extent, and also utilizes a pyrethroid active ingredient, Lambda-cyhalothrin. The label does not state a period of residual control, but allows for reapplication after 7 days if necessary.

The goal of the current re-evaluation was to assess Suspend SC, Polyzone, and Lambda to determine optimal application rates and re-application intervals. Suspend SC and Polyzone and Lambda were applied in the field at mid- and maximum-label rates and residual activity measured over time using a leaf bioassay. Residual activity was measured by mosquito mortality, with products considered active until leaf bioassay mortality dropped to ≤20% (highest mortality observed in untreated controls). Based on WHO guidelines for assessing the residual efficacy of insecticides on wall surfaces, re-application may be necessary when the observed mortality drops below 70% (World Health Organization 2013). Thus in order to compare the products tested, an application was considered effective until leaf bioassay mortality dropped to ≤70%.

MATERIALS

Products and application Products were applied at mid-label (0.75 fl oz/gal/1000 sq ft for Suspend SC and Polyzone, and at 0.4 fl oz/gal/1000 sq ft for Lambda) and maximum-label rates (1.5 fl oz/gal/1000 sq ft for Suspend SC and Polyzone, and 0.8 fl oz/gal/1000 sq ft for Lambda CS). To protect bees the products were only applied to foliage that was not flowering at the time of application. To allow for direct product/application rate comparisons, Suspend SC and Polyzone were applied at mid- and maximum-label rates to adjoining shrubbery of the same species (*Viburnum tinus*) on the Sac-Yolo district premises (Fig 1, site #1; May 2017). An untreated bush of the same species was kept in a pot and was used as an untreated control. Additionally, all product/application rate combinations were applied (June - August 2017) individually to field sites comprised mostly of parks located in Sacramento County (Fig 1). All applications were made with a backpack sprayer (Stihl, Virginia Beach, VA; part number #SR450)

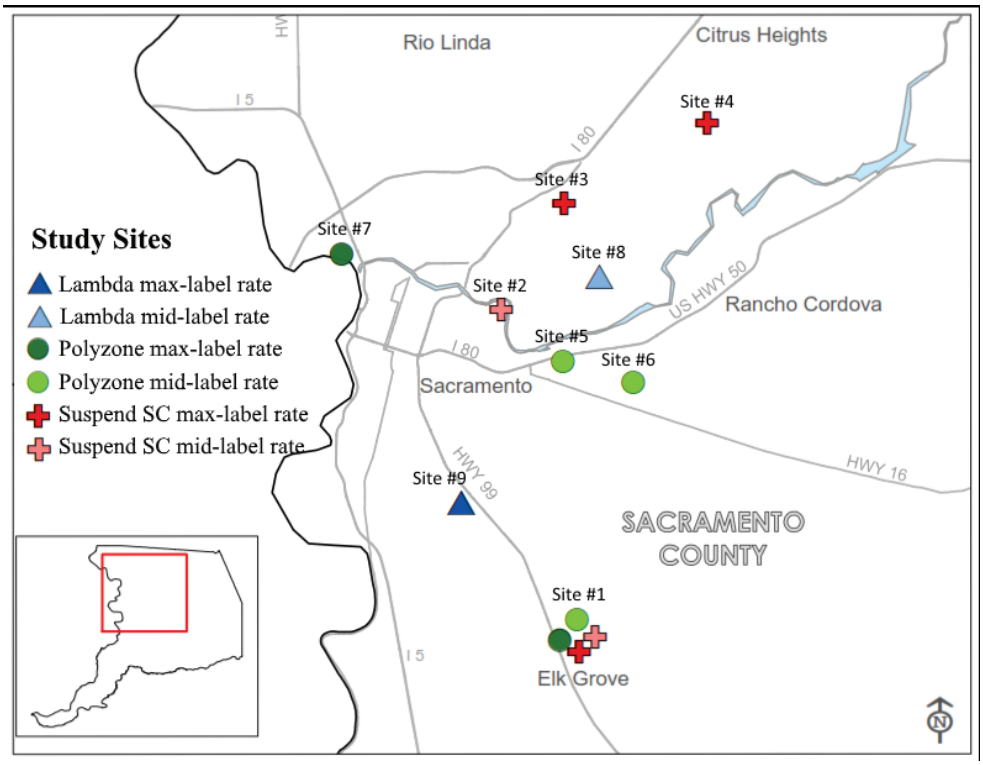

Figure 1 Map of study sites in Sacramento County, California.

with the pressure pump kit and ultra-low volume nozzle installed.

Initially Suspend SC and Polyzone were applied at three different field locations; however, multiple study sites were lost due to trimming of treated foliage. Lambda was evaluated to a lesser extent and each application rate was applied to one study site. The total number of locations where each product/application rate was applied ranged between one and three application sites.

Figure 2 Leaf bioassay cups. Panel A shows the complete cup set-up with a sugar wick placed in the straw opening of the lid. Panel B shows dead mosquitoes in the bottom of assay count that were counted to calculate percent mortality.

Each field site was monitored until the leaf bioassay measured ≤20% mortality for three consecutive sampling events.

Leaf bioassay To assess the residual efficacy of each product/application rate, sites were monitored over time using a leaf bioassay. Leaves were collected from treated foliage on a weekly or bi-weekly basis and stored individually in plastic zip top bags by collection site. Collected plant materials were trimmed into small cuttings that fit into the sample cup and allowed mosquitoes to freely fly around the cup (Fig 2). The assay was performed in 20 oz clear plastic cups with flat snap-on lids and straw openings. Static electricity in the cups was reduced by wiping the inside of the cup with a damp paper towel. Three replicates for each treatment were prepared for each site and time period. Approximately 20 susceptible *Culex quinquefasciatus* colony (CQ1) mosquitoes of mixed sex were introduced into each cup through the straw opening. Mosquitoes were supplied 10% sucrose by sugar wick, 4.5 mL polyethylene tubes filled with sucrose solution and stoppered with a 1 cm segment of dental wick, tubes were inverted and secured in the straw opening with tape. Cups were held at 23°C and ambient humidity.

Mosquitoes were allowed 24 hours of contact time in the assay cup, after which the number of knocked down mosquitoes (any mosquito laying on the bottom of the cup that did not rouse to flight) were counted. To get the final count of mosquitoes per cup, the cups were briefly held at -80°C until all mosquitoes were killed, after which all of the mosquitoes were counted. Leaves were closely inspected to ensure an accurate count. Percent mortality was calculated for each set of replicates.

When first establishing a new site leaves were collected prior to treatment and were bioassayed to ensure mortality observed in bioassay cups was attributable to the applied treatment. Any site that had greater than 20% mortality in the initial bioassay was not included in the study. Each time a bioassay was performed, three negative controls of leaves from an untreated plant and blank cups (mosquitoes only) were included.

Data Analysis The leaf bioassay was used over time to determine the duration of both residual activity and effective control of the assessed products. Mean residual activity was calculated based on the last week that bioassay mortality was >20% before dropping to ≤20% for three consecutive weeks. Mean duration of effective control was calculated based on the last sampling week where bioassay mortality was >70%. Mean residual action and mean effective control were not calculated for Lambda as each application rate was only applied to one site.

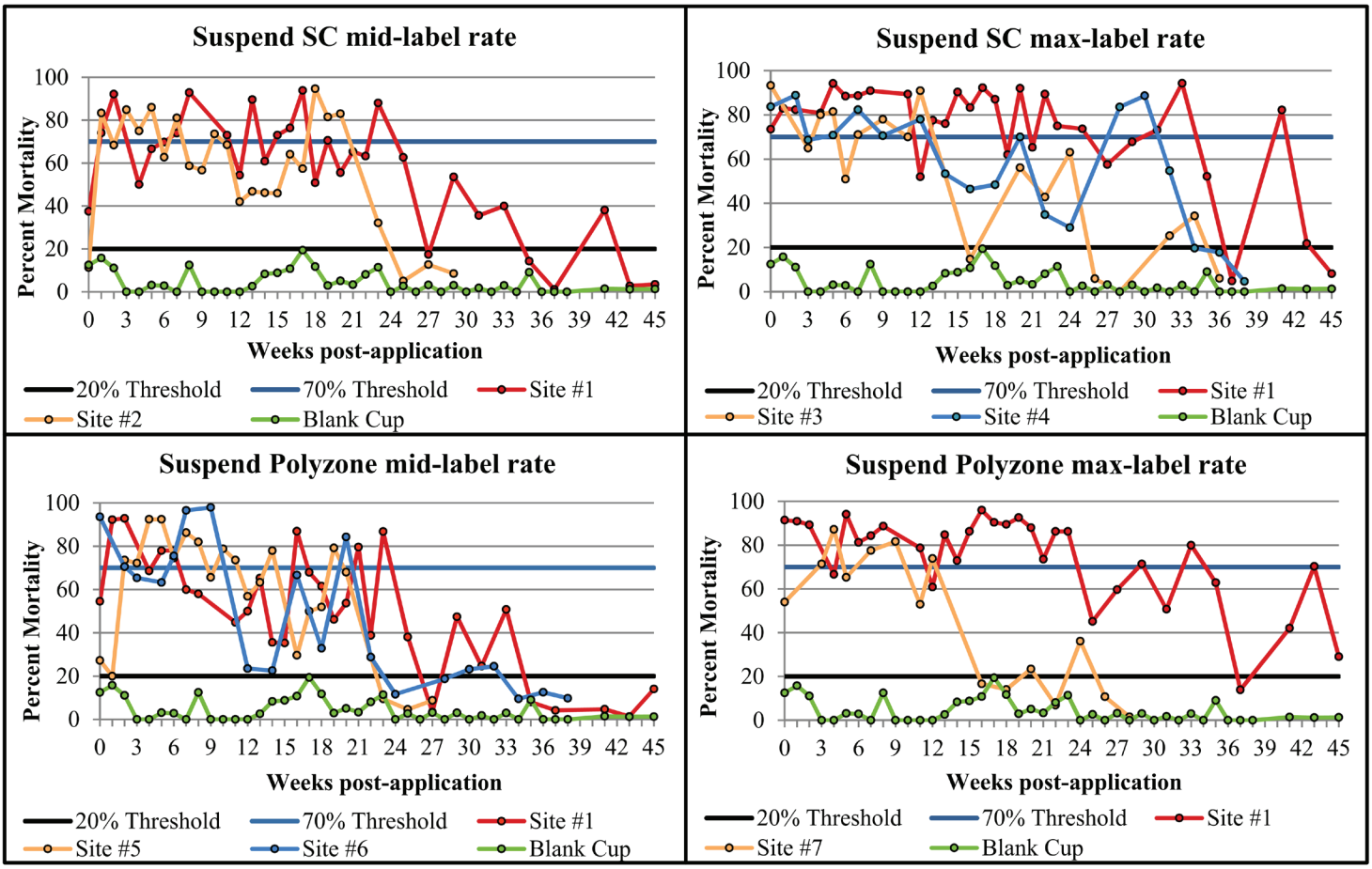

Figure 3 Percent mortality measured across three technical replicate cups performed at each study site for Suspend SC and Suspend Polyzone treatments applied and mid- and maximum-label rates. The 20% threshold for residual activity and the 70% threshold for effective control appears on each figure.

RESULTS

The results of the leaf bioassay indicated that Suspend SC and Polyzone have extended periods of residual action when applied to foliage in the field (Fig. 3). Overall, mean residual action (mortality >20%) was 32 (sd=12.7 weeks) and 36 (sd=5.8 weeks) weeks post-treatment for Suspend SC applied at mid-label (n=2) and max-label (n=3) rates, respectively. For Polyzone the mean residual action was 29 (sd=5.5 weeks) and 34 (sd=10.5 weeks) weeks post-treatment when applied at mid-label (n=3) and max-label (n=2) rates, respectively. Mean residual activity was not calculated for Lambda as each application rate was only applied at one site. The leaf bioassay results for the Lambda applications are shown in Fig 4, for these applications residual activity was detected up to 17 and 31 weeks post-treatment when applied at mid- and maximum-label rates.

Because residual activity is likely to continue beyond the time period of effective mosquito control, mean effective control (in number of weeks) was also calculated (Fig. 3). For Suspend SC the duration of mean effective control when applied at mid-label rate (n=2) was 22 weeks (sd=2.1 weeks); when applied at maximum-label rate (n=3) it was 28 weeks (sd=14.6 weeks). The mean effective control for Polyzone was 21 weeks (sd=1.5 weeks) for mid-label rate (n=3) and 28 weeks (sd=15.0 weeks) for maximum-label rate (n=2). The duration of effective control of Lambda was 5 weeks when applied at mid-label rate and 23 weeks at maximum-label rate.

DISCUSSION

The leaf bioassay results indicated that Suspend SC and Polyzone have similar extended periods of residual activity at both mid- and maximum-label rates. These findings exceeded expectation and led to further questions about how observed residual activity related to the reduction of mosquitoes within the treatment area. To try to infer this relationship, mean effective control durations were calculated based on a 70% mortality threshold in the leaf bioassay. This threshold was selected based on the WHO recommendations for assessing indoor residual insecticide applications. Although there may be marked differences between indoor wall surfaces and foliage applications, the 70% threshold was the selected control threshold in the absence of a more suitable operational recommendation.

The current study was designed as a first step in determining the best product and application rate for our mosquito control needs. Based on the leaf bioassay results a mid-label rate of

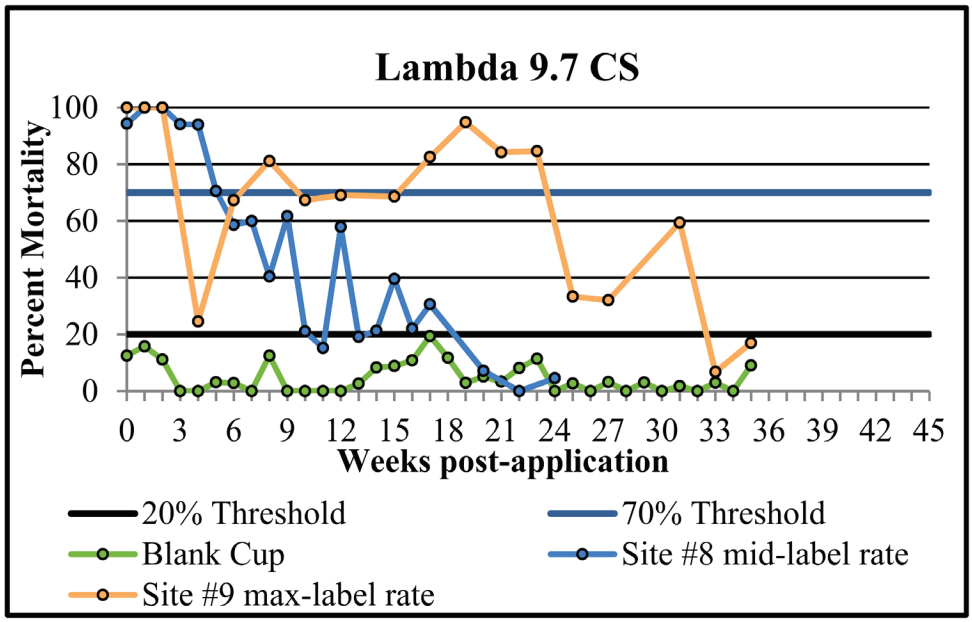

Figure 4 Percent mortality measured across three technical replicate cups performed at each study site for the mid- and maximum-label rate applications for Lambda 9.7 CS. The 20% threshold for residual activity and the 70% threshold for effective control appears on the figure.

either Suspend Polyzone or Suspend SC provided approximately 5 months of effective control, an interval that nearly spanned the entirety of a mosquito control season. Lamda was evaluated at only a single site for each application rate, but this preliminary data indicated that maximum-label rate provided better sustained efficacy in the field than the mid-label rate.

Based on the leaf bioassay data (Fig 3), it is clear that there is a great deal of variation in leaf bioassay data. This may be attributable to leaf selection, where some leaves may have been more heavily treated than others, perhaps due to position of the leaf during application, or because of the growth of new leaves. Even when the same products and application rates were applied there was a high degree of variation in the duration of residual activity and effective control between treatment sites. This variation made long-term repeated sampling important for observing the true residual action of the product and may also indicate a necessity for increasing the number of sampling locations for each site evaluated in the future.

Although the leaf bioassay provided a method for evaluating residual efficacy, this artificial exposure is not necessarily analogous to the exposure of mosquitoes in the field (Lothrop et al. 2003, Lothrop et al. 2008, Britch et al. 2009). First, mosquitoes in the leaf bioassay were exposed to the treated leaves for 24 hours, which may be longer than the exposure time a mosquito would receive in the field. Second, all of the leaf bioassays were conducted with a fully susceptible laboratory colony. With the increased detection of pyrethroid resistance in the Sacramento Valley (Reed et al. 2012), field populations may not be as susceptible as the tested colony. Lastly, as observed by Trout et al.(2007) limitations in application equipment may not allow product to be applied high enough into the trees where important *Culex* vectors rest and host seek, limiting control in these species.

The 24 h exposure interval was used in the leaf bioassay because it allowed for a sufficient exposure time to ensure mortality if the residual insecticide was present and active, thus ensuring that estimates of residual activity were accurate. However, this approach likely overestimated the duration of effective control. Therefore, our next steps will be to evaluate the effect of residual insecticide foliar treatment on mosquito trap counts. We will be performing this evaluation with Suspend Polyzone applied at a mid-label rate. Additionally, because residual treatment can quickly enhance levels of resistance, resistance monitoring will be combined with the treatment assessment. The use of residual insecticides can provide great utility, but increasing insecticide resistance can become a problem especially with such long periods of residual activity beyond the effective control period. Planned field studies will provide more insight into these parameters.

ACKNOWLEDGMENTS

We thank Paula Macedo for her assistance with the study design and the Laboratory and Field Technicians of Sacramento-Yolo Mosquito and Vector Control District who assisted with product application and assessment.

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Modeling the efficacy of aerial spraying on reducing the relative abundance of *Culex tarsalis* and *Culex pipiens*

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INTRODUCTION

Aerial adulticide treatments are effective at rapidly reducing adult mosquito populations during periods of epidemic risk for arboviral diseases (Mount et al. 1996, California Department of Public Health 2017). However, estimates of treatment effects vary widely (Elnaiem et al. 2008, Macedo et al. 2010), because current calculation methods typically rely on comparisons of trap counts before and after single treatment events (Reisen 2010), which are subject to a high degree of short-term volatility. Reliable estimates of the effects of aerial spraying are imperative for appropriate implementation and allocation of control measures and resources. In this study, we aimed to overcome the limitations of shorter-term studies by using a decade of data on aerial treatments and mosquito surveillance to estimate the magnitude of the effect of aerial spraying on adult *Culex tarsalis* and *Culex pipiens* in Sacramento and Yolo counties.

METHODS

Mosquito collection records from CO_2-baited EVS traps in Sacramento-Yolo Mosquito & Vector Control District (SYMVCD) for 2006-2015 were used to estimate the abundance of female *Cx. tarsalis* and *Cx. pipiens*. Aerial spray event polygons from SYMVCD were spatially and temporally aligned with trapping events to identify those potentially impacted by the treatments. The degree to which a treatment impacted a trapping event was characterized by the proportion of a 5-km collection area surrounding the trap that overlapped the spray polygon. Generalized additive models, which are statistical models incorporating smoothed functions of covariates, were used to combine information of the expected abundance and presence of spray events to identify deviations from expected abundance that were attributed to aerial spraying. The magnitude of the deviations was assessed on a weekly timescale.

RESULTS AND DISCUSSION

The abundance of both species was sharply reduced during the week immediately following aerial spraying, with a slightly greater reduction for *Cx. pipiens* as compared to *Cx. tarsalis*. In urban areas *Cx. pipiens* and *Cx. tarsalis* counts were reduced by 78.6% and 61.3%, respectively, during the week following aerial sprays, whereas in agricultural areas, *Cx. pipiens* and *Cx. tarsalis* abundance was reduced by 90.6% and 79.7%, respectively, during the week following aerial spraying. Different bionomics of the species could explain some of the differences in effects between the species. The flight range of *Cx. pipiens* is typically more limited than that of *Cx. tarsalis* and all life stages of *Cx. pipiens* are commonly localized in urban/suburban or dairy-associated areas. In contrast, *Cx. tarsalis* typically emerge from agricultural habitats and may immigrate to urban areas in large numbers (Reisen and Reeves 1990), potentially replacing adults that had been removed by aerial adulticide applications.

CONCLUSIONS

Aerial spraying is effective in rapidly reducing the relative abundance of *Cx. tarsalis* and *Cx. pipiens*, the primary West Nile vectors in California. The effect persists for many weeks, with some variation in the magnitude and duration by land use and species.

ACKNOWLEDGEMENTS

We would like to thank Ruben Rosas, Paula Macedo, and Gary Goodman from Sacramento-Yolo Mosquito & Vector Control District for providing trapping and treatment data used in this study. Funding for the project was provided through the Floyd & Mary Schwall Fellowship in Medical Research awarded to KMH.

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Field Trial of Pyrocide 7396, a PBO Synergized Pyrethrin Adulticide Formulation for Aerial Application

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INTRODUCTION

Abundant vector populations coupled with increased West Nile Virus (WNV) activity during peak season frequently necessitates large scale aerial adulticide applications. Currently in California, Trumpet EC (Naled) is the only adulticide formulation practically available for aerial application. This limits the flexibility of using different classes of pesticides and is not suitable for pesticide resistance management. During the summer of 2017 we evaluated Pyrocide 7396, a piperonyl butoxide (PBO) synergized pyrethrin formulation, which is registered for both ground ULV and aerial application.

METHODS

The pesticide was applied at a rate of 0.67 ounce per acre and at 250-300 feet above ground level by a Cessna 402 aircraft to over 5700 acres of farmland located in the southern portion of San Joaquin County, where vector abundance and WNV activity were high. To monitor efficacy, ten data collection stations spaced a half mile apart were set up along an east to west transect within the application area. At each station, droplets were collected on Teflon coated slides, and one cage of laboratory reared susceptible *Culex quinquefasciatus* and once cage of field- collected wild *Culex pipiens* mosquitoes were used to monitor mortality. Each cage contained 25-35 females 3-5 days old, which were offered sucrose solutionduring the experiment. We also compared the effect of tree

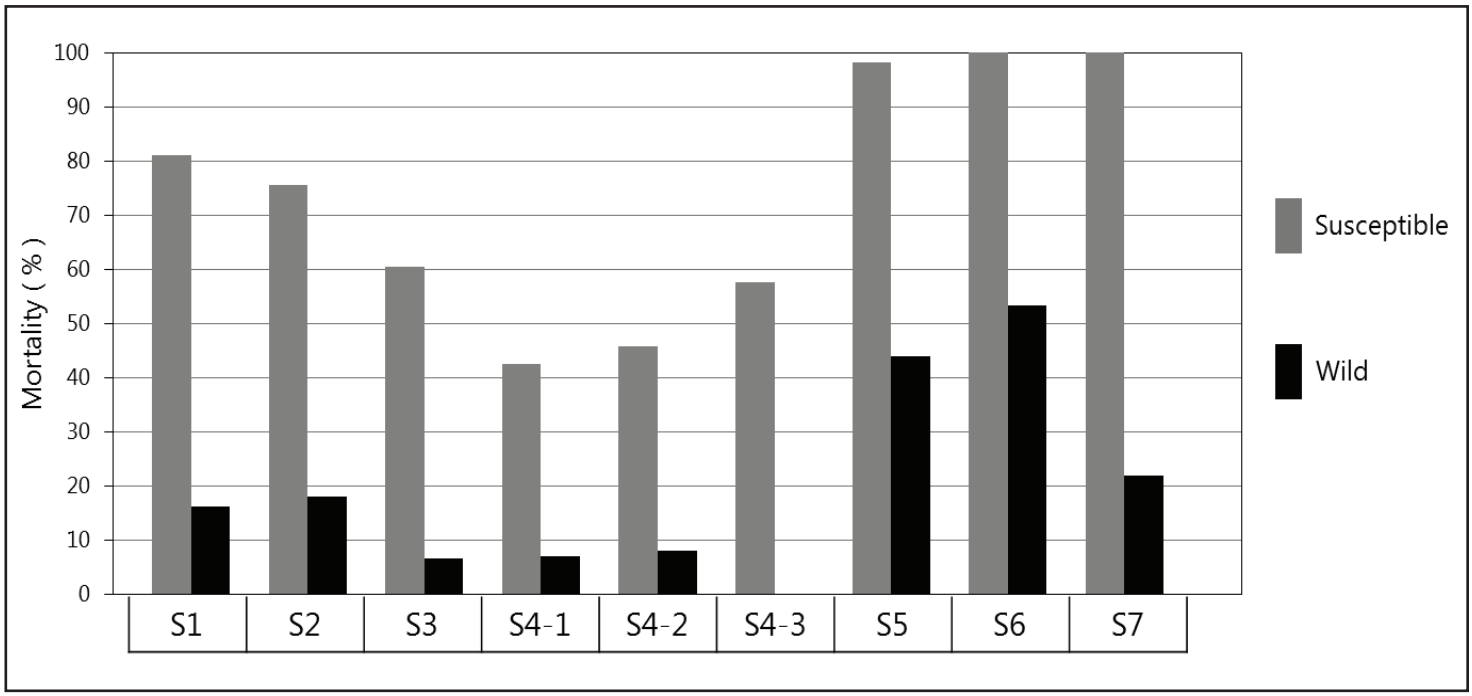

Figure 1 Mortality of caged laboratory susceptible and field-collected wild mosquitoes two hours after aerial application of piperonyl butoxide (PBO) synergized pyrethrin formulation, Pyrocide 7396

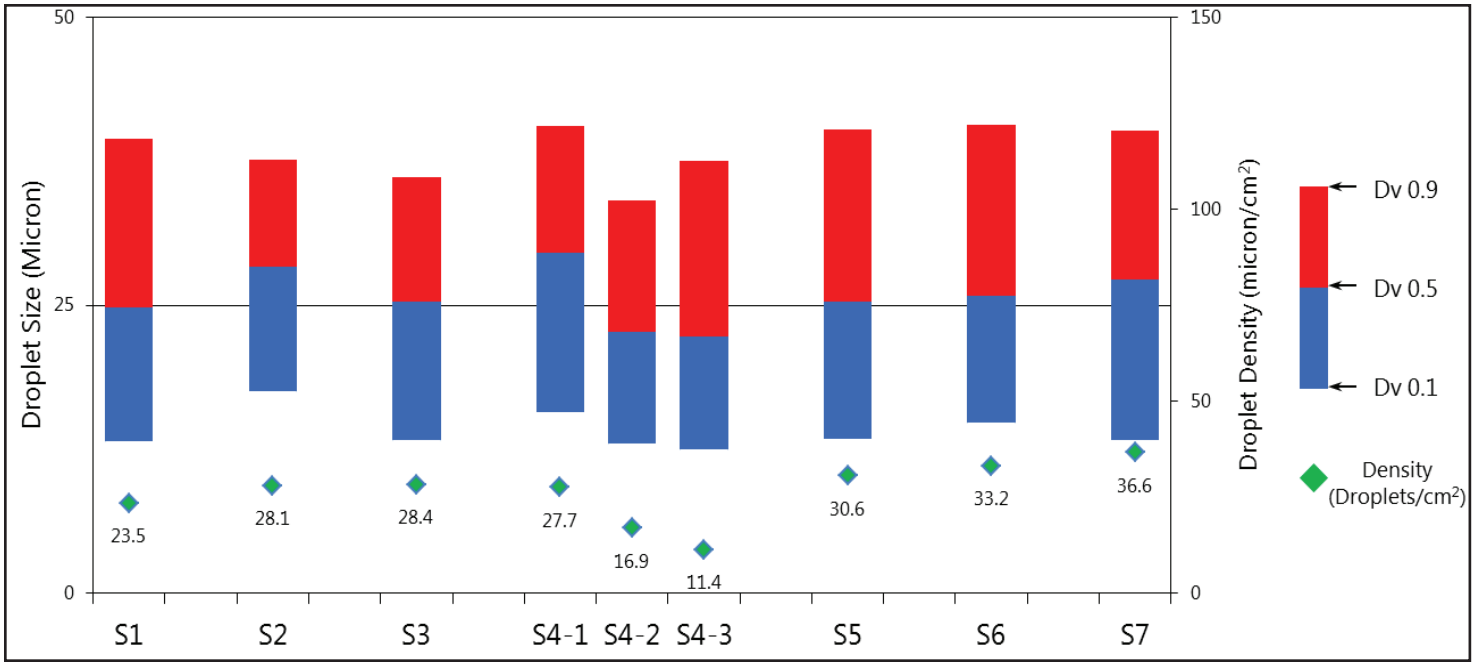

Figure 2 Droplet size and density after aerial application of piperonyl butoxide (PBO) synergized pyrethrin formulation, Pyrocide 7396

canopy on control efficacy. Station S4-1, S4-2 and S4-3 were set 50 feet away from tree canopy, underneath the canopy facing the wind and underneath the other side of the canopy, respectively.

RESULTS AND DISCUSSION

Two hours post spray mortality of susceptible mosquitoes varied from 42.5% to 100% with mortalities being over 95% at station S5, S6 and S7 located in the open field (Figure 1). In contrast, field-caught mosquitoes had very low mortality (range of 0 - 21.9%) except at stations S5 and S6 where mortality was 44% and 53.3%, respectively. Overall, the control efficacy was much lower than the expected 100% mortality in susceptible laboratory mosquitoes and greater than 75% mortality in wild mosquitoes. Droplet Volume Median Diameters (Dv 0.5) at all stations ranged from 22.3 – 29.53 microns (Figure 2), which was comparable to the size of Naled droplets in previous trials. In addition, the droplet densities at all stations ranged from 11.4 to 36.6 droplets/cm^2, which was slightly lower than previously collected for Naled applications but is much lower than that of the same formulation applied by ground ULV. The data suggests that the quantity of active ingredient reaching the mosquitoes was lower than the lethal dose. Results also show that station S4-1, S4-2 and S4-3 had the lowest mortality in both susceptible and field mosquitoes, and that station S4-2 and S4-3 within the canopy cover had substantially lower droplet densities (Figure 2). These results are consistent with other studies that showed tree canopy can create sublethal space for mosquitoes to survive and contribute to resistance development. It is highly recommended that aerial applications are enforced concurrently by a ground ULV application in protected canopy spaces, where surviving mosquito populations are present.

CONCLUSIONS

Pyrocide 7396 is a pyrethrin formulation, different from the organophosphate Trumpet EC, and is a highly needed aerial spray formulation for resistance management. Based on the data from this trial, further studies are required to optimize application rates to achieve effective vector control.

UAS (drone) Meteorological Data Collection in Conjunction with Aerial ULV Events

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ABSTRACT In 2018 the Sacramento-Yolo MVCD (District) utilized two small UAS (Unmanned Aircraft Systems or Drone) units as part of its newly formed program. While the District has participated in third party trials, this past season marked the first District UAS missions, completing several trials and proof of concept operations. One such concept involved mounting a custom engineered temperature sensor to a UAS unit, and reading temperature and relative humidity in real time, while analyzing wind speed and direction at various elevations up to 350 feet above ground level (AGL) during ULV events.

The District began by attaching a data logger style temperature sensor to a DJI Phantom 4 and going up in 50 foot increments and hovering long enough to allow the sensors to acclimate before changing elevation. These readings were taken from ground level up to 300 feet elevation outside of the spray block and out of the aircraft pathway. These readings were taken 30 minutes prior to sunset and 30 minutes post sunset per FAA regulations. Wind speed was also downloaded post application and added to the tables of collected data. Major differences in wind speed and direction existed consistently around 200-250 foot AGL. At the location where the majority of the night time monitoring was conducted, post sunset wind speeds were consistently greater around 200-250 feet AGL, and from a slightly different direction. As the readings were taken above this point up to the airplane's spray height of 350 feet, the wind speeds were shown to decrease to similar velocities and headings as observed below 200 feet AGL. While we observed this trend only at this location, preliminary test flights are showing that other types of consistent wind patterns exist at other aerial ULV application locations. Subsequent trials were refined to capture data closer to application intervals. A waiver from the FAA was granted to the District to fly beyond 30 minutes past sunset under Part 107 regulations. The District also added a more sensitive temperature sensor that repots in real time allowing for shorter acclimation times at each elevation. Readings were captured beginning with spray and for the duration of the application. Readings were taken from 30, 100, 200, and 300 foot AGL.

When looking at all data collected by the UAS units, it was clear that every spray block had consistent differences in temperature and wind speeds at different elevations. Moving forward the District will be lowering the spray application elevation in the block that showed wind speed barriers at 200-250 feet. The District will be conducting additional UAS meteorological trials in all of the aerial spray blocks in 2018, and will make further determinations as data is collected and analyzed.

Technician Boundary Creation and Analysis Using Geographic Information Systems

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INTRODUCTION

The Sacramento-Yolo Mosquito and Vector Control District (District) encompasses two counties covering over 2,000 square miles. This large service area is split into 26 zones, with each zone assigned to a field technician. Since the creation of these zones, urban and suburban development have changed some of the surrounding landscape from rural to residential forcing the District to re-evaluate zone boundaries by investigating the quantitative and qualitative properties of certain zones. Understanding a changing landscape and deciding where to place a technician is a challenge for many mosquito control districts. The purpose of the current presentation was to provide guidance on data analysis and demonstrate how the District delineated new zone boundaries.

METHODS

Using Geographic Information Systems, the District analyzed the timing, densities and spatial distributions of mosquito breeding sites, public service requests, female mosquito abundance and West Nile virus activity. In addition to geographic attributes, technician mosquito control efforts were also analyzed. Technician treatments and inspections for the new modified boundaries were mapped and graphed providing visualization for multiple datasets. Mapping and summarizing the data using ArcGIS geoprocessing tools allowed the District to focus on specific areas of interests and investigate seasonal trends.

RESULTS AND DISCUSSION

The District created a new zone and modified existing zone boundaries to accommodate the changing landscape. Using current high-resolution imagery and GIS data analysis, a new zone boundary was created to encompass urban sprawl. By creating the new zone, it decreased the size of the surrounding rural zones, allowing technicians more time to focus on historically difficult to control mosquito breeding sites. By removing urban development from those zones, technicians were able to inspect and treat up to 20% more of the producing sources such as agricultural ditches and dairy sumps.

CONCLUSION

Identifying changing landscapes and analyzing mosquito control efforts has proven to be a valuable strategy to develop technician service zones. By utilizing GIS, the District was able to map and investigate seasonal trends to find the best allocation of resources to geographic areas.

Evaluation of Adult Mosquito Control ULV Applications in Sacramento and Yolo Counties

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INTRODUCTION

The Sacramento-Yolo Mosquito and Vector Control District (Sac-Yolo) performs over 500,000 acres of ULV (Ultra Low Volume) mosquito adulticiding every year by both ground and aerial applications. Given the amount of resources that such an undertaking requires, routine evaluations of these applications is an important component of a complete mosquito management program. Over the course of the 2017 mosquito season, Sac-Yolo performed evaluations on its aerial ULV applications for both pyrethrum and organophosphate based products. The evaluation of these nighttime ULV events utilized standard metrics such as sentinel cage mortality and droplet density as well as two new tools, the BG Counter trap to monitor mosquito activity and unmanned aerial systems (UAS) to assist with recording atmospheric data. These new tools can assist in maximizing the effectiveness of a ULV application for adult mosquito control. We show examples of the utility of these new technologies as part of an adult mosquito control program.

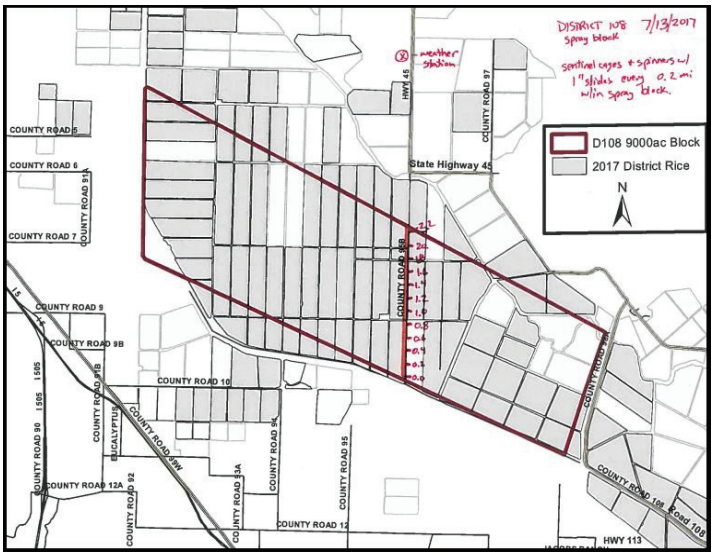

Figure 1 A typical layout of sentinel cage and droplet spinner sites in an aerial spray block (District 108 in Yolo County).

METHODS

Sentinel cages, droplet spinners, ground based and UAS based weather monitoring units were used to evaluate ultra low volume (ULV) adulticide applications. Multiple sites were selected within the spray block and two sentinel cages and one droplet slide spinner unit were placed at each site and multiple sites were deployed in a N-S transect at 0.2 mile intervals. An example of a typical layout of sites in an aerial spray block is shown in Fig. 1.

One sentinel cage contained 20 susceptible colony *Culex tarsalis* [BFS strain] (if sufficient numbers were available), whereas the second cage contained field captured mosquitoes from the target area (again 20 if sufficient numbers were available). The UAS and ground based Kestral weather monitoring was initiated before the spray application began until approximately 30 minutes after completion of the spraying. In addition, the BioGents BG Sentinel Counter Trap was utilized to document mosquito activity levels.

RESULTS AND DISCUSSION

A significant weather anomaly occurred in the northern most rice habitat area of Yolo County, depicted as District 108 in Fig. 1, with a distinctive spike in wind speed at a mid-application altitude between 150' and 250'. The ground based (5') and airplane (300') sensors showed wind speeds that were reasonable for an aerial application to occur, but the mid-altitude showed several mph faster wind currents between the ground and the airplane altitude. This data would not have been obtained without the use of the UAS system for weather monitoring at mid-altitude heights during the application. The BG Counter trap data did show that we had timed our application time to coincide with peak mosquito flight activity, approximately 30 to 60 minutes after sunset.

CONCLUSION

Upon review of the data collected during the 2017 mosquito season, we will continue to gather mid-altitude UAS based weather data. This will be important to maximize the efficacy of our aerial adulticiding program.

ACKNOWLEDGMENTS

Thanks to the Ecological Management Department at the Sacramento-Yolo MVCD for the UAS data obtained during these aerial applications, and to Vector Disease Control International, our aerial applicator.

The Threat from below: Controlling *Aedes aegypti* in underground utility manholes and handholes

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ABSTRACT Since late 2014, *Aedes aegypti* mosquitoes have been expanding throughout San Diego County, although most detections have been in the southern part of the county. Trap counts are usually low with an average of one to two adults per trap in infested areas. In the summer of 2017, however, County of San Diego Vector Control Program (VCP) staff recovered traps with unusually high counts of *Ae. aegypti*, some with over 700 adults per trap night, but typical sources of breeding could not be identified. Further investigations eventually led technicians to suspect that underground utility vaults (including "hand holes" and "man holes") were the culprits. After additional trapping, observing some vaults holding significant amounts of water, and seeing clouds of adult *Ae. aegypti* nearby, VCP was able to work with utility companies to confirm these breeding sources. This presentation focuses on the steps the San Diego County VCP has taken to address *Ae. aegypti* breeding in these below ground sources, the processes and logistics involved, and lessons learned in communicating and partnering with various public utilities to resolve this problem.

Challenges and successes of implementing urban adult mosquito control for WNV suppression in high risk areas of Orange County, California, amid public opposition to pesticides

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ABSTRACT In response to intense West Nile virus (WNV) activity from 2014-2016 (388 WNV cases, 18 deaths) in Orange County, California, the Orange County Mosquito and Vector Control District (OCMVCD) developed a tiered-response, adult mosquito control (adulticiding) program as a part of a newly-adopted WNV Emergency Response Plan (Plan). In early 2017, OCMVCD introduced city administrators from nine high-risk area (HRA) cities to the Plan and requested partnership with each city to effectively educate and notify residents of WNV risk. By August, the mosquito infection rate (Vector Index, VI) in the HRA city of La Habra had escalated to 7.1, well-above CDC-defined levels associated with WNV epidemics, despite increased city-wide larviciding and repeated ultra-low volume adulticiding efforts with AquaDuet™ in city parks and greenbelts. In response, OCMVCD expanded its adulticiding program to weekly (2 nights/week) area-wide truck-mounted applications in three WNV-active residential neighborhoods over a three week period. This presentation highlights the challenges and successes of implementing OCMVCD's IVM-based WNV Emergency Response Plan, which included notification of La Habra residents of the applications and collaboration with public officials and community stakeholders. It also examined the meteorological conditions during applications, pesticide efficacy, and pre- and post-treatment mosquito abundance and infection rates. The lessons learned from the area-wide control effort will help improve the efficacy and efficiency of future residential truck-mounted adulticiding applications in Orange County.

Investigation of sporadic product failures in Sacramento-Yolo catch basin habitats

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INTRODUCTION

Stormwater catch basins are commonly targeted for larvicide applications by mosquito control programs. It is not unusual to have greater than one hundred thousand of these sources within a District's jurisdictional boundaries. Utilization of extended-release products formulated to last 30 days or longer is one approach that makes control of numerous sources manageable. Sacramento-Yolo MVCD applies district made packets of Natular G-30 (spinosad) for this purpose. This granular formulation purportedly provides control for 30 days. However, unfavorable control outcomes began to emerge in some geographic areas during the 2017 season. The current study was undertaken to identify the causative factor(s) for these failures.

MATERIALS AND METHODS

We conducted two replicated larval bioassays in (5-gallon bucket) microcosms. The first bioassay compared efficacy of Natular G-30 removed from district made packets and fresh G-30 obtained from the manufacturer against colony reared, susceptible larval *Culex quinquefasciatus* Say (CQ1). The second compared *Culex pipiens* L. larvae collected from two district locations where product failures were observed and colony reared, susceptible CQ1 against fresh G-30. Buckets were filled with dechlorinated tap water and Natular G-30 was applied at our operational application rate of 10 grams per 50 square feet of water. Three replicates of each treatment for each mosquito population plus three untreated controls were utilized in each bioassay. Following treatment,

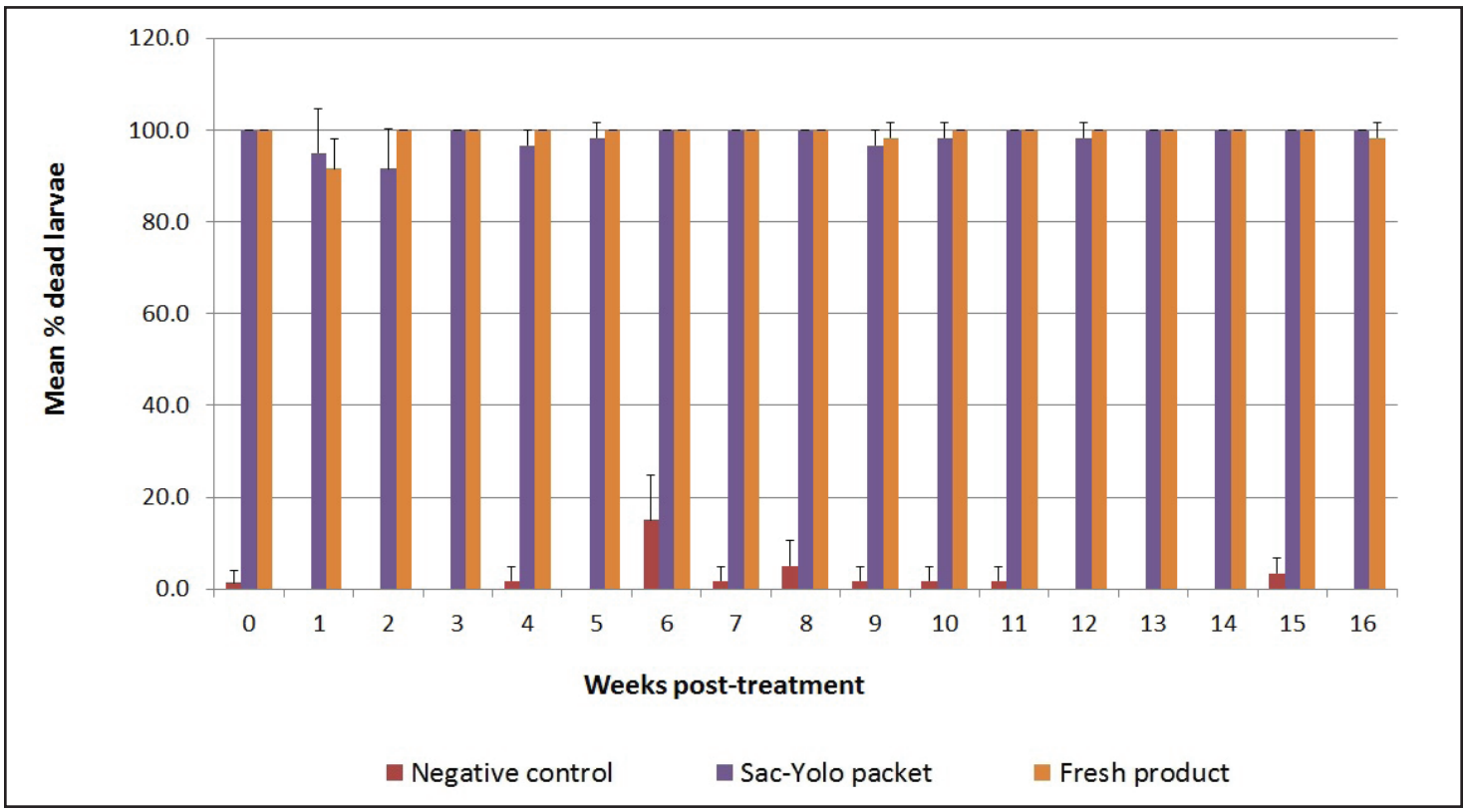

Figure 1 Bucket bioassay of Natular G30 from Sac-Yolo packets vs. fresh product. Mean percent of CQ1 colony mosquito mortality by time post treatment for 3 replicate buckets per treatment (bars are 95%CI)

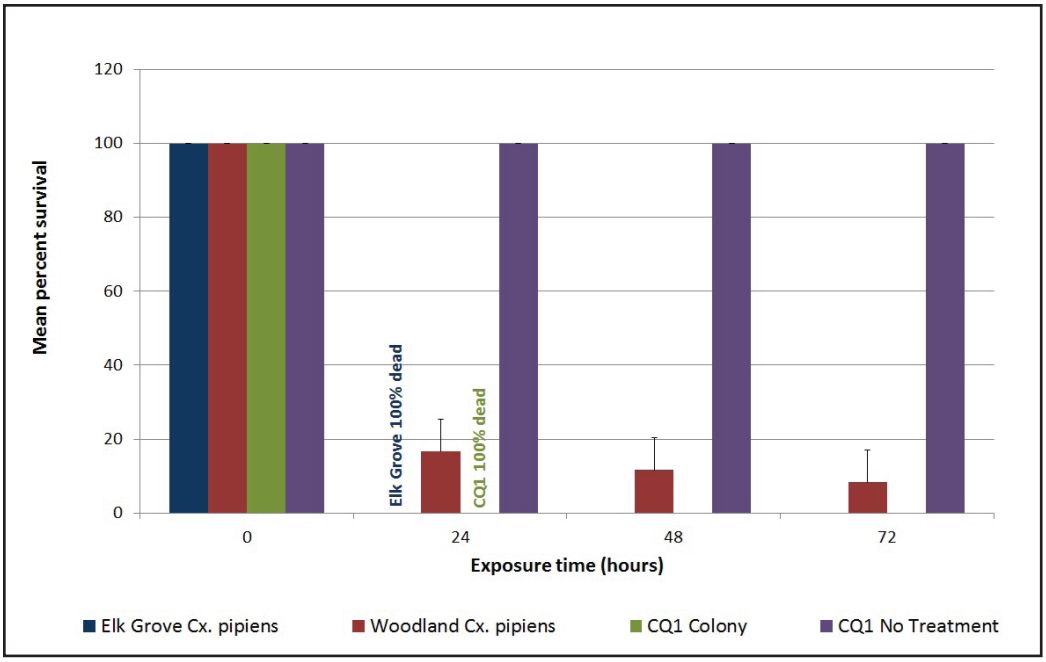

Figure 2 Natular G30 bucket bioassay showing mean percent survival by population as a function of exposure time for 3 replicate buckets per treatment (95% CI)

twenty larvae were introduced into each bucket on a weekly basis and post-treatment counts were done weekly in the first bioassay and at 24, 48 and 72 hours in the second bioassay.

RESULTS

In bioassay 1, no significant difference was found between fresh Natular G-30 obtained from the manufacturer and G-30 from district made packets in either efficacy or duration of control of colony reared, susceptible CQ1 larvae. Indeed, under our ideal, controlled experimental conditions both products produced control for 4 months following a single application (Fig. 1).

In bioassay 2, no survival was found 24 hours post-treatment in either colony reared, susceptible CQ1 or the wild *Cx. pipiens* larvae collected from Elk Grove. However, a mean percent survival of almost 20% at 24 hours post-treatment was exhibited by the population collected from Woodland. Post-treatment counts at 48 and 72 hours continued to show less than 100% mortality for this population (Fig. 2).

DISCUSSION

Natular G-30 provided acceptable control against susceptible larval mosquitoes regardless of how the product was packaged. Trials against wild mosquito populations did reveal differences in efficacy depending upon collection location which suggested causative factors other than those related to the product itself. Research conducted by others has found efficacy of single dose extended-release larvicides can be hindered by site specific characteristics (Harbison et al. 2014). Sacramento-Yolo MVCD records show that catch basins in Woodland are an older design and are highly organic compared to those in Elk Grove. Oxygen is necessary for effective release of spinosad from the granule (Pers. Comm. Clarke) and accumulation of sediment which can bury granules combined with poor water quality in older catch basins may have interfered with product performance by creating anoxic conditions. The city of Woodland is also surrounded by agricultural areas with extensive pesticide use whereas Elk Grove is more: of an urban area. While not common, Su and Cheng (2014) documented the existence of spinosad-resistant CQ1 and explored cross resistance. Future trials by the District will be conducted to define and pursue these and other possible factors responsible for control failures in our catch basin habitats.

ACKNOWLEDGEMENTS

The authors thank Randy Burkhalter for catch basin treatment data, Rajeev Vaidyanathan for his contribution to bioassay design, Clarke for donating fresh G-30, Paula Matney for rearing susceptible CQ1 larvae, and Bret Barner and Kara Kelley for participating in larval introduction and post-treatment counts.

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Evaluations of aerial ultra-low volume mosquito adulticide applications in Placer County, California

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INTRODUCTION

Ultra Low Volume (ULV) aerial adulticide applications can be an effective way to treat large areas of mosquito habitat. To improve the consistency and efficacy of ULV aerial applications made by Placer Mosquito and Vector Control District (District), the authors herein document some of the variables that influenced these applications. We examined variables that influenced droplet deposition, including the effects of environmental conditions (wind speed, wind direction, and temperature inversion) and documented the effectiveness of the treatment using both wild and colony adult mosquitoes. This study analyzed three separate aerial adulticide applications that documented variation of aircraft offsets, presence or absence of inversion layer heights, and the efficacy of Trumpet® EC Insectide (AMVAC Chemical Corporation), with the active ingredient Naled, against wild *Culex tarsalis* compared to susceptible colony *Cx. quinquefasciatus*. These findings were used make informed decisions on when aerial applications will provide the best results in Placer County.

METHODS

Temperature Inversion: To detect the presence or absence of a temperature inversion within the application block, an iMet-XQ UAV Sensor (International Met Systems, Grand Rapids, Michigan) was attached to DJI Phantom 3 unmanned aerial system (UAS). The UAS flew vertically from the launch site and stopped at 25, 100, 200, and 350 feet altitude to stabilize temperature and wind readings. However, the sensor continually took measurements during the flight. Three replicates of this process were completed 30 min before sunset, 15 min after sunset, and immediately after the aerial ULV treatment. The data from the iMet XQ sensor was downloaded post-application and entered into Tableau® software for processing.

Spray Block: A 2.3mi x 2.75mi, 4040 acre target block was treated on 3 separate nights in August, 2017. The block was located in rural rice fields in Placer County, CA. The spray application was made with a Cessna 402 aircraft traveling at a ground speed of 150 knots at an application height of 250 ft above ground level. The aircraft applied an ULV mosquito adulticide Trumpet® EC Insectide (AMVAC Chemical Corporation) with the active ingredient Naled at 1oz/acre.

Droplet Deposition and Product Efficacy: A transect of 12 stations evaluated each application (Figure 1). Each efficacy station consisted of 1 rotary impinger with 2 Teflon® coated slides, 1 cage of 15-20 mixed aged colony *Cx. quinquefasciatus* (CQ1 colony strain), and 1 cage of 15-20 mixed aged wild caught *Cx. tarsalis*. Two efficacy stations were set up outside of the spray block as controls.

For each station, the cages of mosquitoes were positioned next to each other on top of a single 5ft pole. The rotary impinger was placed atop a separate 5ft pole, 3ft-4ft away from the caged mosquitoes. The stations were set up within an hour prior to application, and pick-up started one hour after the completion of the application. The mosquito cages were placed inside plastic bags and stored in coolers to prevent contamination. Mortality was recorded at 1 and 12 h post-spray. The Teflon slides were stored in a slide box and read using DropVison Florescence® (Leading Edge, Fletcher, NC) that same night. The information used from this program included the size or volume of the droplets as Volume Median Diameter (VMD) and droplet density (droplets/mm^2).

Figure 1 Treatment block relative to monitoring station placement during aerial ULV applications of Naled over rice in Placer County, CA.

RESULTS

Temperature Inversion: The before-sunset temperature inversion measurements for the 15 August 2017 application showed the ambient temperature decreased approximately 2-3 degrees Fahrenheit with the 350 ft. rise in elevation. The post-sunset measurements showed an inversion starting to occur with the ambient temperature rising several degrees in the first 0-50ft, and the ambient temperature equalizing from 50-350ft. The post-treatment measurements showed an inversion of an approximate increase of 4-5°F from 0-50ft, and equalized ambient temperature from 50-350ft.

The before sunset inversion measurements for the 16 and 30 Aug 2017 applications showed approximately a 15°F inversion within the first 50ft of elevation, while from 50ft to 350ft the ambient temperatures slightly decreased in temperature, indicating non-inversion conditions. The post-sunset and post-treatment measurements showed an inversion with the ambient temperature rising approximately 15°F within the first 50ft and the ambient temperature equalizing from 50-350ft (Figure 2).

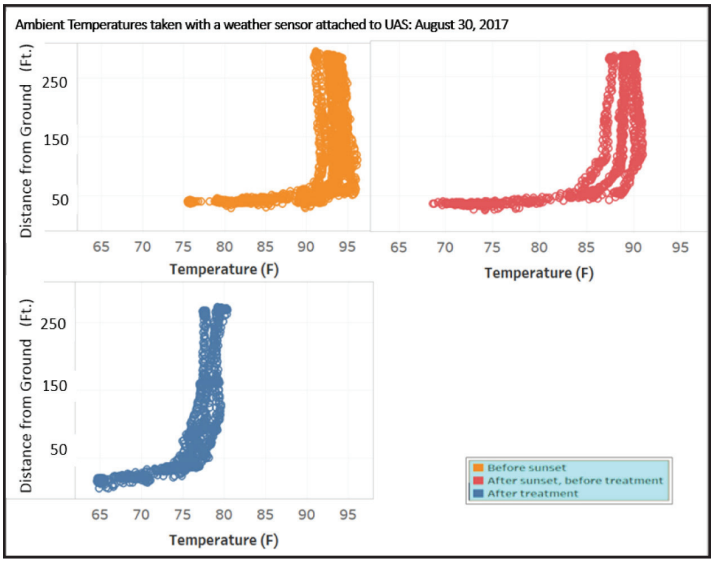

Figure 2 Temperatures taken on 12, 15 and 30 Aug with a weather sensor attached to UAS. Temperatures were taken 15 minutes pre-sunset, 15minutes post-sunset, and immediately after treatment (approximately 1-2 hours post sunset).

Droplet Deposition: All of the test stations collected droplets from the application, with density for the three applications ranging from 0.09 to 2.90 drops/mm^2. Droplet size VMD averaged 31.09 µm, ranging from VMD 18.59 to 75.1µm. Interestingly, the largest mean droplet sizes were collected from sites 1 and 2 located upwind from the treatment block.

Mosquito Mortality: In all three of the aerial applications, the susceptible colony *Cx. quinquefasciatus* caged mosquitoes showed 100% mortality in all of the test cages, including those located outside of the target spray block. The *Cx. quinquefasciatus* control cages showed a mortality of 0%, 5%, and 10% mortality on 15, 16, and 30 August, respectively.

The 15 August application showed 100% mortality for two cages containing wild caught *Cx. tarsalis* located upwind from the treatment block, whereas the six cages within the block showed mortalities ranging from 80% to 100% and four cages downwind from the spray block (starting with the cage 9 closet to the spray block) showed mortality of 76%, 54%, 60%, and 100% (Figure 3).

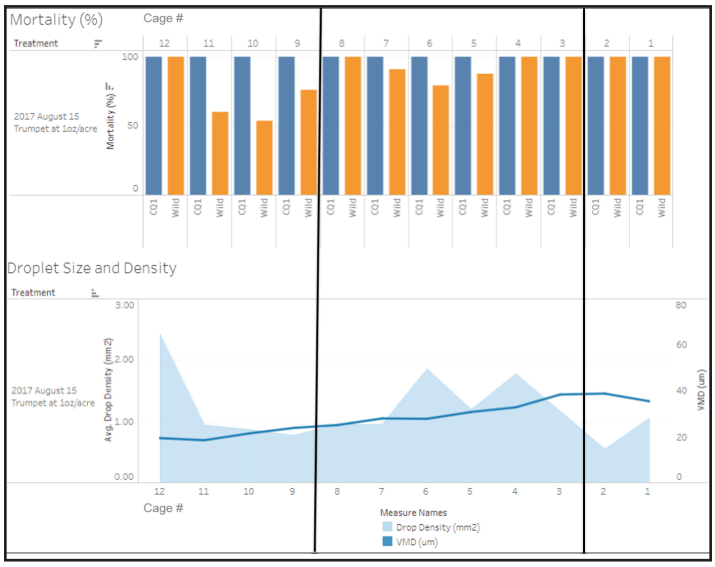

Figure 3 Results for the August 15, 2017 aerial ULV application of Naled over rice in Placer County, CA, including susceptible mosquito mortality (CQ1) compared to wild caught mosquito mortality, with the correlating droplet density (drops/mm2) and droplet size (VMD) for each test station. The vertical black lines indicate the cages that were within the spray block and the cages outside of the spray block.

The 16 August application showed mortality of 55% and 84% for the two cages of wild caught *Cx. tarsalis* located upwind from the spray block. The six cages of wild caught *Cx. tarsalis* within the block showed mortalities ranging from 79% to 100%, whereas the four cages downwind from the spray block, starting with the cage 9 closest to the spray block, showed mortalities of 72%, 100%, 26%, and 25% (Figure 4).

The 30 August application showed mortalities of 80% for both cages of wild caught *Cx. tarsalis* located upwind from the spray block, mortalities ranging from 60% to 100% within the spray block, and mortalities of 42%, 25%, 20%, and 5% downwind of the spray block (Figure 5).

DISCUSSION

There are multiple variables that factor into a successful aerial ULV mosquito adulticide application. We found that the UAS successfully measured temperature inversions which gave us insight into the atmospheric conditions surrounding applications at different altitudes. Although a complete understanding of effects of inversion conditions on droplet deposition was outside of the scope of this study, our data did provide interesting anecdotal results. In particular, the after-sunset data for two of the applications indicated a strong inversion taking place from 0-50 ft above ground level, with the temperature essentially equalizing from 50-350 ft. The next topic for study would be if the point where the temperature starts to equalize has an effect on droplet deposition. Does temperature

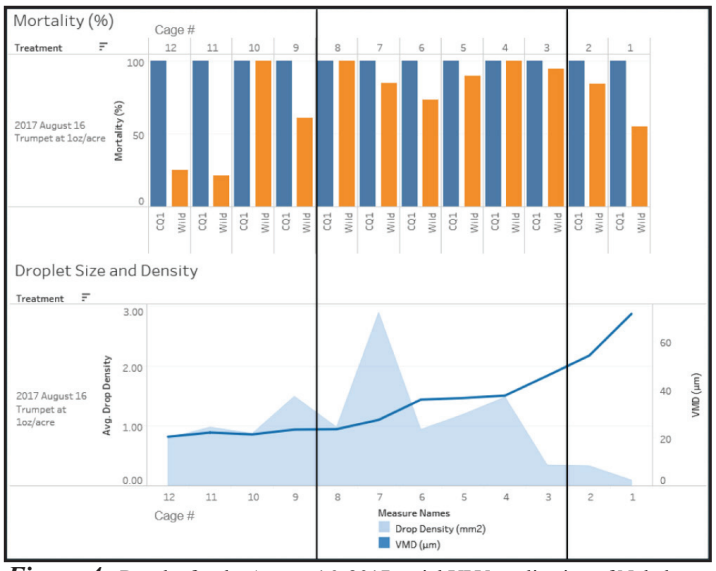

Figure 4 Results for the August 16, 2017 aerial ULV application of Naled over rice in Placer County, CA, including susceptible mosquito mortality (CQ1) compared to wild caught mosquito mortality, with the correlating droplet density (drops/mm2) and droplet size (VMD) for each test station. The vertical black lines indicate the cages that were within the spray block and the cages outside of the spray block.

equalization have a positive or negative effect on the spray cloud? Another factor not examined in this study was the variability of wind conditions and the effect of wind on the spray cloud. To help with this question, we are looking into using a UAS to effectively measure inversion temperatures along with wind speed and wind direction. Based on the use of a fluorescent dye that was added to the product before application and the use of Dropvison Florescence®, all of the stations both inside and outside of the treatment block received a detectable amount of product. The Dropvision® system and fluorescent dye also demonstrated that none of the control stations received a detectable amount of product.

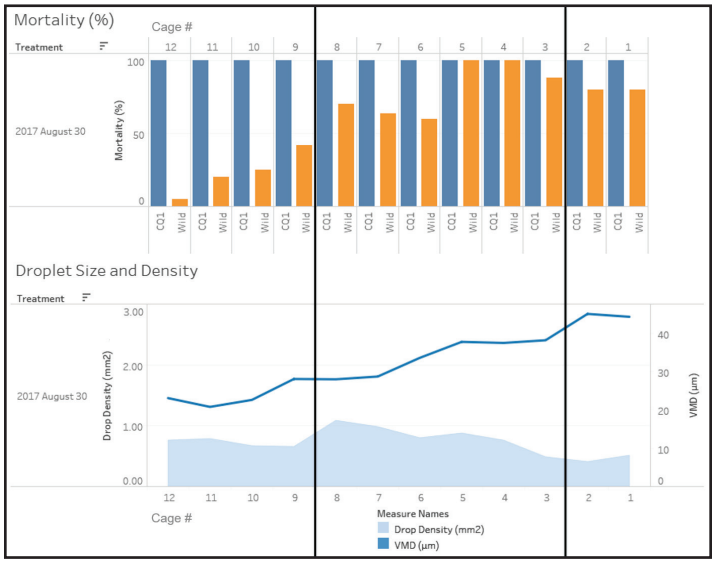

Figure 5 Results for the August 30, 2017 aerial ULV application of Naled over rice in Placer County, CA, including susceptible mosquito mortality (CQ1) compared to wild caught mosquito mortality, with the correlating droplet density (drops/mm2) and droplet size (VMD) for each test station. The vertical black lines indicate the cages that were within the spray block and the cages outside of the spray block.

Droplet densities peaked within the spray block (Fig. 3, 4, 5), illustrating that the spray cloud dispersed through the targeted spray block. The only exception of droplet densities peaking outside the spray block was station 12 on 15 August. For this application, station 12 showed a surprisingly high density of droplets compared to the other stations. The higher droplet density here may be due to unknown environmental conditions or aircraft offset. Although the VMD varied, they were consistent for each application, in that starting with the farthest upwind station and continuing through the block to the farthest downwind stations, the VMDs consistently decreased in size from upwind to downwind. For example, for the first application, site 1 had a VMD of 38.81µm and site 12 had a VMD of 19.51µm. For the second application, site 1 had a VMD of 71.51µm and site 12 had a VMD of 20.44µm. This same trend can be seen for the third application also. This distribution can likely be attributed to the smaller droplets drifting through the spray block, while the larger droplets begin to fall out of the spray cloud upwind from the spray block as the treatments were offset to accommodate wind speed and direction.

Efficacy: There was a measureable difference between mortality rates of the susceptible colony mosquitos and the wild caught mosquitoes (Figure 3, 4, 5). As previously mentioned, all of the colony cages showed a mortality of 100%, while wild caught mosquito mortality varied from 25% to 100%. This difference in mortality led to several questions, including the impact on mortality of droplet density and droplet size, and a comparison of the test mosquitoes themselves in terms of health and vigor. However, one of the main concerns in all pesticide applications is resistance. While organophosphate resistance has been documented, Naled is not known for widespread resistance such as some pyrethroids, but the data nonetheless raised this concern. Our future goal will be to evaluate the potential occurrence of Naled resistance, with continued caged bioassays and controlled laboratory testing such as bottle bioassays.

CONCLUSIONS

For these applications the temperature inversion layer continued from the ground to 350ft. However, there was a substantial difference in the temperature gradient between 0-50ft and 50-350ft. The spray clouds for the most part hit the target block; however, the efficacy data showed that there was a measureable difference between mortality of the colony mosquitoes and the wild caught mosquitoes.

As a result of this study UAS will be a crucial tool in evaluating real-time atmospheric conditions to help increase the efficacy of aerial adulticide applications in the near future. The data provided by the UAS, in conjunction with spray cloud characteristics and deposition, will hopefully increase the accuracy of the current modeling systems used for aerial ULV adulticide applications and the appropriate environmental conditions to achieve the best desired results of these applications in the future. The data collected for this study by the UAS showed accurate inversion layer temperatures and heights for both pre- and post-application.

Mosquito assessment and control using unmanned aerial systems (MAC-UAS): program development at Placer Mosquito and Vector Control District

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INTRODUCTION

The purpose of the Mosquito Assessment and Control Unmanned Aerial Systems (MAC-UAS) Pilot Project at Placer Mosquito and Vector Control District was to identify the technical and operational capabilities of unmanned aerial systems (UAS) in a local government agency mosquito and vector control program. This included training and certification of staff UAS pilots, selection and acquisition of UAS, and identification and evaluation of use types of benefit to mosquito control.

BACKGROUND

Unmanned Aerial Systems (UAS) otherwise known as "drones" have been an up and coming technology for nearly a decade. In 2012 the Federal Aviation Agency (FAA) Modernization and Reform Act was passed, and directed the FAA to develop regulations to integrate unmanned aircraft systems into the national airspace. In August 2016, the FAA released its Small UAS Rule (14 CFR Part 107), the first comprehensive rules governing the commercial and governmental use of small UAS (<55 pounds). These rules, referred to as "Part 107", provided training, operations, and safety guidelines and requirements for UAS in a clear manner that allowed agencies a greater level of comfort in integrating UAS as a tool for mosquito and vector control inspection. While carrying hazardous materials including pesticides is prohibited under Part 107, there are several options ranging from petitioning for exemptions to acquiring a public aircraft certificate of operations that would allow a pathway to be able to carry and apply pesticides from UAS. The leadership of the Placer Mosquito and Vector Control District (District) determined that the potential benefits for mosquito habitat assessment and control were great enough to embark on a pilot project to determine if UAS increased operational efficacy and efficiency. The pilot project was dubbed the Mosquito Assessment and Control- Unmanned Aerial Systems (MAC-UAS) program. The idea of being an early adopter of UAS technology in the mosquito control community appealed to staff, management, and the board of the District. The District board passed a resolution supporting the use of UAS technology in vector control in January 2017. The timeline of events of the MAC-UAS program from its inception in late 2016 through March 2018 (Figure 1) was essentially completed by two District vector control technicians who trained as small UAS pilots under the FAA small UAS rule (14 CFR Part 107), and were supported by District management and scientific staff. The investment of staff time to develop this program was primarily made during the District's off-season, and the financial investment (Figure 2a, b) was deemed to be reasonable based on the likelihood of significantly increasing

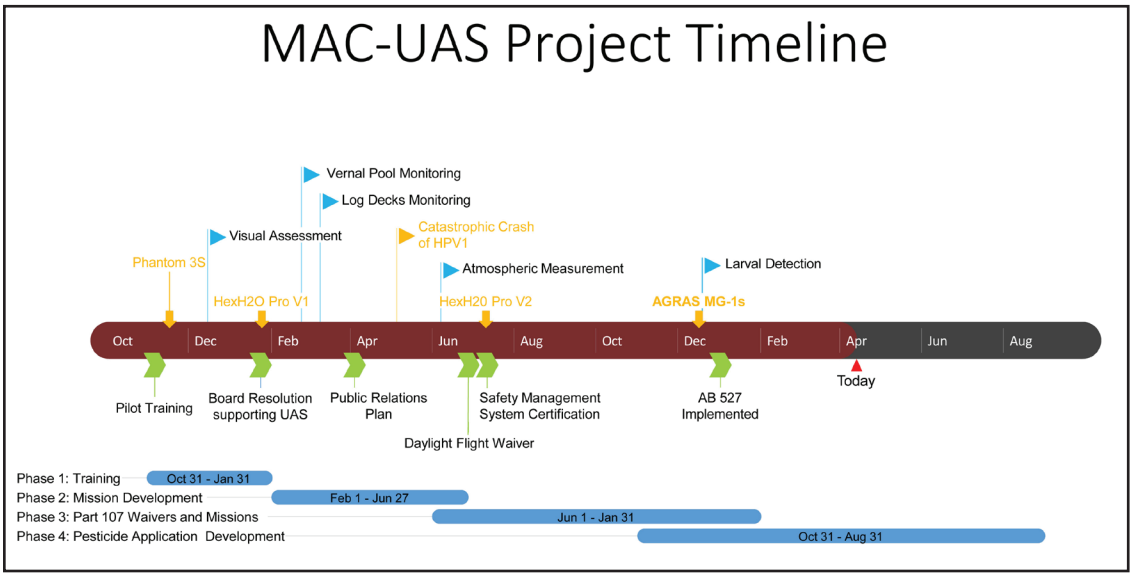
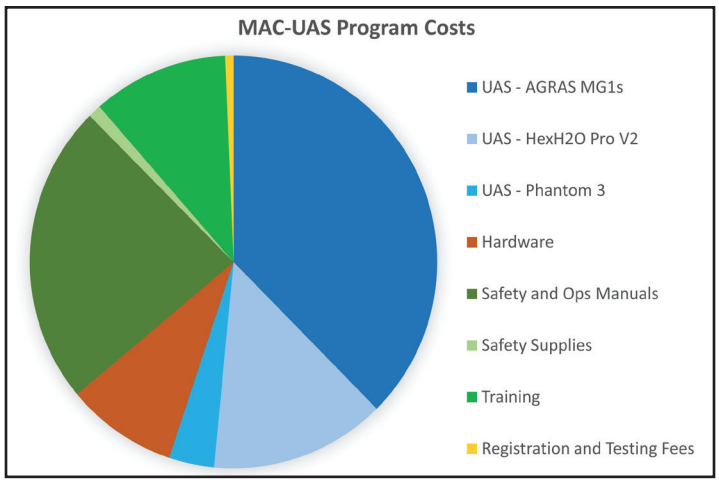


Figure 1: Timeline of events of the development of the Placer Mosquito and Vector Control District (Roseville, CA) Mosquito assessment and control - unmanned aerial systems (MAC-UAS) program from its inception in late 2016 through March 2018.



Category	Cost
UAS - AGRAS MG1s	$18,479.00
UAS - HexH2O Pro V2	$6,850.00
UAS - Phantom 3	$1,765.00
Hardware	$4,329.88
Safety and Ops Manuals	$11,600.00
Safety Supplies	$513.82
Training	$5,297.01
Registration and Testing Fees	$320.00
TOTAL	$48,834.71

Figure 2a & b: Costs associated with the development of the Placer Mosquito and Vector Control District (Roseville, CA) Mosquito assessment and control- unmanned aerial systems (MAC-UAS) program: a) numerical costs and b) relative costs.

District capabilities, inspecting areas previously inaccessible, and improving operational efficiency.

PHASE 1: Training, Certification and Safety, and Operational Procedures

Phase 1 started in November 2016 with the training of two district technicians, Scott Schon and Everardo Ortiz. The District sent Schon and Ortiz to training provided by Drone University USA (Sacramento, CA) for a two-day UAS operations and ground school training. They successfully completed the training, and passed the Part 107 test on November 11, 2016. Following this training, Schon and Ortiz were directed to take the recommendations provided in the training and establish the foundational elements necessary to safely operate UAS as a government agency in compliance with FAA regulations. We quickly determined that a major factor in the safe operations of UAS was pilot experience. Through their training Schon and Ortiz had learned training exercises and safe operation of the DJI Phantom series of UAS. Because of this, we determined that a DJI Phantom 3 Standard was the most cost-effective UAS to purchase to allow for indoor and outdoor training. We also determined that the Phantom 3 Standard would meet the basic requirements for future mosquito assessment missions that involved visual inspection.

The District purchased a DJI Phantom 3 Standard including the necessary accessories on November 16, 2016. This UAS was intended for use as a training platform to allow our pilots to gain flight time, and assess the use of UAS-based imagery as a mosquito habitat assessment tool. During flight training, various maneuvers were tested indoors at the District headquarters including: level flight, turns around cones and pylons, forward and backward flight, take-off and landing. These maneuvers were conducted in different flight modes to further provide pilots with experience and practice.

To address the multifaceted aspects of UAS safety, a new technology with new applications, Harrison Wolf (Wolf UAS, Los Altos, CA), an aviation safety expert specializing in UAS, was hired to develop for the District a set of aviation appropriate operating procedures as well as a safety management system, training manual, and emergency response manual. The process involved providing Mr. Wolf with documentation of the District's mission profiles, UAS specifications, pre-and post flight checklists, and organizational structure. Mr. Wolf provided a custom set of policy recommendations in the form of four manuals, and a day-long training for District management, scientific staff, and UAS crew members. While the cost of this policy development and training was significant, so was the benefit, especially since there were no other mosquito control operations using UAS regularly at the time. We expect that in the future and as UAS operations become more common for vector control and other applications, agencies trying to start a UAS program would have access to a variety of sources for safety, operations, training, and emergency policies and procedures

PHASE 2: Mission Profile Development

Phase 2 began during flight training by the UAS team, and included brainstorming ideas about how specifically to best use UAS to enhance mosquito inspection operations. Since both UAS pilots were experienced vector control technicians, they had the ability to draw on professional training and experience conducting mosquito inspections on the ground as they gained skill and experience flying the UAS. This premise was a major factor in choosing which missions had the most promise. By focusing on UAS uses that extended the real-time reach of the vector control technician with a minimum of post-processing and other data analysis, we hoped to maximize efficiency and minimize the investment of time and effort needed to operate the UAS.

A number of UAS use-types were identified, and the most promising ones developed into mission profiles. For the purpose of this project, we defined a "mission profile" to be a description of a use type; UAS requirements list; description of data viewed, recorded, and stored; data process description; risk management evaluation; and cost return on investment analysis. During this time, the following potential mission profiles were determined:

1. Atmospheric measurements and evaluation (temperature and relative humidity) at various altitudes to detect favorable conditions for aerial adulticide applications from manned fixed-wing aircraft
2. Visual assessment of mosquito habitat
3. Deployment and retrieval of mosquito traps to assess adult mosquito presence and/or abundance in difficult-to-access areas
4. Direct visual assessment of presence of mosquito larvae using submersible camera attached to UAS
5. Liquid larvicide application

At the time of writing, the District had successfully developed and deployed three of the five mission profiles, and was continuing work on the remaining two mission types.

Atmospheric Measurements

For atmospheric measurements, we chose the DJI Phantom 3 Standard platform to carry an iMet-XQ UAV Sensor (International Met Systems, Grand Rapids, Michigan) to detect altitude, temperature, and relative humidity. Because the planned flight times would be near dusk within one hour prior to the flight time for the manned aircraft, anti-collision lights were installed and a waiver for night-time flying was acquired from the FAA. The iMet sensor was the only UAS-specific temperature sensor with GPS receiver identified on the market at the time. Other sensors required the use of the on-board UAS GPS data, which would not allow rapid field-based evaluation of the data. The data from these atmospheric readings will be presented in Hartle et al. (2018).

Visual Assessment of Mosquito Habitat

Numerous visual assessment flights were made with the Phantom 3. Assessment of flood water during the winter of 2017 was helpful to direct where staff should go to perform larval inspections and control. UAS photo monitoring was also used to identify and track a number of flooded areas in open spaces, to count the number of log decks at a sawmill facility, and to assess the proximity of flooded rice fields to urban areas. The visual assessment use type was the simplest and had the most return on investment, because this application allowed the technician to quickly assess large areas in a manner that they were familiar without the UAS, leading to a very natural operational integration.

Deploy and retrieve mosquito traps to assess adult mosquito presence

While the Phantom 3 was not equipped with trap deployment capability (payload release), the idea of picking up an object with a static hook attached to the UAS was contemplated, and scale model testing for the retrieval hook was completed. It was quickly determined that for a payload deployment and retrieval mission a different UAS was necessary.

On April 4, 2017 the District acquired a HexH2O Pro V1 (XtremeVision360 Limited, West Sussex, England). This hexacopter features DJI internals (S600 airframe with A3 flight control system), a waterproof carbon fiber enclosure for the camera and gimbal assembly (GoPro 4 with Zenmuse Gimbal), and a payload drop mechanism. Because this was a completely new system, substantial time was spent reviewing manuals, and bench testing the flight control system and transmitter controls. We discovered that using the GoPro with the A2 flight control system did have some limitations not present on the Phantom 3. In particular, only older versions of the DJI ground station and DJI GO apps were supported. This meant that there was no in-flight camera control nor was there "black box" recording of flight data like we were accustomed to in the Phantom 3. Our requirements for this system were a robust and tested hexacopter with an under-water camera and payload deployment capability. The HexH2O Pro V1was the only system that met all the requirements, unfortunately it was also a four-year old design. We later found that four years in the UAS field means that, while functional, some of the newer safety and performance features common in newer designs were lacking in our HexH2O Pro V1. Numerous take-offs and landings, as well as level flight training missions were made by both pilots. They reported that the controller as well as the handling characteristics of the HexH2O Pro V1 were quite different than the Phantom 3. Two successful tests of payload deployment and retrieval were documented. One small crash of the HexH2O Pro V1 occurred during an early attempt at payload retrieval that resulted in minor damage of two rotors due to the retrieval loop (an approximately 12 inch diameter wire ring) contacting the rotors. A second crash of the HexH2O Pro V1 during a water landing and take-off test flight resulted in total loss of the drone. This crash brought to light the importance of several newer safety and control features available with newer UAS. Because there was no "black box" logging on control inputs and system function, there was no way to definitively determine the cause of the crash. However, during the post-crash investigation, we interviewed the pilot in charge, another pilot that witnessed the crash, and discussed our findings with the UAS manufacturer. The outcome of this investigation was that the UAS likely entered "failsafe mode" upon takeoff which caused an uncontrolled climb of the UAS to approximately 45 feet. The UAS then, still not responding to control inputs from the pilot, lost control and fell to the ground. Radio frequency interference from a nearby cell tower may have played a role. Fortunately there were no injuries and no property damage other than to the aircraft. An insurance claim was submitted and a replacement system, the HexH2O Pro V2, was purchased. Due to the challenges and safety concerns with deploying and retrieving a payload, we have lowered the priority of this mission type until a better trap design is available and experience with the UAS is gained.

Direct visual assessment of mosquito larvae

The newer version of the HexH2O Pro has completely different internals, but retained the same capabilities that were requirements for larval detection when we purchased the HexH2O Pro V1. While the HexH2O Pro V2 looked almost identical as the V1, it had significant upgrades: the DJI N3 flight control system, a

Zenmuse X3 camera with Lightbridge 2, and a channel expansion kit. These upgrades enabled the V2 to use a standard DJI controller and DJI GO app. The HexH2O Pro V2 also uses more powerful motors, the DJI N3 flight control system, and can be used with a second camera controller to allow another crew member to operate the camera while the pilot is flying the aircraft. These upgrades made the HexH2O Pro V2 much easier and safer to operate around and in the water making it a much better platform to use to search for mosquito larvae in their natural habitat. Using the HexH2O Pro V2, with an added 10x macro lens (Polaroid Optics 37mm 4 Piece Close Up Filter Set (+1, +2, +4, +10)), mosquito larvae were successfully detected in a seasonal wetland and a waste water treatment pond. We are currently continuing testing this mission type in other habitats, and working on trying to quantify the visual (video) data of larvae in a way that can assess relative larval density in addition to presence or absence.

Liquid Larvicide Application

In January 2018, the District received the DJI AGRAS MG-1s sUAS (RMUS, Salt Lake City, UT) to begin preparing for liquid mosquito larvicide applications. This particular UAS was chosen based on its weight (<55lbs) qualifying it as a small UAS, its payload capacity (10L of liquid product), and its proven track record of safe and successful use in agricultural settings in Asia. Even though it is new in the US market, the AGRAS MG-1s has been used extensively in Asia to treat rice and other crops. While the District works toward gaining the required regulatory compliance documentation to apply pesticides by UAS, characterization is in progress for the spray droplets and swath for the AGRAS MG-1s using water both with and with out dye and photo paper and water sensitive cards, respectively. Developing a standardized droplet characterization process will enable identification of the effective operational parameters of this UAS with any liquid larvicide that allows aerial application on its label.

PHASE 3: State of UAS technology, regulatory landscape, and future directions

This project is another step toward the mosquito control field using UAS to increase its productivity, safety, response time, and dependability. The FAA Office of UAS Integration continues to work with commercial and government UAS users to improve and develop the UAS regulatory structure. Based on conversations with the FAA, mosquito surveillance and control UAS operations conducted by a governmental entity may operate under two different regulatory structures: 14 CFR part 107 for civil missions, and Public Aircraft Certificate of Authorization (COA). This project's scope was to evaluate missions that would fall under Part 107 and identify missions where a waiver or waivers from Part 107 flight restrictions would be needed. We identified that nighttime flight would be desirable to take atmospheric measurements before and after aerial adulticide application by manned aircraft. The process of developing new and emerging UAS regulations by the FAA is dynamic, and all indications are that mosquito control will be permitted to operate in a manner that satisfies both our mission requirements and the requirements of the FAA. To facilitate make the regulatory process, we will need to develop relationships with FAA officials in our area; learn to clearly articulate our mission goals, risk assessments, and procedures to mitigate those risks; and understand the safety and operational culture of aviators. To achieve this, we will need to explore using outside experts in UAS safety and operations to assist us in developing a robust and safe UAS program that can work with the FAA to refine applicable regulations. We will work with the larger mosquito and vector control community to garner interest and ensure we share information and a culture of safety when starting and operating UAS programs. Recently a UAS committee has been established within the American Mosquito Control Association and will work toward this goal.

FINDINGS

By all accounts, the Mosquito Drone pilot project was a success. We clearly defined four mission types that can be further developed and tested. Through this project, we have also attracted substantial interest from the UAS community, regulators, vector control community, and the public. The need for careful development of training and operational policies and practices, as well as a safety management system is important to identify and manage the risks of operating this new technology. As an early adopter for use of UAS in mosquito control, the District is uniquely positioned to set an example of safe and effective operations of UAS for mosquito habitat assessment. While the flight tests, visual data, and other operational experience is important, equally valuable is the organizational support, staffing, training, and risk management that needs to be established to support a safe and sustainable UAS program. The major lessons learned in this pilot project were:

1. Visual data from a UAS is immediately helpful in a number of ways such as aerial fixed point photo monitoring of flooded areas, lumber mill, and rice fields. Real-time imagery is beneficial for a field technician to assess the extent of flooding from beaver dams, state of irrigation in pastures, rice fields, and other sources. From an efficiency and work-flow perspective, we want to leverage the technical expertise of our Vector Control Technician/ UAS pilots to evaluate the real-time imagery in the field, and avoid post-processing and storing image data if at all possible.
2. The ability for a UAS to drop and retrieve a payload opens a new area of innovation in the design of drone-deployable mosquito detecting sensors (traps, sugar feeding stations, etc).
3. Use of UAS technology coupled with visual and environmental sensors such as temperature, humidity, and pressure can be very useful to generate data that helps to support other mosquito control operations. Other sensors commonly deployed by UAS but not tested in this project include multispectral, LIDAR, and thermal sensors. Again, the focus should be on field data collection, analysis and decision-making, and avoiding post-processing data if

at all possible. Any time UAS acquired data has to go through a lengthy analysis or visualization process the benefits gained from UAS in time and ease of work may be compromised.

4. Each pilot should train and complete a familiarization process on each particular UAS, ideally under the supervision of someone who is familiar in its specific operation and mission requirements. We will continue to develop appropriate risk management and training procedures to ensure District UAS pilots have sufficient technical knowledge, operational skills, and flight experience to safely operate each UAS and execute maneuvers required by each mission.

5. Development of written standard operational protocols (SOPs) to ensure operational success and safety is critical both from a risk management perspective and also from an operational efficiency and success perspective. Keeping detailed notes on each training, testing, or mission flight has been helpful in developing and constantly improving SOPs to achieve better safety and better meet operational goals. Developing a reasonable safety management system that incorporates on-going risk management in flight operations as well as encouraging a safety-centered culture would be a reasonable and beneficial next step.

6. Matching the UAS to the mission requirements through a careful process that examines the capabilities of the UAS with an understanding of what the mission will require is difficult in a new and emerging field. A more formal documented process like a concept of operations (CONOPS) that describes the proposed system from the viewpoint of the user, and communicates qualitative and quantitative system characteristics to all stakeholders may be helpful in the future.

7. Environmental conditions such as wind, heat, cold, RF interference, altitude, size and shape of payload, etc. all have a tremendous effect on the UAS operational characteristics, and need to be considered when developing mission success criteria. During mission testing, environmental parameters should be recorded so that safe flights can be determined and assigned for each mission type.

8. Regulators (FAA) are looking for specific mission or use types for UAS to inform future regulatory changes or refinements. The more precisely we can articulate what we require from UAS to achieve a successful mission, the more likely we can get favorable regulations in the future.

9. UAS technology, let alone its use in mosquito control, is new and constantly changing. As the technology develops, more possibilities open for its use in vector control. We need to establish a way to generate new ideas, discuss the ideas, and test the ones most likely to succeed on an on-going basis.

10. The potential use of UAS as a low-altitude mosquito adulticide and larvicide application platform is clear. This new technology has promise to make public health insecticide treatments faster, more precise, more efficient, and more effective that current technology.

11. The cost versus benefit of UAS operations in the context of a mosquito control program is difficult to ascertain. Developing metrics to measure efficacy or efficiency gains is important to evaluate if the mission profile for the UAS actually results in performance gains or saves resources. There is a fundamental "cool factor" when it comes to new technology, which can cause us to lose sight of the actual gains from the technology.

REFERENCE

Hartle,J., M. Sorensen, M. Boisvert, J. Buettner. 2018. Evaluations of aerial ultra-low volume mosquito adulticide applications in Placer County, California. Proc. Mosq. Vector Contr Assoc Calif. (in press).

Trials of Natular 2EC with a Lite-Foot Track Vehicle

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INTRODUCTION

Natular 2EC is a product that previously had not been used by any mosquito control district in the state of Utah. Magna Mosquito Abatement District (District) was asked by Clarke to conduct a trial to determine the efficacy of Natular 2EC. Four ponds were selected within the District boundaries varying in size from three to five acres. Ponds one, two and three were all spring ponds usually wet from February through June. In any given summer we can see 2-4 broods emerge from these ponds. Pond four was a permanent pond that is wet all year, frozen in winter and producing mosquitoes in summer. This pond usually produces 4-8 broods in a given summer.

METHODS

Natular 2EC is considered to be a single brood liquid larvicide with the active ingredient of Spinosad. The minimum label rate is 1 ounce per acre and the maximum label rate is 2.8 ounces per acre. In ponds 1 and 3, Natular 2EC was applied at the rate of 1.5 ounces per acre. In ponds 2 and 4 we applied 2EC at the rate of 2 ounces per acre. To apply the larvicide we used the District's "Tracked Vehicle." This machine is similar to a snow cat and propelled by hydraulic power wheels. The Track is equipped with a 120-gallon spray tank operated by a hydraulic Hypro pump which is calibrated to treat at 10 gallons per acre. Therefore, to treat an area the size of pond 1 in our study we used 50 gallons of water mixed with Natular 2EC.

All ponds were inspected on or one day before treatment day as well as 24, 48 and 72 hours post treatment. After 72 hours, we inspected every 7 days for two weeks. After two weeks mosquitoes had re-established themselves in all four ponds requiring re-treatment. From our observation, water levels were constant during the time of the trial and as a result[1] Natular 2EC had a two-week effective window, an excellent time frame for a single brood product.

RESULTS

On 9 May2017 pond 1 was treated with Natular 2EC. The pond is five acres and full of thick *Phragmites* with a water depth up to two feet. The mosquitoes found in this pond pretreatment were mostly *Aedes dorsalis* but also some *Aedes vexans,* with counts of 49 larvae found in 15 dips. Post-treatment inspections were similar to pre-treatment inspections. After 24 hours we found 29 live mosquitoes in 15 dips and observed little change after 48 and 72 hours post treatment. As an interesting aside, after 48 hours we observed no larvae smaller than 3rd instar. We did not see dead mosquitoes post-treatment, just fewer live mosquitoes. After one week based on dip counts, we determined that we had achieved approximately 57% mortality in this pond. We determined this mortality based on the fact that the overall number of mosquitoes in pond one was reduced by over 40% just 24 hours post treatment. As the majority of the larvae found pre-treatment were younger 1st and 2nd instar, we determined that we had killed most young mosquitoes. Our evidence for this is that we observed no larvae younger than a 3rd instar after 24 hours. Temperatures in Utah were still cool at this time (67° high temp and still in the 40's at night) preventing marked maturation in this 24 hour time frame. Another important note associated with this pond is the fact that we treated it with 1.5 ounces of Natular 2EC per acre for a total of 7.5 ounces of product in this pond.

Pond 2 also was treated on 9 May 2017 with 2 ounces per acre. Pond 2 is 50 yards to the East of pond 1 and very similar, except that water depth was only one foot. Pond 2 is smaller, measuring approximately 3 acres. Again, mosquitoes were mostly *Aedes dorsalis* and *Aedes vexans* with 91 mosquitoes found in 15 dips pre-treatment. During our post treatment inspection at 24 hours, we observed a few dead mosquitoes, and 14 mosquitoes in 15 dips. After 48 hours we found 8 mosquitoes in 15 dips and after 72 hours we found 4 pupae. One week post treatment we found no mosquitoes in 15 dips and therefore observed 100% mortality. The primary difference in the results of these two ponds, in our opinion, was the amount of Natular 2EC per acre used. In pond 2 we used 2 ounces per acre for a total of 6 ounces in 3 acres.

Pond 3 which is located in West Valley City was treated on 23 May 2017. This pond is 4 acres of salt grass, with the water depth reaching 2 feet in places. During pre-treatment inspection we found 38 mosquitoes in 15 dips. After 24 hours post treatment, we found 2 mosquitoes in 15 dips and after 48 hours post-treatment no mosquitoes were found in 15 dips. We did not observe any dead mosquitoes in pond 3; however, we

did observe 100% mortality after 48 hours, and we didn't find enough mosquitoes to treat the pond again until 6 June. Two weeks after treatment we found 31 mosquitoes in 15 dips. With the thinner/low vegetation in this pond, we used 1.5 ounces per acre for a total of 6 ounces of Natular 2EC in pond 3.

Pond 4 in Salt Lake City was another 5-acre pond reaching a depth of one foot in its deepest areas with a mix of both *Phragmites* and salt grass. We inspected this pond on 28 June 2017 and found exactly 100 *Culex tarsalis* mosquitoes in 15 dips. We treated the pond with the track on 29 Juneabd 24 hours post treatment observed 11 mosquitoes in 15 dips as well as multiple dead mosquitoes in each dip. After 48 hours we observed 98% mortality or a total of 2 mosquitoes in 15 dips. With the mix of vegetation in this pond, we applied 2 ounces per acre for a total of 10 ounces in 5 acres. Two weeks post treatment we inspected the pond and observed re-establishment of *Culex* mosquitoes at35 mosquitoes in 15 dips.

SUMMARY

Overall, the District feels that this trial of Natular 2EC was successful. As with all the products that we use, calibration and rates are very important in effective control. For the most part, a low to medium rates per acre work well to control a variety of mosquito species. As observed in our one failure, a higher application rate is needed in thick vegetation but the rates are still low, even in high vegetation.

The primary objective of this study was to determine the efficacy of Natular 2EC in a variety of environments. We concluded that 2EC efficacy was suitable for our needs and worked well if used appropriately. One negative issue was cost at over $900 a gallon. However, the chemical cost of this trial for all four ponds was only $210 to treat 21 acres or $10 per acre. Therefore, the price was fairly competitive with similar products. We concluded that Natular 2EC is a useful product that can be used in mosquito control programs.

ACKNOWLEDGEMENTS

The author would like to thank Clarke for providing the product used in this study. We would especially like to thank Drew Hunter and Derek Drews with Clarke as well as the Magna Mosquito Abatement District Board of Trustees for their support

A Field Evaluation of VectoPrime® FG Using Low Application Rates at the Baker Valley Vector Control District

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INTRODUCTION

Controlling pasture mosquitoes including *Aedes nigromaculis* and *Aedes melanimon* is an ongoing challenge for mosquito control districts in the Western USA. A limited range of biorational mosquito larvicides are available for treatment of flooded pastures, which is essential to prevent outbreaks of severe mosquito annoyance. Large areas of flooding as well as rapid and asynchronous larval development demand efficiency in application and proper timing when using bacterial larvicides or IGR's. VectoPrime® FG is a granular biorational mosquito larvicide designed for efficient single brood control and flexible application timing. This achieved through a unique combination of *Bacillus thuringiensis israelensis* strain AM65-52 (Bti) and s-methoprene using BioFuse™ technology. The high potency formulation and flash release of an effective s-methoprene dose enables use of low application rates and eliminates concern about late instar larvae escaping the 'Bti effect'. The current paper presents an operational field evaluation of VectoPrime FG conducted in a 40 acre flooded pasture in the Baker Valley of Northeast Oregon.

METHODS

Prior to treatment, six dipping stations were established on a transect at the north end of the pasture where larval activity was identified. Stations were marked with irrigation flags. Adjacent to each flag, either two or three 12.5" diameter plastic pans were placed and filled with clear water to detect deposition of granules. Ten dips were taken at each dipping station, and larval counts were recorded. Instars present were also noted and recorded in order of predominance.

A Polaris® ATV equipped with a Fimco® spreader was used for application of VectoPrime FGat the calibrated rate of 3.5 lbs/acre. The applicator was guided to stay on 20 foot lanes by two flaggers. Temperatures were cool on the day of treatment with a low of 43F and a high of 65F.

RESULTS

Application rate was confirmed to be 3.5 lbs/acre based on the use of 140lbs or product to cover 40 acres. Granule deposit was observed at all sampling stations, indicating an even application rate. Observed larval densities prior to and after treatment are presented in Figure 1. Prior to treatment, the mean larval density was 1.2 larvae per dip (95% CI = 0.18, n=6), and larvae were in the second through fourth instar. Pupae were also present prior to treatment. At 24 hours after treatment, the mean larval density was 0.47 larvae per dip (95% CI = 0.05, n=6). Larvae were in the third and fourth instar. No pupae were detected at 24 hours after treatment. At 48 hours after treatment, the mean larval density was 0.1 larvae per dip (95% CI = 0.02, n=6), and larvae were in the fourth instar. No pupae were detected at 48 hours after treatment.

CONCLUSIONS

Application of VectoPrime FG to this flooded pasture resulted in greater than 90% reduction in larval density at 48 hours post treatment. Post treatment population densities were significantly lower than pre-treatment based on separation of confidence intervals. Cool weather slowed the mortality response. No pupal development was observed after treatment.

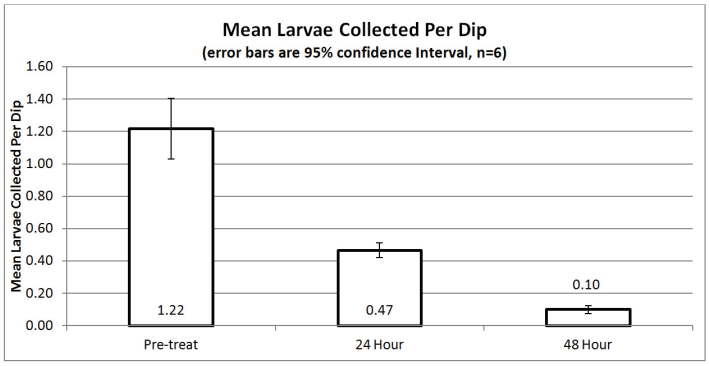

Figure 1 Larval densities prior to treatment and at 24 and 48 hours post treatment

Survey 1, 2, 3: An easy tool to Build Geospatial Application Using ArcGIS

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INTRODUCTION

Location, condition and design of storm water systems can make dramatic differences in the ability of a storm water management structure to hold water and produce mosquitoes. The Shasta Mosquito and Vector Control District (District) routinely requests updated geospatial data from the city and county to better direct our staff to control storm water structures that hold water and breed mosquitoes. However these data are normally incomplete and not categorized by type of structure or even associated with the agency's maintenance schedule.

Beginning in the summer of 2017, District staff started mapping all the storm water structures within the District boundaries and categorizing them according to type of structure, whether the structure was currently wet or dry, and the potential of the devices to breed mosquitoes. This information was entered into a custom built mobile application that the District designed utilizing ESRI's Survey 1,2,3.

METHODS

Geospatial data collection is becoming a necessity in most industries. Collecting data and location information can better inform a researcher or operations department on the response and analysis of the data being collected. Unfortunately, systems or applications that can be hosted on a phone or other mobile data collection hardware can be expensive to have someone else build, or require a high level of technical experience to administer with current staff. Survey 123 provides an easy way for a District to quickly build applications that will collect, store and analyze geospatial information. It is a simple, form-centric data gathering tool that utilizes ArcGIS as a back end system to create useful and timely field applications. In 2017, Shasta MVCD developed a simple application that could be deployed to any hardware system, either as a web application in Android or IOS. Our District has struggled historically with incomplete and out of date GIS records associated with the storm water systems in the District boundaries. So utilizing Survey 1,2,3 we designed a form that collected storm water structure design type, status, location, and vector risk for all storm water structures throughout the District. Survey 1, 2, 3 uses a bundled XLSForm that allows the designer to build questions that auto format in the mobile application. This application is then linked to ArcGIS online (ESRI) to process the data once collected. Between June and September District staff visited each storm water structure and responded to the questions built into the mobile application. At the end of each day the data were uploaded to ArcGIS online for review.

RESULTS AND DISCUSSION

During the course of the project 6,931 storm water structures were catalogued for future District use. The effort has determined that not only were structures being missed, but the percentage of wet versus dry structures was surprisingly low, indicating that mosquitoes originate from a small number of storm water management devices. This knowledge potentially can increase the efficiency of the District's effort inspecting and treating these systems over time as we build a more complete picture of each device's breeding potential.

CONCLUSION

The District will continue to catalogue and update the storm water structure inventory. The more accurate the data the less effort our field staff will have to expend inspecting structures that have a low probability of breeding mosquitoes. In the future we plan to integrate this geospatial information into the District's map-based field collection system and will schedule the inspections and route the inspection path based on a ranking assigned to each device based on the likelihood of mosquito production.

ACKNOWLEDGEMENTS

Rachel Evernden, Kelly Cleland, the SMVCD staff and the SMVCD Board of Trustees for their support. This project was funded by a Shasta-Tehama-Trinity Joint Community College District/Doing What Matters Internship Grant.

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Year two of an expanded West Nile Virus surveillance program

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INTRODUCTION

In 2016, the Delta Vector Control District's West Nile Virus (WNV) surveillance program grew from 43 gravid trap sites surveyed bi-monthly to 172 gravid trap sites surveyed weekly. The 2017 mosquito season was the second year implementing 172 weekly gravid trap sets and was also accompanied by an increase in weekly encephalitis vector surveillance (EVS) trap sets. The overall sustainability of the expanded program, as well as the value of the information generated was evaluated and compared to the first year of implementation.

METHODS

The gravid trapping program is structured using a historical, grid-based geographical system (public land survey system)[1] with four gravid traps evenly dispersed within each square mile of populated area, resulting in 172 total gravid trap sites throughout the District. The sites are fixed throughout the season and provide four measures of mosquito abundance and viral activity per square mile on a weekly basis. To increase understanding of WNV activity in rural regions of the district in 2017, an additional 20 EVS trap sets were assigned to surveillance staff each week, in non-fixed locations, throughout the district, as determined by vector control technician input, and historical records of both rural breeding sources and agricultural areas with previous WNV activity.

RESULTS AND DISCUSSION

The 2017 WNV surveillance season resulted in 4,241 gravid trap sets and 350 CO_2 trap sets. The *Culex quinquefasciatus* collected by the gravid traps were tested in 2,391 mosquito pools and had a minimum infection rate (MIR) of 6.8 WNV infected females per 1,000 tested, whereas *Culex quinquefasciatus*, *Culex tarsalis* and *Culex stigmatosoma* from the EVS traps were tested in 450 mosquito pools and had a a MIR of 7.2 WNV infected

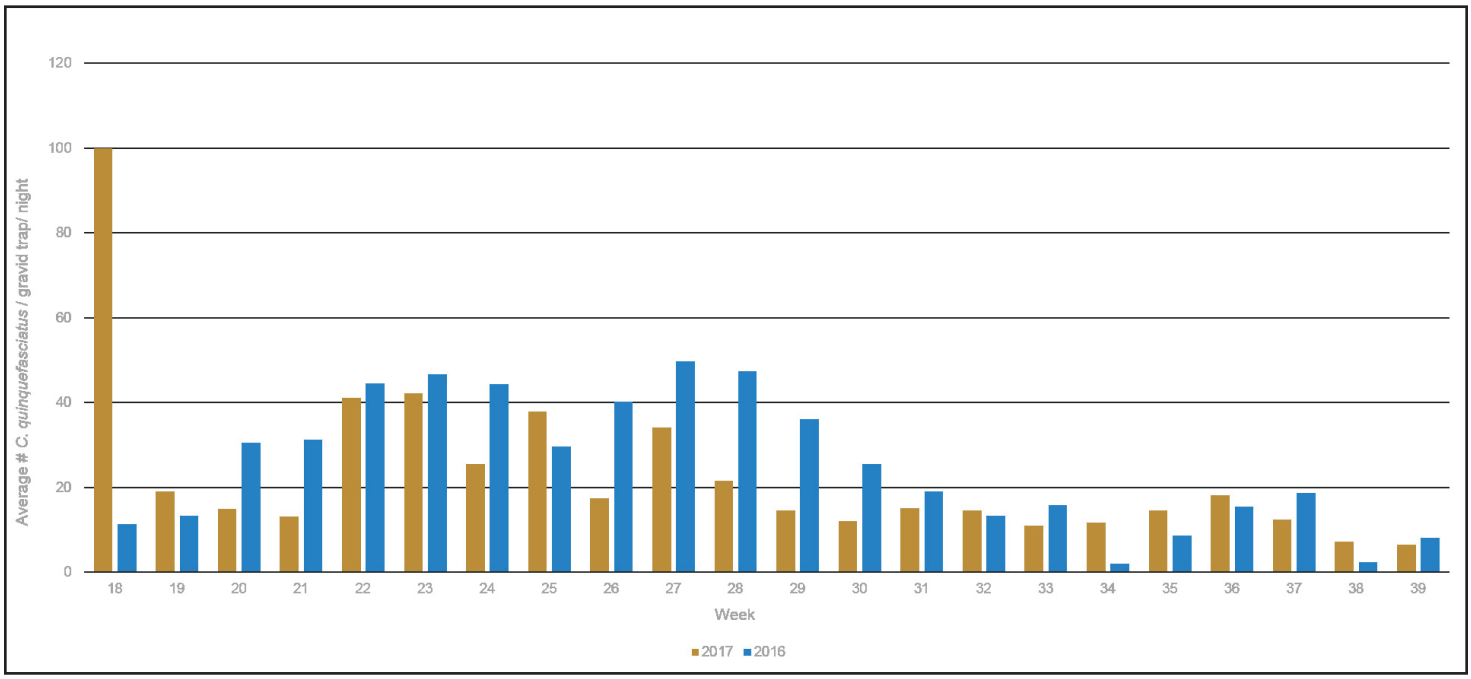

Figure 1 Average number of *Culex quinquefasciatus* collected per gravid trap per night within the Delta VCD during 2017 and 2016.

females per 1,000. The gravid trapping program completed its goal of 172 trap-sets per week, while the EVS trapping program did not consistently result in 20 traps a week, due to the need to relocate staff after an infestation of the invasive species *Aedes aegypti* was detected in the district's most populous city, Visalia. The gravid trapping program was very effective in distinguishing mosquito abundance problems that could then be swiftly addressed by control technicians, and produced a more efficient collaboration between laboratory surveillance staff and vector control technicians. Comparing data between the first and second years of the program, enhanced surveillance and enhanced collaboration potentially helped produce an earlier reduction in domestic mosquito populations during 2017 (Figure 1).

CONCLUSIONS

Delta VCD will continue to implement its expanded WNV surveillance program in years to come to establish a robust baseline of both mosquito abundance and viral activity at a relatively fine spatial scale. Looking forward, the District will use the structure of the gravid trapping program to create an expanded invasive *Aedes* surveillance program that will help measure population abundance and the spread of these species to enhance future control efforts. Furthermore, future seasonal staff will be assigned to either the WNV or invasive *Aedes* surveillance programs which will enhance stability and consistency of data collection.

ACKNOWLEDGEMENTS

The authors would like to thank Delta VCD Manager, Michael Alburn, Superintendent, Paul Jobe, Systems Administrator, Mark Dynge, and Biologist, Mir Bear-Johnson, for their assistance in conceptualizing and developing the WNV surveillance program and constant encouragement throughout.

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Bionomics and phenology of *Culiseta particeps* in Lake County, California

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ABSTRACT *Culiseta particeps* is an uncommon and rarely studied mammalophilic mosquito species with a known range along the west coast of the United States and Canada, with some collections made in Arizona and Alaska. One 1989 southern California study determined host seeking activity to be highest in the spring, but otherwise very little research has been done on the seasonality or overall bionomics of this species. The goal of this study was to address gaps in previous research and investigate aspects of the bionomics of *Cs. particeps* including host selection, parity, overwintering behavior, and larval and adult seasonality in Lake County, California. Adult collections were made to target both host-seeking and resting mosquitoes using CO2–baited traps and vacuum aspiration collections from underground, human-made environments. Larval collections were made via dip sampling from two locations at Highland Springs Reservoir. All mosquitoes were identified to species, where possible. Determination of aestivation or overwintering status was evaluated through trapping and weather associations and dissections of ovaries of empty (non blood-fed or gravid) adult females. A total of 59 blood-fed *Cs. particeps* were collected in Lake County and bloodmeals identified to determine host selection. All blood fed females had fed on mammals and 71% fed on black-tailed deer. True diapause was unlikely for this species but collections of host seeking females during unexpected periods of warmth in winter and follicle degeneration associated with periods of extreme heat in summer suggest periods of aestivation during extreme temperatures.Host-seeking activity of *Cs. particeps* in Lake County appears highest in the late summer and early fall months. This study will provide future investigators with additional knowledge of this species aiding in the broader understanding of its biology and ecology.

The use of sugar-feeding by individual mosquitoes for arbovirus detection in the laboratory

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ABSTRACT Many species of mosquitoes consume sugar for survival, and because they salivate during sugar feeding, they may expectorate virus at the same time. As a result, sugar feeding has been used for mosquito control, as well as for arbovirus detection in field and laboratory settings. In this study, we used the expectoration of virus into sugar wicks to estimate the extrinsic incubation period of West Nile virus in individual *Culex tarsalis* females. After feeding on an infectious bloodmeal, individual *Cx. tarsalis* females were offered a sucrose-soaked cotton wick, which was collected daily and tested for West Nile virus. This method of collecting longitudinal samples from individual mosquitoes yielded similar results to those of the standard capillary tube method, while allowing for a more direct and precise estimation of the extrinsic incubation period using fewer mosquitoes. We expect that this approach may prove valuable for future virological studies to characterize variation in the amount and diversity of expectorated virus over mosquitoes' life spans.

Attractive toxic sugar bait stations for control of *Culex quinquefasciatus*

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INTRODUCTION

Sugar meals are essential to adult mosquito nutrition and only recently have been incorporated into systems that blend sugar bait with insecticides for mosquito abatement. Attractive toxic sugar baits (ATSBs) with multiple insecticides have been suggested to reduce resistance and enhance control agent efficacy against multiple mosquito life stages. We report here on laboratory and field assessments of ATSB stations with the insect growth regulator pyriproxyfen (PPF) and the entomopathogenic fungus *Beauveria bassiana* (Bb) against *Culex quinquefasciatus* mosquitoes.

METHODS

ATSBs with different PPF and Bb contact zones were tested on host-seeking or gravid female mosquitoes feeding on attractive bait. Autodissemination of PPF/Bb by contaminated adults to glass bowls holding early fourth instar larvae was assessed. Exposure periods were 2 d in mesh cages (laboratory) or 5 d in mesh enclosures (field). Post-exposure, larvae were reared for up to one week to assess emergence rates and surviving adults transferred to rearing chambers were monitored for mortality for up to 21 days. *B. bassiana* infection was identified from adult mosquito cadavers incubated in 24-well plates in a high humidity box for up to one week. In gravid adult assays, egg rafts were enumerated and the total number and hatching rate of eggs were determined. The persistence of mosquitocidal effects was examined for different ATSB designs aged up to 2 weeks under laboratory or field conditions. In the field, *Cx. quinquefasciatus* from laboratory colonies were released within replicated pyramidal mesh PVC enclosures on top of fiberglass tubs (total height = 2.5 m), and dead individuals were collected daily over a 5-day period and live individuals were removed from enclosures on day 5. Each enclosure contained a single hanging ATSB with either a combination of PPF/Bb or water control and glass water bowls with larvae. Two release/recapture trials, one with fresh and one with aged (1 or 2 weeks) ATSB stations, were performed in spring and autumn of 2017. The spring trial included host-seeking adult females (n = 75) and fourth instar larvae (n = 75, three glass bowls) and the autumn trial involved gravid adult females (n = 40) with fourth instar larvae (n = 40, two glass bowls).

RESULTS

Laboratory results linked PPF (with or without Bb) to significant larval and adult mortality compared to controls. Average emergence was reduced by 90% (fresh) to 50% (1 week aged) and average adult mortality ranged from 30-75%. ATSB designs that stored more PPF per station and resisted desiccation for longer periods were more effective overall against adults and larvae. Compared to PPF alone, the Bb/PPF combination produced slightly enhanced adult mortality when the ATSB was aged for a week. Bb used alone in an ATSB station was a potent delayed-onset adulticide against host-seeking females, producing greater than 60% average mortality and infection rates over a 2 week post-exposure period; however, Bb lethality against gravid females averaged less than 20%, with high rates of sublethal infections evident post-mortem. Sugar deprivation may be a key factor in determining PPF/Bb ATSB efficacy. Host-seeking adults sugar-starved for 24 hours displayed higher mortality and infection rates than non-starved cohorts. The two field trials resulted in similar trends for mosquito mortality: PPF/Bb enclosures greatly reduced adult (86 ± 11%) and larval (59 ± 12%) abundance compared to controls (adults: 21 ± 17% and larvae: 16 ± 13%). Field-acquired Bb infection was detected in an average of 10% of recaptured adults. Egg raft production by field-exposed gravid adults was reduced, on average, by nearly half in PPF/Bb enclosures (15 ± 9 rafts) compared to control enclosures (28 ± 3 rafts). Overall, an ATSB with pyriproxyfen and *B. bassiana* provided sustained control of adult and larval *Culex quinquefasciatus* and future field deployments targeting wild populations are the next step to further determine the usefulness of this system for mosquito abatement operations.

Metagenome Sequencing Based Identification of Arboviruses in Field-Captured *Culex* Mosquitoes from the San Gabriel Valley, California

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INTRODUCTION

In California, local vector control agencies monitor *Culex* mosquitoes for endemic arboviruses such as West Nile virus (WNV), Saint Louis encephalitis virus (SLEV), and Western equine encephalomyelitis virus (WEEV). In 2017, 1,522 pools of *Culex* mosquitoes from 23 cities within the San Gabriel Valley, Los Angeles County, California were tested for the presence of viral RNA (WNV, WEEV, and SLEV) using real-time polymerase chain reaction (RT-PCR). Of those, 153 (10%) were positive for WNV during the 2017 season. In the current study, we applied next-generation sequencing (NGS) to 81 (53%) of these pools positive for WNV to reveal any novel arboviruses previously undetected in the San Gabriel Valley, Los Angeles County, California.

METHODS

Eighty one pools (11 *Culex tarsalis* and 70 *Cx. quinquefasciatus*) were selected for further analysis by metagenomic sequencing based on an initial positive RT-PCR WNV test with a Ct score between 13 and 30, and a sample date of June through September, 2017 (Table 1). These selected mosquito pools were then subjected to random reverse transcription and PCR amplification to extract the nucleic acids from the original homogenates (Hang et al. 2016). The amplicon libraries were sequenced using an Illumina MiSeq sequencer and reagents (Illumina, Inc., San Diego, CA). The sequence data were analyzed utilizing a bioinformatics pipeline which involves data quality processing, *de novo* assembly, comparing the assembled contigs and unassembled reads with sequence databases using two BLASTN programs (megablast and discontiguous megablast), and sequence-based taxonomic classification (Kilianski et al. 2015, Hang et al. 2016).

RESULTS AND DISCUSSION

Metagenome sequencing using MiSeq generated a total of 18,709,956 raw reads for the 81 samples. The percentage composition of sequence reads that belonged to eukaryote, bacteria, viruses or others, i.e. novel sequences from unknown organisms or artificial sequences from PCR, were determined (Figure 1). A considerable amount of arboviral sequences were identified in many samples, specifically, 50 out of the 81 mosquito pools (61.7%) that had ≥ 5% of non-human sequence reads were viral sequences. WNV sequences were found in 31 out of the 81 sequenced pools (38.3%). The Ct scores from the original RT-PCR WNV test in general had a negative correlation with the numbers of WNV sequence reads from the metagenome sequencing (p-value < 0.05). We noticed that the number of WNV sequence reads was highly variable among the samples. As an example, for the seven samples with Ct scores ranging from 16 to 17 (16.4 ± 0.4), the number of WNV sequence reads varied from 0 to 8,444 (1806 ± 3201). This observation is consistent with previous studies which have shown that metagenome sequencing by NGS has limitations in the quantitative analysis of viruses from complex specimens (Ozsolak and Milos 2011, Yang et al. 2015, Greninger 2018). With PCR being target based, its increased sensitivity allows it to be specific and quantitative. On the other hand, the complexity of NGS data, with the high abundance of host nucleic acids (from tissue and body fluids) has been known to mask the sequencing of viral RNA species, and the issue of sensitivity and the number of reads for RNA may not correlate with their actual abundance (Linsen et al. 2009, Ozsolak and Milos 2011, Wang 2011, Yang 2015, Greninger 2018). The discrepancies also could have been introduced during sample preparation and sequencing steps (Ozsolak and Milos 2011). The multiple freeze-thaw cycles of the nucleic acids can lead to sample degradation which could have been reduced by aliquoting samples to reduce the number of freeze-thaw cycles, but the subsequent NGS testing was decided after the 2017 sampling season.

Metagenome sequencing is a technical approach that can be used to discover novel viral and non-viral sequences, including unknown arboviruses. A number of other viruses were discovered in the *Culex* WNV positive mosquito pools tested. Among the assembled sequence contigs with the lengths of ≥ 500 bp, 59 out of the 81 pools (72.8%) were found to contain viral sequences other than WNV. These viral sequences shared similarities with known viral sequences in GenBank, with nucleotide identities ranging from ≥ 99% to approximately 65%. These potential arboviruses belong to a variety of taxonomic groups of viruses, including Alphamesonivirus (Hang et al. 2016), Biggievirus (KX924639), Bunyavirus (Chandler et al. 2015), Cordoba virus

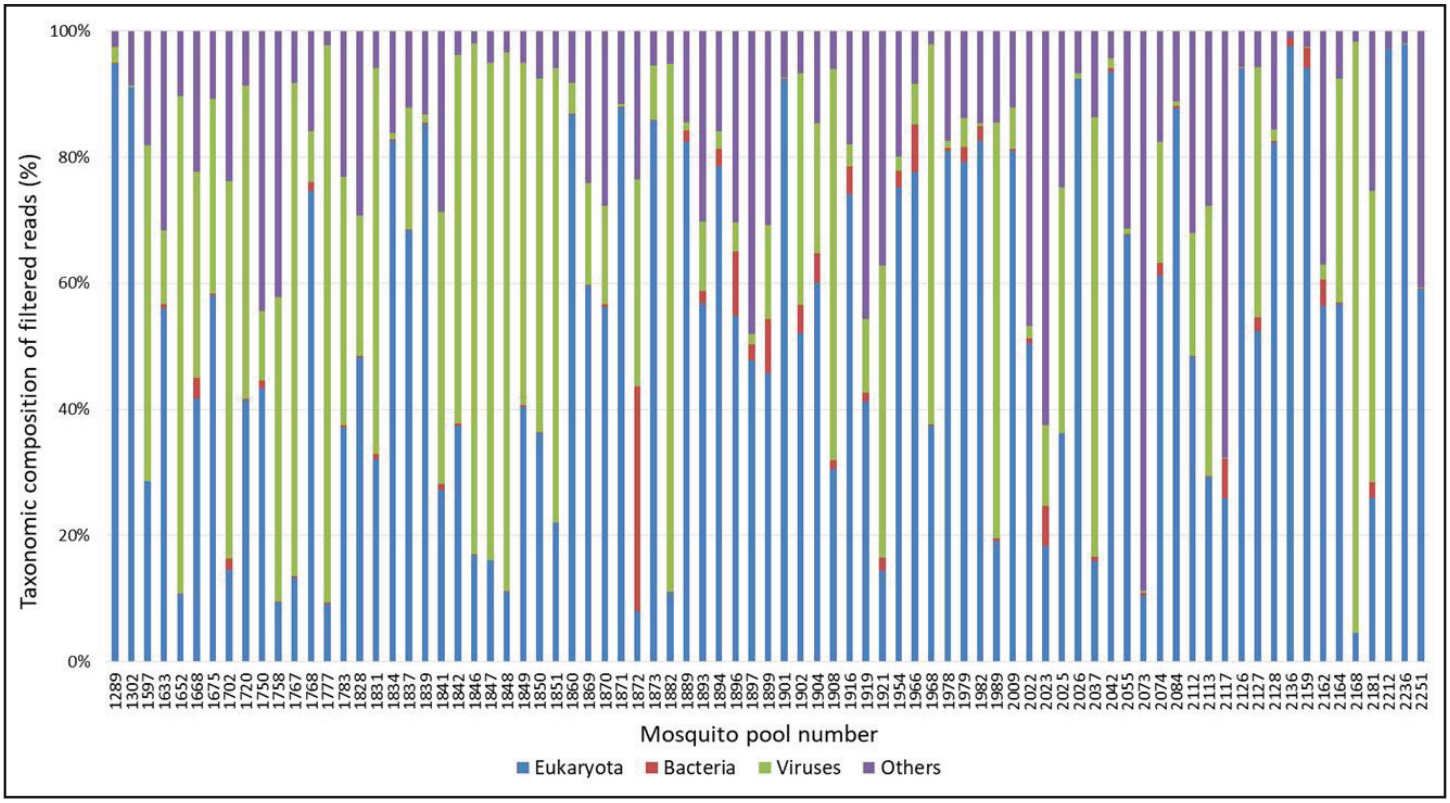

Figure 1 The taxonomic composition of the sequence reads from the metagenome sequencing. Sequence reads were classified to the group of eukaryote, bacteria, or viruses. Others, i.e. novel sequences from unknown organisms or artificial sequences from PCR, were determined.

(Nunes et al. 2017), *Culex* flavivirus (Kim et al. 2009), Marafivirus (Glasa et al. 2015), Houston virus (Vasilakis et al. 2014), and Negevirus (Vasilakis et al. 2013). Further analyses of the metagenomic sequence data will shed light on the presence of arboviruses carried by *Culex* mosquitoes in the San Gabriel Valley, and genetic, phylogenetic and taxonomic characteristics of these arboviruses. Specifically, the genetic variation of mosquito-borne WNV and the relationship with WNV in avian and human clinical specimens in California and from other locations will be analyzed (Di Giallonardo et al. 2015, Grinev et al. 2016, Duggal et al. 2015, Grinev et al. 2018).

CONCLUSIONS

Los Angeles County is an international hub. It is important to identify novel viruses as well as new genetic variants of viral pathogens. Metagenomic information represents a valuable addition to our current District surveillance program and will enhance our ability to anticipate the possibility of novel infections in residents of San Gabriel Valley.

ACKNOWLEDGEMENTS

Funding: Armed Forces Health Surveillance Branch Global Emerging Infections Surveillance and Response System (GEIS). We thank J. Wakoli Wekesa, Sam McKeever, Gimena Ruedas, and the 2017 West Nile virus team (Winifred Khakali, Tyler Laird, and Sarah Miller) from the San Gabriel Valley Mosquito and Vector Control District.

DISCLAIMERS

The views expressed here are those of the authors and do not reflect the official policy of the Department of the Army, the Department of the Navy, Department of Defense or U.S. Government.

Table 1 Number of mosquitoes and mosquito pools submitted for metagenome sequencing by San Gabriel Valley Mosquito and Vector Control District in 2017.

Species	Total Number of Mosquitoes	Number of EVS Traps	Number of Mosquitoes in EVS Traps	Number of Gravid Traps	Number of Mosquitoes in Gravid Traps	Number of BG Sentinel Traps	Number of Mosquitoes in BG Traps	Number of Pools
Culex quinquefasciatus	1,334	2	25	63	1,212	5	97	70
Culex tarsalis	142	0	0	11	142	0	0	11
Total	**1,476**	**2**	**25**	**74**	**1,354**	**5**	**97**	**81**

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Metagenomic sequencing of *Culex tarsalis* from the field

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INTRODUCTION

Culex tarsalis is a vector of West Nile virus and other arboviruses that can breed in landscapes with pooled rain water and in urban settings that have uncovered water containers such as fouled swimming pools or flood control canals. The lack of a complete genome or even subgenomic mitochondrial genome sequence for this vector has hampered our ability to monitor insecticide susceptibility. Moreover, its full microbial payload has not been extensively examined. To address these gaps, we carried out a metagenomic next-generation sequencing (mNGS) pilot study to capture both the *Cx. tarsalis* sequences and non-host microbial sequences from *Cx. tarsalis* collected under standard conditions at a single site during peak abundance in fall of 2017.

METHODS

Mosquitoes were captured at a marsh habitat located in Fremont CA (USA) using EVS CO_2-baited traps and frozen using dry ice or immobilized using triethylamine (TEA). Mosquitoes were sorted on a chill table, female *Cx. tarsalis* collected, and separated into head and abdomen pools (n=5 and n=20) before storage at -80 ^{o}C. DNA and total RNA were extracted from homogenized *Cx. tarsalis* specimens using ZR duet DNA/RNA miniprep plus kit. No significant differences in RNA or DNA yields were detected between specimens frozen on dry ice or treated with TEA. NGS libraries were prepared for each pool and sequenced on an Illumina NextSeq 550 sequencer. An average of 30M reads (range 17M – 41M) were obtained for RNA samples, and an average of 38.6M reads (range 26M-50M) were obtained for DNA samples. *Cx. tarsalis* sequences were identified through nucleotide sequence similarity to a compilation of all publicly available non-redundant mosquito sequences present in the NCBI GenBank repository. A near complete draft of the 14,850bp mitochondrial genome and transcriptome has been assembled for *Cx. tarsalis*. For each specimen pool, we assessed the presence of mutations in genes known to confer insecticide resistance such as the voltage-gated sodium channel (kdr). DNA sequence and RNA transcriptome sequences obtained from these studies will be made available to the community for further study.

RESULTS

Between 0.6 % - 1.2 % of the total RNA reads corresponded to non-host sequences, which were investigated further for the presence of mosquito food sources, commensals, and potentially pathogenic microbes. Alignment to the NCBI set of non-redundant nucleotide (nt) and protein (nr) databases revealed the presence of known and potentially novel viral and non-viral microbes of mosquitoes. These microbes partitioned differentially across the head and abdomen fractions. Our results highlight the potential for mNGS approaches to accelerate our understanding of vector dynamics and insecticide resistance in California. Whether and how these results may vary according to environmental context and time of year has yet to be determined.

BG-Counter: a very efficient multi-task tool

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INTRODUCTION

The BG-Counter (Biogents) is a relatively new auto-counting adult mosquito device that is designed for use with a BG Sentinel Trap. Objectives of our experiments were 1) to determine the accuracy of the counter by comparing the number of adult mosquitoes recorded by the counter to actual counts of mosquitoes in the trap, and 2) to assess if the BG-Counter could determine peak(s) of adult mosquito abundance in the field.

METHODS

The BG-Counter is designed to count mosquitoes using an infrared sensor and also possesses environmental sensors that can record temperature, relative humidity and ambient light. The counter is positioned on the top of a BG-Sentinel Trap (Biogents) and can use CO_2 (or not) to attract mosquitoes. The CO_2, fan and counter can all be activated **remotely** to operate during different time durations over a 24-hour period, and the mosquitoes are counted when entering the trap while the counter is "on". The BG Counter samples host-seeking mosquitoes periodically and reports numbers by increment of 15 minutes. (Figure 1).

For our experiments, the BG-Counter was set up to release CO_2 at a rate of 420 ml per minute (preset by Biogents) and count mosquitoes from 1900 to 0900 h the next day. The fan was running over a 24-hour period for both experiments (accuracy and peaks of activity). After a few days of operations we have noticed some issues with trap counts (mosquitoes escaping from the trap). To remedy the situation we installed a bigger catch bag to give more room and allow the catch of more mosquitoes. We also moved the catch bag from underneath the counter and relocated it underneath the fan within the trap to improve the count accuracy by having less mosquitoes escaping the trap. We tested the counter over 17 consecutive days in an agricultural environment (rice) where *Culex tarsalis* are predominantly found to assess the accuracy of the counts (counter vs actual counts).

For that experiment, the catch bag was collected every day and replaced by a new one. The catch bag was brought to the laboratory where technicians accurately counted the numbers of mosquitoes to compare to the number recorded by the counter.

For the second experiment (peaks of activity), the BG-Counter

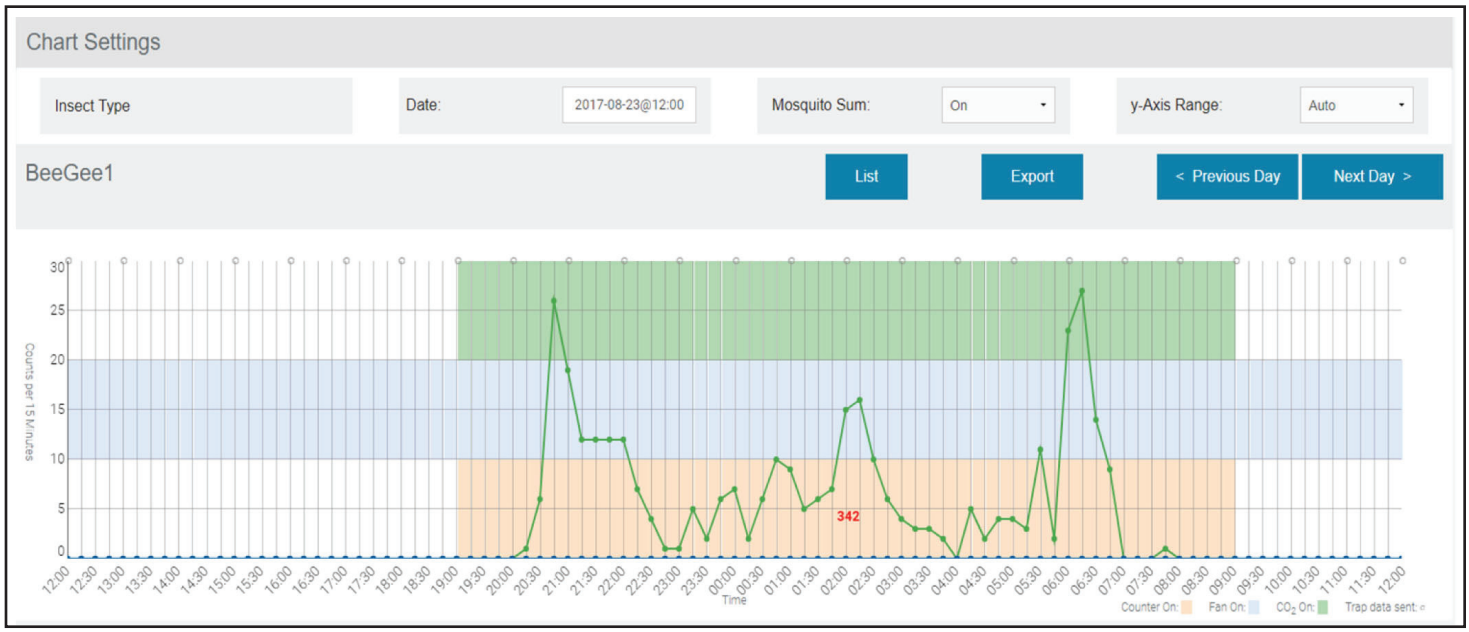

Figure 1. Example of the graph provided on Biogent's website after 24 hours of data collection. The graph indicates when the CO_2, counter and fan were "on" and "off" and shows the total number of mosquitoes counted during the period the counter was "on".

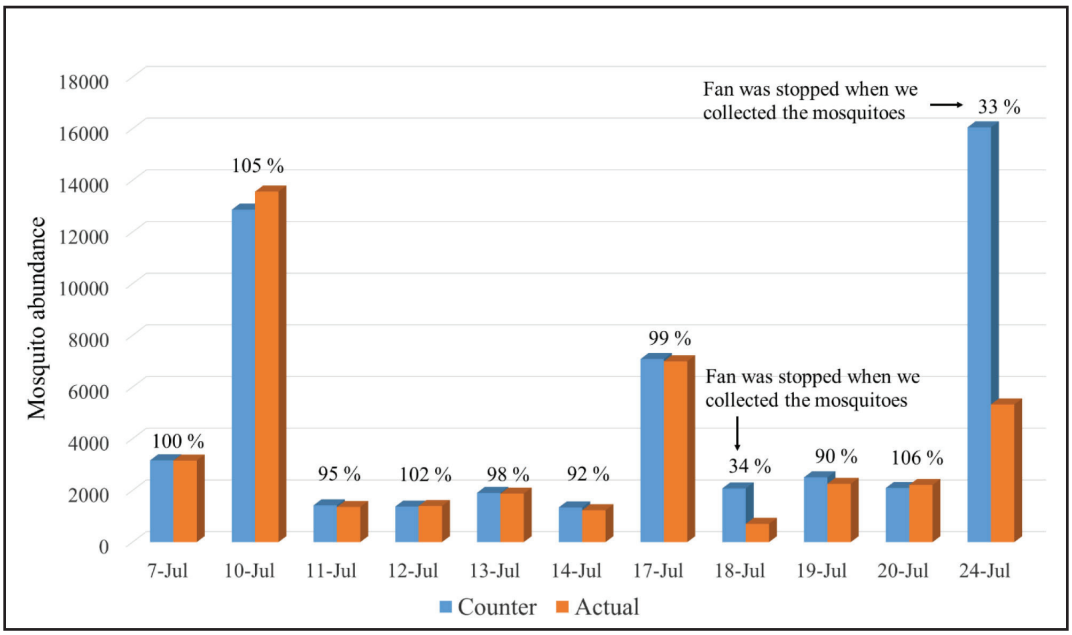

Figure 2. Comparison of counter vs actual counts in the BG-trap located in a rice field over a 17 day-period

was set up at the same location and operated over a 3-month period (July to September). The accuracy being proven by the previous experiment, the catch bag was no longer collected on a daily basis and was emptied once a week. The 20 lb CO_2 cylinder was also replaced every two weeks and the battery recharged if needed.

RESULTS

By operating the fan all the time and modifying the placement of the catch bag within the trap, the number of the mosquitoes collected represented, on average, 98.5% (90% - 106%) of the number recorded by the counter (data when the fan was stopped are not included) (Figure 2). Those results were very significant since the instruction manual from Biogents stated that "*if you make sure that mosquitoes do not leave the catch bag after they have been sucked in the accuracy of the BG-Counter for correctly counted mosquitoes is between 80% and 90% correctly counted mosquitoes*" (Biogents, 2017). Our modification relocating the catch bag within the trap limited mosquitoes from escaping the trap and explained our greater accuracy.

Peaks of activity for *Culex tarsalis* were readily apparent from the BG-Counter data. The peaks of activity and the abundance varied during the 3-month period from July to September. As expected the abundance of mosquitoes decreased significantly in August and September compared to July, but peaks of activity, although less well defined, were still apparent (Figure 3). At least four different peaks of activity are apparent during the month of July with a very high abundance of mosquitoes during the whole month. Results were very consistent during that month with both curves in July almost overlapping perfectly. We also observed that the main peaks of activity at sunset and sunrise changed with the time of sunset and sunrise during the course of the season (Figure 3).

The peaks in the evening and the morning occurred at a consistent time interval either after sunset or before sunrise. To verify this hypothesis we changed the scale of our graphs (X axis) according to the time of sunset and sunrise instead of the time of the day. With the new scale, all the first peaks observed at sunset over the 3-month period were always present in a time period of 30 to 45 minutes after sunset while at sunrise all the peaks were observed in a time period of 45 to 60 minutes before sunrise (Figure 4). The onset of mosquito activity at those specific times were related to luminosity. Another peak in the evening was also present for a 2-month period (July and August) two hours after sunset but the factors stimulating this renewed activity were not clear.

The CO_2-baited BG-Counter trap caught thousands of mosquitoes every day; much more than commonly used traps for West Nile surveillance. This new abundant source of live mosquitoes allowed us to do more pesticide resistance and efficacy trials than we would have been able to do otherwise. Because the BG-Counter can also record temperature, relative humidity and luminosity every 15 minutes, we will eventually use those data to determine if any of those variables (or the combination of those variables) could explain the presence of the peaks of activity during certain periods of the season or even on a daily basis.

CONCLUSION

Based on the quantity and the quality of data collected during the different trials in the summer 2017, we consider the BG-Counter as a very efficient multi-task tool. We documented different peaks of activity over the season. The other data collected by the counter (temperature, relative humidity and luminosity) can also help us determined how these factors affect the behavior of the mosquitoes. Overall, the BG-Counter is an effective and useful surveillance tool that holds great promise in helping provide a more robust assessment of mosquito abundance and activity upon which treatment decisions can be made with better efficacy and efficiency.

LITERATURE CITED

Biogents, 2017. BG-Counter Instruction manual – English. 17 pages.

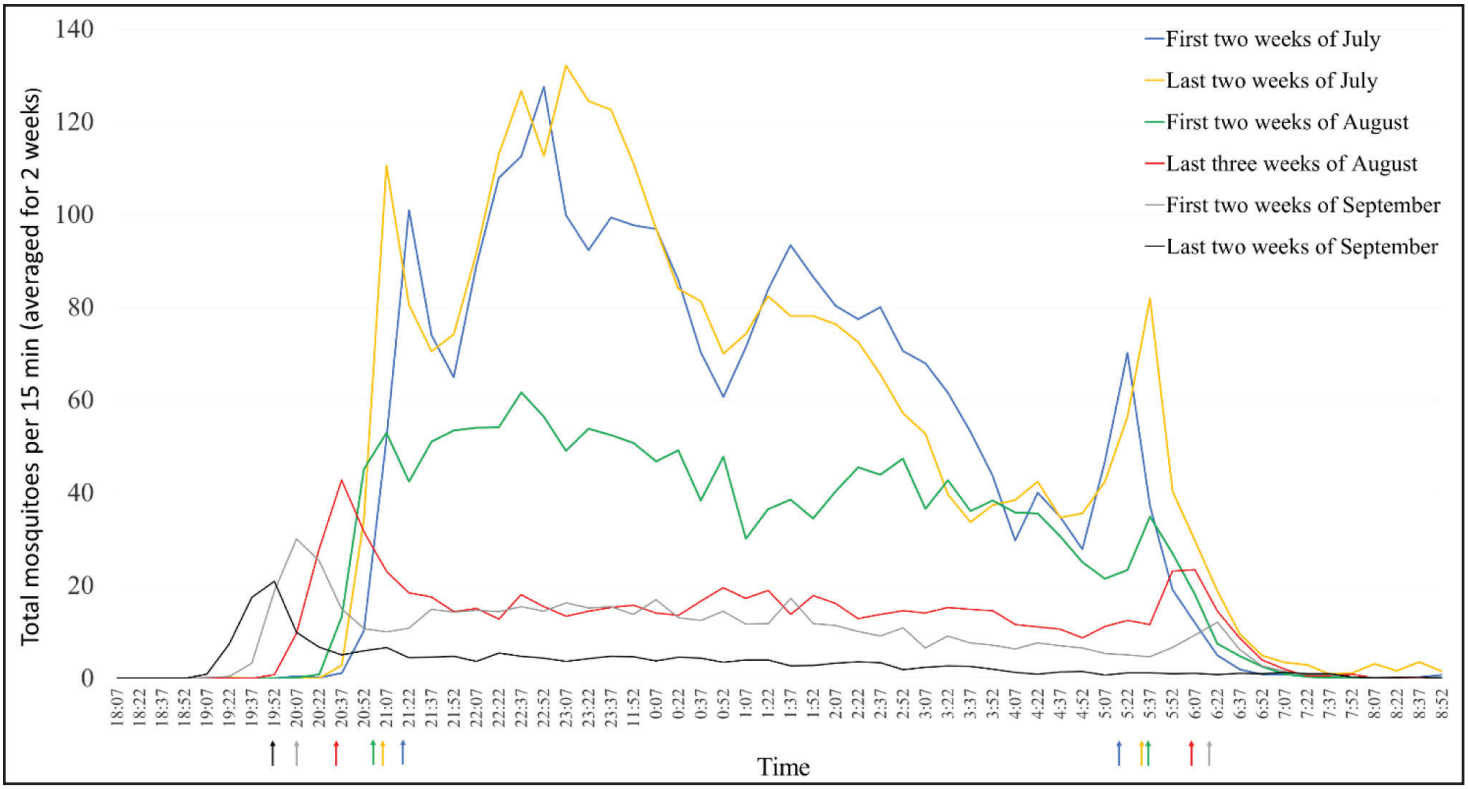

Figure 3. Peaks of activity of *Culex tarsalis* (average of 2 weeks) in a rice field over a 3 month-period (July to September)

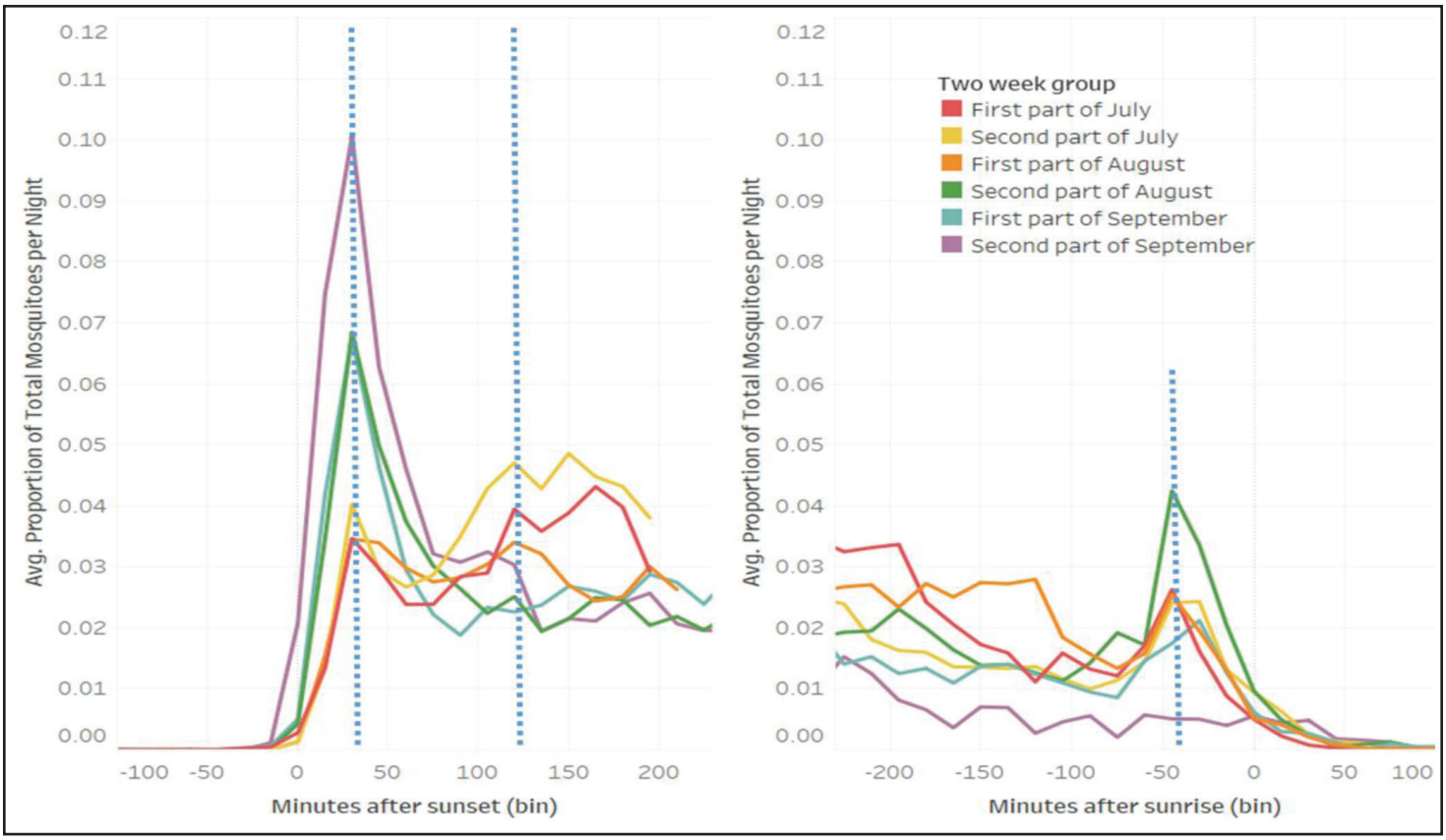

Figure 4. Graphs showing consistency of mosquito activity over a 3-month period at sunset and sunrise.

Current Status of *Aedes aegypti* and *Aedes albopictus* Mosquitoes in California

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ABSTRACT *Aedes aegypti* and *Ae. albopictus* have become firmly established in many areas of California. These mosquitoes can create extreme biting nuisance during daylight hours as well as elevating the risk of local transmission of several arboviruses including Zika, dengue, and chikungunya. Discoveries of one or both species have been made in over 160 cities and census-designated places in 14 counties since 2011 with evidence of thriving populations in many of these locations. Despite aggressive local efforts to eradicate or slow the spread of these invasive mosquitoes, the known infestation areas continue to expand outward and new sites are being reported at an accelerated pace. This presentation provides current information on the spread and distribution of *Aedes aegypti* and *Ae. albopictus* and discusses some of the key lessons learned thus far.

Invasive *Aedes* Surveillance Network in Alameda County

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INTRODUCTION

Surveillance for invasive *Aedes* mosquitoes in urban landscapes is challenging because they tend to disperse short distances from sites of emergence, may not be strongly attracted to traps that are commonly used to monitor *Culex spp.* abundance, and breed cryptically using small ubiquitous containers. Accurately defining the geographic distribution and abundance of invasive *Aedes* mosquitoes is critical for effective control efforts. Traps that are currently used for surveillance by mosquito control agencies can be burdensome because they are costly to purchase (e.g. BG-Sentinel®) or require a large workforce to replace mosquito attractants (e.g. EVS dry ice-baited traps and water-baited oviposition cup traps (OCT).

METHODS

To overcome the large workforce needed for invasive *Aedes* surveillance using OCT, we developed a modified oviposition trap that contains a larger volume of water and Altosid® 30-day briquettes to prevent larval development. Total cost for manufacturing each OBT (oviposition bucket trap) was $2.80 and 3 minutes of labor. The OBT can be inspected less frequently than OCT because the water attractant is retained in the trap for at least 30 days.

RESULTS

The modified OBT enabled the Alameda County Mosquito Abatement District to deploy an *Aedes* trap network that consisted of 660 OBTs that were distributed throughout Alameda County where people reside or work (land area of 2,130 km^2, of which approximately 800 km^2 contain residences). The trap network was deployed by two seasonal lab technicians over the course of one month. More time was needed to identify trap locations using Google Maps compared to finding trap locations while driving, except in highly urbanized areas where first identifying the trap sites using Google Maps was more efficient, potentially because of the challenges of finding spaces for truck parking in dense urban settings. Trap site locations and photos of trap placements were mapped using Google Maps. OptiMap web services were used to optimize driving time between trap sites. Approximately 200 h of work effort, or 25 full-time work days, was used to inspect and service the *Aedes* trap network each month (20 min per trap, inclusive of driving time). Use of OBT in high density trap networks for monitoring invasive *Aedes* mosquito abundance in highly populated and large landscapes where these species have not been established may be an economical and effective alternative to OCT.

Stable isotopes to assess the dispersal of *Aedes aegypti* in an urban landscape

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INTRODUCTION

Aedes aegypti disperse in the urban environment to search for food, mates and oviposition sites. The dispersal of this vector species has impacts on its population dynamics with ramifications for gene flow, insecticide resistance, and pathogen transmission as well as vector-control and public-health activities. Although at least 25 mark-recapture (MR) experiments have been performed to gather information on *Ae. aegypti* dispersal, all of them were carried out at tropical or subtropical latitudes between 22.92° S and 28.68° N (Guerra et al. 2014). The lack of studies in the U.S. is particularly surprising due to the long history of outbreaks caused by *Ae. aegypti*-borne pathogens in human populations in the southern USA, dating back at least to the first half of 17th century (Eisen and Moore 2013), and the more recent spread of *Ae. aegypti*, with established populations documented up to 39° N (Washington, DC; Lima et al. 2016). Moreover, the dispersal estimates of many MR studies in the literature may be biased due to manipulation of the mosquitoes prior to mosquito release, limitation of recapture effort to small areas, or low numbers of recaptured individuals (Maciel-de-Freitas and Lourenço-de-Oliveira 2009). Due to the risk of importation of *Aedes*-borne tropical pathogens into the southwestern U.S. and recent trials of *Wolbachia*-based control strategies that depend on mating of released male *Ae. aegypti* with wild females, it is important to characterize *Ae. aegypti* dispersal accurately for the southwestern U.S. and to link dispersal patterns with environmental contexts, including socio-economic factors. In the current study, we exploited the potential to alter carbon (C) and nitrogen (N) isotopic signatures in mosquito tissues to mark *Ae. aegypti* during the larval stage (Hamer et al. 2012). This marking technique limits the manipulation of mosquitoes and avoids the need to apply markers to the cuticle of adult mosquitoes after emergence (e.g., dusting with fluorescent powder). Stable isotopes are forms of chemical elements that occur naturally in the tissues of organisms at a ratio (δ) that is relatively stable within a limited geographic area and time period. The alteration of the isotopic ratio in mosquitoes can be achieved by enriching the larval water with a tiny amount of ^{13}C or ^{15}N, which are then integrated into the mosquito's tissues during development and carried over transstadially into the adult stage. The aim of the current study was to use this marking technique to characterize *Ae. aegypti* dispersal in California's Central Valley, where invasive *Ae. aegypti* has been established since 2013 .

METHODS

Study area. We performed the MR study in Madera, a city in central California with a population of 61,000 people. Since the first *Ae. aegypti* detections in a small area of the city in 2013, the population has spread across most of the urban area. We located two (one for each marker) partially overlapping circular study areas which comprised the location of *Ae. aegypti* first detection. Each study area had a radius of 500 m and was divided into five 100-m concentric annuli. After identifying households within each concentric annulus to achieve uniform trapping density throughout the study area, residents were contacted by telephone to identify participants willing to allow placement of mosquito traps within their properties. A total of 77 households were selected as trapping sites and each was equipped with either 1 Biogents (BG) Sentinel trap, 1 autocidal gravid ovitrap (AGO) or 3 BG Gravid Adult Traps (GAT), for a total of 144 traps deployed. GATs were filled with 1 L of hay-infused water, and a Biogents clear sticky card was hung from the clear lid of each trap to retain trapped mosquitoes. Sticky cards were replaced once per week to maintain trap efficacy throughout the study. *Marking methods*. Following the protocol reported in Hamer et al. (2012), we prepared water with altered isotopic signatures in two different containers with 10 L of deionized water, the first amended with 51.6 mg of 99% glucose (source of ^{13}C) and the second amended with 38.3 mg of 99% KNO_3 (source of ^{15}N). We then added 6.4 g of food consisting of 4.2 g of Defatted Liver Powder (BioServ) and 2.2 g of Brewer's Yeast (BioServ) to each container. On 7 Aug 2017,

a screened outdoor mosquito enclosure was set up adjacent to the district headquarters. Inside the enclosure, eight 4.92 L plastic trays were filled with 1 L of ^{13}C-enriched water, and approximately 300 F_1 eggs, originating from nearby Clovis, California, were placed in each tray, for a total of 2,457 eggs. The following day, eight additional trays were prepared using ^{15}N-enriched water and 2,539 *Ae. aegypti* eggs collected from Madera during the previous three weeks. Starting from 13 Aug 2017, each tray was inspected visually once per day for the presence of pupae, which were then transferred carefully from the trays to a 200 mL water container and placed in insect rearing cages. Pupae from ^{13}C and ^{15}N-enriched water containers were kept in separate cages, and different sets of tools were used for all operations involving ^{13}C and ^{15}N-enriched water to avoid any cross-contamination between C and N isotopes. Emerging adults were offered a 10% sucrose solution *ad libitum* using 60 mL glass jars with cotton wicks inserted into the jar containing the sugar solution. Adults were released for six days between 19:00 and 20:30 at defined release points at the center of the two trapping areas. The number of adults released each day was assessed by counting the pupal exuviae in the cages after release. Traps were checked daily for four days during the first trapping week, and afterwards three days/week until 13 Sep 2017. Catch bags in BG Sentinels were replaced upon each trap check and the bags with caught mosquitoes were stored at -80°C at the district headquarters. The day after collection, mosquitoes in each bag were identified to species using a dissecting stereoscope. All individuals identified as *Ae. aegypti* were counted by sex and put into 2 mL vials, while all other individuals were counted and discarded. In the same way, all *Ae. aegypti* found on sticky cards in GATs were counted by sex and transferred into 2 mL vials using forceps and then stored at -80°C. All of the tools used to collect mosquitoes, handle or identify them were thoroughly cleaned using 99% methanol between traps. At the end of the MR study, all vials with *Ae. aegypti* were transported to the UC Davis insectary and stored at -20°C. Later, *Ae. aegypti* were pooled in groups of 1-4 individuals and placed in tin capsules stored in 96-well trays for isotopic analysis, performed through mass spectrometry at the UC Davis Stable Isotope Facility. Thirty additional *Ae. aegypti* individuals, 15 for each sex, were collected in the study area one month after the end of the MR study and analyzed for isotopic signature. This step allowed us to develop reference distributions of isotopic delta (δ^{15}N and δ^{13}C, normalized ratio of the heavy to the light isotope) background values within the study area. These two reference distributions then were used to determine whether the pools of trapped *Ae. aegypti* contained marked individuals. We considered a mosquito pool as marked when its isotopic delta (δ) was more than 10 SDs above the mean of the reference distribution. Reference samples were processed following the exact same laboratory protocol as all other samples.

RESULTS

A total of 1,394 isotope-marked adults, 896 ^{13}C and 498 ^{15}N marked, were released over six days. Approximately 13,000 *Ae. aegypti* were collected over 15 trapping days following release (1,810 trap-nights). To date, 3,707 mosquitoes (2,992 females, 715 males), divided into 1,173 pools, have been analyzed for isotopic signature, corresponding to the first 8 trapping days. Among the analyzed pools, 147 were marked, 82 with ^{13}C and 65 with ^{15}N. Pools marked with ^{15}N had a mean δ^{15}N of 15.4 (SD = 3.1), whereas the average for reference mosquitoes was 8.2 (SD = 0.4). Using data from an ad-hoc laboratory experiment (not shown), we estimated that the 65 ^{15}N-marked pools contained 69 marked mosquitoes, 46 females and 23 males. The recapture percentage for ^{15}N-marked *Ae. aegypti* was 13.9%. The mean δ^{13}C of ^{13}C-marked pools was 527.2 (SD = 405.7), whereas the mean for the reference mosquitoes was -21.4 (SD = 1.2). The estimated number of marked mosquitoes in the 82 ^{13}C-marked pools was 118, consisting of 79 females and 39 males. The recapture percentage for ^{13}C-marked *Ae. aegypti* was 13.2%. The overall recapture percentage was 13.4% (187 individuals). Combining data from the two study areas, 67% of the recaptured individuals were females and 33% were males. Of the 125 recaptured females, 61% were trapped in BG Sentinels and 39% in GATs, whereas 9.7% of males were trapped in GATs, and 90.3% in BG Sentinels. Males showed a mean isotopic delta higher than females for both ^{15}N and ^{13}C enriched samples. The shift between the male and female distributions was statistically significant for ^{13}C (Wilcoxon signed rank-test, W=830, P<0.001) but not for ^{15}N (W=450, P=0.3).

The maximum distance at which a marked mosquito was recaptured was 636.3 m for males, 1-5 days after release, and 635.5 for females, 1-2 days after release. The timing of recaptures is reported as a range because releases continued over multiple days. The overall mean distance traveled (MDT) by recaptured mosquitoes from the release location was 285.0 m, with MDT of 292.5 m for males and 280.9 m for females. Females collected in GATs had a MDT of 316.8 m, whereas the MDT for females collected in BG Sentinels was 252.6 m. The average daily direction (clockwise from North) of dispersal ranged between 59° and 218°, with an overall average of 155.3°. The daily average wind direction and speed varied from 277° to 304° (WNW) and 6.3 to 10.8 kph, respectively. Wind speed had a regular cyclic pattern, generally peaking between 1600 and 1800 h (maximum speed of 15 kph) and being calm during the night.

DISCUSSION

The distribution of both δ^{13}C and δ^{15}N in positive control *Ae. aegypti* individuals had high precision, which enhanced our ability to discriminate between unmarked and marked individuals. The overall recapture percentage of 13.2% was slightly above the median for *Ae. aegypti* MR studies (except for some MR studies in equatorial latitudes, Guerra et al. 2014).

Results revealed that recaptured *Ae. aegypti* dispersed on average 285.0 m (MDT) from the release location, a distance farther than those typically reported in literature (e.g., Muir and Kay 1998, Harrington et al. 2005, Russell et al. 2005, Maciel-De-Freitas et al. 2007, but see Maciel-de-Freitas and Lourenço-de-Oliveira 2009). This result may be due to the optimal conditions during the MR study, performed when *Ae. aegypti* population had the highest fitness and when virtually no mosquito control activities performed within the study area. Overall, males dispersed farther than females, a trend reported previously for *Aedes albopictus* in urban areas (Medeiros et al. 2017). Among females, those collected in GATs, presumably seeking oviposition sites, were recaptured at greater distances (MDT = 316.8 m) compared to those collected in BG Sentinels (MDT=252.6 m). The greater recapture distances probably reflected a delay to oviposition after initial host-seeking and acquisition of at least one blood meal. Moreover, in urban contexts, the availability of hosts is high, whereas habitats suitable for oviposition may be more difficult to locate, requiring farther dispersal. Wind appeared to have strong effects on the direction of dispersal. In the study area, wind originated from the WNW, and the average dispersal direction of the recaptured *Ae. aegypti* was SSE. We found that mosquitoes dispersed mainly downwind or orthogonal to the wind direction when the daily average wind speed was higher than 6.4 kph. Below this wind speed, mosquitoes dispersed in all directions, a pattern previously reported (Clements 1992). Because releases occurred over multiple days, the wind-related dispersal pattern became harder to resolve as the study progressed. Among the recaptured individuals, a male was recaptured at 636.3 m from release point 1-5 days after release, opposite to the main wind direction and separated from the release site by a major road (about 20 m wide). We hypothesize that the movement of this male was likely the result of human transport, maybe in a motorized vehicle, which is a dispersal means that invasive *Aedes* have been known to use (Eritja et al. 2017).

CONCLUSION

Despite the relatively small size of the recapture areas (500 m radius), we found that marked *Ae. aegypti* dispersed rapidly and further than distances reported in earlier studies (Guerra et al. 2014), generally following the main wind direction and potentially exploiting human movements. Nevertheless, given the recapture of *Ae. aegypti* beyond the 500 m radius study area, the MDTs reported in the current study may still underestimate the true dispersal capacity of this species. These preliminary results have important implications for understanding *Ae. aegypti* invasion dynamics and containing the spread of potential outbreaks caused by *Aedes*-borne pathogens in urban areas of the southwestern U.S.

ACKNOWLEDGEMENTS

We thank Leonard Irby, Alex Scalzo, and the other staff of the Madera County Mosquito and Vector Control District for their support of this study. We also acknowledge funding support from National Aeronautics and Space Administration's Applied Sciences Program in Health and Air Quality (Grant NNX15AF36G) and 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).

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Closing the suitcase on Zika: The end of a two-year journey?

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INTRODUCTION

The first human cases of Zika virus (ZIKV) infection was reported from the Americas in May 2015 from Brazil. In the span of less than a year ZIKV had spread across South America, Central America, the Caribbean and parts of Mexico. As with other tropical diseases, travel-associated cases of Zika soon were reported from the United States, and local transmission was ultimately detected in both Florida and Texas. States such as California, with expanding infestations of Aedes aegypti and Ae. abopictus and close proximity to the Mexican border, are at ongoing risk for autochthonous ZIKV transmission. This presentation will review the epidemiological data for travel-associated Zika cases reported in California between November 2015 and September 2017 and discuss how that data can be used to evaluate cases of Zika and other exotic arboviruses in California.

METHODS

Enhanced surveillance methods have included: 1) review of human cases focusing on travel history, symptoms, outcomes, and demographics; 2) review of testing results from human cases tested at the CDPH Viral and Rickettsial Disease Laboratory (VRDL), local public health laboratories, and commercial testing facilities; and 3) assessment of risk for local transmission given co-location of *Aedes* mosquitoes and viremic human cases.

RESULTS

Between November 2015 and September 2017, California reported 589 confirmed and probable Zika infections, all contracted while traveling to a Zika affected area, through sexual contact with a Zika-infected returned traveler, or congenitally from a Zika-infected mother. An analysis of the data associated with these cases has identified the most frequent travel locations, testing methods, symptoms, and outcomes.

CONCLUSION

While the risk for autochthonous transmission of Zika remains low, several of the critical factors required for localized transmission now exist in California. Reviewing what we have learned about Zika can help inform planning and response for future outbreaks of exotic arboviruses and address the risk of local transmission.

Effect of temperature on the extrinsic incubation period of Zika virus in *Aedes aegypti*

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INTRODUCTION

Aedes aegypti, the primary vector of Zika virus (ZIKV), has established populations in California, leading to the potential for ZIKV transmission in the state (Pless et al. 2017). All published research to date on ZIKV transmission has been conducted under tropical conditions (26-28°C); however, this temperature range does not fully represent the potential for transmission in subtropical regions such as California. Understanding how geographic and seasonal variation in temperature may alter ZIKV transmission potential is crucial to determining mosquito-borne ZIKV transmission risk in California. To better understand how temperature affects ZIKV transmission in *Ae. aegypti*, we evaluated the extrinsic incubation periods (the time from ingestion of Zika virus to transmission, EIPs) in *Ae. aegypti* in the laboratory at 4 constant temperatures that approximate the range of temperatures to which *Ae. aegypti* is exposed throughout its geographic range.

METHODS

Female *Ae. aegypti* (F4, originally collected as eggs in Clovis, California) were fed on interferon-knockout mice infected with a ZIKV strain isolated from human serum in Puerto Rico in 2015 (PRVABC59) in accordance with UC Davis IACUC protocol #19404. Blood-fed mosquitoes were sorted into half gallon ice cream cartons (175-200 mosquitoes/carton) and held at one of 4 temperatures: 18°C, 22°C, 26°C, or 30°C with 80% humidity, 12:12 L:D cycle, and constant access to 10% sucrose. At timepoints bracketing transmission results in previous EIP studies using other flaviviruses (Chan and Johansson 2012; Reisen et al. 2006; Danforth et al. 2015), mosquitoes were cold anesthetized for 5 minutes, their legs and wings removed with forceps, and saliva collected using the capillary tube method (Aitken 1977). 20 mosquitoes were collected at each temperature/ timepoint combination. RNA was extracted from leg/wing, body, and saliva samples, and RT-qPCR was done to test for ZIKV RNA. Samples with a cycle threshold (Ct) of 38 or below were considered positive for ZIKV; this limit of detection was determined by prior testing using the same extraction and RT-qPCR reagents, protocols, and equipment (Stone et al. 2017). Positive bodies indicated ZIKV infection, positive legs/wings indicated virus dissemination from the midgut, and positive saliva indicated potential transmission.

RESULTS AND DISCUSSION

In total, 320 mosquitoes blood-fed and survived the duration of the study. ZIKV infection, dissemination, and transmission rates varied with temperature and time; dissemination and transmission rates increased over time at each temperature, except at 18°C, where overall dissemination and transmission was low (40% dissemination and 15% transmission at day 31 at 18°C). Dissemination increased from 55% at day 10 to 90% at day 25 (22°C), from 70% at day 5 to 100% at day 20 (26°C), and from 75% at day 3 to 100% at day 21 (30°C). Transmission increased from 5% at day 10 to 50% at day 25 (22°C), from 30% at day 5 to 95% at day 20 (26°C), and from 25% at day 3 to 100% at day 21 (30°C). The median time from ZIKV ingestion to transmission (median EIP, EIP_{50}) at each temperature was estimated by fitting a generalized linear mixed model. EIP_{50} ranged from 5.1 days at 30°C to 25.0 days at 22°C. At 26°C, EIP_{50} was 9.4 days. At 18°C, only 15% transmitted by day 31 so the EIP_{50} could not be estimated.

CONCLUSIONS

As expected, ZIKV extrinsic incubation period in *Ae. aegypti* varied with temperature. The EIP is shorter at high temperatures, and longer at low temperatures. Median EIPs for ZIKV in *Ae. aegypti* vary slightly from those of dengue in *Ae. aegypti* and West Nile virus in *Culex tarsalis* (Chan and Johansson 2012; Reisen et al. 2006; Danforth et al. 2015): at 30°C, the EIP_{50} of ZIKV is shorter (5.1 days) than for dengue and West Nile (~7 days), however at 26°C, EIP_{50} is more similar among all three viruses (~9 days West Nile, ~10 days dengue, 9.4 days ZIKV). At 22°C, ZIKV EIP_{50} in *Ae. aegypti* is longer (25 days) than EIP_{50} of West Nile (~16 days). This

information is critical for modeling ZIKV transmission dynamics and to understand geographic and seasonal limits of ZIKV risk. It is especially relevant for determining risk in subtropical regions with established *Ae. aegypti* populations such as California.

ACKNOWLEDGEMENTS

The authors thank Sarah Karels, Sunny Anh, and Jackson Stuart for their help rearing mosquitoes and preparing materials, Lark Coffey for her guidance, and Aaron Brault of the CDC for providing the ZIKV strain. Financial support for this study was provided by National Aeronautics and Space Administration's Applied Sciences Program in Health and Air Quality (Grant NNX15AF36G). CMB also acknowledges support from and 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). Mouse work was done in accordance with UC Davis IACUC protocol #19404.

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Vector competence of California mosquitoes for Zika virus

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ABSTRACT Zika virus (ZIKV) has recently emerged as a significant global threat to human health following outbreaks in Micronesia and rapid spread in the Americas in 2015 and 2016. Understanding which mosquito species transmit ZIKV efficiently and determining whether recent ZIKV strains are more infectious to mosquitoes is important for estimating regional outbreak potential and for prioritizing local mosquito control strategies. In this study, we present the results of vector competence studies of several mosquito species with the potential to become infected with ZIKV in California: *Aedes aegypti*, *Aedes albopictus*, *Aedes sierrensis*, *Aedes notoscriptus*, *Culex tarsalis*, and *Culex quinquefasciatus*. This work, supported by several MVCAC member agencies,showed that of the species studied, only *Ae. aegypti* and *Ae. albopictus* were competent for transmitting ZIKV.

Quantifying socioeconomic heterogeneities in the risk of local Zika and dengue outbreaks in California

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INTRODUCTION

Aedes aegypti, the primary vector of Zika virus, was first detected in Los Angeles County, California in 2014. Since that time, *Ae. aegypti* have continued to spread and have now been detected in 62 cities in the county. As of March 2018, 145 cases of Zika also have been identified in Los Angeles County, all of which were either acquired while traveling or via sexual transmission (California Department of Public Health, 2018). With Zika-infected travelers arriving at Los Angeles and other U.S. regions with *Ae. aegypti*, there is a need to understand the potential for local chains of transmission and whether socio-demographic factors can predict risk for Zika and other urban *Aedes*-borne viruses.

METHODS

During summer 2017, we surveyed 163 households in six communities in Los Angeles County: East Los Angeles, Boyle Heights, Commerce, La Mirada, Whittier, and Downey. At each household, we conducted surveys on lifestyle and household characteristics, and performed standardized 10-minute indoor and outdoor adult mosquito collections using a hand-held aspirator. The socio-demographic context of each household was determined using census tract data from the U.S. Census Bureau. We identified predictive variables associated with *Ae. aegypti* abundance at the household level using multiple Poisson regression models, adjusting for potential confounders. To determine whether predictor variables varied heterogeneously across cities enrolled in this study, we used the Kruskal-Wallis rank sum test.

RESULTS AND DISCUSSION

In total, 543 adult *Ae. aegypti* were collected from the participating households, with an average of 3.33 mosquitoes (range=0-35) collected per household. Adult *Ae. aegypti* were detected at 71.7% of households and were found indoors at 12.3% of households. We found that higher household income (measured as the median income per census tract) was associated with fewer *Ae. aegypti* outdoors but was not associated with indoor abundance. Among the household characteristics and lifestyle factors, we found that the density of containers holding water per residential property was a significant predictor for increased *Ae. aegypti* abundance both indoors and outdoors. We adjusted for median income per census tract, mosquito collector, and the average temperature for the previous seven days in the final models. The mean ranks of the density of standing water containers did not vary significantly among cities (Kruskal Wallis P-value = 0.20).

Our study was the second to find an association between low income and increased *Ae. aegypti* abundance in the continental U.S. In Tucson, Arizona, Walker et al. (2011) found an inverse relationship between income and *Ae. aegypti* abundance, suggesting that lower-income households provided more breeding habitat for mosquitoes. Along the Texas-Mexico border, however, *Ae. aegypti* abundance was significantly greater in the high-income neighborhoods on the U.S. side, although dengue transmission was greater in the low-income neighborhoods of Mexico (Reiter et al. 2003). These apparent contradictions suggest that the relationship between socioeconomic status and *Ae. aegypti* abundance could vary regionally in the U.S.

CONCLUSIONS

We found that low income was associated with increased abundance of *Ae. aegypti* in Los Angeles County. We hypothesize that this relationship is driven by heterogeneities in lifestyle factors and household characteristics. We found that the household variable most closely associated with the abundance of *Ae. aegypti* was the density of outdoor water-holding containers . This suggests that small properties in low income areas with high numbers of containers of standing water are at risk of having higher abundance of *Ae. aegypti* and should be targeted for source reduction and vector control in Los Angeles.

ACKNOWLEDGEMENTS

We thank undergraduate assistants Tiffany Tan (UC Irvine), Khin KyiSin (UCLA), and Chelsea Galicia (Whittier College) for their help with household surveys. We also thank the staff at the Greater Los Angeles County Vector Control District (GLACVCD) for their logistical support and laboratory space. We also thank Harold Morales, Tanya Posey, and Steve Vetrone from the GLACVCD for their help with mosquito identifications. We especially thank Susanne Kluh at the GLACVCD for overseeing field work and logistics that made this study possible. This research was

supported by the National Aeronautics and Space Administration's Applied Sciences Program in Health and Air Quality (Grant NNX15AF36G) and funding to CMB from the School of Veterinary Medicine, UC Davis. CMB also acknowledges support from and 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). This study was exempt from IRB under exemption 2.

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West Nile Virus in California: A Summary of the First 15 Years of Human Infections

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ABSTRACT The first human case of West Nile virus (WNV) in California was reported in Riverside County in 2003. Since 2003, there have been more than 6000 human infections in the state, posing a significant burden on medical and public health systems during arboviral transmission season. From 2003-2015 (the latest date for which nationwide data are available), California has contributed approximately 12% of the national burden of WNV human cases, ranging from a low of 6.5% in 2006 to 36.3% of the national WNV case burden in 2015. This presentation reviews the epidemiological data for West Nile virus infections in California between 2003 and 2017 and discusses how these data can be used to minimize the risk of West Nile virus human transmission in future seasons.

Trials and Use of New Technology to Improve Surveillance and Control of Mosquitoes at the Salt Lake City Mosquito Abatement District

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ABSTRACT For a mosquito control district to stay efficient and effective it is important to constantly strive to make improvements. New technologies and innovations have been appearing in the mosquito control field as well as other sectors. In the past year the Salt Lake City Mosquito Abatement District (SLCMAD) performed several trials to look at ways of improving surveillance and control of mosquitoes. Some of the trials included designing new adult mosquito traps, trying new traps designed by other researchers, experimenting with different sources of CO2, determining if mosquito populations that developed resistance to *Lysinibacillus sphaericus* pesticide increased in susceptibility after a year of using different active ingredients, and determining the feasibility of new hetero-dissemination techniques. New technologies incorporated at SLCMAD in 2017 included unmanned aerial vehicles and 3-D printers. Several of these trials with new technologies gave very promising results for improving the work at SLCMAD.

Evaluation of Biogents Sentinel 2 and Biogents-Bowl Traps to Collect *Aedes sierrensis* Mosquitoes in an Suburban Setting in the Greater Salt Lake Area of Utah

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ABSTRACT Salt Lake City Mosquito Abatement District (SLCMAD) conducted trials with Biogents traps (BGS-2 and BG-Bowl) to determine the efficiency of the traps at collecting the Western Treehole Mosquito, *Aedes sierrensis* (Ludlow). This mosquito is a major nuisance to residents, causing many service requests at SLCMAD and is also a vector of *Dirofilaria immitis*, dog heartworm. Trap styles and lure combinations included: (1) BGS-2 (Biogents) trap with BG lure; (2) BGS-2 trap with BG lure and CO_2; (3) BG-Bowl trap with BG lure; and (4) Mosquito Magnet traps with CO_2. Mosquito Magnet traps were used as controls, as these traps were used in the past in by SLCMAD to collect *Ae. sierrensis* mosquitoes to reduce biting pressure to residents. The traps were set once a week at three different locations with historically high numbers of *Ae. sierrensis.* Traps were positioned at least 30 feet apart from each other at each site, and each week the traps were rotated through the four different areas within each site so that trap placement would not bias the average mosquito catch results. The trial lasted for 12 weeks. Results showed that the traps which used CO_2 as an attractant, the BGS-2 trap with BG lure and with CO_2 and the Mosquito Magnet traps were the most effective at collecting *Ae. sierrensis* mosquitoes. Our results show that the BGS-2 trap may be an efficient collection tool for the surveillance of *Ae. sierrensis.*

Patterns of virus activity in the Coachella Valley

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ABSTRACT The Coachella Valley Mosquito Control District (CVMVCD) began controlling mosquitoes in 1955, following the completion of the All American Canal bringing water to the area for farming. Since then, the District has had endemic cycles of Western equine encephalomyelitis virus, St. Louis encephalitis virus (SLEV), and West Nile virus (WNV). Since 2015, the District has detected SLEV in mosquito samples regularly after not detecting this virus since 2003. This report discusses the patterns of co-circulating WNV and SLEV, and using previous work on virus activity, discuss how having two viruses in circulation may impact control efforts in the Coachella Valley.

Analysis of West Nile Virus activity and correlation with varying levels of drought in San Joaquin County, CA, from 2004-2017

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ABSTRACT Changes in climatic conditions may have an effect on the activity of West Nile Virus (WNV) and mosquito abundance. It is known that temperature plays an important role in the amplification of WNV but another type of climate condition which severely affected the state of California over a several year period is drought. San Joaquin County which is made up of agricultural land, waterways, as well as urban areas, experienced various levels of drought since the arrival of WNV in 2004 as well as fluctuations in WNV activity and mosquito abundance. To determine if there may be a correlation between drought, WNV activity, and mosquito abundance, in-house testing results from San Joaquin County Mosquito and Vector Control District were reviewed from 2004 to 2017 and compared to the drought levels of the respective years.

INTRODUCTION

Climatic conditions may play an important role in the activity of West Nile Virus (WNV). Temperature may affect WNV by increasing the rate of mosquito development and viral replication (Reisen et. al. 2006) but another condition which commonly affects the state of California is drought. The National Oceanic and Atmospheric Administration (NOAA) defines drought as "a deficiency in precipitation over an extended period, usually a season or more, resulting in a water shortage causing adverse impacts on vegetation, animals, and/or people." The state of California periodically experiences periods of drought which may last for several years and are characterized by low reservoir levels, low ground water levels, and reduced volume and velocity in rivers and streams. Although drought conditions may result in a decrease in mosquito abundance due to the reduction of mosquito oviposition and larval sites, conditions favorable for mosquitoes and West Nile Virus (WNV) also may be created which did not exist during normal or wet years and potentially may result in an increase of WNV activity (Paull et. al. 2017).

The lack of rainfall, reduced snowpack, and resulting snowmelt can contribute to slower-moving rivers and streams, resulting in the creation of new breeding sources through pooling. Normally-persistent water sources stocked with *Gambusia affinis,* mosquito fish, may have a decrease in water levels and an increase in organic content, killing off the mosquito fish. In agricultural areas, although water usage may be less frequent, flood irrigation can still provide mosquito breeding sources. In urban areas, even when water restrictions are in place, landscape may be overwatered and items in the vicinity of sprinkler systems as well as catch basins can be filled with water and serve as an oviposition site. In many rural areas, natural water sources may be limited which can result in the congregation of birds and wildlife to the few water sources available, putting hosts in closer proximity to the vectors which may feed on them, improving success of the mosquito as well as WNV transmission.

San Joaquin County has experienced several drought years since the arrival of WNV in 2004, with drought conditions taking place between 2007 and 2011 and most recently, between 2012 and 2016. The county is located in the Central Valley of California and due to being comprised of agricultural land and urban areas, both its farmers and urban residents were subject to water restrictions such as a reduction in usage and designated water-use days during the droughts. San Joaquin County is also traversed by many rivers and waterways which are influenced by precipitation, snowmelt, and upstream reservoir releases, variables which can affect drought status in the valley. As all of these conditions may have an effect on mosquitoes and WNV activity, this paper takes a retrospective look at data from 2004 to 2017 and the corresponding drought conditions to determine if there may be a correlation between drought and WNV activity in San Joaquin County.

METHODS

A self-calibrated Palmer Drought Severity Index (PDSI) was obtained from the Western Regional Climate Center which quantified the level of drought in San Joaquin County for each water year (Fig. 1). The PDSI designates a negative number for drier-than-average or drought conditions and a positive number for above-average or wet conditions, with average conditions identified by a value of zero. Pearson's correlation coefficient was used to calculate the correlations between PDSI values and measurements of WNV activity collected during each mosquito season.

During the months of April through the end of October, dead

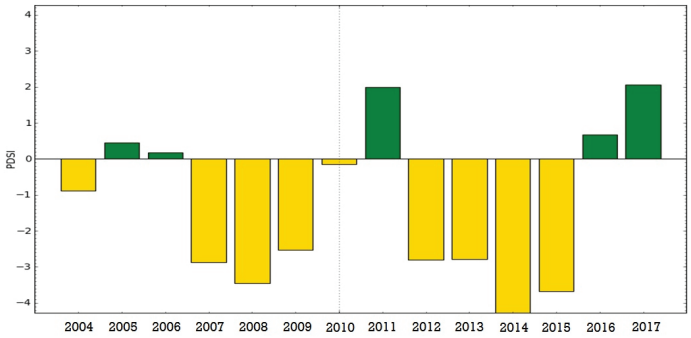

Figure 1 Palmer Drought Severity Index (PDSI) for San Joaquin County, CA for 2004-2017

birds reported by the public are retrieved by the San Joaquin County Mosquito and Vector Control District (District) and mosquitoes are collected throughout San Joaquin County for WNV testing. Encephalitis Virus Surveillance (EVS) and gravid traps were deployed for mosquito collections and from each trap, *Culex pipiens* and *Culex tarsalis* mosquitoes were pooled into groups of five-50. Mosquito pools are tested in-house by RT-qPCR for WNV, with samples prior to 2010 tested in-house by RAMP®. For dead birds, brain, ocular or oral samples were taken and tested in-house, with birds prior to 2013 tested by UC Davis. All testing results were entered into the CalSurv Gateway database. For retrospective analysis, mosquito species abundance data, WNV testing results, and minimum infection rates (MIR) from San Joaquin County for the years 2004 – 2017 were obtained from the CalSurv Gateway database and used in Pearson's correlation calculations with PDSI.

RESULTS AND DISCUSSION

A Pearson's correlation coefficient was calculated to determine whether there was a relationship between PDSI and WNV activity by using data for WNV positive birds, mosquitoes, human cases, and the MIR. The total number of WNV positive birds per year and the corresponding PDSI values demonstrated a possible inverse relationship (Fig. 2), with a Pearson's correlation coefficient (r) value of -0.43 ($p > 0.05$); indicating a possible moderate negative correlation, where the number of WNV positive

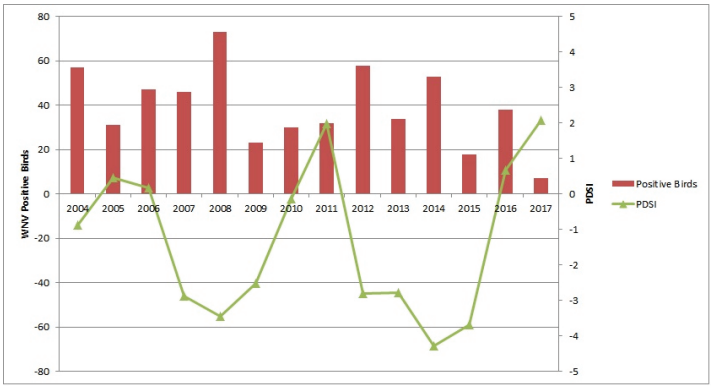

Figure 2 West Nile Virus (WNV) positive birds and Palmer Drought Severity Index (PDSI) in San Joaquin County, CA, from 2004-2017

birds was higher in years when PDSI values were lower due to drought. The total number of West Nile Virus positive mosquito pools per year and corresponding PDSI also demonstrated a possible weak inverse relationship (Fig. 3) with a Pearson's correlation coefficient (r) value of -0.23 ($p > 0.05$). The MIR and PDSI also demonstrated a possible inverse relationship, with a weak negative correlation of -0.34 ($p > 0.05$) (Fig. 4). However, there is likely no correlation between the number of human cases per year and PDSI as the Pearson's r-value was 0.05 ($p > 0.05$) (Fig. 5); this suggests that although WNV activity may be higher in the typical vector-host transmission cycle during drought years, this may not result in increased WNV transmission to humans. As mosquito control treatments are scheduled in response to collection and testing results, efficient mosquito control likely plays an important role, quickly reducing the public's health risk even when vector potential is high in the population.

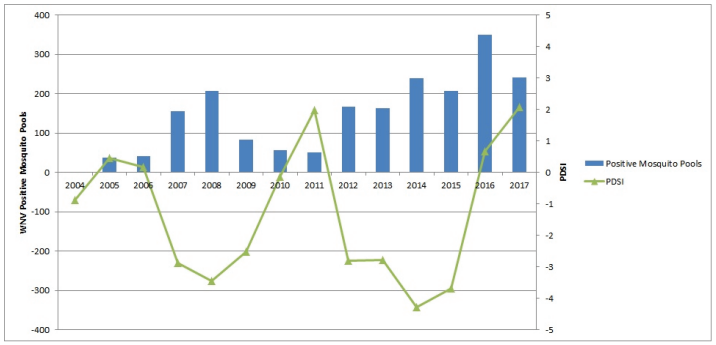

Figure 3 West Nile Virus (WNV) positive mosquito pools and Palmer Drought Severity Index (PDSI) in San Joaquin County, CA, from 2004-2017

CONCLUSION

Pearson's correlation determined that in San Joaquin County there may be a moderate negative relationship between drought as measured by the PSDI and WNV in dead birds, with more cases of WNV positive birds during years of drought. There also was a possible weak negative relationship between drought and WNV positive mosquitoes and the MIR, indicating that there may more WNV positive mosquito pools and a higher MIR during drought years. There was no correlation found between drought and the number of human WNV cases per year. These data suggest that WNV activity in the natural host-vector transmission cycle may be greater in drought years; however, this does not always result in more human cases. It is likely that responsive and efficient mosquito control practices play an important role in preventing transmission to the public during drought years when vector potential may be higher. With the possibility of drought conditions occurring more frequently, it will be important for mosquito and vector control to maintain its efficiency and responsiveness in order to protect the public.

Figure 4 Minimum Infection Rate (MIR) and Palmer Drought Severity Index (PDSI) in San Joaquin County, CA, from 2004-2017

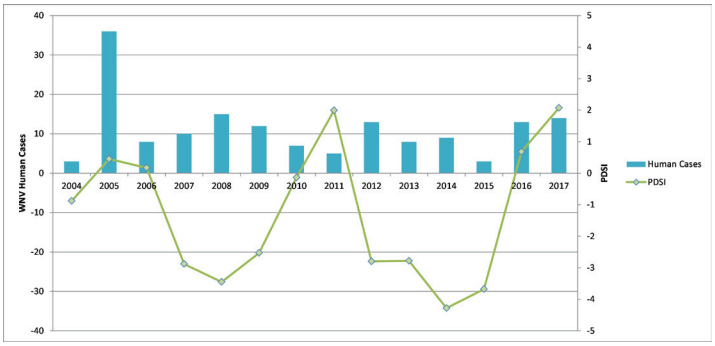

Figure 5 West Nile Virus (WNV) human cases and Palmer Drought Severity Index (PDSI) in San Joaquin County, CA, from 2004-2017

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Current West Nile virus dead bird surveillance practices in the United States

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ABSTRACT West Nile virus (WNV) dead bird testing is an important component of WNV surveillance, and along with mosquito surveillance, focuses mosquito control efforts in time and place. Nevertheless, dead bird testing has decreased since the initial spread of WNV. We investigated WNV surveillance practices in each state to better understand the role of dead bird testing in WNV surveillance throughout the United States today. Thirty-three out of the forty-eight contiguous states with WNV (Alaska and Hawaii have not detected WNV) currently test dead birds for WNV surveillance, although involvement ranges from occasional testing to large-scale integrated testing efforts accompanied by public outreach. After speaking with officials from nearly all 48 continental states, the authors conclude that despite limited resources and diminished interest, dead birds remain a useful WNV surveillance tool in many U.S. states.

A Look at Trap Height and Its Effects on *Culex tarsalis* Abundance and Minimum Infection Rates

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ABSTRACT *Culex tarsalis* is a known vector of West Nile virus (WNV) in California. Studies done in the northern United States have examined the effects of trap height on the number of mosquitoes collected and Minimum Infection Rates (MIR). However, no studies have compared the effect of trap height on MIR for *Cx. tarsalis* in California. Two locations in Lake County, CA were compared during this study: a riparian corridor and an oak woodland. Each site had a triplicate of dry ice-baited traps per 1.5m height and 4m height. The study compared the number of *Cx. tarsalis* and the MIR for each location and trap height.

The vector index: improving the evidence basis for adult mosquito control

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INTRODUCTION

The vector index (VI) is used to estimate the relative abundance of arbovirus-infected mosquitoes within an area as the product of two commonly monitored quantities, mosquito trap counts and mosquito infection prevalence (Gujral et al. 2007). The VI is used increasingly to guide control programs as an entomological indicator of human infection risk for arboviruses, especially for zoonotic pathogens such as West Nile virus (WNV) (Kilpatrick and Pape 2013). However, there is no established VI threshold below which transmission to humans would not be expected. Our study characterized the relationship between VI and human WNV disease incidence and identified optimal thresholds for broad-scale mosquito control actions.

METHODS

Using surveillance data from several collaborating mosquito control agencies spanning northern and southern California, we considered whether the VI could accurately predict the occurrence of human cases over a subsequent three-week period. We performed the analysis for a range of spatial scales to determine whether VI is capable of providing predictions of human WNV disease incidence at scales fine enough to be actionable for mosquito control. We used Poisson regression models to adjust for demographic factors, and we compared VI thresholds using receiver-operating characteristic (ROC) curves.

RESULTS AND DISCUSSION

Overall, a higher VI was associated with elevated incidence of WNV disease in all counties. The positive predictive value (PPV) of the final model using city-level data was 34%, indicating that cases did occur during three-week periods when cases were predicted approximately one-third of the time. Adjusted for demographic factors (percentage of the population over 65 years of age and percent male) resulted in improved prediction of case occurrence compared to VI alone. Predictions were more reliable at coarser spatial scales (census county division, mosquito control district, or county), but these scales were too coarse for targeted implementation of mosquito control. The resulting actionable VI threshold ranged from 0.178 to 4.015 infected female mosquitoes per trap-night for a typical city with a population of 55,000 and 10% of the population over age 65, highlighting the variability in this relationship depending on a county's ecology or differences in approaches to mosquito control and surveillance. These results expand on the findings of other studies showing that VI is predictive of human WNV disease occurrence (Kwan et al. 2012) and human WNV disease incidence (Kilpatrick and Pape 2013; Colborn et al. 2013).

CONCLUSIONS

There was an overall association between a higher VI and an elevated incidence of WNV disease in humans. After applying models to adjust for demographic factors, predictions of human disease occurrence were most reliable and actionable at the city scale. Additionally, there was heterogeneity in the VI thresholds that maximize concordance between the predicted and observed occurrence of cases among counties, which is likely attributable to differences in local ecology and in control responses among the corresponding MVCDs.

ACKNOWLEDGEMENTS

The authors would like to thank Robert Cummings of Orange County MVCD for coordinating the funding for this study and Jacklyn Wong and Anne Kiemtrup, Vector-Borne Disease Section, California Department of Public Health for providing the data on cases of WNV disease in humans. We acknowledge funding from Orange County, Greater Los Angeles County, San Gabriel Valley, Sacramento-Yolo, Placer, and Turlock MVCDs. CMB also acknowledges support from and 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).

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Comparison of MagMAX™-96 Viral RNA Isolation Kit with MagMAX™ Core Nucleic Acid Purification Kit in RT-qPCR to detect WNV, SLEV and WEEV in mosquitoes

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ABSTRACT The use of RT-qPCR (reverse transcription quantitative polymerase chain reaction) has become more accessible and therefore, an increasing number of vector control agencies are beginning to do in-house testing to detect mosquito-borne viruses. There are several available reagent kits on the market for both extraction and PCR. Here we evaluated and compared two RNA extraction kits for the detection of WNV, SLEV, and WEEV, the original MagMAX™-96 Viral RNA Isolation kit (MagMAX™-96 Viral) and the new MagMAX™ Core kit. The MagMAX™-96 Viral extraction kit is the current kit that has been optimized and used in our PCR laboratory. The new MagMAX™ Core kits recently has been released by Life Technologies (now Thermo Fisher Scientific). Results using the new MagMAX™ Core kits for PC1 (SLEV, WEEV and WNV mixture), and the 10^1 pfu/ml concentration of SLEV had higher C_t values when compared to samples extracted with the original MagMAX™-96 Viral kit. The new MagMAX™ Core kit samples also showed a trend of having higher C_t values for samples that had been stored for 3 – 4 months at -80°C. At this time, we recommend that laboratories that are currently using the MagMAX™-96 Viral RNA Isolation kit should continue to do so.

INTRODUCTION

Vector surveillance and pathogen detection are essential for assessing the risk of vector-borne diseases. Therefore, vector control agencies routinely monitor local mosquito species, population abundance and pathogen infection. Routine surveillance of disease occurrence in humans, dead birds and sentinel animals, along with mosquito activities such as population abundance and distribution, are used by vector control agencies to make decisions in control operations. Currently there are three arboviruses that are monitored in southern California, which include West Nile virus (WNV), St. Louis encephalitis virus (SLEV), and western equine **encephalomyelitis** virus (WEEV). All three viruses are endemic within California and occasionally cause seasonal outbreaks, typically during summer months.

Since its introduction in 2003, WNV has been consistently detected within both dead bird and mosquito samples, while both WEEV and SLEV historically exist at much lower prevalence. WEEV has not been detected since 2007 in California (Feiszli et al. 2008). However, SLEV was recently detected in 2015 in the Coachella Valley for the first time since 2003 (White et al. 2016) and continues to be detected on a regular basis. The reemergence of SLEV highlights the importance of testing for viruses such as WEEV, even if their detection has not been observed in recent years.

Quantitative Polymerase Chain Reaction (qPCR), due to its high sensitivity and specificity compared to immunoassays, is an ideal tool for pathogen detection (Su and Cheng 2012). Additionally, in recent years, qPCR has become more accessible, affordable and feasible for most laboratories. Therefore, many mosquito districts now do in-house testing using qPCR. Currently there are several kits of reagents for both nucleic acid extraction and qPCR amplification. In general, kits are usually uniform and consistent; however, there may be slight differences between kits and/or manufacturers. Additionally, it is essential to use the kits that are best optimized for the system and protocols currently in use within the laboratory. In the current study, we compared the original MagMAX™-96 Viral RNA Isolation kit, which is the current extraction kit used in our PCR laboratory, to the recently released MagMAX™ Core Nucleic Acid Purification kit.

MATERIALS AND METHODS

RNA extraction was conducted using the original MagMAX™-96 Viral RNA Isolation kit (MagMAX™-96 Viral) (Ambion AMB1836-5) or the new MagMAX™ Core Nucleic Acid Purification kit (MagMAX™ Core kit) from applied Biosystems (Life Technologies, now Thermo Fisher Scientific) according to the manufacturer's protocols. RNA was extracted from lysates of WNV, SLEV and WEEV. Positive control standards of WNV, SLEV, and WEEV were provided by the Davis Arbovirus Research

Table 1 Known West Nile virus positive mosquito pools from 2017 chosen for a comparison between original MagMAX™-96 Viral RNA Isolation and new MagMAX™ Core extraction kits. Original Ct values were from samples extracted using the MagMAX™-96 Viral RNA Isolation kit.

Mosquito Pool Number	West Nile Positive Original C_t
17-1401	28.15
17-1400	23.79
17-1402	16.61
17-1934	25.23
17-2066	19.5

and Training (DART), at University of California, Davis. For triplex qPCR, a mixture of SLEV, WEEV and WNV from the DART panel was used as positive control 1 (PC1) [Vial 6 (WNV): Vial 5 (SLEV + WEEV) = 1:1] (Su 2017). SLEV and WNV samples containing 10^6 PFU/ml of inactivated virus were diluted to 10^1 PFU/ml to evaluate the ability to detect low virus copy numbers after extraction by both kits. Known positive mosquito pools from 2017 samples with C_t values ranging from 16.61 to 28.15 were used (Table 1). One set of mosquito pools were known positives from the same week of testing (pool #1400-1402). The second set of mosquito pools were known positives from 2017 that had been stored in a -80°C freezer for 3 – 4 months (pools 1934, 2066, 2136).

Mosquito pools were homogenized in 800 µl of PBS (1x) for 5 min at 3,000 rpm by Tissue Disruptor Genie (Scientific Industries; Bohemia, NY) and centrifuged for 5 min at 15,000 rpm at 4° C (Eppendorf Centrifuge 5452R; Hauppauge, NY). Lysates of WNV, SLEV and WEEV were used as received from DART or diluted using nuclease-free water. All extractions were eluted in 50 µl of elution buffer.

For samples extracted using the original MagMAX™-96 Viral kit, a deep well extraction plate was loaded with 20 µl of bead mixture (beads: lysis/binding enhancer = 1:1). To the bead mixture, 70 µl of sample were added, followed by 131µl of lysis/binding solution (65 µl of lysis/binding concentrate + 65 µl of isopropanol + 1 µl carrier RNA). Wash plates consisting of two each of Wash 1 and Wash 2 were loaded with 150 µl per well. Washing solutions were prepared by adding isopropanol (Wash 1) or ethanol (Wash 2) to the wash concentrate according to the manufacturer's instructions. Extractions were then processed using the MagMAX™ Extraction machine system according to manufacturer's protocols.

For extractions processed with the new MagMAX™ Core kit, a deep well plate was loaded with 30 µl of bead mixture (beads: proteinase K = 2:1). To that, 70 µl of sample was added followed by 700 µl of lysis/binding solution (350 µl of lysis concentrate + 350 µl of binding concentrate). Wash plates consisting of one plate of each of Wash 1 and Wash 2, were loaded with 500 µl per well. Extractions were then processed using the MagMAX™ Extraction machine system according to manufacturer's protocols.

After elution, qPCR was carried out in a separate room. In total, 10 µl of template extract was added to 15µl of qPCR mixture. The qPCR mixture contained the following: 2.5 µl of RNAse free water, 2.5 µl each of forward and reverse primer(s) (10K pmol/ml) (Table 2) (Brault et al., 2015), 6.25 µl of Taqman 1-Step Fast Viral Master Mix, and 1.25 µl of probe(s) (6K pmol/ml). Controls included PC1, NC1 and NTC1 (as previously described). Additionally, a PC2, NC2 (previously extracted PC1 and NC1) and NTC2 (nuclease-free water) were also included. The PCR plate was then sealed and centrifuged for 2 min at 500 rpm to remove air bubbles. The sealed PCR plate was loaded in the 7500 Fast RT-PCR system for amplification using the following protocol: Holding Stage I (50°C x 5 min), Holding Stage II (95°C x 20 seconds), Cycling (95°C x 3 seconds, 60°C x 30 seconds) for 40 cycles (Su 2017).

RESULTS AND DISCUSSION

For the extractions of PC1 containing all three targets (SLEV, WEEV and WNV mixture, and the 10^1 pfu/ml concentration of SLEV, the C_t values of the new MagMAX™ Core kit extractions ranged from 0.88 – 2.43 higher for the new MagMAX™ Core kit when compared to the original MagMAX™ 96 – Viral kit (Table 3) (Paired *t*-test $P < 0.05$; $n = 4$). For 10^1 pfu/ml concentration of WNV, the C_t values were slightly lower for the new MagMAX™ Core kit extractions when compared to the MagMAX™ 96 – Viral kit (Table 3) (Paired *t*-test $P = 0.034$; $n = 4$). The PC1 extraction results for SLEV also showed significantly higher C_t values in the new MagMAX™ Core kit ranging from 3.00 – 4.08 C_t values higher in comparison to the MagMAX™ 96 – viral (Table 3) (Paired *t*-test $P = 0.0011$; $n = 4$). The new MagMAX™ Core kit has a reduced amount of washes, with only one wash 1 and one wash 2. Possibly this reduction in wash steps was not sufficient in removing all inhibitors from the RNA sample, thus inhibiting amplification and increasing C_t values.

Table 2 Sequences for primers and probes, dyes and quencher for probes for WNV, SLEV and WEEV.

	WNV
Forward primer	5'-TCA GCG ATC TCT CCA CCA AAG-3'
Reverse primer	5'-GGG TCA GCA CGT TTG TCA TTG-3'
Probe	**6FAM**-TGC CCG ACC ATG GGA GAA GCTC-**QSY**
	SLEV
Forward primer	5'-CTG GCT GTC GGA GGG ATT CT-3'
Reverse primer	5'-TAG GTC AAT TGC ACA TCC CG-3'
Probe	**VIC**-TCT GGC GAC CAG CGT GCA AGC CG-**QSY**
	WEEV
Forward primer	5' – AGG TAA ACT GCA CAT TCC ATT CC - 3'
Reverse primer	5' – TTC GTG ACT GTA GGC GTG TGA - 3'
Probe	**ABY**-CCG ACA GTC TGC CCG GTT CCG-**QSY**

Table 3 Comparison of Ct values between original MagMAX™-96 Viral RNA Isolation and new MagMAX™ Core extraction kits for WNV, SLEV, and WEEV lysate standards from DART. Positive difference in Ct values indicates higher Ct values in RNA extracted with the new MagMAX™ Core Kit samples. Negative difference in Ct values indicate lower Ct values in MagMAX™ Core Kit samples (For all samples, paired t-test $P < 0.05$; $n = 4$).

	MagMAX™-96 Viral RNA Isolation	Core Kit	Diff. in C_t
SLEV in WSW mixture			
Test 1	21.93	24.95	3.03
Test 2	25.68	28.67	3.00
Test 3	23.70	27.78	4.08
Test 4	24.80	27.85	3.05
WEEV in WSW mixture			
Test 1	26.03	27.01	0.98
Test 2	28.30	29.76	1.45
Test 3	26.85	29.02	2.17
Test 4	27.99	29.85	1.87
WNV in WSW mixture			
Test 1	25.69	26.57	0.88
Test 2	26.93	28.78	1.85
Test 3	22.03	24.46	2.43
Test 4	27.56	29.41	1.85
WNV 10^1 (PFU/ml)			
Test 1	30.59	30.22	-0.38
Test 2	27.04	26.46	-0.58
Test 3	28.44	27.68	-0.76
Test 4	27.76	27.59	-0.17
SLEV 10^1 (PFU/ml)			
Test 1	29.98	31.99	2.01
Test 2	28.82	30.22	1.40
Test 3	30.94	32.04	1.10
Test 4	27.99	29.76	1.77

Additionally, positive mosquito pools from 2017 were used for comparison. These pools were tested to compare "real world" samples to evaluate how each extraction kit performed when biological materials, such as mosquito DNA, RNA and proteins, were present. Mosquito pools 1400 – 1402 had been stored in a -80°C freezer for less than one week prior to use (Table 1). The C_t values between the extraction kits for these pools appear to be very similar, with less than 0.52 difference between C_t values (Table 4) (Paired *t*-test $p > 0.05$; $df = 0$). Mosquito pools 1934, 2066, and 2136, were stored at -80°C for 3 – 4 months before use in this comparison. For both extraction kits, the C_t values were higher than when the pools were first tested (Table 4). Additionally, the difference of C_t values between the two kits were different. For the high and middle C_t value pools, 1934 (C_t 25.23) and 2066 (C_t 19.5), respectively, the new MagMAX™ Core kit extraction samples were 3.72 – 5.41 C_t values higher than the MagMAX™ 96-viral kit extraction samples (Table 4) (Paired *t*-test $p > 0.05$; $df = 1$). Between the two extraction kits, it appears that the new MagMAX™ Core kit has a trend to give slightly elevated C_t values compared to the original MagMAX™ 96-viral extraction samples, particularly for older samples. However, more replications need to be done for statistical analysis.

Although RNA is typically stable at -80°C, there may have been slight RNA degradation within the stored mosquito pool homogenate, which could account for the higher C_t values (Shabihkhani et al. 2014). Furthermore, as the positive mosquito pools were stored as mosquito homogenates rather than pure extracted RNA, there may be RNAses that were present within the mosquito tissues that may have contributed to possible RNA degradation. There is also the possibility that the additional freeze-thaw cycle may have contributed to the degradation of RNA (Hu et al. 2017, Yu et al. 2017). Additionally, although the -80°C freezer the samples were stored in is designed to maintain constant temperature, slight temperature variations may have occurred, resulting in slight RNA degradation. Between the two kits, the original MagMAX™ 96-viral kit produced lower C_t values than the new MagMAX™ Core kit for samples that had been stored for a few months. However, the C_t values were equivalent between the two kits for samples stored for less than one week.

In conclusion, the new MagMAX™ Core kit tended to have higher C_t values than the MagMAX™ 96-viral kit. This was particularly noticeable for SLEV, both when combined with WNV and WEEV in PC1 and when diluted to 10^1 pfu/ml. There was also a trend for both kits to have higher C_t values when retesting older samples. However, the new MagMAX™ Core kit produced markedly higher C_t values compared to original MagMAX™ 96-viral kit values for samples that had been stored for more than 3 months. This could possibly become an issue should any sample that tested positive with a high C_t value need to be retested as the new MagMAX™ Core kit might not extract sufficient RNA. The MagMAX™ Core kit does offer convenience with the inclusion of ready-to-use wash solutions and quicker processing times due to decreased wash steps. However, more reagent is used in the MagMAX™ Core kit protocol than the MagMAX™ 96-viral kit.

Ideally, more mosquito pool samples should be tested to further confirm the performance of the MagMAX™ Core kit. Additionally, variations on storage temperature and length of storage time would give a better understanding on the extraction efficiency of the MagMAX™ Core kit. However, with the current data, it is recommended for laboratories that have already optimized their RT-PCR protocol with the original MagMAX™ 96-viral kit to continue with this extraction method.

ACKNOWLEDGEMENTS

The authors are grateful to Mr. Justin Liao and Mr. Chris Sabin with Applied Biosystems (later Thermo Fisher Scientific) for their support. Additionally, the authors duly acknowledge the valuable help from UC Davis DART, and constructive discussion with Dr. Shaoming Huang with San Joaquin County Mosquito and Vector Control District.

Table 4 Comparison of Ct values between original MagMAX™-96 Viral RNA Isolation and new MagMAX™ Core extraction kits for known WNV positive mosquito pools collected during 2017. Original Ct values were from samples extracted using the MagMAX™-96 Viral RNA Isolation kit. Positive difference in Ct values indicate higher Ct values in MagMAX™ Core extraction kit samples. Negative difference in Ct values indicate lower Ct values in MagMAX™ Core extraction kit samples.

Pool #		Original Ct	MagMAX™-96 Viral RNA Isolation	MagMAX™ Core Kit	Diff. in Ct
17-1402		16.61	16.44	16.86	0.41
17-1400		23.79	23.19	23.70	0.52
17-1401		28.15	28.59	28.29	-0.29
17-2136	Test 1	9.61	15.45	17.69	2.24
	Test 2	9.61	15.45	17.36	1.91
17-2066	Test 1	19.50	22.05	27.13	5.09
	Test 2	19.50	22.39	26.11	3.72
17-1934	Test 1	25.23	27.24	32.65	5.41
	Test 2	25.23	28.14	31.80	3.65

*For statistical analysis additional tests are needed.

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Comparing a Commercial Kit versus Standard Guanidine Thiocyanate Extraction Protocols for Nucleic Acid Extraction and Purification

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ABSTRACT Accurate detection of arthropod-borne pathogens from field-collected specimens is a central component of vector control programs and detecting pathogen RNA or DNA using polymerase chain reaction (PCR) is probably the most common method for detecting pathogens. Many public health testing facilities utilize commercially available RNA/DNA extraction kits for isolating and purifying RNA/DNA, because they offer ease of use and reliability; however, these kits are also relatively expensive. Most commonly used commercially available RNA/DNA extraction kits are based on standard guanidine thiocyanate extraction protocols that were developed several decades ago in academic or other public research laboratories (i.e. Chomczynski and Sacchi. 1987). Here, we compare a commercially-available RNA/DNA extraction kit with a typical guanidine thiocyanate extraction protocol from the literature and found little difference in results from subsequent RT-PCRassays. Utilizing standard guanidine thiocyanate extraction protocols thus appears to offer equivalent results but with tremendous cost saving versus commercially-available kits.

INTRODUCTION

Accurate detection of pathogens depends upon extracting RNA and DNA of sufficient quantity and quality for subsequent RT-PCR assays. There are numerous methods for isolating and purifying RNA/DNA including alcohol precipitation, spin columns etc. but using paramagnetic beads in conjunction with chaotropic salts is a widely used method for isolating nucleic acids (Lanciotti et al. 1992; Tan et al. 2009).

Extraction protocols based on chaotropic salts, typically guanidine thiocyanate (GT), in combination with solid phase reversible immobilization (SPRI) paramagnetic beads are highly efficient and commonly used by public health agencies. Commercially available SPRI-based viral RNA extraction kits utilizing GT chemistry (hereafter: kit) are widely used because of their ease of use and reliability, but can be relatively expensive and offer little flexibility for experimentation and problems arising from contamination or accidental spillage, because kits contain individually packaged components (i.e. lysis solution, wash solutions etc.). Therefore, if one component is spilled or contaminated then the entire kit would be compromised. Conversely, if the lysis solution from a GT protocol is spilled or contaminated, then this component can be easily replaced. In addition, kits are usually based on existing GT protocols developed in research laboratories, and there are numerous different GT protocols in use among the laboratories of the world. Therefore, GT protocols offer an alternative to kits. Further, GT extraction protocols can be significantly less expensive than but may or may not be as efficient as kits at recovering nucleic acids. In some situations a decrease in efficiency is acceptable, but for human pathogen testing any loss in efficiency would be unacceptable. Here, we compare the extraction/purification efficiency of a popular kit vs a GT protocol from the literature for extracting and purifying viral RNA from mosquito pools (*Culex* spp.). The relative amounts of recovered RNA was assessed by comparing cycle threshold (Ct) values from downstream real-time RT-PCR results.

MATERIALS AND METHODS

For these experiments, we created a single large volume of known positive control sample by combining multiple pools that tested positive for West Nile Virus (WNV); therefore all extraction templates (kit and GT protocol) were taken from this aggregate sample. In addition, we performed a serial dilution experiment where a sample of inactivated WNV at a concentration of 10^6 pfu was diluted 10x from 10^6 to 10^0, and resulting the dilution series was extracted using both the kit and GT protocol. The inactivated WNV sample was supplied by the University of California Davis Arboviral Research and Training (DART) laboratory. For the commercial kit, we used the MagMAX™ -96 viral isolation kit following the manufacture's recommended protocol and for the GT protocol, we followed the approach of Lijtmaer et al. (2012). However, Lijtmaer et al. (2012) did not utilize SPRI bead technology, thus we utilized a protocol from P. Jolivet and J. W. Foley (Ludmer

Centre for Neuroinformatics and Mental Health, available at http://www.openwetware.org/wiki/SPRI_bead_mix#Nucleic_acid_binding_bead_mixes) to generate SPRI beads "in house". Buffer recipes for the GT protocol closely followed Lijtmaer et al. (2012) except we modified the binding buffer from 6 molar (M) to 3M concentration of GT. He et al. (2017) determined that a 3M concentration of GT was optimal and our own optimization experiments supported these results (not shown). Extractions were carried out using a MagMAX™ express 24-sample magnetic particle processor. For the kit, we followed the manufacturer's recommended protocol and we adapted the GT protocol for use in the MagMax™ express machine. Extraction component volumes and MagMAX™ plate layout are shown in Fig. 1.

The relative performance of each extraction method (i.e, kit vs GT) was assessed by performing RT-PCR experiments using a custom WNV TaqMan probe designed by Lanciotti et al. (2000). In addition, we used TaqMan™ Fast Virus 1-Step Master Mix, RT-PCR and an ABI 7500 RT-PCR machine using the Fast setting. Cycle threshold values generated using the kit vs GT protocol were compared using a paired t-test.

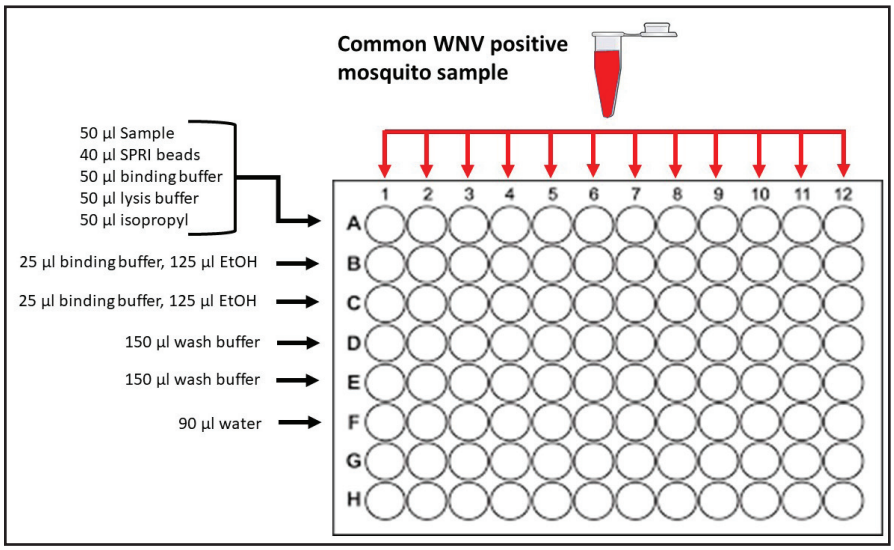

Figure 1 Figure showing volumes of extraction reagents for each row of a MagMAX™ 96-well extraction plate (two plates can be processed simultaneously in the MagMAX™ express 24-sample magnetic particle processor). A common sample (positive for West Nile Virus was used for all extractions and recipes for binding buffer, lysis buffer and wash buffer can be found in Lijtmear et al. (2012). Note-we modified the binding buffer to 3 molar concentration (see text). Directions for making SPRI beads can be found at http://www.openwetware.org/wiki/SPRI_bead_mix#Nucleic_acid_binding_bead_mixes

RESULTS

We performed 52 extractions for the kit and GT protocol (104 extractions total). We did not quantify the concentration of virus in the aggregate extraction template, but the lowest Ct values from paired kit vs GT undiluted extractions were 25.28 vs 25.86, respectively, whereas the highest values from the 100x dilution extractions were 34.77 for the kit and 33.19 for the GT protocol. Across samples, $\overline{XX}$ kit = 28.31, whereas $\overline{XX}$ GT = 27.69, SEM kit = 0.4027, SEM GT = 0.5104. Overall, Ct values from samples extracted using either method were very similar, but slightly lower for the GT protocol (two-tailed P value = 0.0002, df = 51, standard error of difference = 0.155). Results from the serial dilution experiments were similar in that Ct values for the kit vs GT protocol were very similar (Table 1).

DISCUSSION

As the number of pathogens and vectors continues to increase, developing cost-efficient testing protocols becomes ever more important. For example, the spread of invasive *Aedes aegypti* and *Ae. albopictus* mosquitoes, and their associated viral pathogens such as Zika, chikungunya, and dengue fever viruses, compels public health agencies to conduct surveillance for these pathogens. Therefore, developing more cost-effective testing methods will enable additional testing to be completed without incurring dramatic increases in costs.

The extraction protocol described here is adaptable and we continue to assess additional components that might offer improved efficiency. For example, although our initial experiments indicated that an excess of SPRI beads compensated for exclusion of an RNA carrier (results not shown), including a non-RNA carrier like poly acryl or glycogen might increase RNA recovery. Future experiments will determine the efficacy of these additional components for recovering low quantities of nucleic acids. Finally, although not shown here, the GT protocol appears to work equally well at extracting and purifying bacterial DNA from hard ticks (*Ixodes sp.*). The only change necessary is generating SPRI beads designed for binding DNA and instructions for generating DNA SPRI binding beads can also be found at http://www.openwetware.org/wiki/SPRI_bead_mix#Nucleic_acid_binding_bead_mixes.

CONCLUSIONS

Determining the distribution of viral pathogens is an integral component of mosquito control programs and appropriate mosquito control measures can be accelerated by rapidly detecting pathogens in field-collected specimens which can help prevent disease transmission because detecting WNV can lead to the application of pesticides to control adult mosquito populations and help prevent the spread of WNV (Lanciotti et al. 2000). Our results demonstrate the feasibility of using standard GT viral RNA extraction protocols that are equal in efficiency to commercially-available viral RNA extraction and purification kits, but at a fraction of the cost. For example, the cost for extraction chemistry using a kit is >$3.00/sample compared to ~$0.25/sample for a standard GT protocol.

Table 1 Results of the serial dilution experiment for the kit vs GT protocol. Values are cycle threshold (Ct) scores.

	WNV concentration						
	10^6	10^5	10^4	10^3	10^2	10^1	10^0
MagMAX™ -96 viral isolation kit	14.19	17.68	20.96	24.24	27.54	29.91	0
GT protocol	14.73	17.96	20.76	24.00	27.61	29.81	0

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Not the usual suspects: The trials and tribulations of a hantavirus case investigation

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ABSTRACT A case of hantavirus pulmonary syndrome (HPS) was reported to the California Department of Public Health in August 2017. Beginning six weeks and continuing to two weeks prior to illness onset, the case-patient had extensive travel history through northern California, Idaho, and Montana to rural areas including camping and staying in rustic cabins. Based on travel locations and time to disease onset, initial suspicion focused on Montana as a likely source of exposure. However, PCR and sequencing of Sin Nombre virus from a respiratory sample from the case-patient suggested that the patient's isolate of hantavirus was genetically more similar to hantavirus sequences obtained from the Lake Tahoe region rather than Montana. The patient's stay in northern California included a private residence and tent camping in the Truckee area. An investigation of these areas and the patient's activities supports the Tahoe area exposure location and the possibility of an atypical, outdoor hantavirus exposure. The ability to get an ideal sample to genetically sequence the patient's hantavirus isolate facilitated the follow-up investigation shift from what seemed like an obvious exposure to a more unusual one.

Vector competence of Northern California mosquitoes for *Dirofilaria immitis*

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INTRODUCTION

Dirofilaria immitis, commonly known as dog heartworm, leads to several fatal cardiovascular diseases in domestic canines and felines. Recently, California has experienced an increase in the prevalence of heartworm, including in San Joaquin and Lake Counties (Huang et al. 2013). To prevent the spread of heartworm infections, it is necessary to focus on vector competence, which is the ability for the mosquito vector to cultivate and aid *D. immitis* in its development to the infective (L3) stage (Ledesma and Harrington 2011). The current study investigated the vector competence of common Northern California mosquitoes, including *Aedes sierrensis*, *Culex pipiens* complex, and *Culiseta incidens* for D. immitis

METHODS

Mosquitoes were collected as larvae in the field, raised to the age of 3-7 days old in an insectary, and experimentally infected by feeding on canine blood with varying titers of D. immitis (2.5, 5, and 10 microfilariae(mff)/µL). Infected canine blood was provided by TRS Labs Inc. in Athens, GA, which contained a strain of D. immitis (Wildcat strain) isolated from Kentucky. Upon arrival of the infected blood, the titer of microfilariae was determined by diluting the blood (1:10) and transferring 20 µl to three dual-chamber Cellometer Couting Chambers (Nexcelom Bioscience, Lawrence, MA). Each chamber was observed under a compound microscope and an average was calculated of all six chambers to determine the titer (mff/µl). Once the initial titer was determined, the infected blood was diluted in a heparinized tube with non-infected sheep blood to reach the desired titer of D. immitis. Mosquitoes were then offered blood using a Hemotek membrane feeder, or hanging blood droplets through a mesh-topped, gallon-sized feeding container. Females were subsequently held for 15-25 days post-infection, and then decapitated at 15, 18, 21, and 25 days post-infection for evidence emerging larvae. Infective rates were then calculated, and comparisons were made at different time points as well as between different species.

RESULTS AND DISCUSSION

Overall, Ae. sierrensis displayed the highest vector competence, based on the average number of L3s emerging by day 15 (10.1), with infective rates reaching a maximum of 93.7% at a titer of 5 mff/µL. Although infective rates of Cx. pipiens were significantly less than Ae. sierrensis, this species was still considered to be a competent vector , with the emergence of L3s by day 18 post-infection, as well as infective rates reaching 24% at a titer of 5 mff/µL. Even though the titer for Cx. pipiens complex was increased to 10 mff/uL with an additional time point (25 days post-infection) added to the experiment, neither increase made a significant difference in infectivity for this species. Cs. incidens were reluctant to blood feed using our experimental protocol, therefore data was not presented for this species.

CONCLUSIONS

Ae. sierrensis is a highly competent vector D. immitis, and this species is probably important wherever it is abundant. Additionally, *Cx. pipiens* complex may also be an important vector of *D. immitis* due to its relatively high abundance in areas of interest, and its ability to cultivate the parasite to its infective stage. Such information may aid local vector control agencies in identifying important vectors, and should be extended to a wide range of mosquito species in the future.

ACKNOWLEDGEMENTS

This study would not have been possible without the contributions of Lake County VCD, San Joaquin County MVCD, Marin-Sonoma MVCD, and University of the Pacific.

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A History of Rickettsiosis in California and the San Gabriel Valley, Los Angeles County, California

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ABSTRACT Rickettsial diseases have been documented in the United States since the mid-1800s, but were not defined by the number of cases until around 1925. Furthermore, the distinction between louse-borne and flea-borne rickettsiosis did not occur until the 1940s. Once diagnostic tests began to differentiate among bacterial species, and antibiotics and pesticides became readily available, the number of cases substantially declined and this decline continued for the next few decades. In the 1990s, the number of human cases began rising again and there have been more than 500 cases in southern California alone over the last 20 years. Seventy-five percent of these cases were in Los Angeles County, with at least one-third occurring in the San Gabriel Valley. Our paper presents the history of rickettsiosis in the United States, and a summary of past work in the District to show how this vector-borne disease posed a major public health risk to residents within the boundaries of the San Gabriel Valley Mosquito and Vector Control District in Los Angeles County from 2014- 2016 (ACDC 2005, Nelson et al. 2016, Foo et al. 2017, Nelson et al. 2017, Nelson et al. 2018).

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The path to the detection and identification of rickettsial pathogens responsible for human flea-borne rickettsiosis in the Los Angeles County, California

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ABSTRACT Flea-borne typhus in the United States is distributed in localized areas of California, Hawai'i and Texas. In California, the overwhelming burden of this disease is carried by residents of Los Angeles County (LAC), especially those in downtown Los Angeles, and north and east into the San Gabriel Valley (Wekesa *et al*. 2016). This disease is classically associated with *Xenopsylla cheopis* (Oriental rat flea) which parasitize commensal rats, and is caused by *Rickettsia typhi*. Recently, multiple species of *Rickettsia* have been found circulating within populations of opossums and *Ctenocephalides felis* (cat flea); potentially enhanced by high populations of feral cats and small mammals in peri-domestic environments. In addition, cases of flea-borne typhus have increased in nearby Orange County. Human cases are diagnosed with serological tests that do not differentiate between *Rickettsia* species circulating in southern California. The quantative polymerase chain reaction (qPCR) and sequencing have been used during investigations of some clusters of human cases to detect several *Rickettsia* species from cat fleas and tissues from opossums and cats (Maina *et al*. 2016, Nelson *et al*. 2018, Mullins *et al*. 2018). The causative agent *Rickettsia* in these cluster cases remains unconfirmed.

We propose a collaborative study aimed at identifying the causative agent of flea-borne typhus in the San Gabriel Valley by identifying the Rickettsial agent circulating in cat fleas, opossums, cats and human hosts, concurrently. The study would require taking samples from infected patients as well as sampling fleas and host animals from the most likely place of exposure. Patients presenting to their local health care providers in LAC with typhus-like febrile illness would be identified and asked to participate in the study by contributing paired samples of blood sera, blood clots, skin swabs or eschars. All samples would be tested by molecular and serological methods to identify the rickettsial agents present. New isolates shall be sequenced, typed and analysis done to determine the identity of circulating Rickettsia, especially the type causing disease in people.

Disclaimer Statement

The views expressed in the presentation and this abstract are those of the authors and do not necessarily represent the official policy or position of the Department of the Navy, Department of Defense or the U.S. Government.

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Reducing the risk of flea-borne typhus: Strategies for area-wide control of fleas in residential neighborhoods

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ABSTRACT The suburban transmission cycle of flea-borne typhus in Southern California involves opossums, feral cats, domestic pets, and cat fleas. The suburban transmission cycle has been studied extensively since it was first described from Los Angeles County, in the 1980s. The Orange County Mosquito and Vector Control District (OCMVCD) has responded to over 130 human cases of flea-borne typhus in Orange County, CA, since 2006. This presentation will summarize the components of the integrated vector management strategy used by OCMVCD to respond to and prevent human cases of flea-borne typhus. The OCMVCD area-wide flea control program consists of the following: 1) determination of a target area to focus prevention and control activities based on flea and host animal surveillance, 2) outreach to local animal control and city jurisdictions to promote collaboration opportunities to prevent and control fleas, 3) distribution of an educational postcard to parcels within the target area, 4) distribution of topical flea control medication to residences with dogs and cats in the target area, 5) application of residual insecticides targeting larval and adult fleas, and 6) vertebrate pest management. The well-documented increase of flea-borne typhus cases in Texas and California highlights the need for a novel, area-wide flea control strategy targeting suburban wildlife. The fast and rapid reduction of fleas on suburban wildlife and domestic pets is necessary in order to prevent human disease.

Jumping into the Future: An Analysis of Fifty Years of Flea Data from Urban Wildlife in Orange County, 1967-2017

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ABSTRACT The Orange County Mosquito and Vector Control District has been conducting flea surveillance on urban wildlife in Orange County for fifty years. This study will highlight the medically important species of fleas found on skunks, opossums, raccoons, roof rats, squirrels, coyotes, and feral cats collected in residential neighborhoods of Orange County from 1967 to 2017. Fleas collected from backyard wildlife have been epidemiologically linked to flea-borne rickettsial disease transmission in urban and suburban areas of Orange County. To understand the risk of flea-borne disease transmission in an area, surveys of wildlife are conducted to determine species distribution, population size, ectoparasite population size, and vector-borne pathogen diversity. This information is used to guide decisions about risk management and potential intervention strategies to reduce and prevent flea-borne disease. Results from this study show that the average number of fleas per opossum has increased significantly from 1967 to 2017 in the County. The increase in the abundance of medically important flea species is correlated with the increase in human flea-borne rickettsial disease cases reported to the California Department of Public Health over the last fifty years in Orange County. This study will also highlight OCMVCDs' detections of *Orchopeas howardii* on eastern fox squirrels and the potential for enzootic plague transmission.

Rickettsial Infections among Cats and Cat Fleas in Riverside County, California

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ABSTRACT Few studies have investigated the role of domestic cats (*Felis catus*) in the recurrence of flea-borne rickettsioses in California and the southern United States. In this study, we investigated the presence of *Rickettsia typhi* or *R. felis* in domestic cats (*Felis catus*) and the fleas (primarily *Ctenocephalides felis*, the cat flea) associated with these cats in Riverside County, California. Thirty (30) cats and 64 pools of fleas collected from these cats were tested for rickettsial infections. Three (3) cats and 17 flea pools (from 10 cats) tested positive. PCR and DNA sequencing indicated that 1 of the cats was positive for *R. felis,* whereas 2 were positive for *Candidatus* 'R. senegalensis' infection. Additionally, 12 of the flea pools were positive for *R. felis*, while 5 were positive for *Ca.* 'R. senegalensis'. In contrast, no cats or their associated fleas tested positive for *R. typhi*. Finally, 8 sera from these cats contained spotted fever group rickettsia (SFGR) antibodies. The detection of *R. felis* and SFGR antibodies and the lack of *R. typhi* and typhus group rickettsia (TGR) antibodies indicated that *R. felis* is the main rickettsial species infecting cat fleas. The detection of *Ca.* 'R. senegalensis' in both fleas and cats also provides additional evidence that cats and their associated fleas are infected with other *R. felis*- like organisms highlighting the potential risk for human infections with *R. felis* or *R. felis*-like organisms.

The role of the deer mouse (*Peromyscus maniculatus*) in sylvatic plague transmission in California

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ABSTRACT It has long been theorized that deer mice (*Peromyscus maniculatus*) are a primary reservoir of *Yersinia pestis* in California. However, recent research from other parts of the western United States has implicated deer mice as spillover hosts during epizootic plague transmission. This retrospective study analyzed deer mouse data collected for plague surveillance by public health agencies in California from 1971 to 2016 to help elucidate the role of deer mice in plague transmission. The fleas most commonly found on deer mice were poor vectors of *Y. pestis* and occurred in insufficient numbers to maintain transmission of the pathogen. In addition, fleas whose natural hosts are deer mice were rarely observed and even more rarely found infected with *Y. pestis* on other rodent hosts. These analyses indicated that it is unlikely that deer mice play an important role in maintaining plague transmission in California. Although they may not be primary reservoirs, results supported the premise that deer mice are occasionally exposed to and infected by *Y. pestis* and may be spillover hosts.

A Review of Plague in California from 1983 - 2016

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ABSTRACT Plague, caused by the bacterium *Yersinia pestis*, was introduced to California in 1900 via commensal rodents and their fleas. Plague subsequently spread to native rodents establishing a sylvatic (rural) cycle in the western United States. Excluding the Central Valley and southeastern desert regions, plague is endemic in many parts of California today. The California Department of Public Health – Vector-Borne Disease Section and partner agencies maintain a statewide surveillance system that monitors humans, susceptible mammals, and fleas for exposure to plague. From 1983 through 2016, 5% (n=27,549) of rodents and 8.7% (n=13,595) of carnivores tested were positive for *Y. pestis* or *Y. pestis* antibodies. During this time, 26 human plague cases were reported with likely exposure in California; recreational areas represented the most common exposure location. In 2015, two human plague cases, the first reported in 9 years, were associated with visits to Yosemite National Park. These cases coincided with increased detections of plague-positive rodents in the Sierra Nevada. This presentation provided a review of plague activity in California with a focus on the utility, limitations, and biases of the surveillance program and the ability to detect changing plague activity over time.

Flea'in Around: Creating New Tools for an Arcane Discipline

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ABSTRACT Fleas are remarkable and highly specialized insects, with no part of their external anatomy being easily mistaken for that of any other arthropod. Due to their small size, the subtle differences among the distinguishing morphological characteristics of each species, and complexities of preparing specimens, identifying, and working with fleas is challenging. Various documents and taxonomic keys are available that discuss mounting procedures and the identification of medically important fleas for large regions of the world including the United States; however, many of these have become antiquated over time and little has been done to update the format and presentation of these various tools by using modern advances in photography and computer technology. Some of the key characters used to distinguish flea species are presented in older keys in the form of line drawings and written descriptions of highly specialized characteristics, which are accurate but can be difficult to use when comparing these to structures on a whole specimen when viewed through a microscope. The current paper presents a guide which describes in detail techniques for the preservation, preparation, clearing, and mounting of Siphonaptera specimens. In addition, we also present an easy to use photographic key of twelve flea species collected from common mammals and pets in Orange County, California. This key, which is freely available online at Orange County Mosquito and Vector Control District's website, is an effective tool for the identification of common flea species found in southern California. Using the key in conjunction with the mounting guide will provide users with a guide to preserving, mounting, and identifying flea specimens. Flea genera included are *Cediopsylla*, *Ctenocephalides, Echidinophaga, Hoplopsyllus, Leptopsylla, Nosopsyllus, Orchopeas, Oropsylla, Pulex*, and *Xenopsylla* from hosts including cats, coyotes, dogs, mice, opossums, rabbits, raccoons, rats, skunks, squirrels, and woodrats.

INTRODUCTION

Fleas are remarkably specialized arthropods that live as ectoparasites within the fur, hair, feathers, and burrows/nests of many mammals and birds. Every morphological development, from the many seta, bristles, and combs that line the surface of their exoskeleton, to laterally compressed bodies, is adapted for living in the highly unusual microenvironment created by their hosts. Living within their host's pelage makes fleas very cryptic, often causing them to go unobserved by those not trained to look for them. Having such a close relationship with their host, either living on the host directly or within their nests, combined with the need for both males and females to metabolize blood for sustenance, has enabled species within the order Siphonaptera to become key vectors of pathogens causing diseases such as the plague (*Yersinia pestis*), where their role in transmission has been documented since the late 1800s (Herms 1961). Being of medical importance, it is essential to keep individuals within the public health field properly trained in the identification of fleas, as well as the anatomy, taxonomy, microscopy, and systematics that play a part in the creation of the resources used in identification. However, it can be difficult for professionals and students new to the study of Siphonaptera to become proficient in identifying specimens to species or subspecies level due to a combination of uniquely specialized characters presented in flea anatomy, the new and odd terminology that goes with these characters, and the difficulty of locating and/or accessing resources on the subject. To work with fleas many specialized technical skills are required that are not covered in most educational institutions, organizations, or agencies. Finding references, especially current ones, covering the nuances of working with fleas is difficult, and these specialized references contain mainly written descriptions and line drawings of highly specialized and unique structures. Proper identification of most flea species relies on clearing and mounting the specimen for examination under a compound microscope. Finding resources that describe clearing and mounting fleas can be difficult, but these are essential for species identification using the external and internal anatomy of the flea. Our current paper describes, *How to Mount Your Flea: A Guide to the Preservation, Preparation, Clearing, and Mounting of Siphonaptera*, and the *Pictorial Key to Some Common Fleas of Southern California*, two newly created documents that can provide a solid starting point or resource for clearing and mounting, as well as identifying flea specimens. For those working in public health, these two documents may be

useful in Integrated Pest Management (IPM) programs that manage flea populations and can be fundamental tools for any plague or typhus surveillance projects and response plans implemented by a state or local agency. With the last known human-to-human and pneumonic plague outbreak in the U.S. occurring in Los Angeles, Calif. in 1924, and only 63 human plague cases being directly or indirectly linked with exposure to sylvatic rodents in California during the succeeding years, the likelihood of a plague outbreak is low (CDPH 2016). However, there is still the possibility of enzootic transmission and proper plans and precautions should be in place. California's Plague Surveillance and Control Program, is an inter-agency program that relies on the involvement of state, federal, and local agencies under the direction of the California Department of Public Health (CDPH 2016). In the *California Compendium of Plague Control, "*Part III" covers the surveillance and control of plague (CDPH 2016). The newly created identification key and mounting guide are tools that can be included in sylvatic plague epizootic response plans, where it is necessary to assess the rodent flea population to determine their species composition, medical importance and potential to transmit pathogens. With bites from plague infected fleas being the most frequent route of plague transmission to humans in California (CDPH 2016), it is important to estimate an accurate flea index (the number of fleas per host in a given area) and have the capability to accurately and timely identify the species of fleas collected from hosts or burrows/nests. Because most agencies and professionals are going to have limited practice and experience working with fleas, the newly created pictorial key and mounting guide can be implemented as a starting point to train technicians in the skills required to perform accurate and timely identifications in any surveillance program tasked with monitoring fleas.

With the abrupt end of Carl F. Baker's work on Siphonaptera in 1905, the death of Nathaniel C. Rothchild in 1923, and the slowdown of Dr. Karl Jordan's work in 1938, the efforts from some of the original and pioneering researchers of Siphonaptera ended (Hubbard 1947). Due to a boom in research on medically-important species, stemming from several human plague outbreaks in the United States starting in 1904 (Hubbard 1947), research on anatomy and morphology had advanced at a swift rate as the medical importance of fleas became increasingly established. The 1940s marked one of the most prolific eras in flea research in northwestern America, including work from researchers such as George Holland at Kamloops Laboratories in Vancouver, British Columbia, Ruth Svilha at the University of Washington, Seattle, Clearance Hubbard at Pacific University, Forest Grove, Oregon, M.A. Stewart at U.C. Davis, as well as Gus Agustin, and Irving Fox (Hubbard 1947). During this era, M.A. Stewart described two new genra and eight species, Hubbard described two new genra along with several new species, George Holland with M.A. Stewart described one new genus, Irving Fox created descriptions of four undefined species from museum specimens, and Gus Auguston described one new genus and several new species (Hubbard 1947). By 1943, the need for people to serve during World War II depleted the " flea men of the west", as well as researchers from the rest of the world (Hubbard 1947). There wouldn't be such comprehensive studies done again untill 1953 when Mariam Rothchild alongside G.H.E. Hopkins would produce the *Illustrated Catalogue of the Rothschild Collection of Fleas (Siphonaptera) in the British Museum* (Rothschild 1953), an extensive 6 volume piece that was published by the museum over the years of 1953 through 1981. Some of the largest accomplishments of this work include the cration of new descriptions for insect tissues associated with fleas, and covers over 7,000 drawings generated from the largest collections of fleas in the world. In the 1960s, Cluff E. Hopla performed extensive research on fleas in the artic and subartic regions of America, which was one of the first to be performed in these areas and important in understanding the capability of the free-living larval stage of fleas to adapt to environmental changes, and the effects of the environment on population distribution (Hopla 1965). In the 1980s, Robert E. Lewis authored *The Fleas of the Pacific Northwest* (Lewis et al. 1988), which is only the second comprehensive reviewof fleas in northwestern America since Hubbard's piece in 1947, *Fleas of Western North America* (Hubbard, 1947).

Despite the many advances that have been made in the study of Siphonaptera, little innovation or change has been applied to update the tools and resources used to identify fleas. Recent advances in microscopy, photography, computer, and wireless technology has made viewing, studying, and photographing the anatomy of small to microscopic arthropods, under high-powered microscopy, readily accessible and plausible for experts, professionals, or enthusiasts alike. With most flea species being described at a time lacking such technology, some questions remain on the nuances of flea anatomy and taxonomy, especially on a species and subspecies level. The genus *Orchopeas*, consisting mainly of parasites found on tree squirrels, is one of the best examples of the difficulties that have been encountered when describing and identifying species within a given flea genus. With a high number of subspecies and substantial variation in characteristics (some being very obscure and most being described from individual specimens rather than a series of individuals) has caused confusions to arise when attempting to identify species (Hubbard 1947).

THE CREATION OF A PICTORIAL KEY

Due to the lack of advances and resources in the study of Siphonaptera, the identification of fleas can be difficult for new students to the field. Photographs of flea species, and specific anatomical features are usually not readily available, and when accessible, are often taken of a specific species to demonstrate generalized anatomical structures for the entire order. These are printed onto the page of a book, thereby limiting the user's access and ability to manipulate or magnify the image to obtain a better view. With the high variation of characters even among species or subspecies, it is common to use multiple references to aid in identifying a given species or a single morphological feature. This is mainly due to the unique terminology used to

help relate the unusual anatomy of fleas to that of other insects, resulting in body parts having multiple terms associated with them, which may vary depending on the author or authority. One of the goals of our project was to create a new pictorial key, entitled *Pictorial Key to Some Common Fleas of Southern California,* that uses color photographs of physical specimens alongside short written descriptions to aid in the identification of the common flea species associated with locally abundant backyard mammalian wildlife and pets in southern California.

Table 1 List of host mammals examined for fleas.

Common Name	Species
Cat	*Felis catus*
Coyote	*Canis latrans*
Dog	*Canis lupus familiaris*
Mouse	*Mus musculus,* Peromyscus californicus, Peromyscus fraterculus, Peromyscus maniculatus
Opossum	*Didelphis virginiana*
Rabbit	*Sylvilagus audubonii*
Raccoon	*Procyon lotor*
Rat	*Rattus rattus, Rattus*
Skunk	*Mephitis mephitis*
Squirrel	*Sciurus niger, Spermophilus beecheyi*
Woodrat	*Neotoma bryanti,Neotoma lepida, Neotoma macrotis*

Orange County Mosquito and Vector Control District (OCMVCD) maintains flea species and host mammal data spanning from 1997 to the present. Data from 1997 to 2016 was used to determine the most commonly found flea species present on mammalian wildlife and pets from Orange County, California. To obtain the data, OCMVCD examined host animals, usually postmortem, for the presence of ectoparasites (Table 1). Any fleas found on the host were collected, identified, sexed, and enumerated before either being tested for infection or added to a reference collection (Pennicks et al. 2018). After reviewing the nearly twenty years of data, twelve common flea species from wildlife and pets were identified (Table 2).

Once the species list was determined, multiple references, such as *Fleas of Western North America* (Hubbard 1947) and *the Fleas of the Pacific North West* (Lewis et al. 1988) were used to determine the best anatomical features to use in the identification of each species. The main inspiration for the structure, or layout, of the new key was a series of "pictorial" keys created by the U.S. Department of Health and Human Service's Center for Disease Control and Prevention (CDC), entitled *Fleas: Pictorial Key to Some Common Species in the United States*, and *Fleas: Pictorial Key to Species Found on Domestic Rats in Southern United States* (Fritz 1947, Pratt 1947). Both keys are found in the *Pictorial Keys: Arthropods, Reptiles, Birds and Mammals of Public Health Significance* (CDC 1966), a collective reference covering multiple insects and other medically important animals. Even though these two keys were last revised in 1954, they give an excellent framework for a pictorial key, and features for identifying common fleas.

Table 2 Twelve common flea species found on backyard wildlife and pets in Orange County, Calif.

Scientific Name	Common Name
Cediopsylla inaequalis interrupta	Common black rabbit flea
Ctenocephalides felis	Cat flea
Diamanus montanus	Ground squirrel flea
Echidnophaga gallinacea	Sticktight flea
Hoplopsyllus anomalus	N/A
Hoplopsyllus glacialis foxi	N/A
Leptopsylla segnis	Mouse flea
Nosopsyllus fasciatus	Northern rat flea
Orchopeas sexdentatus sexdentatus	N/A
Orchopeas howardii	Squirrel flea
Pulex irritans	Human/house flea
Xenopsylla cheopis	Oriental rat flea

With the structure of the key in place, the next task became obtaining mounted specimens of each species and capturing photographs of all the key features used to identify them. Many of the common specimens, such as the cat flea, *Ctenocephalides felis* (Buche), and the sticktight flea, *Echidnoghaga gallinacea* (Westwood), were obtained from the reference collection maintained at OCVMCD. To acquire photographs of mounted specimens for species not available in OCVMCD's reference collection, the author was invited to the Natural History Museum of Los Angeles County's Entomology Department and allowed access to their extensive flea collection. Photographs of specimens collected by Clarence Hubbard while writing *Fleas of Western North America*, were used in constructing our key.

Photographs for the key were made using a Samsung® Galaxy Note5 smart phone, and a scope mounting device (Phone

Figure 1 Phone Skope® smartphone mounting device.

Skope®, Beaver, UT, USA), which allows a smart phone to be attached to the eyepiece of a microscope to acquire pictures. The mounting device consists of a case built for the specific model of smartphone the operator owns/uses, and a universal mount that attaches to the back of the case, which is tightened around the eyepiece of the microscope (Figure 1). This device was chosen due to its low cost (compared to microscope cameras) and ease of use which allows portability to any location that has specimens and a compound microscope to acquire pictures.

The greatest difference between our pictorial key and those used as its reference is the digital design. As a file that can

Figure 2 Comb arrangement found on fleas in southern California. Left: both genal and pronotal comb present; Middle: only pronotal comb present; Right: no genal or pronotal comb present.

be downloaded onto a smart phone, tablet, or laptop/desktop computer, the key works best with a touch screen device that allows the user's hand to easily magnify and move to any portion of the key that they have been directed towards. The *Pictorial Key to Some Common Fleas of Southern California* (http://www.ocvector.org/fk/) starts by having the user determine the presence or lack of genal and/or pronotal combs by grouping fleas into three main categories: 1) both genal and pronotal combs present; 2) only the pronotal comb present; 3) no genal or pronotal comb present (Figure 2). Once it is determined that the flea fits into one of these three main categories, the key moves the user through branches of more specific anatomical features that are easily reached by tracing or tracking arrows provided to guide the user from one key character to another. At the very end of each branch of the key, the user will be provided with an overall picture of both the female and male of the species, and the full scientific name.

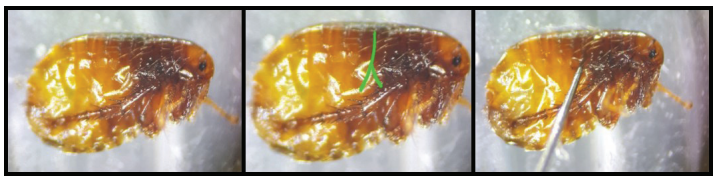

Figure 3 Series of color pictures that display the proper placement of incisions made for potassium hydroxide soaks.

MAKING A GUIDE TO MOUNTING FLEAS

Many species of fleas must be cleared and mounted onto a microscope slide and viewed under a compound microscope to clearly see the characters needed for proper identification. Clearing and mounting fleas is not a new subject, but protocols can be difficult to locate. Clearing and mounting a flea can be difficult, due to the specialized tools required for the work, the small size of the insect, and a body which is laterally compressed. Another goal of our project was to create a guide entitled, *How to Mount Your Flea: A Guide to the Preservation, Preparation, Clearing, and Mounting of Siphonaptera* (http://www.ocvector.org/fk/). This guide combines steps/procedures from multiple historic and current references to describe the entire process from preserving an unmounted specimen to sealing and labeling a finished slide. Users of the guide will find color pictures accompanying most of the sections and steps to make it easier to conceptualize the tasks that need to be performed (Figure 3).

To make the procedure comprehensive, the guide breaks the clearing and mounting process into multiple smaller sections/steps. The first section discusses the various materials and tools needed for the work, with pictures of each included. Homemade versions of some tools are also included in the first section. The second section unveils the proper way to preserve specimens prior to clearing and mounting. The third section, "The Four Steps to Clearing Your Flea", is the main portion of the guide and will require the most time. Steps found in this section include: 1) potassium hydroxide (KOH) soak; 2) evacuating internal contents; 3) spreading and hardening the flea; 4) cellosolve soak. Once users have cleared their flea, the guide will discuss the fourth section, "Mounting Your Flea", which describes how to mount the flea on a microscope slide. The final sections address how to seal and label the slide, thereby completing the process (Figure 4).

CONCLUSION

Due to their medical importance, professionals within the public health fields should be educated on the identification of fleas. Despite their historical significance, and ability to captivate researchers over the years, the format of the tools and materials created on the subject have become antiquated with time. The key and mounting guide presented in this paper were produced to create new, easy, effective, and accessible references for professionals and enthusiasts alike, while updating the format/presentation of the tools associated with the study of Siphonaptera by using modern advances in technology. Both the *Pictorial Key to Some Common Fleas of Southern California*, and *How to Mount Your Flea* are available for download at OMVCD's website.

The future of this project includes the refinement and expansion of both the identification key and the mounting guide. The first plan is to add a new 13th species, *Pulex simulans* (Baker), to the pictorial key . With the key being in a digital format, any edits and additions can be performed with relative ease. Because both *P. irritans* (L.) and *P. simulans* (Baker) are morphologically identical externally, and the only differentiating characters being the sclerite and crotchet on the aedeagus or external portion of the male's intromittent organ (Snodgrass 1946), it is common to misidentify these species unless males are cleared and mounted.

Hopefully, discrepancies regarding these two species will be addressed in future work. The mounting guide will be updated in the future to include more technical procedures such as dissection methods for the male's genitalia and female's spermatheca, which is necessary for the comparison and identification of certain species. The use of histology techniques to stain specimens may also be included in future updates/revisions of the mounting guide.

Figure 4 Series of pictures showing how to properly label a slide. Top image: unlabeled slide with information locations marked. Bottom Image: Properly labeled and sealed slide.

ACKNOWLEDGEMENTS

The authors would like to thank the entire staff of OCMVCD for all their work and support during the completion of this project, the Natural History Museum of Los Angeles County's Entomology Department for granting us access to their extensive flea collection and microscopes, and all the researchers that laid the ground work for the study of Siphonaptera.

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Soft ticks and tick-borne relapsing fever in the Eastern Sierra

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ABSTRACT In 2016 and 2017, the California Department of Public Health-Vector Borne Disease Section Southern Region office responded to confirmed, probable, and suspect cases of tick-borne relapsing fever (TBRF) at several locations in Mono County. Two of these locations had experienced previous cases of TBRF over the past decade, while others were novel exposure sites. This presentation highlighted the challenges of finding and controlling soft ticks and preventing future cases of TBRF in areas of high endemicity.

Species composition and temporal distribution of adult *ixodid* ticks at a regional park in Los Angeles County, California

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ABSTRACT Beginning in January 2017, tick sampling was conducted at San Dimas Canyon Community Regional Park in Los Angeles County, California to assess the species composition and temporal distribution of adult ixodid ticks. Previous collection efforts at this location demonstrated that the park harbors a high density of *Ixodes pacificus* and *Dermacentor occidentalis*, two tick species that can potentially serve as vectors of several zoonotic pathogens. The site was visited approximately once every four weeks and ticks were collected using a drag cloth method. The results of tick collection and their public health importance were discussed.

Phylogeography of Borrelia Spirochetes in Ixodes Ticks Highlights Differential Risk of Tick-borne Disease Transmission in Northern versus Southern California

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ABSTRACT The common human-biting tick, *Ixodes pacificus,* is an important vector of the Lyme disease spirochete, *Borrelia burgdorferi* sensu stricto (ss) in western North America and has been found to harbor other closely-related spirochetes in the *B. burgdorferi* sensu lato (sl) complex. Results from this study indicate genetic diversity and geographic structure of *B. burgdorferi* sl in California ticks, with *B. burgdorferi* ss, the agent of Lyme disease, found in only northern California *I. pacificus* and not detected in any southern California ticks. In contrast, the *B. burgdorferi* sl spirochete, *Borrelia bissettiae*, was detected in both *I. pacificus* and a commonly collected wildlife tick, *Ixodes spinipalpis*, in both northern and southern California.

Diversity of *Borrelia* Species in San Mateo County

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ABSTRACT While the Lyme disease group *Borrelia burdorferi sensu lato* (Bb sl) complex continues to grow in number of known species, little research is being done into its diversity in specific regions of the United States. We tested *Ixodes pacificus* ticks and small mammals for the presence of borreliae from parks in San Mateo County between 2015 and 2017. Overall, 86 rodent and tick samples that tested positive for Bb sl, but negative for *Borrelia burdorferi sensu stricto* (Bb ss), were further analyzed by sequencing of the 12s rRNA region to determine species. Our results determined the diversity and distribution of borreliae in small mammals and ticks that are closely associated with urban and suburban environments in San Mateo County.

Reducing *Culex erythrothorax* at a freshwater marsh using larvicide, physical control, and traps

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INTRODUCTION

Culex erythrothorax Dyar, the tule mosquito, is a competent vector of West Nile virus and can be highly abundant in marsh habitats with dense stands of bulrush and other vegetation (Walton and Workman 1998, Tietze et al. 2003, Counts and Peavey 2006). Aquatic vegetation serves as a refuge for larval and adult *Cx. erythrothorax*, but can obstruct liquid or granular larvicides from entering the water, thereby limiting their impact on reducing mosquito abundance. Environmental temperatures below 50 °F slow mosquito development substantially (Bar-Zeev 1958, Loetti et al. 2011). Growing Degree Days base 50 °F (GDD) is a summary heat index that measures heat accumulation in the environment when surface temperatures exceed 50 °F (McMaster and Wilhelm 1997), allowing for mosquito growth.

METHOD

The study site was a 28 acre freshwater marsh that abuts the San Francisco Bay (GPS coordinates near the midpoint of the study site are 37.629926, -122.139293). Each week, when weather conditions were favorable, 8.8 lbs / acre of VectoMax, VectoBac G, or VectoLex CG was applied at the site to reduce *Cx. erythrothorax* abundance. To determine if one product was more efficacious than another, and to limit the potential for insecticide resistance, the larvicide products were rotated each week (Figure 1A). To evaluate the efficacy of EVS CO_2 and Mosquito Magnet (MM) traps in attracting *Cx. erythrothorax*, EVS traps were placed approximately 50 m on either side of a MM that was positioned in the freshwater marsh. EVS traps were provisioned with dry ice, and the trap contents of the EVS and MM collected daily. The collected mosquitoes were identified to species using a dissection microscope. Mosquito abundance was reported as females per trap-night +/- standard error of the mean (SEM). GDD values were calculated from temperature data collected at a weather station located at the Hayward Executive Airport, approximately 2 mi northeast of the study site (Weather Underground)

RESULTS

During weeks 17 – 26 of 2016, *Cx. erythrothorax* were highly abundant at the marsh site (521 +/- 319 mosquitoes / trap night; Figure 1A, left y-axis). After two larvicide applications, there was a 23-fold reduction in adult *Cx. erythrothorax* abundance at the site (Figure 1A). The reduced mosquito abundance from week 30 – 34 did not coincide with a reduction in GDD (Figure 1A, right y-axis). Although larvicide applications continued for subsequent weeks, mosquito abundance returned to pre-treatment levels by week 37 (Figure 1A). High *Cx. erythrothorax* abundance at the site (1780 +/- 360 mosquitoes / trap night) encouraged East Bay Regional Park District (EBRPD) to remove most of the tule plants from the marsh (Figure 1B). Removal of the tule from the site in combination with a reduction in GDD correlated with a 20-fold reduction in *Cx. erythrothorax* abundance during weeks 48 – 52 (91 +/- 43 mosquitoes / trap night; Figure 1A). During the subsequent year (2017), *Cx. erythrothorax* abundance at the study site remained low (71 +/- 19 mosquitoes / trap night). Comparison of the EVS CO_2 to MM traps showed the MM caught significantly more *Cx. erythrothorax* (Figure 1C).

DISCUSSION

Tule plant removal from a 10 foot wide ditch successfully limited *Cx. erythrothorax* breeding when the use of larvicides alone failed (Counts and Peavey 2006). However, physical removal of emergent vegetation to control mosquitoes in large ecologically sensitive habitats presents challenges to landowners and managers that seek to protect habitats which support threatened or endangered species. Intensive monitoring of mosquito abundance at the study site provided the data that motivated discussions with EBRPD staff, resulting in an outcome that substantially reduced *Cx. erythrothorax* in the area that could not be accomplished with larvicide applications alone. To suppress *Cx. erythrothorax* abundance in habitats where removal of emergent vegetation is not permitted or feasible, MM may be more effective than EVS CO_2 traps for reducing biting pressure from adult female mosquitoes.

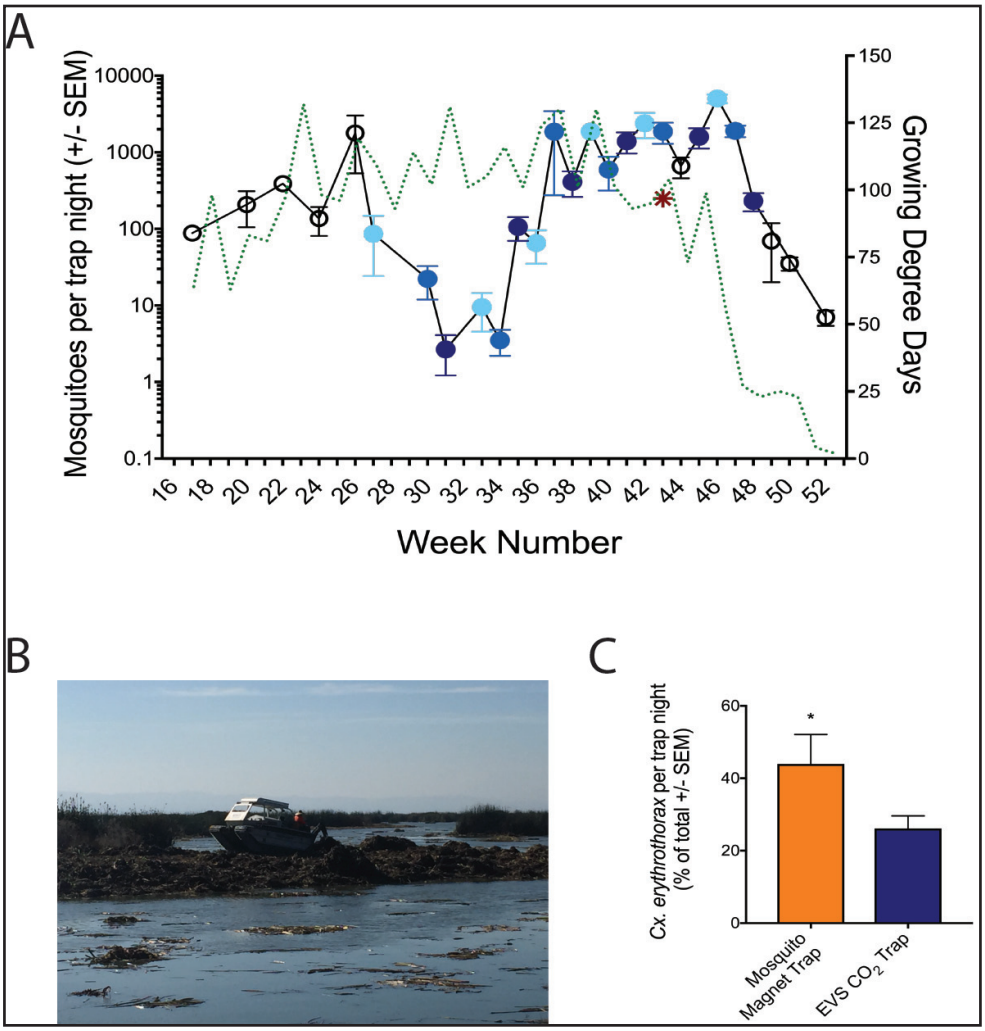

Figure 1 **Larvicide impact on *Cx. erythrothorax* abundance in a freshwater marsh and comparison of MM with EVS CO_2 traps for capturing *Cx. erythrothorax*.** (A) Larvicide applications reduced *Cx. erythrothorax* abundance for several weeks (left y-axis), but abundance increased until emergent vegetation was removed (indicated by red asterisk) and was followed by fewer growing degree days (right y-axis). (B) Physical removal of emergent vegetation from the freshwater marsh by EBRPD contractors. (C) MMT captured significantly more adult *Cx. erythrothorax* relative to CO_2 traps (paired t test, $P < 0.05$).

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North America's Oldest Lake and Mosquito Control in the Constructed Clearlake Oaks Keys Community

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Clear Lake is the central feature of Lake County, and most of the county's 65,000 residents live close to its shores. It's about 4.5 million years old, warm, shallow, eutrophic (rich in nutrients) and has a robust fishery. It's basically the opposite of Lake Tahoe which is cold, deep and oligotrophic and has a nominal fishery.

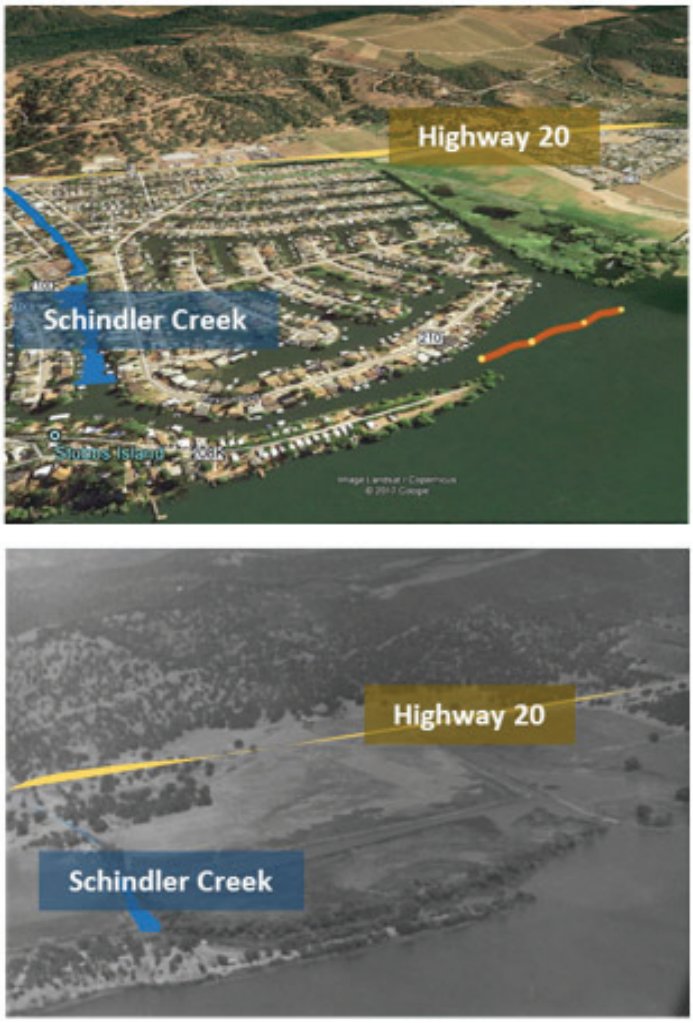

Figure 1 The Clearlake Oaks Keys (left) with the floating boom (illustrated in orange) designed to prevent algal mats from entering the channels and the same view (right) pre-development.

The Clearlake Oaks Keys community was constructed sometime after 1950 at the extreme eastern end of Clear Lake (Fig. 1) This shore has particularly sluggish currents because it lacks an outflow. The Keys development exacerbated the natural cumulative effects of the eastward wind (Fig. 2). Its four miles of intricate, shallow channels, act like a net collecting anything that blows in.

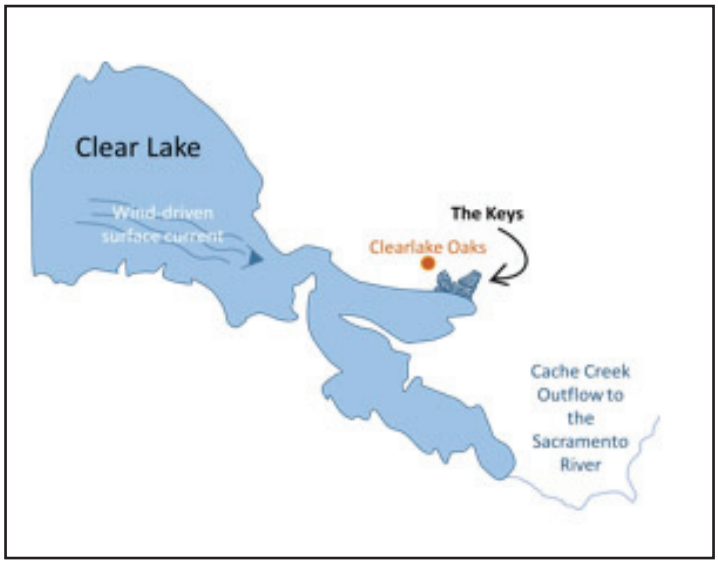

Figure 2 The typical wind direction across Clear Lake is from west-to-east. Floating matter (from wayward kayaks to cyanobacteria mats) are wind-driven toward the eastern arms of the lake and collect there.

Dominant aquatic plant life in the Keys may be partially determined by lake level and water clarity and varies unpredictably each year. If the water column is cloudy or surface plants occlude sunlight, seedlings rooted at the lake bottom lack sufficient light to grow. The Keys' Property Owners Association (POA) has previously herbicided and harvested weeds in the channels, but their efforts in mitigating aquatic plants haven't been comprehensive.

The planktonic cyanobacteria, *Limnoraphis* (formerly *Lyngbya*), is intermittently detectable in Clear Lake. In 2009, it was the dominant plankton species, packing into the Keys and creating an excellent larval habitat for mosquito species

that are competent vectors of West Nile virus. Since then, the Lake County Vector Control District (LCVCD) has regularly monitored larval and adult mosquito abundance in that community. *Culex tarsalis* is the most abundant mosquito in dip samples and frequently the only species collected in adult traps.

Adult surveillance is conducted with up to three CO_2-traps placed at set locations biweekly. Although we have yet to collect *Cx. stigmatosoma* adults by trapping, they consistently made up a significant proportion of the larval collections. Prior to 2012, adulticiding was conducted as necessary depending on service requests.

We have examined three recent mosquito seasons and the unique plant-types that were most dominant in the Keys: a planktonic cyanobacteria, a rooted emergent plant and a floating plant. The relative mosquito control challenge and the effect larval treatment had on adult *Cx. tarsalis* abundance each year are presented.

2012. The year of the cyanobacteria, *Limnoraphis*

This microscopic plant is planktonic. In 2012, it was visually the most dominate plant in the Keys. Unlike most phytoplankton, the cells of plants in this genus are enclosed in a protective sheath, so the filament looks much like a roll of quarters (Fig. 3). As they die, the filaments amass on the surface in 'haystack-like fashion', increasing in size until they resemble broken pieces of asphalt. Because they float and protrude from water, they were wind-driven into the Keys and became trapped in the channels (Fig. 4).

Figure 3 The filament oriented from the lower-left toward the upper-right corner is a cyanobacteria in the genus *Limnoraphis.*

Mats of *Limnoraphis* decomposed in the channels, reeked like sewage, loaded the water with nutrients and lowered the dissolved oxygen content. The aquatic environment had too little oxygen to support fish (predatory on mosquitoes), but abundant food for larval mosquitoes. Because of these changes, larvae that would typically be confined to a narrow band of habitat along the edge of the channel were surviving across the width of the channel; one dip taken among the mats could contain hundreds of larvae.

Figure 4 Mats of *Limnoraphis* sp. cyanobacteria cover most of the open water in front of homes in the Clearlake Oaks Keys, expanding mosquito habitat from the edge of the channel across its entire width.

On 17 August, a three-person crew used a Maruyama backpack sprayer operated out of the District's 22-ft boat to treat eleven acres of the channels with VectoMax FG (10lbs/ac). On 25 September, a second application with VectoBac G (10lbs/ac) treated 18 acres. Biomist 4+12 was applied by truck-mounted ULV twice weekly (1.65 oz/acre). Despite these treatment efforts, *Cx. tarsalis* adult counts rose steadily to a peak in the first week of October, with 531 females/trap-night (Fig. 5) Adult counts declined only after the onset of autumn weather, as air temperature dropped and relative humidity climbed. West Nile virus was detected in adult mosquitoes, but not in humans. Residents may have stayed indoors; avoiding both mosquito bites and the noxious odor. Prevention of *Limnoraphis* mat accumulation is probably the best method to minimize mosquito production in the Keys. In 2013, the POA installed a floating boom system to prevent mats from floating into the channels (see Fig. 1). It was installed with assistance from District staff and maintained through 2015. However, *Limnoraphis* was detectable but not prevalent in any part of Clear Lake during those years.

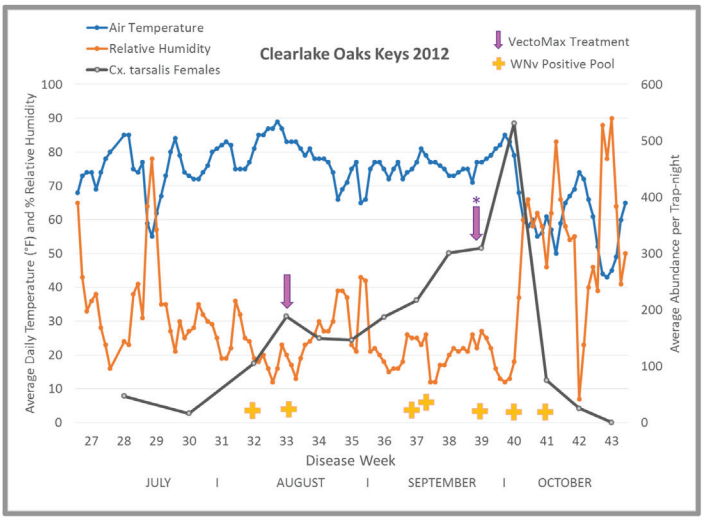

Figure 5 Mean *Culex tarsalis* female abundance per trap-night and temperature and humidity plotted weekly during 2012. *Larval treatment on week 39 was with VectoBac rather than VectoMax..

2015. The year of the aquatic weed, *Ludwigia* (Creeping Water Primrose)

Ludwigia grows from the shore in shallow water and rarely reaches across the width of a channel (Fig. 6). It can exclude fish and provide immature mosquito habitat. In 2015, the dip counts in this habitat were ten-fold higher than usual; approximately 30-40 larvae/dip. The first larvicide treatment on 8-9 July was based on larval counts and preceded a spike in adult abundance. VectoMax FG (14.6 lbs/ac) was applied to 18 acres of *Ludwigia* using a three-person crew, a Maruyama backpack sprayer, and a 22-foot boat.

Figure 6 Lake County Vector Control Technician, Brad Hayes, dips for subadult mosquitoes in *Ludwigia* sp. (Creeping Water Primrose) habitat along the shore of a channel in the Clearlake Oaks Keys.

Culex tarsalis adult collections peaked at 553 females/trap-night three weeks after the initial VectoMax FG application and then declined for most of the rest of the season(Fig. 7) . Two more applications of VectoMax FG (10 lbs/ac) occurred on 4-5 August (24 acres) and 24-25 August (16 acres). The acreage treated reflected the increased coverage of *Ludwigia* as the season progressed and the subsequent loss of access to channels as lake level dropped. Biomist 4+12 was applied on a twice weekly basis by truck-mounted ULV (1.65 oz/acre).

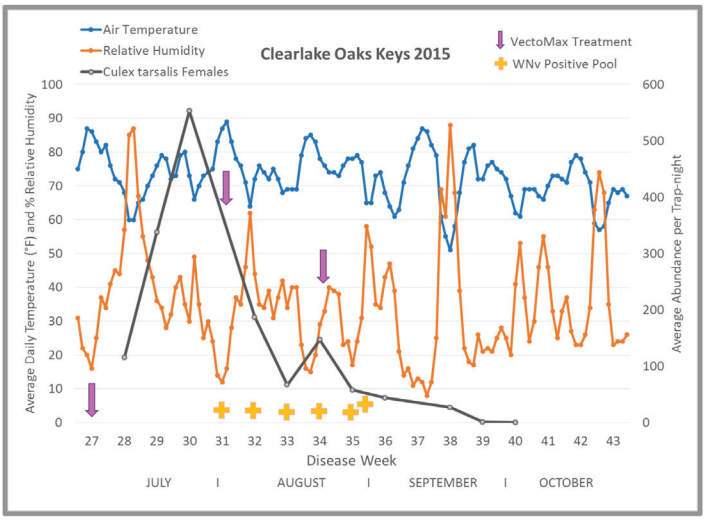

Figure 7 Mean *Culex tarsalis* female abundance per trap-night and temperature and humidity plotted weekly during 2015.

2017. The year of the tiny aquatic weed, *Lemna* (Duckweed)

This floating plant (Fig. 8) can fill a channel from one shore to the other as though it's been converted to a golf putting green. It can be a boon for mosquito control. *Lemna*'s roots are suspended in the water column, removing nutrients. Fewer

Figure 8 The tiny plant *Lemna* sp. (Duckweed) is free-floating and may be so abundant as to cover a channel completely, eliminating the free water mosquito sub-adults require to breath.

nutrients could mean less food for mosquito larvae and the better water quality supports the fish population. Additionally, a covering of *Lemna* may be dense enough to prevent immature mosquitoes from breathing at the water's surface.

In contrast to the 2012 and 2015 seasons, *Cx. tarsalis* adult counts remained low (Fig. 9). Peak abundance was modest, 79 females/trap-night. No larviciding applications were conducted. Biomist 4+12 ULV truck mounted applications were conducted on a per service request basis (1.65 oz/acre).

CONCLUSION

Dead-end channels can present unique mosquito control challenges year-to-year even if the larger body of water is choppy, predator-filled and unlikely to ever become mosquito habitat. Of the plant species found in the Keys, the cyanobacteria *Limnoraphis*, presented the most significant obstacle to mosquito control efforts. The floating mats of cyanobacteria prevented much of the granular pesticides VectoBac G and VectoMax FG from making contact with the water. Additionally, the high non-lethal bacterial load present in the water likely was in direct competition with the larvicides. Mosquitoes produced in *Ludwigia* habitat did decline with larvicide treatment. The product was able to penetrate the plant canopy and was present at a high enough concentration in the water to reduce larval abundance. In contrast, *Lemna* did not seem to support production of large numbers of *Cx. tarsalis* and didn't require larvicide application.

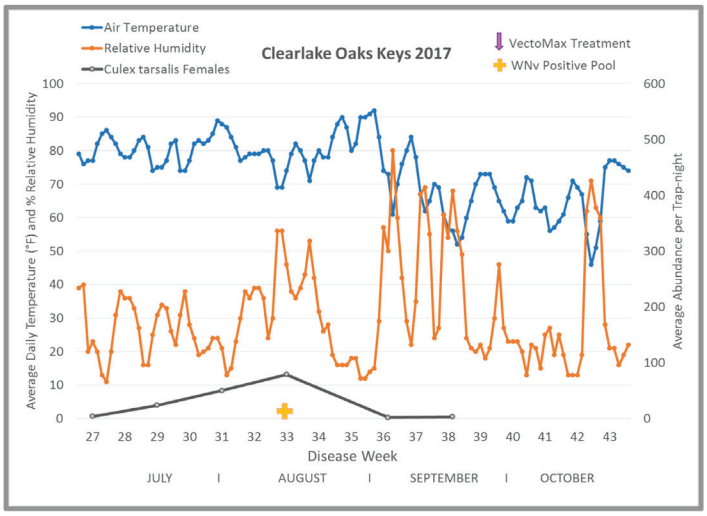

Figure 9 Mean *Culex tarsalis* female abundance per trap-night and temperature and humidity plotted weekly during 2017.

One Fish, Two Fish: A Better Way of Counting Mosquitofish Fry

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ABSTRACT Accurate enumeration of data can be difficult when managing large numbers of live aquatic specimens, such as the western mosquitofish, *Gambusia affinis*. Manually weighing or counting such organisms is inefficient, prone to bias, and may result in prolonged handling stress. The Orange County Mosquito and Vector Control District's mosquitofish program has explored a practical, non-invasive, and cost-effective method of tallying daily mosquitofish fry production using a simple combination of a cellphone camera and MS Paint®, a free software pre-loaded onto any Windows® operating system. This software allows staff to easily and expediently collect reproductive output data with precision and accuracy.

INTRODUCTION

The western mosquitofish, *Gambusia affinis*, is the most widely-used organism for the biological control of immature mosquitoes (Swanson et al. 1996). These livebearing fish are prolific, feed voraciously, and are extremely hardy (Pyke 2005, Walton 2007). When used properly, these fish can be effective at reducing the emergence of adult mosquitoes.

Many statewide mosquito and vector control agencies have dedicated captive-rearing aquaculture programs (Programs) for the production of these fish. Such Programs, if designed well and maintained in a controlled environment, allow staff to maximize fish production, operate within budget, and enhance quality control. When compared to acquiring mosquitofish by other means, such as field collection or purchase, these Programs provide staff with a more consistent and reliable in-house supply of high quality product to meet demand.

Enumeration of newly produced young fish (i.e., fry) during collection can help Program staff determine and inventory mosquitofish production. Traditionally, fish counts are estimated based on weight and require manual manipulation of fish. These methods are cumbersome, inaccurate, and induce unnecessary stress on fish.

Our objective was to identify an alternative means for counting total fish numbers that avoided weight estimates and minimized fish handling stress. The enumeration method discussed here allows Program staff to assess daily fish production more precisely and efficiently as compared to the traditional weight-based method.

METHODS

First, obtain a white, plastic bucket (for maximum color contrast) and hand net for collecting mosquitofish fry. Harvest the fry and place them into the bucket in as little water as possible to increase picture clarity and prevent the overlapping of fish (Figure 1a). Any detritus or biotic material should be removed at this point. Next, position the cell phone camera above the white bucket, assure proper lighting, and take the photograph (Figure 1b). Place fry into their designated tank immediately after taking the photograph to minimize stress and holding time.

Download the photograph onto a computer and open it in MS Paint® (Figure 1c). Select a brush style and color from the menu options (preferably, use a brush style with a bright color for easy visibility). Begin marking each fish with the brush tool while simultaneously recording with a hand-held tally counter, being sure to tally one fish per mark (Figure 1d). It is highly recommended to place the mark between the fish eyes to best demarcate each fish. Save the picture as needed and record fish count data.

CONCLUSIONS

The mosquitofish enumeration method described here greatly reduces fish handling time, fry stress and mortality, and errors that may result from human bias. Unlike traditional fry estimates that rely on staff handling and weighing live fish, this method uses a photograph of the sample, which may be archived and used by other individuals for verification. Once fry quantity is tabulated, each photograph can be used as a standard for future comparisons. While comparisons between this method and traditional counting

were not included here, the traditional counting method is no longer used in the Orange County Mosquito and Vector Control District's (District) mosquitofish program. The District has shown that the former method was inaccurate and invoked unnecessary handling stress. Furthermore, when fry counts for the same harvests were performed by several staff using the methods described here, discrepancies were greatly reduced. Currently, comparative fry counts for precision and accuracy between the MS Paint® method and free imaging software, such as ImageJ, are being explored.

Total fry biomass may also be indirectly estimated by multiplying fry count data with average fry mass, which is very useful information for Program staff for determining initial fish feeding rates, stocking density, and production. Biomass data can also help determine if current fry production can meet future needs.

Because mosquitofish experience high levels of mortality during their first year, largely due to competition and cannibalism by larger fish, immediate harvesting of fry can be very important for maximizing fish survival and use. This method offers Program staff a simple, precise, and low-cost way to estimate mosquitofish fry production.

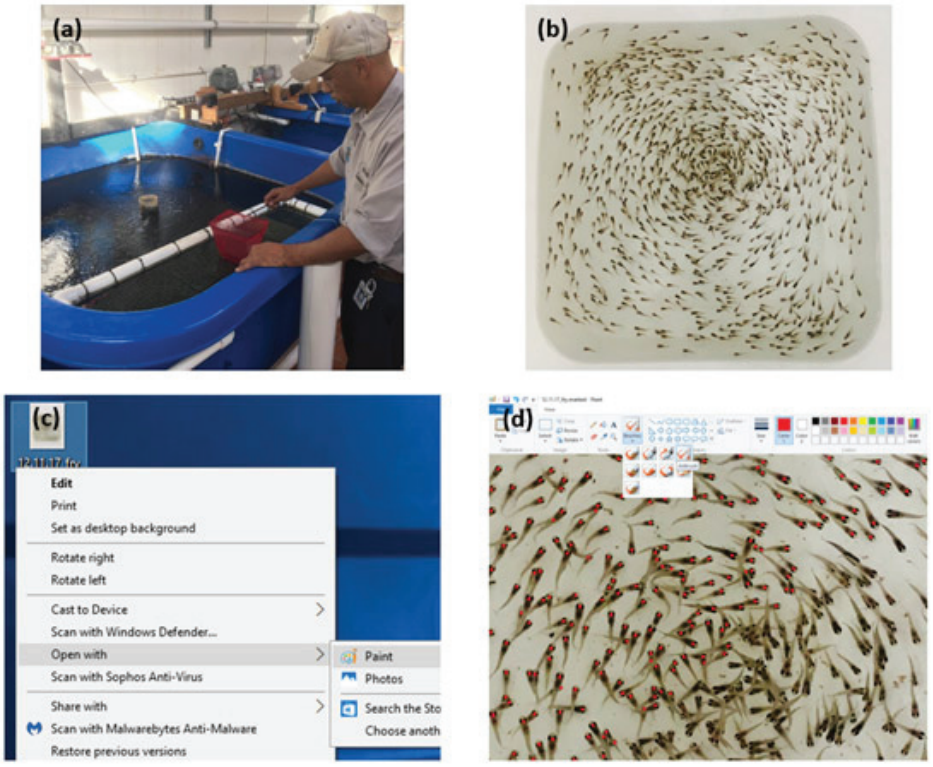

Figure 1 The process of enumerating mosquitofish fry (a) beginning with fry collection and (b) photographing the daily harvest, then (c) opening the image in MS Paint®, and (d) tallying the fry as they are counted.

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Mosquito Control and FEMA

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INTRODUCTION

In the early spring of 2017, the San Joaquin County Mosquito and Vector Control District (District) was faced with the challenge of responding to mosquito breeding in the areas affected by the aftermath of the January and February storms. The affected areas were expansive, and required numerous aerial larvicide applications and subsequent adulticide applications to control the mosquitoes found in these areas. The District's work was increasingly important, due to the fact that many of these affected sites were at the epicenter for West Nile virus activity during the three previous years. Because San Joaquin County was included in a major disaster declaration (FEMA-4308-DR-CA), the District applied for reimbursement from the Federal Emergency Management Agency (FEMA) for costs incurred to carry out the actions necessary to control these mosquitoes.

DISTRICT'S RESPONSE

The District initiated contracted aerial spraying and in-house ground - based applications to abate the increased threat of mosquito-borne diseases. The District used mobilizedforce account labor and equipment and utilized associated materials to carry out these mosquito abatement measures. The necessity to educate our public regarding the mosquito control efforts along designated areas most affected by these storms and related flooding was crucial. Simultaneous to the District's control efforts, Governor Brown included San Joaquin County in his proclamation of a State of Emergency for FEMA consideration.

The District applied for Public Assistance reimbursement funding for the emergency protective measures utilized to mitigate the threat of mosquito-borne diseases to the residents and visitors of San Joaquin County. We were required to provide FEMA with documentation of all the work completed to avert a public health threat. The costs incurred by the District totaled $381,429.35, approximately 10% of the District's operational budget to control the mosquitoes in and around these affected sites.

DISCUSSION

The District's proof used to qualify for reimbursement for mosquito abatement measures through FEMA; included, letters of cooperative support received from our local public health officer, San Joaquin County Office of Emergency Services (San Joaquin County OES), and California Department of Public Health Vector Borne Disease Section. In addition, we provided the damage description and dimensions of the area affected, cost documentation, charts and graphs of mosquito counts, description and costs of pesticides used, and maps illustrating the pesticide application areas. District staff continued to work closely with representatives from FEMA, California Office of Emergency Services (Cal OES), and San Joaquin County OES representatives through the end of September 2017. Through this process, additional information was requested that included copies of contract agreements with our aerial applicators, pesticide application cost comparisons to the average costs of the three previous years March thru June, and copies of news releases and other forms of public outreach conducted by the District. The application process was laborious and required long hours to achieve the requirements of the FEMA application process. Although we provided all the necessary documentation necessary to qualify for FEMA public assistance, the District received preliminary information through Cal OES that mosquito control reimbursement requests may be deemed ineligible on a State-wide basis for the FEMA-4308-DR-CA storm event.

CONCLUSION

Federal report stated that 2017 shattered U.S. damage record for natural disasters. Costs incurred by Hurricane Harvey, Irma, and Maria totaled $292 billion and the northern and southern California fires totaled $14 billion. It could be said that in any other year, the District may have been in a more favorable position in its request for FEMA reimbursement.

Update from the Pacific Southwest Center of Excellence in Vector-Borne Diseases

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ABSTRACT The Pacific Southwest Center of Excellence in Vector-Borne Diseases addresses the urgent public health challenges presented by ongoing spread of invasive vectors, exotic pathogens such as Zika virus, and several endemic pathogens transmitted by mosquitoes and ticks. UC Davis and UC Riverside, along with MVCAC, CDPH, and other public health and vector control partners, aim to increase the capacity of the United States to respond to vectorborne disease threats by (1) conducting applied research to develop and validate effective prevention and control tools and methods to anticipate and respond to invasive mosquitoes and disease outbreaks, (2) training vector biologists, entomologists, and physicians in the knowledge and skills required to address vectorborne disease concerns, and (3) strengthening and expanding already effective collaboration between researchers and public health organizations for surveillance, prevention, and response. This presentation included a brief overview of the center and highlighted emerging research on invasive *Aedes aegypti* and *Aedes albopictus*, which have continued to spread throughout urban southern California.

Defense resources available for emergency vector control with Hurricane Harvey as a case study

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ABSTRACT The U.S. Department of Defense maintains a large and robust mechanism to protect troop health against insect vectors worldwide. When a disaster or epidemic occurs within the U.S. or its territories, these assets may be employed as part of Defense Support of Civilian Agencies (DSCA). This paper discussed the various entomological and public health related resources that are potentially available from the 3 military services (Army, Navy, and Air Force) under DSCA. After looking at the scope of what is available, the recent response of the Air Force Aerial Spray Unit in Texas, post-Hurricane Harvey, was examined as a case study.

An overview of the statewide *Aedes aegypti* pesticide resistance program in California

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ABSTRACT The global spread of Zika virus in 2015 and subsequent local transmission in the United States in 2016 brought the vector potential of *Aedes aegypti* to the forefront as a public health concern. Traditional control methods have been ineffective due to a variety of ecological and biological factors, including various resistance mechanisms to major classes of insecticides that have been described in some populations of *Ae*. *aegypti*. In 2017, the California Department of Public Health, Vector-Borne Disease Section established a pesticide resistance monitoring program for *Ae*. *aegypti* submitted by local mosquito control agencies combating the spread of these mosquitoes. Currently, this program focuses on genetic screening of *Ae*. *aegypti* for two mutations known to confer knockdown resistance to pyrethroids/pyrethrins. Program goals include establishing resistance profiles in California, providing comprehensive reports of laboratory testing to participating agencies, developing enzyme activity and live mosquito assays of pesticide resistance, as well as expanding testing to include *Ae*. *albopictus*. The information generated by these assays will be useful to local agencies for developing control strategies against these invasive mosquitoes, especially regarding arboviral response plans.

Impact of Management Practices on Mosquito Abundance in Wetlands Managed for Wildlife

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ABSTRACT The management of seasonal wetlands for waterfowl and other wetland-dependent wildlife has the potential to produce mosquito abundance that surpasses abatement thresholds of local mosquito control districts. To help minimize the impact of wetland management activities on public health and safety, best management practices (BMPs) were developed by the Central Valley Joint Venture in coordination with the California Department of Public Health and the Mosquito and Vector Control Association of California. In the current study, we evaluated the effect of two BMPs (discing and establishment of predator reservoirs) on the presence of mosquito larvae in wetlands managed by the California Department of Fish and Wildlife (CDFW) at two wildlife areas (Gray Lodge and Los Banos). Larval mosquito abundance during early summer in disced treatments at both study sites (1.6 and 0.15 larvae per dip at Gray Lodge and Los Banos, respectively) was much lower compared to two other management strategies – traditional (6.02 and 3.23 larvae per dip) and optimal seed production (10.85 larvae per dip at Los Banos) and were lower than in wetlands with reservoirs for mosquito predators (2.58 and 1.65 larvae per dip). Immature mosquito abundance was similar in areas treated by both BMPs during July at Gray Lodge Wildlife Area, and the lack of irrigation in the traditional control wetlands meant that no mosquitoes were collected. When financially feasible, discing has the potential to both enhance wetland quality for waterfowl and decrease mosquito production in units managed for wintering waterfowl, but there may be occasions when wetland units utilizing these BMPs require insecticide treatment because mosquito abundance has surpassed abatement thresholds. Further evaluation is needed to determine the overall cost-effectiveness and long-term applicability of these control strategies.

INTRODUCTION

The Central Valley of California is one of the most important areas for migrating birds in North America (Heitmeyer et al. 1989), exhibiting some of the highest concentrations of waterfowl anywhere in the world. Since the Gold Rush Era, approximately 90% of the wetlands in this region have been lost to agriculture, water diversions, and flood control (Gilmer et al. 1982). This loss places an ever-increasing importance on the few remaining wetlands in this region.

Irrigating seasonal wetlands during the summer months is one of the most effective tools for increasing the carrying capacity of a seasonal wetland for waterfowl. On average, irrigating seasonal wetlands during the summer months doubles the carrying capacity for wintering waterfowl as compared to Central Valley wetlands without summer irrigation (Naylor 2002, Olson 2010). Irrigating wetlands during this time period also has been shown to produce larval mosquito abundances that surpass abatement thresholds of local mosquito and vector control districts (Washburn 2012). There is no universal abatement threshold for mosquitoes, but discussion among members of the AB 896 working group found that thresholds for treatment of *Aedes* mosquitoes were between 1 and 3 larvae per dip while 1 *Culex* mosquito found in 10 dips indicated that treatment was needed (unpublished discussion December 2017).

To minimize the impact of wetland management strategies on public health and safety, the Central Valley Joint Venture, in coordination with the California Department of Public Health and the Mosquito and Vector Control Association of California, developed guidelines for best management practices (BMPs) for mosquito control in managed wetlands (Kwasny et al. 2004). This guide is readily used by wetland managers, but the effect of these BMPs on larval mosquito numbers and whether these strategies can reduce adult mosquito abundance below current abatement thresholds rarely has been investigated. The current study evaluated the efficacy of different BMPs in wetlands at wildlife areas of the Central

Valley that are managed primarily for wintering waterfowl.

In this initial study, we evaluated the impact of two BMPs for limiting mosquito abundance and enhancing wildlife values. Discing has been shown both to reduce mosquito production in managed wetlands (Lawler et al. 2007, Washburn 2012) and to increase the carrying capacity of seasonal wetlands for waterfowl (Gray et al. 1999, Naylor 2002). This is done largely by reducing the presence of perennial emergent plant species and promoting more productive annual species of moist-soil vegetation. The presence of semi-permanent water within the swales and potholes of wetland units offers a habitat that is generally lacking in the Central Valley of California, but has been suggested to reduce mosquito production in irrigated seasonal wetland units (Washburn 2012).

MATERIALS AND METHODS

Our evaluation was conducted on Gray Lodge Wildlife Area (Butte County) in the Sacramento Valley, and Los Banos Wildlife Area (Merced County) in the San Joaquin Valley. Both of these properties are owned and operated by the California Department of Fish and Wildlife and are actively managed to promote plant communities beneficial to wintering waterfowl. Wetland units that were evaluated were chosen in coordination with area managers, and ranged between 6.9 and 29.1 ha (mean=13.8 ha).

Within these wetland cells, we evaluated the effect of two BMPs that have been suggested to reduce mosquito larval densities within managed wetlands and provide benefits to wildlife using those wetlands. These treatments included: 1) discing wetland units prior to irrigating, and 2) maintaining water in swales between irrigations to serve as a reservoir for maintaining native invertebrate predators. Larval mosquito abundance in the wetlands managed using BMPs was the compared to traditional and optimal management methods, described below.

The discing treatment entailed drying seasonal wetland units in March, and discing all undesirable or residual vegetation using two passes with a stubble disc, and then one pass with a finish disk approximately 4-6 weeks after drawdown. Operators used stubble disks with 71.12-91.44 cm (28-36 in.) blades that cut a minimum of 15.24-20.32 cm (6-8 in.) below ground level. Following discing, units were flash irrigated (for not more than 5 days) to germinate moist-soil seeds, and irrigated 1 or 2 more times (4-7 days for each irrigation) to bring plants to maturity. Wetlands were monitored for mosquito larvae during each irrigation period, using the sampling method described below. Wetlands with predator reservoirs were drawn down in April. About 4 weeks after drawdown, managers conducted any pre-irrigation treatments they felt were necessary other than discing (e.g. mowing of cocklebur, spraying of jointgrass, etc). Following this, swales and potholes were flooded within the unit to establish the desired predator base. Units were then irrigated 2-3 times to get moist-soil plants to maturity. Water levels in swales and potholes were maintained between irrigations to maintain predator populations. The predator populations were not sampled, but were expected to be similar in nature to Washburn's study (2012). Wetlands were monitored for mosquito larvae during each irrigation period, using the sampling method described below.

Traditional wetland management (= control treatments) entailed drawing down the water in wetlands in April. About 4 weeks after drawdown, managers conducted any pre-irrigation treatments they felt were necessary other than discing as described above. Units were then irrigated once, approximately 6 weeks after drawdown for 14-21 days. This is the method of moist-soil management that has historically been used by wetland managers to achieve their target plant species composition and productivity. This is by far the simplest (and cheapest) to employ without considering costs associated with mosquito abatement.

The optimal management treatment entailed drawing down water in wetland units in April. Approximately 4 weeks after drawdown, managers conducted any pre-irrigation treatments they felt were necessary other that discing as described above. Units were then irrigated 2-3 times (initial irrigation approximately 6 weeks after drawdown), with the last irrigation being approximately 2 weeks in duration. This method typically results in the highest carrying capacity for wintering waterfowl.

Wetlands were monitored weekly during irrigation periods, starting 2 days after wetland units were filled. Mosquito larvae were sampled within three 200-meter sections along the perimeter of each wetland that were identified as being representative of vegetation occurring throughout the unit. We took 20 dips per section (10 meters between each dip), for 60 total dips per wetland unit. At each location we sampled the best available habitat within reach, and collected larvae with a standard, white, 400-ml dipper. Sampling was conducted by technicians of Butte County Mosquito and Vector Control District or CDFW staff trained by Fresno Westside Mosquito Abatement District. When able, laboratory technicians from Fresno Westside MAD aided in verifying the genera of captured mosquitoes.

The abundance of mosquito (*Culex* and *Aedes*) larvae per dipper sample was compared among three treatments at a particular wildlife area using a Kruskal-Wallis one-way analysis of variance for each sampling date. Pairwise comparisons of mosquito abundance between treatments were made using the Mann-Whitney test. Dipper counts from the three sampling areas in each wetland were treated as independent estimates of mosquito abundance: 60 samples per wetland per date were ranked regardless of sampling area. The three sampling dates at each wildlife area were mid-June, mid-July and late July at Gray Lodge WA and late June, late July and mid-August at Los Banos WA.

RESULTS

Gray Lodge In mid-June, significantly more mosquito larvae (*Culex* and *Aedes*) were collected in control and predator reservoir treatments than in wetlands that had been disced ($\chi^2 = 13.14$, df = 2, $P < 0.001$) (Figure 1A); the majority of the mosquitoes collected were *Aedes*. Discing reduced total immature mosquito

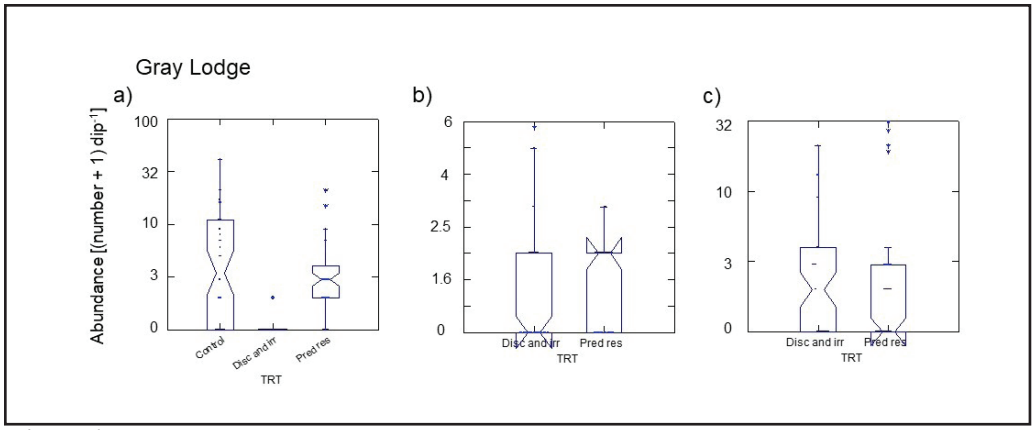

Figure 1 Larval mosquito abundance in wetland management treatment areas at Gray Lodge Wildlife Area. a) mid-June, b) mid-July, c) late July

abundance by 3.8- and 1.6-fold as compared to traditional management practices (control) and to a wetland with predator reservoirs, respectively (Table 1). In mid- and late July, larval mosquito abundance did not differ significantly between wetlands that had been disced or had predator reservoirs ($P > 0.06$) (Figures 1B and 1C). The wetlands with traditional (control) management practices had been irrigated in June but were not irrigated in July. No standing water was found, and so no samples were taken.

Los Banos The sites were more variable in Los Banos, in that not every treatment was present at each of the sampling points. In late June, larval mosquito abundance differed significantly among three treatments ($\chi^2 = 40.53$, df = 2, $P < 0.0005$) (Figure 2A). The optimal management sites did not have water and were not sampled in late June. Dip samples from the traditionally managed wetland and the wetland with predator reservoirs harbored 16- and 9-fold more immature mosquitoes per dip sample, respectively, than did the disced wetland (Table 1). However, the sampling stations in areas A and B in the disced wetland contained very little water.

In late July, approximately 5-times more mosquito larvae were collected from a wetland under optimal management than from the control wetland treatment ($\chi^2 = 64.14$, df = 2, $P < 0.0005$) (Figure 2B). There was not enough water in the wetlands under the disced treatement to be sampled. The number of mosquito larvae per dip sample from a wetland with predator reservoirs was intermediate to the wetland under optimal management and the disced wetland (Table 1).

In mid-August, only the wetland under optimal management and the wetland with predator reservoirs contained enough water for sampling. The wetland under optimal management produced 7 times more larvae per dip sample than did the wetland with predator reservoirs ($\chi^2 = 32.39$, df = 1, $P < 0.0005$) (Figure 2C). The optimal management wetland contained *Aedes* larvae, whereas the wetland with predator reservoirs contained a mixture of *Culex* and *Aedes*.

DISCUSSION

Implementing established BMPs has significant potential to reduce mosquito production in managed wetlands and improve habitat for waterfowl. At both of our study sites, disced wetlands were shown to have substantially fewer mosquitoes than traditionally managed and optimally managed wetlands early in the year. Most of the mosquitoes collected at both areas were *Aedes* mosquitoes (2,699 *Aedes* and 109 *Culex*). At Gray Lodge, as the season progressed, there was not a significant difference between the two BMP strategies implemented – both had low larval mosquito counts in the middle of June and numbers that exceeded the threshold for treatment in late June. At Los Banos, disced units typically had fewer mosquito larvae per dip than other treatments, with units with predator reservoirs having fewer larvae than traditional or optimal vegetation management strategies. The benefit to using one of the two BMPs to reduce numbers of mosquito larvae was seen over practices for traditional or optimal vegetation management in this single study year. This was due in part to the amount of time that water was permitted to stand within the treatments. The traditional wetland management (control treatments) was kept to a two irrigation events, where the second event was a long flooding of 14-21 days. The number of irrigation events was more similar between the management to produce the optimal vegetation and the two BMPs (2-4 total irrigation events), but the optimal management included a long flooding of 14 days.

There are potential drawbacks to the BMPs. Discing is expensive (approximately $50/acre), and not all wildlife areas have the capacity to implement discing at a large scale. Most wildlife areas

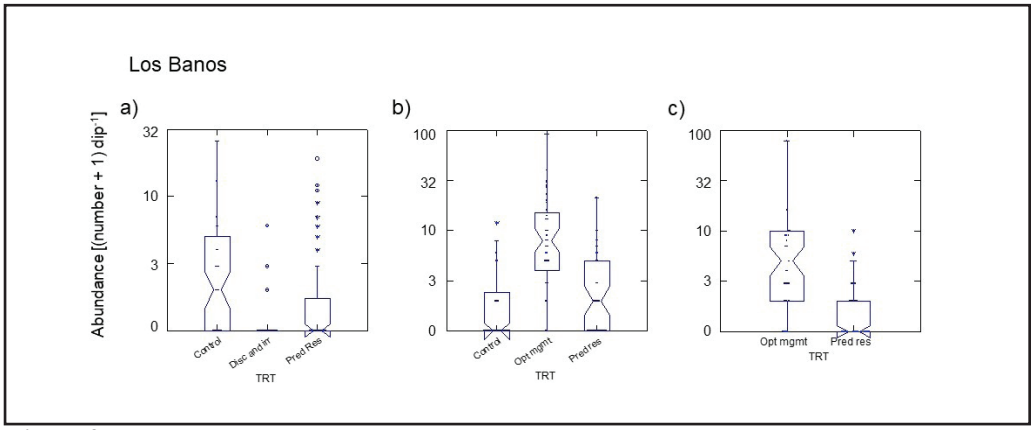

Figure 2 Larval mosquito abundance in wetland management treatment areas at Los Banos Wildlife Area. a) late June, b) late July, c) mid-August

Table 1 Comparisons of immature mosquito abundance among best management practices treatments in wetlands at two wildlife areas. 20 dips were taken in 3 separate areas of each wetland for a total of 60 dips per sampling date per wetland.

Refuge	Date	Treatments	Larval mosquitoes [median (lower, upper quartile) dip^{-1}]
Gray Lodge	mid-June	control = predator reservoirs > discing	2.5 (0, 10) = 2 (1, 3) > 0 (0, 2.5)
	mid-July	predator reservoirs = discing	1 (0, 1) = 0 (0, 1)
	late July	predator reservoirs = discing	0 (0, 2) = 1 (0, 3)
Los Banos	late June	control > predator reservoirs > discing	1 (0, 4) > 0 (0,1) > 0 (0, 0)
	late July	optimal mngmt > pred reservoirs > discing	7 (3, 14) > 1 (0, 4) > 0 (0, 2)
	mid-Aug	optimal mngmt > predator reservoirs	4 (1, 9) > 0 (0, 1)

also lack the water conveyance capabilities to get water on and off in 5-7 days, and it is unrealistic for many properties to achieve this. Although not as effective as discing, adding swales and potholes to act as mosquito predator reservoirs, supporting Coleoptera (Dytiscidae and Hydrophilidae), Hemiptera (Notonectidae and Belostomatidae), and Odonata (Coenagrionidae), may be beneficial in some areas (Washburn 2012). This technique is less cumbersome to wildlife area managers than discing, and provides additional semi-permanent wetlands within the boundary of a seasonal wetland. Quantifying the impact of these smaller semi-permanent wetlands on the recruitment of locally breeding waterfowl and other wetland dependent wildlife would be of benefit to wetland managers.

Implementation of these BMPs resulted in an approximate 25% decrease in seed yield in target plan species compared with optimal management strategies. However, most of the negative impacts of these BMPs on wildlife are indirect, in that implementing the BMPs takes time away from other wildlife management activities. Staffing and funding levels at many wildlife areas are approximately ¼ to ½ what is required to optimally manage these lands. Wetland managers in California have more to do than time available to accomplish in a given year, and any additional effort (or reduction in scale of activities) requires more time per acre for management activities. Additional funding and staffing on these wildlife areas would directly increase their ability to implement BMPs that reduce mosquito production and enhance wildlife values.

A limitation to this work is that it focused on a single year. A second trial of the BMPs is planned in similar locations to examine if the trends seen in this study are repeated in 2018.

ACKNOWLEDGEMENTS

This work benefited greatly from the discussions had by the AB 896 working group. Members are, in alphabetical order: Elizabeth Andrews, CDPH; Matt Ball, Butte County MVCD; Peter Bonkrude, Shasta MVCD; Joel Buettner, Placer MVCD; Brad Burkholder, CDFW; Erika Castillo, Alameda County MAD; Jennifer Henke, Coachella Valley MVCD; Brian Olson, CDFW; Conlin Reis, Fresno Westside MAD; Marty Scholl, Sacramento-Yolo MVCD; and William Walton, UC Riverside. We also thank Andy Atkinson, Dave VanBaren, and staff at Gray Lodge Wildlife Area; and Bill Cook, Sean Allen, and staff at Los Banos Wildlife Area for their coordination and efforts in implementing these BMPs. Matt Ball and Eric Dillard at Butte County MVCD; Conlin Reis, Chance Rowan, and staff at Fresno Westside MAD; and Jessica Nesbit and Ashley Wilson at the California Department of Fish and Wildlife's Comprehensive Wetland Habitat Program conducted monitoring and identification of mosquito larvae, and without them, this work could not have been completed.

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Patterns of St. Louis encephalitis virus activity in the Coachella Valley

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ABSTRACT In the fall of 1933, more than 1,000 cases of a new encephalitis were diagnosed in St. Louis, Missouri. Previous cases of encephalitis had occurred in 1919, 1924, and 1932, but none had produced as many cases as this outbreak (Editorial 1933, Lumsden 1958). The onset of the disease was between 5-12 days, and patients fortunate enough to not succumb often recovered quickly. The etiological agent was distinct from the mosquito borne encephalitis viruses known at that time (Cox and Fite 1934). Mosquitoes were suspected to be vectors, because very few cases were found to have occurred in the same house (Editorial 1933). 1933 was the fourth year of a severe drought, and the few places where water was found included the open sewer system. "The Health Officer states that anywhere along this stream where there is a quiet place, one may dip up a mixture which is three-fourths larvae and one-fourth water – as he expressed it, 'a living soup of larvae.'" (Editorial 1933).

St. Louis encephalitis virus (SLEV) has been consistently found in the Coachella Valley since surveillance was initiated in 1977 (Emmons et al., 1978). In 1984, a resident from Indio became ill in September while a second resident in Palm Springs became ill in October (Murray et al. 1985). As these two individuals were physically removed from the Salton Sea shoreline where virus activity was thought to have been limited, further investigations on the spatial and temporal patterns of SLEV activity in the Coachella Valley were conducted to see if there were additional methods of virus maintenance (Reisen et al. 1995a,1995b). Later serological surveys found that humans were frequently infected with this virus in Coachella and Imperial valleys (Reisen and Chiles 1997, Reisen et al. 1996). West Nile virus (WNV) was first detected in the Coachella Valley in 2003, which is also the last year that SLEV positive samples of mosquitoes were detected until 2015 (White et al. 2016) (Table 1). SLEV positive samples were detected from July 28 through October 6, 2015, from June 28 through November 10, 2016, and from July 26 through September 19, 2017. This is a slightly longer detection period than was seen in 1993, when the transmission rates were highest during July-September (Reisen et al. 1996). Positive virus samples were also detected further north than in previous years. Before WNV, SLEV detections followed a pattern of starting on the east side of the Salton Sea, with subsequent detections being found to the northwest, typically stopping in Mecca and Thermal. Movement was gradual (<1.3 km/day; Reisen et al. 1995b) and thought to

Table 1 Numbers of pooled mosquito samples tested and the number of positives detected for West Nile virus (WNV), St. Louis encephalitis virus (SLEV) and western equine encephalomyelitis virus (WEEV) from 2003-2017.

	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Mosquito pools tested	1,803	1,840	2,758	2,731	1,774	2,088	1,918	3,322	2,996	3,408	2,045	2,130	3,903	4,645	5,148
Positive pools															
WNV	22	104	83	39	26	54	14	69	43	118	43	66	99	19	120
SLEV	3	0	0	0	0	0	0	0	0	0	0	0	37	92	23
WEEV	0	0	19	0	0	0	0	0	0	0	0	0	0	0	0

be within the flight range of female *Culex tarsalis,* the principal vector in this area. In 2016, positive SLEV samples were detected northwest of Mecca and Thermal in La Quinta; the two positive samples were from pooled *Cx. quinquefasciatus* mosquitoes and were found on October 20 and November 10. Three other samples of *Cx. quinquefasciatus* mosquitoes were also positive for SLEV in 2016, indicating that *Cx. tarsalis* remains the primary vector, with the potential for spillover into *Cx. quinquefasciatus.*

Reisen et al. (1993) calculated the duration of the extrinsic incubation period of SLEV and estimated that the number of degree days from oral infection to median transmission was 115.2 degree days. Their work indicated that high temperatures would be needed for an outbreak to overcome the slow replication of the virus in the female mosquitoes. When examining droughts like the one outlined by Bredick (1933) in the origins of SLEV, the winters of 2014-2015 and 2015-2016 were exceptionally dry (KTRM station in Thermal, California).

How WNV and SLEV will co-circulate in the Coachella Valley remains to be seen. Previously, SLEV was found to persist over winter, but periodic increases in activity were associated with the introductions of new SLEV strains (Reisen et al. 2002), although surveys of northbound migrant birds failed to detect active arbovirus infections (Reisen et al. 2010). A small number of pooled *Cx. tarsalis* mosquito samples containing both arboviruses have been detected concurrently in time and space (2015 – 4; 2016 – 1; 2017 – 4). Because pools contain up to 50 female *Culex* mosquitoes each, it is possible that different individual mosquitoes within these pools were positive for SLEV and WNV, and that there was not a co-infection of an individual female. There have been no known cases of SLEV in humans in the Coachella Valley since SLEV was detected in 2015, although there were 3 cases in California in 2016 and 23 cases in Arizona in 2015 (CDC 2018). WNV cases have continued to be reported in Coachella Valley (2 in 2015 and 5 in 2017). That SLEV is continuing to circulate in the Coachella Valley after being undetected for 12 years highlights the importance of continuing to monitor for likely pathogens in an area. Had the District not been testing for SLEV as part of a triplex RT-PCR assay in 2015, its presence in the valley may have been missed and appropriate mosquito control measures would not have been implemented.

ACKNOWLEDGEMENTS

The author is grateful for the discussions had with colleagues regarding SLEV, as these greatly assisted in clarifying her thoughts. These colleagues include Greg White, Kim Hung, Melissa Snelling, Chris Cavanaugh, Gabriela Harvey, Gerald Chuzel, Arturo Gutierrez, Marc Kensington, Mike Esparza, Charles Rodriguez, and Jeremy Wittie. Additionally, the author thanks William K. Reisen, Hugh Lothrop, and Branka B. Lothrop, as their work made this manuscript possible.

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Orange County is No Match for Invasive *Aedes*!

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ABSTRACT *Aedes aegypti* and *Ae. albopictus* were discovered in several small foci totaling approximately 4.5 km^2 in Orange County, Calif., during 2015. Within two years of discovery, the combined area with these invasive *Aedes* mosquitoes increased 15.25 times to a projected 69.2 km^2 by the end of 2017; a single find of the Australian backyard mosquito, *Ae. notoscriptus*, was also made in 2017 within the existing *Aedes* infestation area. Despite the Orange County Mosquito and Vector Control District's (OCMVCD) intensive education and control efforts, invasive *Aedes* have continued to spread and pose new health risks to people and animals in the county. In response to their expansion, OCMVCD augmented its mosquito surveillance program to include the use of equipment and methods specialized for collecting invasive *Aedes*, such as Biogents Sentinel (BGS) traps, BG Lure®-enhanced gravid and encephalitis virus traps, battery-powered backpack aspirators, and ovitraps; immature invasive *Aedes* were collected during focused residential and commercial property inspections by "door-to-door" surveillance teams. Geographic information systems software applications, such as ESRI's Arc Desktop 10.2 and ArcGIS Online, and Google Earth, were used to delineate the infestation areas and target invasive *Aedes* hotspots for control and public education efforts. The current paper describes the rapid expansion of the infestation from 2015 to 2017, and the general strategy used to address areas where invasive *Aedes* have had a direct impact on the quality of life for residents.

INTRODUCTION

Aedes aegypti and *Ae. albopictus* transmit arboviruses that are acknowledged as increasing threats to human health in the Americas, particularly dengue, chikungunya, and Zika viruses (Hahn et al. 2016). The emerging health threats caused by these anthroponotic arboviruses and their vectors have highlighted the need for accurate records of the extent of *Ae. aegypti* and *Ae. albopictus* (collectively, invasive *Aedes*) infestations to guide local efforts for routine mosquito surveillance and control (WHO 2009). In 2014, driven by the re-discovery of *Ae. albopictus* in Los Angeles County in 2011 (Zhong et al. 2013) and the detection in 2013 of *Ae. aegypti* in several central and southern California counties (Gloria-Soria et al. 2014, Hahn et al. 2016, Yoshimizu et al. 2016), the Orange County Mosquito and Vector Control District (OCMVCD) enhanced its mosquito surveillance program by incorporating methods specialized for detecting invasive *Aedes*. These methods included the use of mosquito traps designed to attract invasive *Aedes* and employing "door to door" (D2D) teams to inspect properties for their presence in neighborhoods. Following increased surveillance, D2D inspections, and public awareness campaigns, both species were found separately in several locations in Orange County during 2015 (CDPH 2016). Our report highlights some of the tools used and methods developed by OCMVCD to track the invasion of *Aedes* mosquitoes and define areas for focused control efforts to reduce the threat of mosquito-borne pathogen transmission.

MATERIALS AND METHODS

OCMVCD's surveillance tools for the detection of invasive *Aedes* mosquitoes included Biogents Sentinel (BGS) traps (Biogents AG, Regensburg, Germany), BG Lure®-enhanced gravid traps (Cummings 1992), encephalitis virus surveillance (EVS) traps (Rohe and Fall 1979), battery-powered backpack aspirators (Prokopack Model 1459, John W. Hock Co., Gainesville, FL), autocidal gravid oviposition (AGO) traps (BioCare® AGO, SpringStar Inc., Woodinville, WA), and ovicups (Fay and Eliason 1966). Collections of adult and immature mosquitoes (all species) were made weekly at >200 locations in Orange County and were processed by laboratory personnel. Field-collected mosquito larvae were placed in vials of 70% ETOH and identified to species. Eggs of invasive *Aedes* laid on wetted seed paper (Anchor Seed Co., Saint Paul, Minnesota) in ovicups were dried briefly on the paper, immersed in water, and reared to adults for identification. Adult mosquitoes collected in traps or aspirators were killed and kept on dry ice, identified to species, enumerated, pooled for arbovirus testing when appropriate, and recorded. Field-captured invasive *Aedes* adults were sent to UC Davis (DART Laboratory) for testing of chikungunya, dengue, Zika, and West Nile viruses. Locations of invasive *Aedes* were geocoded and added to an ArcGIS (ESRI, ArcMap ver. 10.2, 2017) shapefile for mapping, geoprocessing, and spatial statistical analysis.

Using ArcMap 10.2, OCMVCD mapped all detections and

Figure 1 A) Orange County map showing location (green star) of first *Ae. aegypti* detection in Orange County (Anaheim), 2015. B) Neighborhood proximity map with initial detection of invasive *Ae. aegypti* (green star); circle indicates 300 m (0.2 mi) radius buffered zone and outlined parcels.

delineated infestation areas through the use of the buffer tool from the geoprocessing toolset as new detections were made. New detections were defined as an area where an invasive *Aedes* was detected for the first time and was at least 800 m (0.5 mi.) from an area where detections were previously made. Buffer parameters were set to a 300 m (0.2 mi) radius from each point/dataset based on reported flight ranges of *Ae. aegypti* in an urban setting (Reiter et al. 1995). Buffered areas around data points that overlapped with other buffer areas were dissolved into a larger contiguous feature. Curved edges of infestation areas of each resulting polygon buffer were squared-off to the nearest street intersection. The "Measure Polygon" tool in ArcMap 10.2 was used to calculate the area for each polygon. Further analyses with Google Earth Pro (Google, Mountain View, CA) were performed to audit area calculations for a particular projection. Once updated with newly identified infestation areas, the shapefile was then converted to a Keyhole Markup Language File (KML) for OCMVCD technicians and D2D teams to utilize in Google Earth. The KML file allowed users without access to ArcMap software to upload and save the polygons to Google Earth and access them in the field on hand-held tablet computers.

A species distribution model (SDM) utilizing invasive *Aedes* abundance, collection location, and environmental data (temperature, vegetation index, and precipitation) as covariates was created using the "Spatial Analyst Tools" and "Spatial Statistics Tools" within ArcMap 10.2 to determine the potential for further spread of invasive *Aedes* within Orange County. As more sites with invasive *Aedes* were discovered, multiple models were created using presence data as the main covariate, similar to Kraemer et al. (2015). Euclidean distance modeling and buffering were applied to display the distribution. A homogeneous habitat throughout Orange County's urban areas was assumed and used with temperature suitability modeling (Brady et al. 2014, UCANR 2017) for projecting species expansion.

RESULTS

Initial Detection of Invasive *Aedes* in Orange County, 2015

The first detection of an invasive *Aedes* (*Ae. aegypti*) occurred in April, 2015, during a service request for mosquitoes biting occupants inside a single family home located 11.25 km (7 mi) from the Los Angeles/Orange county border within the city of Anaheim (Fig 1, A). . Within 48 hrs. of the discovery, OCMVCD mobilized 30 personnel and placed a combination of 80 mosquito traps (gravid traps, CO_2-baited BG Sentinel Traps, and ovicups) on and within a 300 m (0.2 mi) radius of the index home (Fig. 1, B), and treated the exterior of the property with an ultra-low-volume pyrethroid adulticide. OCMVCD monitored the area weekly for six months. Adult *Ae. aegypti* only were captured during the first week at the index home, and none were detected elsewhere in the surrounding area during the monitoring period. OCMVCD found "Lucky bamboo" (*Dracaena* spp.) plants in the house and successfully reared *Ae. aegypti* adults from eggs collected from the resident's Lucky bamboo plants. These plants had been purchased from a Vietnamese product market in Garden Grove (Orange County) that obtained its supply of Lucky bamboo from a wholesaler in San Gabriel (Los Angeles County), where invasive *Aedes* were already established (CDPH 2016). No adult *Ae. aegypti* were recovered at the Garden Grove market.

OCMVCD's second discovery of *Ae. aegypti* occurred on October 15, 2015, in Mission Viejo during routine adult mosquito trapping. The collection site was approximately 32 km (20 mi) south of the first detection in Anaheim and was believed to be unrelated to the earlier find (Fig. 2, A). A subsequent door-to-door investigation and mosquito surveillance effort in Mission Viejo revealed that, within a 400 m (0.25 mi) radius area of the

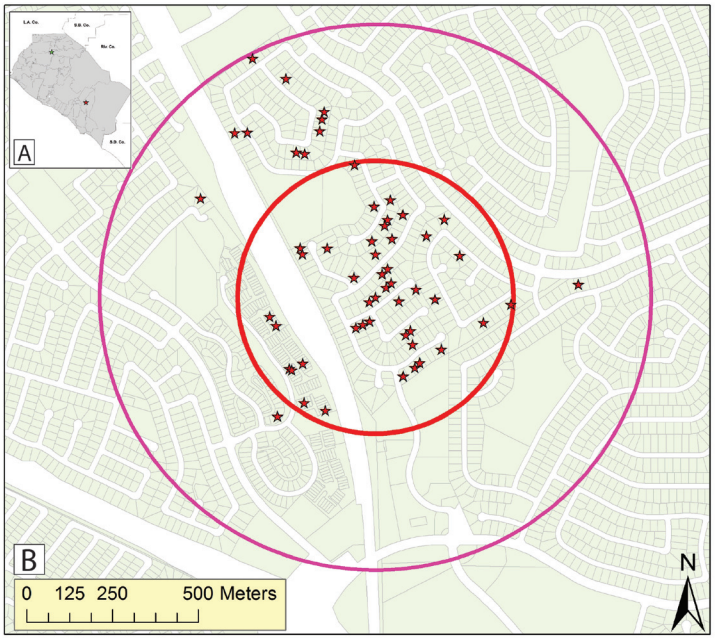

Figure 2 A) Orange County map with location (red star) of second detection of *Ae. aegypti* in Orange County (Mission Viejo), 2015. B) Proximity map of the Mission Viejo infestation with 400 m (0.25 mi) and 800 m (0.5 mi) radii buffer rings.

Table 1 Infestation area estimates for (a) *Ae. aegypti* and (b) *Ae. albopictus* by city and year.

Estimated Invasive *Aedes* Infestation Area (km^2) per City/Community by Year			
City	**2015**	**2016**	**2017**
Anaheim	0.4 (a)	1.26 (a)	7.1 (a,b)
Brea			1.03 (a)
Buena Park		0.55 (a,b)	1.21 (a,b)
Costa Mesa	0.39 (a)	0.51 (a)	1.27 (a)
Cypress			0.24 (a)
Fullerton			1.28 (a)
Garden Grove	0.59 (a)	1.7 (a,b)	9.09 (a,b)
Huntington Beach	0.34 (b)	0.34 (b)	0.81 (a,b)
Irvine			1.17 (a)
La Habra		2.26 (a)	14.82 (a,b)
La Palma			0.34 (a)
Lake Forest	0.09 (a)	0.09 (a)	0.39 (a)
Los Alamitos	0.59 (b)	0.66 (b)	4.58 (b)
Mission Viejo	0.86 (a)	1.85 (a,b)	2.39 (a,b)
Newport Beach		0.12 (b)	0.12 (b)
Orange	0.24 (a)	0.44 (a,b)	3.41 (a,b)
Santa Ana/Unincorp. North Tustin	1.11 (a)	3.35 (a,b)	19.59 (a,b)
Stanton			0.34 (a)
Total Infestation Area (km^2)	**4.59**	**13.13**	**69.18**

index property, 57% (46/80) of all inspected properties were found to be occupied by adults and/or larvae *Ae. aegypti* (Fig. 2, B). Additional detections were made on properties up to 800 m (0.50 mi) from the index home (Fig. 2, B). OCMVCD initiated door-to-door mosquito control (source reduction, larviciding, and adulticiding) and public education measures immediately in an attempt to eradicate the mosquitoes from the neighborhood. After these first two detections of invasive *Aedes* in the county, OCMVCD responded to eight more geographically-distinct discoveries of invasive *Aedes* during October - November, 2015, in the following cities: Huntington Beach (*Ae. albopictus*), Los Alamitos (*Ae. albopictus*), Anaheim (*Ae. aegypti*), Costa Mesa (*Ae. aegypti*), Garden Grove (*Ae. aegypti*), Lake Forest (*Ae. aegypti*), Orange (*Ae. aegypti*), and Santa Ana (*Ae. aegypti*) (Fig. 3, A). At the end of 2015, the total countywide infestation area for both invasive *Aedes* species, based upon flight range estimates, was approximately 4.59 km^2 (1.77 mi^2) distributed over nine of the 34 cities in the county. Within the known infestation area, *Ae. aegypti* was estimated to infest 78.3% [3.6 km^2/4.59 km^2 (1.39 mi^2/1.77 mi^2)] of the total, and *Ae. albopictus* comprised the remainder (Table 1); their distributions did not overlap in 2015 (Fig. 3, A).

Expansion Years, 2016 - 2017 During 2016-2017, counts of invasive *Ae. aegypti* collected in BGS traps increased from a countywide average of 0.1 *Ae. aegypti*/trap-night (n= 58 *Ae. aegypti*/554 trap-nights; range 0-11) to 0.51 *Ae. aegypti*/trap-night (n = 325 *Ae. aegypti*/633 trap-nights; range 0-13), while numbers of *Ae. albopictus* declined from a countywide average of 0.32 *Ae. albopictus*/trap-night (n = 179 *Ae. albopictus*/554 trap-nights; range 0-14) to 0.02 *Ae. albopictus*/trap-night (n = 13 *Ae. albopictus*/633 trap-nights; range 0-4). In Santa Ana, the county's most heavily infested city with *Ae. aegypti* (Table 1), BGS trap counts increased from 0.1 *Ae. aegypti*/trap-night (n = 9 *Ae. aegypti*/90 trap-nights; range 0-3) to 0.98 *Ae. aegypti*/trap-night (n = 176 *Ae. aegypti*/179 trap-nights; range 0-6) during 2016-2017. These changes in trap counts were the result of increased surveillance in *Ae. aegypti*-infested areas and indicate a higher relative abundance and greater distribution of *Ae. aegypti* compared to *Ae. albopictus* in Orange County.

In 2016, 20 new infestation areas were identified (Fig. 3, B), and the total number of cities with invasive *Aedes* increased 50% from 9 to 12 cities (Table 1). The total infestation area nearly tripled from 4.59 km^2 to 13.13 km^2 (1.77 mi^2 to 5.05 mi^2) during 2016, with the majority of the increase occurring from August to November. In 2017, the expansion of invasive *Aedes*- infested areas increased more than five times over the amount of 2016, from an estimated 13.13 km^2 to 69.18 km^2 (5.05 mi^2 to 26.7 mi^2)

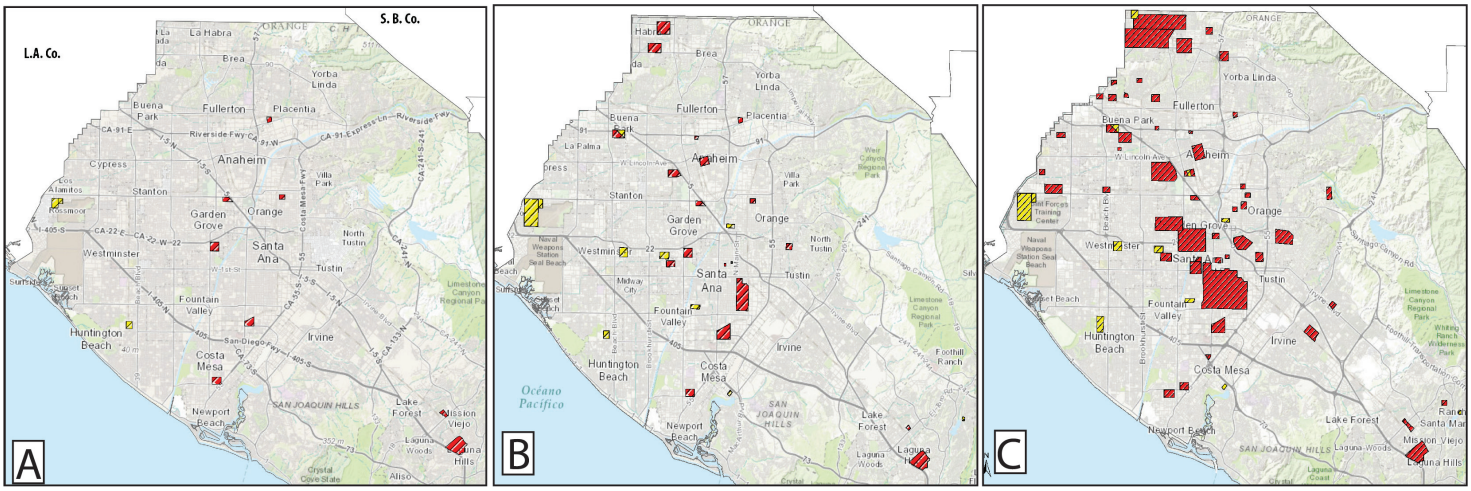

Figure 3 A) 2015: Initial detection of invasive *Aedes* mosquitoes [total infestation = 4.5 km^2 (1.75 mi^2)]. B) 2016: Second year of detections and expansion of invasive *Aedes* mosquitoes [total infestation area = 13.1 km^2 (5.1 mi^2)]. C) 2017: Third year of detections and expansion of invasive *Aedes* mosquitoes [total infestation area = 69.1 km^2 (26.7 mi^2)].

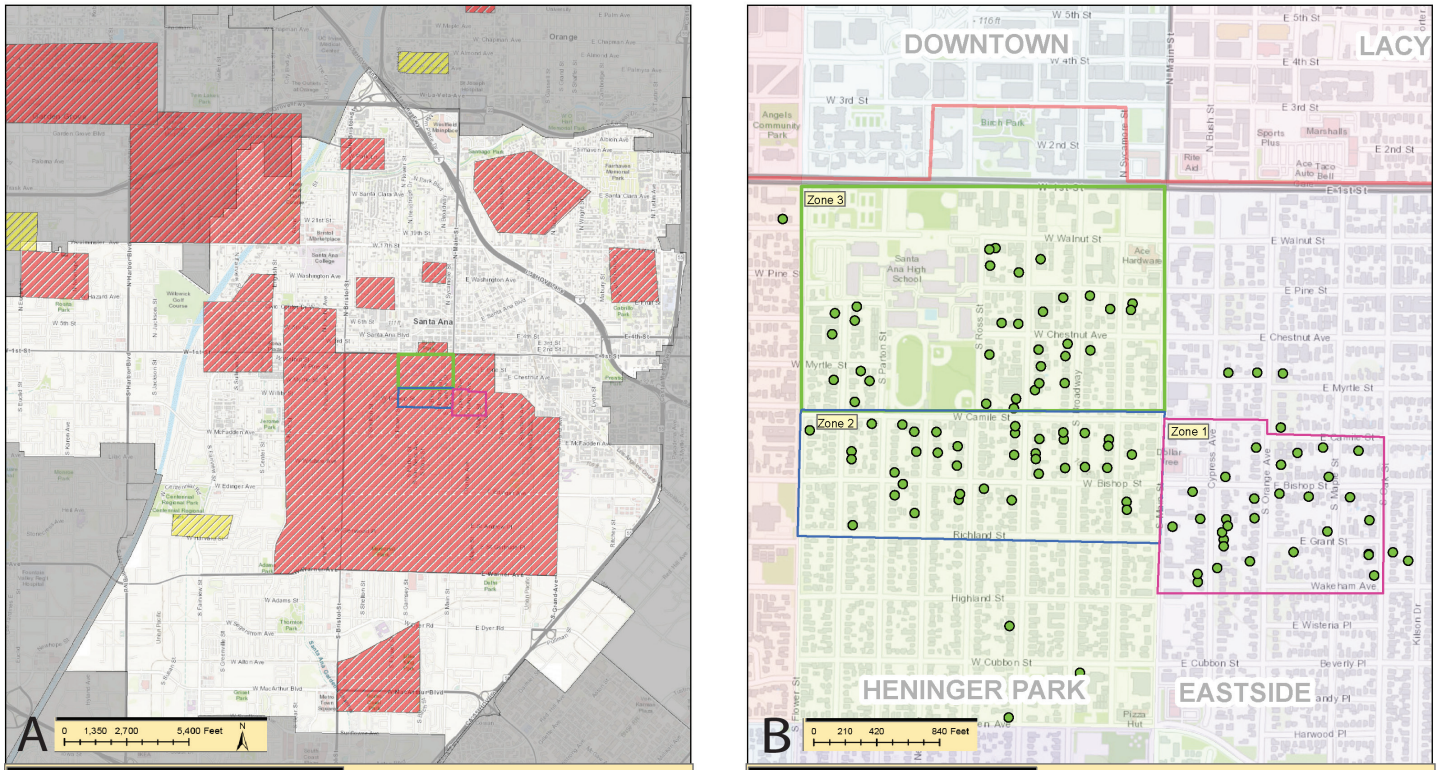

Figure 4 A) Estimated *Ae. aegypti* infestation areas (red) in Santa Ana (white). B) Highest *Ae. aegypti* infestation zones in Santa Ana. Green dots indicate properties with *Ae. aegypti*.

in total area (Table 1). The number of distinct infestation areas and cities with invasive *Aedes* expanded from 30 to 50 and from 12 to 19, respectively, by the end of 2017 (Fig. 3, C; Table 1). *Aedes aegypti* accounted for about 77% of the total invasive *Aedes* infestation area [10.1 km^2/13.13 km^2 (3.9 mi^2/5.1 mi^2)] by the end of 2016, similar to 2015's value (78.3%). In 2017, however, new detections of *Ae. aegypti* increased relative to *Ae. albopictus*, resulting in *Ae. aegypti* accounting for approximately 90% [62.2 km^2/69.1 km^2 (24.0 mi^2/26.7 mi^2)] of the combined invasive *Aedes* infestation area by the end of the year. Overlap of *Ae. albopictus* and *Ae. aegypti* distributions occurred in two and seven areas of Orange County at the end of 2016 and 2017, respectively (Fig. 3, B, C). Of 496 newly-discovered locations with invasive *Aedes* (larvae and/or adults) in 2017, *Ae. aegypti* was found in 471 (95.0%); 313 (63.1%) of the new sites with invasive *Aedes* were found in Santa Ana.

The largest area infested with *Ae. aegypti* is currently located within Santa Ana and occupies an estimated infestation area of 412.7 km^2 (159.3 mi^2) (Fig 4, A). Due to an increase of service requests attributed to *Ae. aegypti* biting pressure on the residents of Santa Ana during 2017, OCMVCD initiated an area-wide control plan to alleviate the level of mosquito annoyance. Staff were deployed three times, 2 days each, over a three week period in September, 2017, to three adjacent, *Ae. aegypti*-infested neighborhoods in the city (Fig. 4, B). Of 677 targeted homes, OCMVCD accessed 383 (57%) for inspection and treatment; *Ae. aegypti* was found breeding in 23% (89/383) of the inspected properties.

Aedes aegypti was also found in an underground stormwater drain during routine mosquito surveillance with EVS traps for *Cx. quinquefasciatus* in the city of Cypress during 2017. Although not considered optimal habitat for invasive *Aedes*, *Ae. aegypti* breeding has been documented in stormwater drainage systems in Mexico (Arana-Guardia et al. 2015) and observed in Los Angeles County (S. Kluh, pers. comm.).

In addition to *Ae. aegypti* and *Ae. albopictus*, another invasive *Aedes*, the Australian backyard mosquito, *Ae. notoscriptus*, was found in Anaheim during 2017 in a larval sample mixed with immature *Ae. aegypti*. The known distribution of *Ae. notoscriptus* in Orange County was limited to this single location at the end of 2017.

Arbovirus Test Results, 2016-2017 OCMVD submitted 242 pools consisting of 419 *Ae. aegypti* and 64 pools with 163 *Ae. albopictus* females for arbovirus testing to UC Davis (DART Laboratory) during 2016 – 2017. None tested positive for an anthroponotic arbovirus; however, one pool each of *Ae. albopictus* and *Ae. aegypti* tested positive for West Nile virus in 2016 and 2017, respectively.

DISCUSSION

The findings from the 2015 discoveries of invasive *Aedes* in Orange County suggested that some of the introductions were associated with the movement of plant products purchased in other southern California counties and by adult dispersal via vehicles. The Lucky bamboo plants found in the 2015 Anaheim infestation originated from an ornamental plant wholesaler located in an invasive *Aedes*-infested region of Los Angeles County. The *Ae.*

aegypti infestation in Mission Viejo may have originated from a member of a bromeliad plant club, who grew over 10,000 potted bromeliads on the property and readily traded them throughout southern California. This site is located about 200 m (0.125 mi) south of the index property and was heavily infested with immature and adult *Ae. aegypti*. OCMVCD also observed invasive *Aedes* fly into passenger cars for potential dispersal to other locations during investigations in heavily infested neighborhoods.

After analyzing data from 2015 through 2017, results from the SDM suggests that most of the projected spread of invasive *Aedes* will be confined to north Orange County and that the majority of the region will be inundated with *Ae. aegypti* within several years (Fig. 5). These results also demonstrated that *Ae. aegypti* is expanding faster than *Ae. albopictus*. *Aedes aegypti* is considered to be better suited than *Ae. albopictus* for dry climates and is expected to occupy more areas of the southwestern U.S. and California (Hahn et al. 2016, Lounibos et al. 2016). In addition, the transmission potential for anthroponotic arboviruses is considered greater with *Ae. aegypti* than *Ae. albopictus* (Campbell et al. 2015, Lounibos et al. 2016).

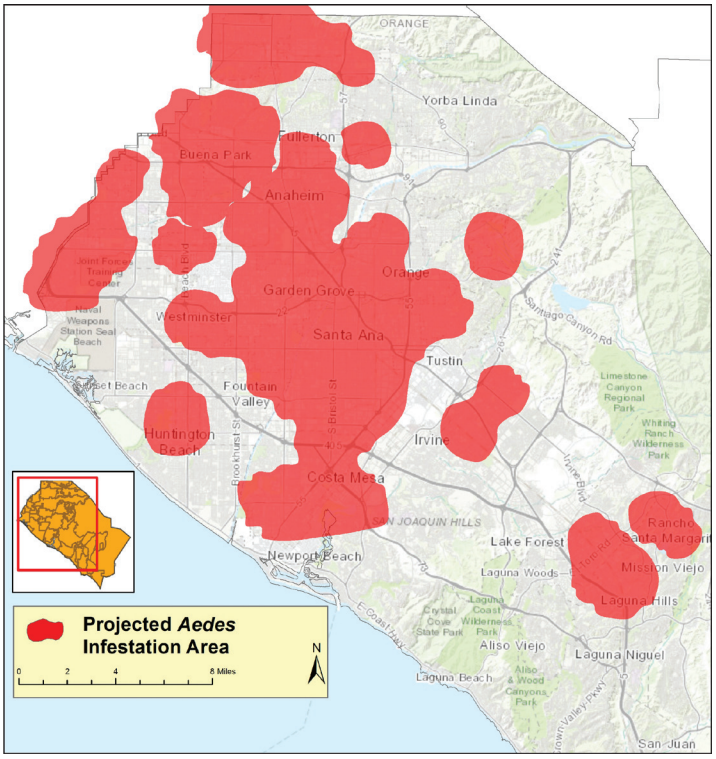

Figure 5 Projected invasive *Aedes* distribution using data from 2015, 2016, and 2017.

CONCLUSION

The control methods needed to combat *Ae. aegypti* and *Ae. albopictus* are different from the approaches used to suppress *Culex* vectors in urban areas of southern California. OCMVCD has adjusted its control program accordingly to include specialized "door-to-door" teams to aid in the control of numerous small breeding sources and public education campaigns within invasive *Aedes*-infested neighborhoods. Furthermore, OCMVCD's use of a species distribution model has allowed for fine-scale tracking of invasive *Aedes* and helped delineate areas where they are expected to spread in Orange County. This approach is essential in suppressing arbovirus transmission by invasive *Aedes* through geographically-focused applications of adulticides and larvicides. New methods under development for invasive *Aedes* control, such as the release of *Wolbachia*-transfected invasive *Aedes* males (Dobson et al. 2004, Xi et al. 2005) and pyriproxyfen-infused oviposition traps (Unlu et al. 2017), will be evaluated in the future.

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Limited specificity of a TaqMan PCR assay developed to screen for *Borrelia burgdorferi sensu stricto*

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ABSTRACT *Borrelia burgdorferi* sensu stricto, the spirochete that causes Lyme disease, is a member of the *B. burgdorferi* sensu lato group. While the western blacklegged tick, *Ixodes pacificus*, is the vector of Lyme disease to people in western North America, many genomospecies within this group have been detected in California *I. pacificus*. In the past few decades, more specific molecular protocols have been developed to streamline testing and surveillance for *B. burgdorferi* ss, with results being used to assess prevalence and human disease risk. In this poster we highlight the limited specificity of a TaqMan PCR assay that was originally developed and used to determine tick infection with *B. burgdorferi* ss. This assay captures other members of *B. burgdorferi* sl group, but does not amplify *I. pacificus* infected with *Borrelia miyamotoi*.

Mosquito Magnet and BG-Sentinel Traps Baited with BG-Lure for Collecting *Aedes aegypti*

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INTRODUCTION

Mosquito Magnet Traps (MMT) are used to suppress the abundance of *Culex* and *Aedes* spp. mosquitoes that are native to the USA. BG-Sentinel traps were developed to trap *Aedes aegypti* and *Aedes albopictus*, which are not native to the USA. The geographic expansion of invasive *Aedes* mosquitoes into habitats previously dominated by *Culex* spp. brings attention for a need to control the abundance of mosquitoes from both genera in urban and suburban landscapes.

METHODS

We compared the number of *Ae. aegypti* collected by MMT and BG-Sentinel traps that were both baited with a BG-Lure at sites in the City of Madera (California, USA) where *Ae. aegypti* were prevalent during the study period. Briefly, traps were placed at least 100 m from each other at sites in the City of Madera with known *Ae. aegypti* infestations, trap contents were collected weekly, and trap location randomly reassigned each week (n = 3 independent trials at 12 trap locations).

RESULTS

The MMT captured 8-times more *Ae. aegypti* compared to the BG-Sentinel trap, suggesting that the former is more effective for invasive *Aedes* surveillance and abundance suppression.

Blood-meal analysis of *Culex erythrothorax* collected in a marsh habitat

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INTRODUCTION

Culex erythrothorax mosquitoes can transmit arboviruses such as West Nile virus (WNV) and may be highly abundant in marsh habitats that contain tule or bulrush. Knowing the species of animal that infectious mosquitoes take blood from can inform vector control workers of whether there is increased risk of transmitting the virus to humans. For example, if WNV-infected mosquitoes also contain human blood, there may be an increased risk for human infection in the area where the mosquitoes were collected.

METHODS

Nested PCR was used to amplify the *mitochondrial gene cytochrome c oxidase I* (*mtCOI*) to identify the species of animal that the mosquito had fed upon. To validate the PCR primers, DNA was extracted from 50 µl of blood collected from rooster, bovine and horse, the *mtCOI* gene amplified using nested PCR, the resulting PCR products separated using gel electrophoresis, the DNA extracted from the gel and sequenced. BLAST queries of the DNA sequences that were extracted from the blood of known species against the NCBI Nucleotide collection (nr/nt) database returned the *mtCOI* gene of the corresponding species (PCR products were > 500 bp; BLAST Expect values (E) were 0). Of note, as little as 0.5 µl of blood could be amplified using nested PCR and sequenced to determine the species of animal from which the blood was collected DNA was then extracted from blood-engorged *Cx. erythrothorax* that were collected in marsh habitats bordering the San Francisco Bay (California, USA) to determine the species of animal that the mosquitoes had fed upon.

RESULTS

Culex erythrothorax collected at the Hayward Regional Shoreline had fed upon mourning dove, California towhee, Wild Turkey, American crow, and human. The finding that human blood was found in *Cx. erythrothorax* collected at a site that is infrequently visited by people suggests that this WNV vector may disperse from breeding sites to seek blood meals.

Innovative application technology proven to break DENV and ZIKV transmission

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INTRODUCTION

A cornerstone of the successful response to a 2016 outbreak of Zika virus in Florida, USA, was wide area larvicide spray (WALS™) of VectoBac® WG (Bti strain AM65-52 WG) to control container mosquitoes, specifically *Aedes aegypti* and *Ae albopictus*. This technology was first developed by the Institute of Medical Research (IMR) in Kuala Lumpur, Malaysia where backpack sprays were demonstrated to reduce dengue incidence (Lee et al. 2008, Tan et al. 2012). The technology has been used for several years by the Singapore Ministry of Defense (MINDEF) for troop protection (Lam et al. 2010, Lee et al. 2010), and was successfully evaluated in the USDA Asian Tiger Mosquito (ATM) control project (Williams et al. 2014). Following the re-introduction of dengue virus to Florida, WALS application methodologies were expanded using forest protection aerial spray methods, and successfully integrated into dengue and Zika virus control programs (Pruszynski et al. 2017, Likos et al. 2016). Here we present application and evaluation methods for WALS, and highlight its impact within an IVM response to a Zika virus outbreak.

WALS TECHNOLOGY

The objective of WALS application of VectoBac WG is to distribute spray drops containing this target-specific larvicide over a wide area in a manner which deposits effective doses into the small and hard-to-find habitats of container mosquitoes. Drop size and coverage are critical to success of WALS application. Drops in the extra fine to very fine (VF) size class (ASABE Standard S-572.1) ranging from 40 to 140 μm are utilized to achieve both coverage and container deposit. Three spray platforms have been developed for WALS application (Table 1). In order to apply WALS rapidly over large areas, both aerial and advanced vehicle mounted application technologies were developed starting in 2010 (Table 1). Aerial application methods were based on forest protection spray platforms designed for control of lepidopteran

Table 1 WALS Application Platfor

Platform	Utility	Equipment	Remarks
Backpack Sprayers	Protection of zones around houses where known disease cases have been reported. Targeted treatment of known container accumulations.	Power Backpack sprayers capable of generating VF drop spectra (60-80μm)	Sprays can be targeted to accumulations of trash, tires, bromeliads, and other known or suspected container accumulations within properties.
Vehicle Mounted Sprayers	Wide area coverage of residential blocks.	Modified cold foggers and air blast machines capable of generating VF drop spectra (40-120μm depending on equipment)	Application is limited to streets and highly dependent on wind for drop distribution across blocks.
Aerial Application	Rapid coverage of large areas.	Helicopters and fixed wing aircraft equipped with atomizers capable of generating drop spectra from 80-140μm.	Not dependent on streets or property access.

Figure 1 Backpack spraying in Malaysia

pests. These platforms have the capacity to provide rapid coverage of large spray blocks with drop spectra that penetrates vegetative canopy, and deposits into small containers with sufficient coverage and dose to control mosquito larvae.

WALS IMPACT ON ZIKA TRANSMISSION

During the 2017 Zika virus outbreak in the Wynwood neighborhood in Miami, Florida, USA, VectoBac WG was repeatedly applied using aerial WALS to a two square mile area surrounding the outbreak. Aerial ULV applications of the mosquito adulticide naled were concurrently applied to a 10 square mile area which included the transmission zone. *Aedes aegypti* in BG Sentinal® trap counts decreased to one per trap per day after the second aerial spraying with naled. Counts then gradually returned to high levels (>20 per trap per day) in the adulticide-only spray area, but were maintained at about 5–10 per trap per day for at least 1 month in the area treated with both adulticide and WALS (Likos et al. 2016). The integrated approach including WALS application stopped Zika virus transmission (US CDC 2016).

Figure 2 Truck mounted spraying Singapore

LARVICIDAL EFFICACY

Larvicidal efficacy of WALS is monitored by measuring spray deposit and by changes in adult mosquito trap counts. Spray deposit is monitored by placement of sentinel jars in the treated area before spraying. Six ounce polystyrene jars (US Plastics) are opened and placed upright inside the jar lid for exposure to sprays. Four different exposure conditions are selected to confirm good coverage, including: open to the sky; light vegetative cover (<50% coverage); heavy vegetative cover (50-100% coverage); and completely obstructed (under a table or porch). Typically >10 replicate houses are selected for placement of Jars in the four conditions. Jars are collected after spraying and sent to Benzon Research, Carlisle, PA for bioassay using 20, laboratory-reared, third instar *Ae. aegypti* in 100ml of water placed in the jar. Percent mortality is read at 2, 4, 6, 24, and 48 hours after infestation. During the 2016 Zika virus outbreak

Figure 3 Dengue Response Key West, Florida

Figure 4 Zika virus response Miami, Florida

in Florida, these bioassays were conducted for Miami Dade, Volusia, Broward, and Brevard Counties. Representative results for both mean percent mortality and percent coverage (percent of jars showing any mortality) are presented in figures 5 and 6. Both helicopter and fixed wing applications provided acceptable coverage, even when obstructed by canopy and cover.

CONCLUSIONS

- Wide area larvicide spray (WALS) of VectoBac WG has been successfully developed for control of dengue, Zika and chikungunya virus vectors.
- WALS offers efficient control of container mosquitoes in small containers which are hard to find and hard to treat.
- Disease transmission has been interrupted in multiple settings using WALS either alone or in integrated programs involving other interventions.
- WALS application platforms including backpack sprayers, aircraft spray systems and vehicle mounted sprayers have been successfully employed for control of container mosquito vectors.

ACKNOWLEDGEMENTS

The authors would like to thank the following organizations and individuals who have contributed to recent development and operational implementation of WALS in the Americas:

Benzon Research: Chad Finkenbinder; Danielle Costigan

Cayman Islands MRCU: Alan Wheeler; Fraser Allen; Bill Petrie; Richard Clough; and Angela Harris.

Coachella Valley MVCD: Jeremey Whittle; Jennifer Henke; Rodney Chamberlain; and Gabriella Harvey

Clarke Mosquito Control: Clark Wood; Griffith Lizarraga; Jake Hartle; Amy Solis; Bill Jany; and Derek Drews

Dynamic Aviation: TK Rosalina; Kurt Friedemann; Dan Shenk; and Dustin Hill

Florida Keys MCD: Ed Fussell; Steve Bradshaw; Andrea Leal; Catherine Pruszynski; Larry Hribar; James Zdan; Kurt Joseph; and Curtis Libby.

Helicopter Applicators Inc. Glenn Martin; Dan Rudisill; and Mark Bunch.

REMSpC Consulting: Bob Mickle and Lynn Mickle

Special thanks to Candace Royals of Valent BioSciences LLC for her countless hours in the field supporting spray operations and evaluations.

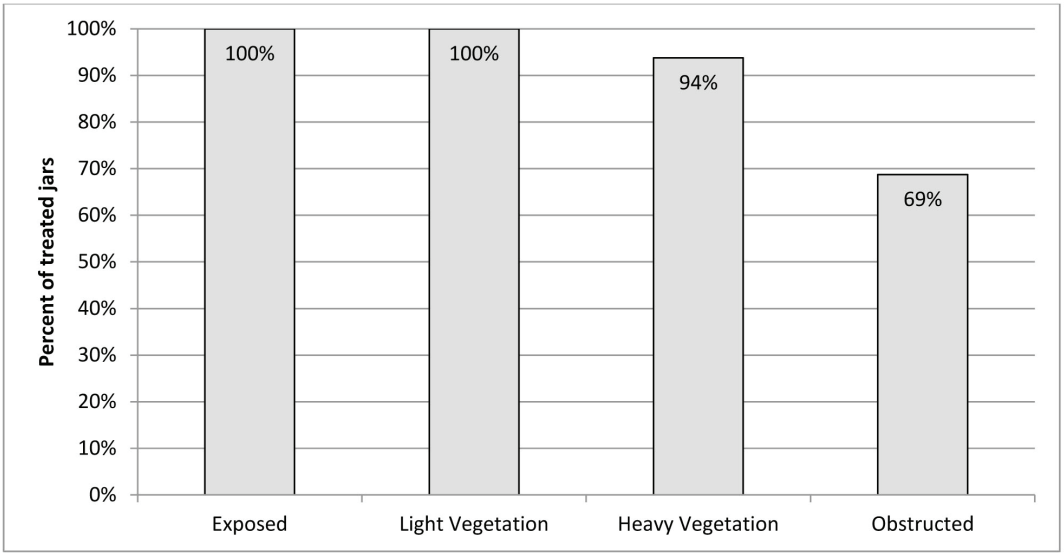

Figure 5 Percent of jars showing at least 20% mortality in bioassay after exposure to a VectoBac WG WALS helicopter application in four different conditions in Broward County, Florida . (n=34 exposed jars; n=16 jars under light cover, 16 jars under heavy cover, and 16 completely obstructed jars)

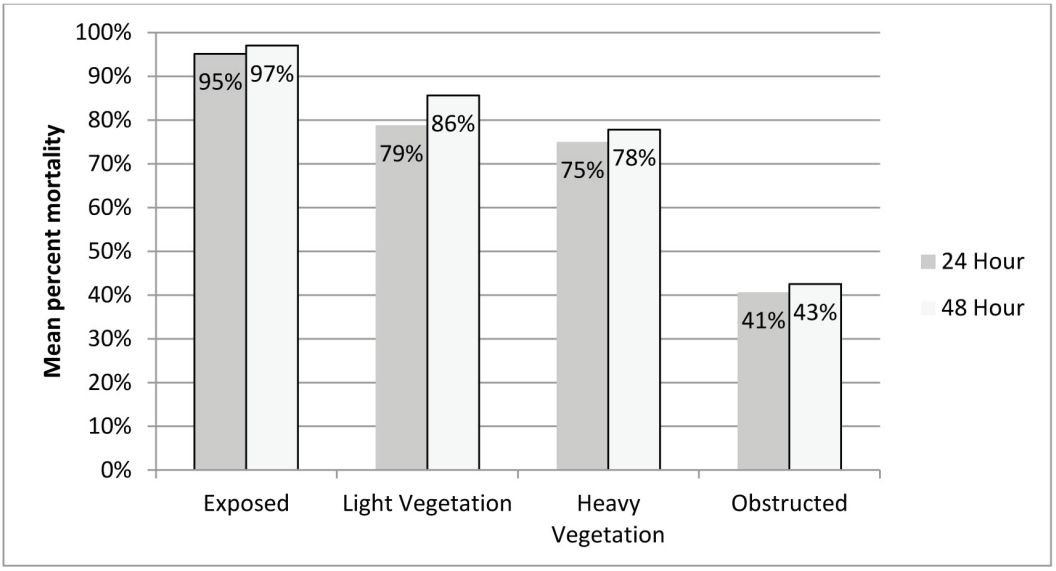

Figure 6 Mean 24 and 48 hour percent mortality measured in bioassay of sentinel jars placed in four cover conditions during an aerial spray in Broward County Florida. (n=34 exposed jars; n=16 jars under light cover, 16 jars under heavy cover, and 16 completely obstructed jars)

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Elucidating a possible genetic component underlying host preference in *Culex tarsalis*

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INTRODUCTION

In the western United States, *Culex tarsalis* is an important bridge vector of arboviruses, including West Nile, western equine encephalomyelitis, and St. Louis encephalitis viruses (Reeves et al. 1990; Reisen 2012). West Nile virus has caused epidemics in the U.S. every summer since 1999, resulting in 48,088 cases and 2,017 deaths as of January 9, 2018 (CDC). Although considerable research has been done on *Cx. tarsalis* ecology (Reisen 2012) and blood-feeding behavior (Thiemann et all. 2012), genetic analyses are scanty. Using microsatellite markers, at least three distinct populations have been identified in the US(Venkatesan and Rasgon 2010), but these populations have not been compared with respect to phenotypes such as vectorial capacity, insecticide resistance, or behavior. Within other species, for example, genetic variation has been linked to insecticide resistance (Main et al. 2015) and host preference in multiple mosquito species (Main et al. 2016; McBride et al. 2014). *Culex tarsalis* is an opportunistic feeder, and host availability may influence host selection (Campbell et al. 2013). However, over-utilization of some hosts and under-utilization of others has been reported, and the frequency of mammalian blood meals varies seasonally (e.g. 0 to 42% in 2008), with a peak in late summer (Thiemann et al. 2011). Whether host selection has a substantial genetic basis has not been explored.

METHODS

In this study, we sequenced the genomes of 12 individual adult female *Cx. tarsalis* collected at a farmstead in north Davis in 2008, as described in Thiemann et al. (2011). Individuals were selected for genome sequencing based on whether they fed on mammalian (n=6) or avian (n=6) hosts. The combined genomic sequencing data was used to assemble a draft *Cx. tarsalis* reference genome using the software MEGAHIT (v1.1.2). The nuclear assembly was highly fragmented, but the mitochondrial genome was nearly complete (15,566 bp), which was 92% identical to the *Cx. quinquefasciatus* mitochondrial genome.

RESULTS AND DISCUSSION

We identified 1,044 biallelic SNPs in the mitochondria. A lower nucleotide diversity was observed among mammal-fed (π =0.001) versus bird-fed (π =0.002) *Cx. tarsalis*. Using a principal-components analysis, we found evidence suggestive of population structure.

CONCLUSIONS

The results of this mitochondrial study were consistent with the hypothesis that specific *Cx. tarsalis* genotypes were more likely to feed on mammalian than avian hosts. However, additional genetic analysis involving genome-wide nuclear markers and larger sample sizes are needed to confirm this pattern.

ACKNOWLEDGEMENTS

We thank the Vector-Borne Disease pilot grant program of the UC Davis School of Veterinary Medicine for funding. The sequencing was carried by the DNA Technologies Core and Expression Analysis Core at the UC Davis Genome Center, supported by NIH Shared Instrumentation Grant 1S10OD010786-01. CMB also acknowledges support from and 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).

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Re-Charging the EVS-CO2 Trap

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ABSTRACT Surveillance of adult mosquitoes plays an essential role in mosquito control programs by providing a way to track mosquito-borne virus activity, mosquito abundance, and target areas for control. The non-rechargeable battery-powered (three D-cells) Encephalitis Virus Surveillance (EVS) carbon dioxide-baited trap has been widely-used by mosquito control agencies as a surveillance tool since its introduction in 1979. Although improvements to the original design have been made over time, the reliance on D-cell battery power makes the trap prone to operational failure and produces copious battery waste when utilized in a large-scale mosquito surveillance program. The Orange County Mosquito and Vector Control District (OCMVCD) modified its stock of EVS traps and replaced the old D-cell battery unit with a rechargeable 6-volt, sealed gel-cell, lead-acid battery to improve reliability, reduce operational costs, and minimize battery waste. Enhancements also included hanging a BG-Lure® at the side of the trap near the trap entrance and adding white tape to the black body of the trap; both modifications were made to increase host seeking cues that are particularly attractive to invasive *Aedes*. OCMVCD is using this new trap design as part of its mosquito surveillance program with promising results.

INTRODUCTION

Mosquito traps have been an integral part of mosquito and arbovirus surveillance since the introduction of the CO_2-baited, 6 volt-powered CDC miniature light trap (Sudia and Chamberlain 1962, Newhouse et al. 1966). In California, mosquito control agencies use a variety of custom-designed and commercially-produced versions of this trap, with some designs powered by disposable D-cell batteries mounted on the trap (4.5 – 6V) or by an unattached, 6V rechargeable gel cell battery connected to a power cable, with or without light (Resien et al. 2000). One CDC-style trap, the 4.5 volt driven Encephalitis Virus Surveillance (EVS) mosquito trap (Rohe and Fall 1979), has been widely-used with CO_2 bait by a variety of mosquito and public health agencies worldwide to assess temporal and spatial distributions of mosquitoes, black flies, and other biting arthropods. It features an "all in one unit" trap design with three non-rechargeable D-cells mounted on top of the trap frame.

Attaching the power supply to the EVS trap allows for a single point to hang the trap in the field without needing a second spot for battery placement, as is required with other CDC-style versions where the power supply is separate from the trap. This single unit design has proven to be very convenient when setting traps in underground stormwater drains for mosquito surveillance (Su et al. 2003), or at locations where a trap stand with a battery holder would be difficult to position. When considering applications of traps and batteries, selection should be based on the most practical design for the task they will fulfill (Weber 1988).

The Orange County Mosquito and Vector Control District (OCMVCD) has constructed its version of the EVS trap since the 1980s using readily-available materials.

The results of several trap comparison studies have suggested that the configuration of the trap inlet and direction of mosquito entry plays a larger role in determining catch size than supply voltage, airflow, or CO_2 presentation (Cummings and Meyer 1999, Klein 1999, Resien et al. 2000). Following these studies, OCMVCD made several design improvements to its version of the EVS trap, including removing a 4.5 in. dia. rain shield over the trap inlet that reduced mosquito access. Improvements also included encasing the trap's power supply in a water-resistant, plastic-cover to prevent electrical problems due to condensation. For ease of use in the field, the power supply is still mounted to the EVS trap frame; the rain shield was considered the primary structure that reduced mosquito access to the trap.

Although the EVS trap is relatively easy to build and deploy, disposal and replacement of the batteries is both expensive and environmentally unsound when used in a large-scale mosquito surveillance program (Williams et al. 2009). To overcome these limitations, OCMVCD recently modified its version of the EVS trap into a more cost-effective design by replacing the D-cell battery holder with a small rechargeable 6-volt, sealed gel-cell, lead-acid battery (SLA). This presentation reports on the benefits of the new design.

Problems with the Three D-Cell Battery-Powered EVS CO_2–Baited Trap

- Frequent electrical shortages due to battery housing design
- Requires frequent removal and replacement of batteries
- Produces excessive battery waste

MATERIALS AND METHODS

Construction Steps:

1) Deconstruct an old D-cell powered EVS Trap (Fig. 1).
2) Fasten the top side of a soap dish container (Item 1) to the top of the EVS metal frame.
3) Crimp two 22 gauge primary wires to a 22-18, 6 stud, ring terminal. Thread the terminal through a 10-32 bolt with lock washers (Item 3), and drill into the underside of the soap container. Repeat the crimp and ring terminal step and drill alongside the previous bolt on the underside of the soap container. These bolts hanging through the soap container will act as the positive/ negative terminals for re-charging the 6V SLA battery (Item 4). Once installed, removal of the battery for recharging is not required.
4) Take one of the loose wires from one of the ring terminals and crimp to a 16-14 female 0.250 quick disconnect terminal (Item 5). Repeat with the other loose wire from the other ring terminal. These quick disconnect terminals attach to the respective 6V SLA battery terminals.
5) Take the second loose wire from the positive ring terminal and solder to the SPST mini toggle switch (Item 6). Solder a new wire from the switch to the positive terminal on the motor. Take the last loose wire from the other ring terminal and solder to the negative terminal on the motor (Item 7).
6) Use heat-shrink tubing (Item 8) appropriately to cover all exposed wires. Place a Velcro® strip (Item 9) in the soap dish container (Item 1) to hold the battery (Item 4) in place. Fix the top half of the soap dish container to the bottom half using zip ties.
7) Attach a 3 in. fan (Item 10) to the motor (Item 4) and place the completed frame (Item 11) into a 4 in. x 3 in. ABS DWV Hub x Hub Reducing Coupling (Item 12).

Materials List Items:

1. Two soap dish container, tops only (Item 1)
2. Two 22-18, 6 stud, ring terminal (Item 2)
3. Two 10-32 bolts, 0.75 in. long and 6-32 lock washers
4. One rechargeable sealed lead acid 6V 4.5 amp-hr. battery (PowerSonic® Model PS640, San Diego, CA)
5. Two 16-14 female 0.250 quick disconnect terminal
6. One SPST mini toggle switch, 6 amp, 125 VAC rating
7. One Mabuchi motor, RF-500TB-14415, brush motor
8. 1ft., 1/8 heat-shrink tubing
9. 1 ft., Velcro® strip
10. One 3 in. dia. fan blade
11. One EVS metal frame
12. One 4 in. x 3 in. ABS DWV hub x hub reducing coupling

Battery Charging Station:

Arrange a series of 6V battery chargers (500 or 1,000 mA output, depending on the type) with alligator clips and connect to standard 120V AC electrical outlets (Fig. 2). Connect each charger's alligator clips to the designated polarity (+/-) of the two recharging bolts projecting below an EVS trap's power unit. Recharging a trap's 6V SLA battery takes about 4 hrs. using a charger with a 500 mA output (less with a 1,000 mA output). 6V battery chargers can be purchased at retail electronic supply stores or online (e.g., PowerSonic® model PSC-61000A-C).

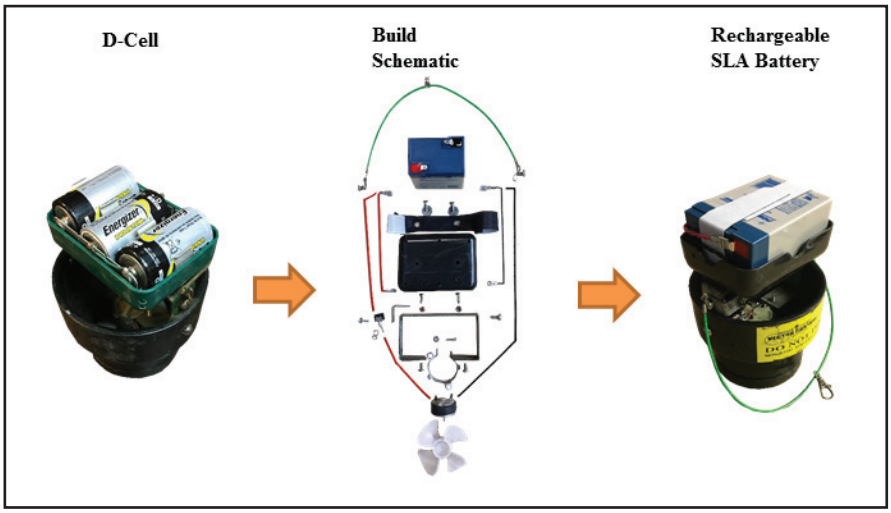

Figure 1 Build diagram of the 6V rechargeable EVS trap.

Figure 2 Recharging Station

DISCUSSION

Converting an existing 4.5-volt, D-cell battery powered trap into one with a mounted rechargeable battery consists primarily of replacing the housing for the D-cell batteries with a rechargeable 6-volt SLA battery. This modification eliminates the often faulty D-cell battery housing, adds user-friendly charging connection

terminals, and increases motor speed due to an extra 1.5 volts (Bhattacharya 2008). A single conversion can be done in approximately two hours. Since OCMVCD completed its trap conversion of 105 EVS traps in 2017, only two rechargeable SLA EVS trap failures have been recorded compared to 31 failures during a previous year with the D-cell battery design. The cost of operating the old D-cell and new rechargeable SLA EVS traps were compared and indicated a savings of $0.68 per use (Fig. 3). The rechargeable SLA battery has an expected lifespan of up to five years. At the OCMVCD, the use of each EVS trap once a week equates to ~50 cycles a year. After taking into account the cost of the rechargeable SLA battery ($8.19), battery charger ($20 - $40.00), and electricity per charge ($0.32), each converted EVS trap will save OCMVCD approximately $121/trap unit throughout the five-year lifetime of the rechargeable SLA battery (Fig. 4). In total, OCMVCD projects to save over $12,000 during the next five years as a result of the modifications to all 105 traps in its inventory.

An optional adaptation that OCMVCD is currently evaluating with the rechargeable battery conversion involves attaching a human scent (Takken 1991) mimic lure [Biogents (BG)-Human Lure Cartridge, Biogents AG, Regensburg, Germany] at a cost of $28.30/unit. This modification involves using a wire and one chain link to hang the lure from the side of the trap near the trap entrance. The lure has an expected average life of four months. Therefore, the cost of just replacing the lures will be $424.50/unit over five years (Figure 2). Although the preliminary results indicate that the BG lures are attracting invasive *Aedes*, not enough data have been collected to show if these results ultimately justify the additional cost of adding the lures.

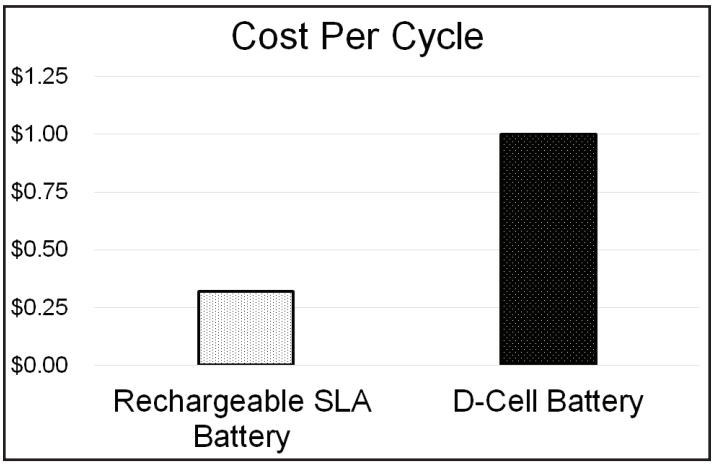

Figure 3 Comparison of the cost of running a rechargeable, 6-volt SLA battery trap ($0.32) versus a three D-cell battery trap ($1.00) for one trap night (24 hours).

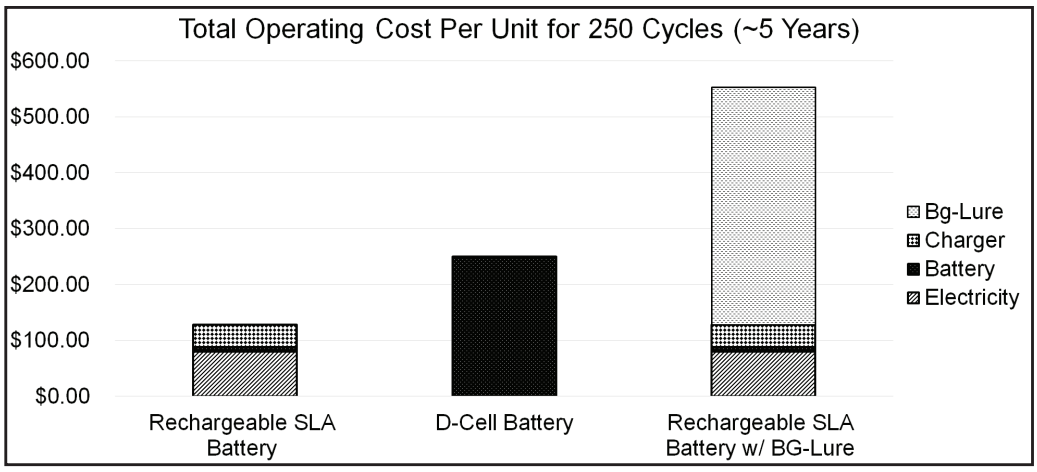

Figure 4 Comparison of total operating costs (including hardware such as battery, and charger for SLA battery) for 250 cycles of a rechargeable SLA battery trap without BG-Lure ($128.18), a D-cell battery trap ($250), and a rechargeable SLA battery with BG-Lure ($552.68).

CONCLUSION

After one year of use, the reconfiguration of the three D-cell EVS trap to a rechargeable 6-volt SLA battery power system has resulted in fewer trap failures and produced less battery waste. Although no trap comparison studies have been done, this new version of the EVS trap has shown to be as effective at collecting host-seeking mosquitoes from multiple genera consisting of *Culex, Aedes, Culiseta, and Anopheles* as the former design. OCMVCD predicts that these remodeled EVS traps will last multiple years, require fewer repairs, and save money. Further refinements are under consideration.

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Saving resources by utilizing a novel reel dipper to inspect out-of-reach sources

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INTRODUCTION

Dipper tools to survey water sources for mosquito larvae are typically comprised of a white cup that can hold up to 330 ml of water and is attached to a handle of 90 – 175 cm in length. Traditional dippers are highly effective to inspect water sources for mosquito breeding if the water is within easy reach of the mosquito control worker. However, some water sources, such as open settling tanks at waste water treatment plants, are difficult or dangerous to access when using traditional dippers. Consequently, workers may be inclined to apply larvicide at high-risk out-of-reach breeding sources without having an accurate estimate of mosquito larvae density.

METHODS

We employed a short fishing pole and reel with the line attached to a 150 ml plastic vial that was weighted with a metal washer to dip tanks at waste water treatment facilities. To dip with the reel-based dipper, the fishing pole was suspended over the water and the weighted vial quickly released directly downward, into the water. Upon striking the water, the vial filled rapidly and was immediately retrieved by winding the spool of the fishing reel. The retrieved water was deposited into a traditional dipper to estimate larval density.

RESULTS

When tested at a waste water treatment facility during 2017, the reel-based dipper retrieved fewer larvae from hard-to-reach breeding sources than collected during the prior year, resulting in less work effort and lower quantities of larvicide (Vectorbac G) (reduced by 9 h and 119 lbs, respectively). This study highlights the value of developing novel approaches and equipment to inspect atypical and hard to access mosquito breeding sources.

Effectiveness of Broadcast Treatments of Extinguish Plus® on Red Imported Fire Ant Infested Golf Courses of the Coachella Valley

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ABSTRACT Due to daily irrigation creating humid conditions, golf courses with acres of turfgrass and planted trees provide suitable conditions for massive *Solenopsis invicta* colony formations. Golfers and maintenance crews are attacked frequently receiving painful stings from the aggressive ants. Increased mosquito surveillance and control needs during 2017 led the Coachella Valley Mosquito and Vector Control District to hire seasonal employees to conduct inspections and treatments of red imported fire ants, *S. invicta*. To ensure that treatments were as effective as those made in previous years, we examined golf courses prior to treatment, approximately 4 weeks after treatment, and 4 months after treatment. Broadcast treatments of Extinguish Plus® Insecticide Fire Ant Bait (active ingredients: 0.365% hydramethylnon and 0.250% s-methoprene) were made at 1.5 lbs. per acre using a Seed Herd Spreader. To determine ant activity, the District used a baiting survey procedure that required placing hot dog slices around the tee, fairway, and green of six non-consecutive holes at each 18-hole golf course (Figure 1). After 60 minutes, the number of positive baits was recorded (Henke and Perezchica-Harvey 2017). Extinguish Plus® reduced ant activity on 13 of the 15 golf courses treated. The overall percentage of baits positive for fire ants pretreatment was reduced from 58% (522 of 900 baits)to 40% of baits 4 weeks after treatment, a 31% reduction. At 4 months after treatment, the percentage of positive baits was 51% among all golf courses, similar to the pretreatment data. These results were similar to reductions seen in previous years.

ACKNOWLEDGEMENTS

The authors are grateful for work done by Seasonal Vector Control Technicians who treated the golf courses; in alphabetical order, they were: Elias Casarrubias, Manuel De La Torre, Guillermo Delgado, Heriberto Dismaya, Jose Maldonado, Albert Martinez, Marcos Martinez, Marco Medel, Mario Munoz, Cesar Ponce, Rosendo Ruiz, Jesus Vasquez, and Jose Zavala. We also thank the staff at the golf courses for their cooperation during our surveys.

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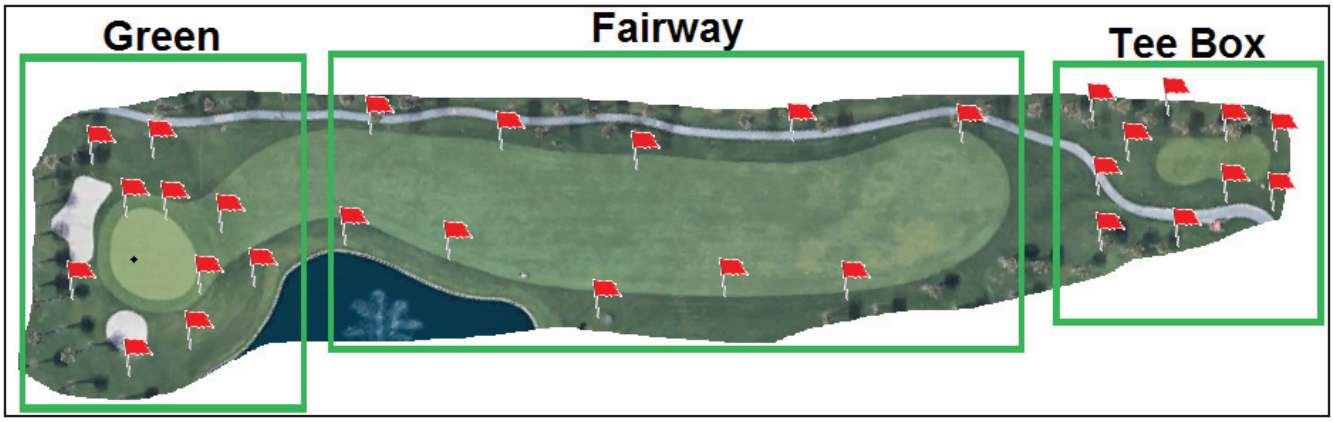

Figure 1 Typical layout of baits at a hole on a golf course where the flags represent the approximate location of the hot dog slices (bait). Six non-consecutive holes were assessed at each 18-hole course.

Permethrin Resistance in *Culex pipiens*

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INTRODUCTION

Culex pipiens is a vector of West Nile virus (WNV) that is broadly distributed in urban and suburban landscapes. Vector control agencies routinely employ insecticides to reduce the abundance of infected *Cx. pipiens*, and often use those with pyrethroid-based chemistries because of the relatively low residues and safety properties. We sought to employ the MVCAC Integrated Vector Management Committee's recommendations on testing *Cx. pipiens* that were collected in Alameda County for insecticide resistance by examining the activity of permethrin-metabolizing enzymes and the *kdr* loci of the *voltage gated sodium channel 1* (*vgsc-1*) gene using quantitative polymerase chain reaction (qPCR). Mutation of L1014F at the *kdr* loci of *vgsc-1* in *Cx. pipiens* contributes to pyrethroid resistance. A goal of the study was to determine the baseline of permethrin resistance in *Cx. pipiens* in Alameda County.

METHODS

The activity of alpha-esterase (AE), beta-esterase (BE), oxidase (OX), glutathione-s-transferase (GST), acetylcholinesterase (AChE) and insensitive acetylcholinesterase (iAChE) were evaluated. Enzyme activity was normalized to protein content of mosquito homogenates.

RESULTS

There was no significant difference in the activity level of AE, BE, OX, iAChE or AChE in adult *Cx. pipiens* that were collected near a wastewater treatment facility (WWTF) and a large tidal lagoon (TL) in Alameda County relative to *Cx. pipiens* from a susceptible laboratory colony. GST activity was significantly reduced in *Cx. pipiens* that were collected at the tidal lagoon (TL) relative to the lab colony. A total of 80 field-caught *Cx. pipiens* were tested for the mutant allele in the *kdr* loci of *vgsc-1* that is associated with pyrethroid resistance. The majority (70%) were homozygous for the susceptible *kdr* allele, 26% were heterozygous, while 4% of the *Cx. pipiens* were homozygous for the resistant allele.

DISCUSSION

This study was the first assessment of insecticide resistance markers for *Cx. pipiens* that were collected in Alameda County. While the activity of insecticide-metabolizing enzymes in the field-collected *Cx. pipiens* were similar to those from a susceptible lab colony, 30% of the mosquitoes that were collected in the field contained at least one copy of the *kdr* allele that is associated with resistance to pyrethroid insecticides. Because vector control agencies in Alameda County rarely apply pyrethroid insecticides to control mosquitoes (e.g. twice during the prior three years), the presence of resistant *kdr* alleles in mosquitoes suggests that structural or household insecticide use may contribute to insecticide resistance in these mosquitoes.

Insecticide Resistance in *Culex tarsalis*

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INTRODUCTION

Insecticide resistance in mosquitoes is a public health threat that limits the products that can be used to kill virus infected mosquitoes. *Culex tarsalis* transmits several arboviruses, including West Nile virus, and can breed intensively in natural habitats with pooled rainwater, such as marshes. Large anthropogenic containers such as fouled ornamental ponds can support *Cx. tarsalis* breeding in urban landscapes. Although *Cx. tarsalis* may prefer birds when taking a blood meal, they are not fastidious in the preference, and bite humans as well. When arbovirus-infected mosquitoes are discovered where humans cohabitate, insecticides may be used by vector control workers to reduce mosquito abundance and the risk of arbovirus transmission. Knowledge of insecticide resistance would inform vector control workers of which products should be used to protect public health from arbovirus transmission. Insecticide resistance can be detected by increased activity of enzymes that quickly metabolize the insecticide to reduce its effect on the mosquito. Insecticide resistance also can be tested using a CDC Bottle Assay, which is used to calculate percent mortality at different concentrations of insecticide, over time.

METHODS

The CDC Bottle Assay was performed using permethrin and mosquitoes collected from a coastal regional park (RP) and from a nearby coastal national wildlife refuge (NWR).

RESULTS

Of the mosquitoes collected at the RP, 74 % were *Cx. tarsalis*. This mosquito population had an LD_{50} of 9.17 mg/bottle after 90 minutes and an LD_{50} of 1.78 mg/bottle after 24 hours. Mosquitoes collected from NWR were 97 % *Cx. tarsalis*. This mosquito population had an LD_{50} of 53.4 mg/bottle after 90 minutes and an LD_{50} of 1.69 mg/bottle after 24 hours. The RP is located approximately 1.8 miles from the NWR. Evidently, mosquito populations in close proximity to one another can differ significantly in their susceptibility to permethrin. Such data may be useful for tailoring appropriate insecticide concentrations to discrete regions for controlling mosquitoes.

Using Autocidal Gravid Ovitraps (AGO Traps) to Capture *Aedes aegypti* in the Coachella Valley

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ABSTRACT *Aedes aegypti* was first detected in the Coachella Valley in May 2016. Since then, the Coachella Valley Mosquito and Vector Control District has been examining trapping methods to improve its surveillance efforts. In particular, we looked for a trapping technique sensitive enough to detect low levels of *Ae. aegypti* activity. We opted to construct our own Autocidal Gravid Ovitraps (AGO) traps using the design outlined in Barrera et al. 2014 (Figure 1). We built our own AGO traps at a low cost and have been deploying them since April 2017. Bait was made using a hay infusion (1 kg of Bermuda hay per 120 l (32 gal) of water). The infusion was allowed to ferment for a week and then was diluted 1:1 with clean water when added to the trap. The final mixture added to each trap was 9.5 l (2.5 gal). In 2017, we deployed 38 traps distributed in six cities throughout the Coachella Valley. The AGO traps used in our surveillance program were successful at detecting *Ae. aegypti* activity in our known infestation areas (Figure 2). We also found that gravid *Culex quinquefasciatus* were attracted to the bait used. The traps caught large numbers of house flies. While this was good for the residents, it interfered with the capture of mosquitoes on the glue boards and made identification of mosquitoes difficult. The glue boards we used were exceptionally sticky, which complicated preparation of the traps and impeded insect identification. Overall, we found that the traps did detect *Ae. aegypti*, even when they were not collected in other traps. We plan to continue using these traps in the future.

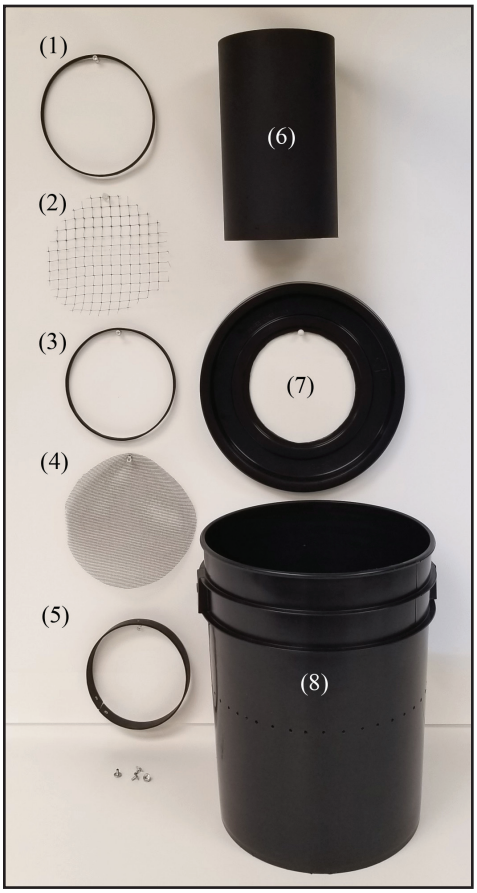

Figure 1 The trap was made with a black 5-gallon bucket (8) with a lid (7). To create the resting and capture arena, a 10" length of a 6" diameter PVC pipe was used (6). To hold fabric in place, rings were cut from a separate 6" diameter PVC pipe in ¼" (1 and 3) and ½" lengths (5). A 1-in section was removed from the ½" rings. The rings were heated in an oven at 392°F until softened to allow for stretching around the 6" PVC pipe perimeter. Fiberglass window screen was added to one end (4 – held in place with the ½" ring), and ½" bird netting was added to the opposite end of the pipe (2 – held in place with the ¼" rings). All PVC pipe pieces were painted matte black.

ACKNOWLEDGEMENT

We thank Jazmin Valop and Jazmin Carbajal for their time and assistance in assembling the traps.

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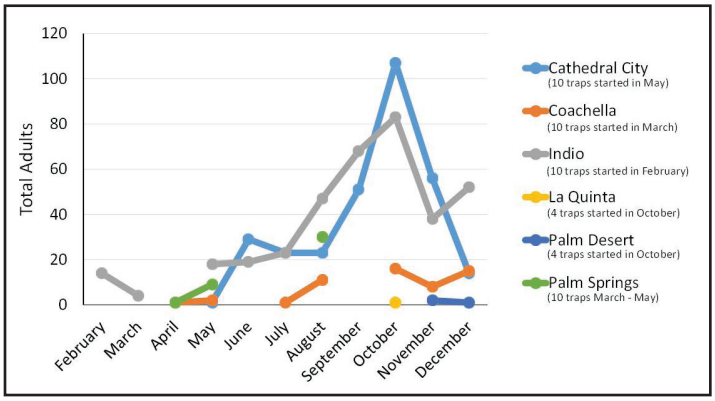

Figure 2 Total number of *Ae. aegypti* collected in AGO traps per month in 6 cities. Different numbers of traps were used depending on the size of the area where *Ae. aegypti* had been detected.

Rabies in Alameda County and California: 1960 to 2017

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INTRODUCTION

Alameda County, like all California Counties, is a declared rabies area, and is home to many animals subject to rabies, as well as some animal species that are reservoir hosts for rabies. Over the decades, there has been a change in the wildlife rabies dynamics, or sampling to detect these infections. Looking at the rabies testing data from California Veterinary Public Health Section (VPHS) (acquired through a 'Infectious Diseases Branch Surveillance Data Request', October 2017), we see a shift from predominantly skunk rabies being the most commonly detected in the 1960's to the present where skunk rabies has not been detected in Alameda County in the last 10 years (Fig. 1).

METHODS

Rabies surveillance relies upon what animals people report, what is testable, and what is tested. Presently, most of animal control agencies in Alameda County have far less interaction with wild animals than in past decades; when a resident could trap a nuisance animal, the local animal control agency would pick up the animal, usually for euthanasia or sometimes relocation, and if acting unusual would submit the animal for rabies testing. In this past situation, local animal control agencies were able to submit more skunks and other wildlife for testing than presently (Fig. 2). Currently the Alameda County Public Health Laboratory only accepts heads (or whole bats); therefore, suspect animals must be prepared for testing by decapitation at the local animal control agency or veterinary clinic, and only the heads submitted for direct fluorescent antibody (DFA) test on brain tissue.

RESULTS

During 1997-2008 in Alameda County, we tested 594 bats, averaging 49.5 tested per year, with 5.75 positive per year (ranging from 1-10) (Fig. 3). During 1997-2008, we also tested 369 skunks, averaging 30.75 tested per year, with 2.25 positive per year (ranging from 1-6). During 2009-2015, we tested 237 bats, averaging 33.9 (about 1/3 less bats tested) tested per year, with 5.14 positive per year (ranging from 2-13).We also tested 74 skunks, averaging 10.6 per year, with zero positive for rabies. Currently, on average, we test about 2/3 less skunks than we have in past decades, which may have contributed to the decline of skunks detected with rabies.

Looking at the overall wildlife rabies picture in California, we have succeeded in reducing rabies in several species, such as dogs, cows, horse, and skunks. Although the practice of vaccinating pets

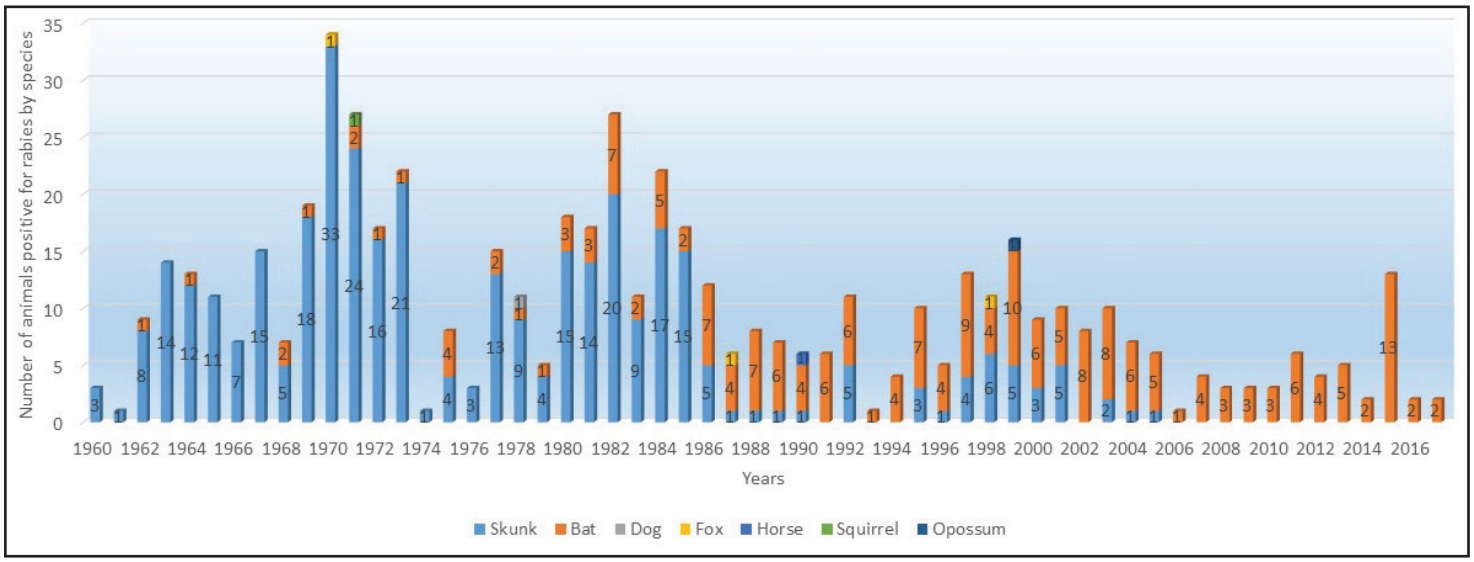

Figure 1 Animal Rabies Detected in Alameda County 1960-2017 Data from California Veterinary Public Health Section

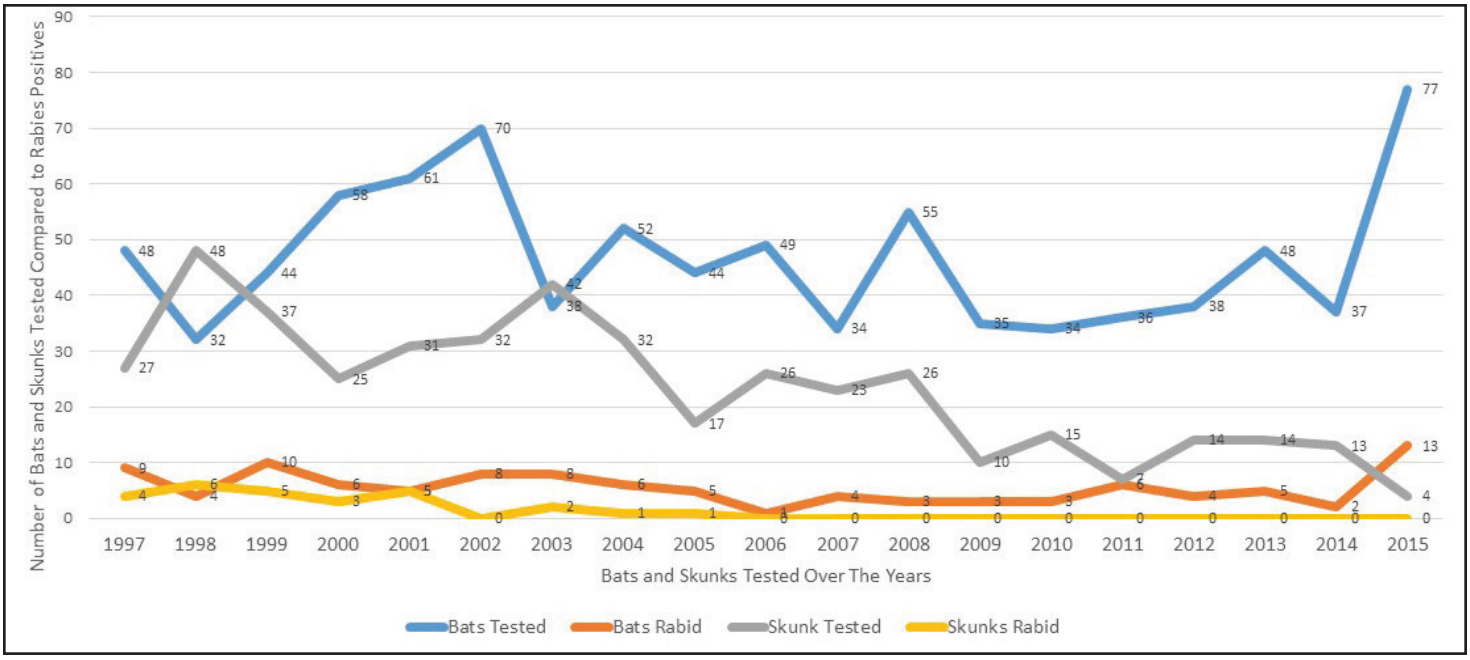

Figure 2 Bats and Skunks Tested for Rabies Compared to Rabies Positive 1997-2015

and livestock against rabies has shown success, it is still unclear what has caused the decline in the detection of skunk rabies in California. In 1963 we had 86 dogs detected with rabies in California, and after 1980, dog rabies was only detected in single digits per year. Cumulatively, the following numbers of animals tested positive for rabies in California during 1960-2017: Skunks 9,997, Bats 7,341, Fox 440, Dog 392, Cow 215, Cat 105, Horse 65, Bobcat 39, Coyote 29, Raccoon 28, Opossum 14, Sheep 12, and all others are <10.

DISCUSSION

Nationally, since 1960 there have been 122 human fatalities due to rabies, and 2 survivors (Fig. 4). Fifty-six (46%) of the fatal cases acquired their infection from exposure from bats, while there were 43 (35%) fatalities due to dog bites, though 30 (70%) of these exposures occurred outside of the U.S. Five cases of human rabies were due to human error in transplants, or laboratory exposure, and six fatalities from unknown sources (Anderson et al. 1984, Petersen and Rupprecht. 2011, and (CDC) Centers for Disease Control. 2017). It is clear that the main risk for rabies exposure is from bats (the list of involved

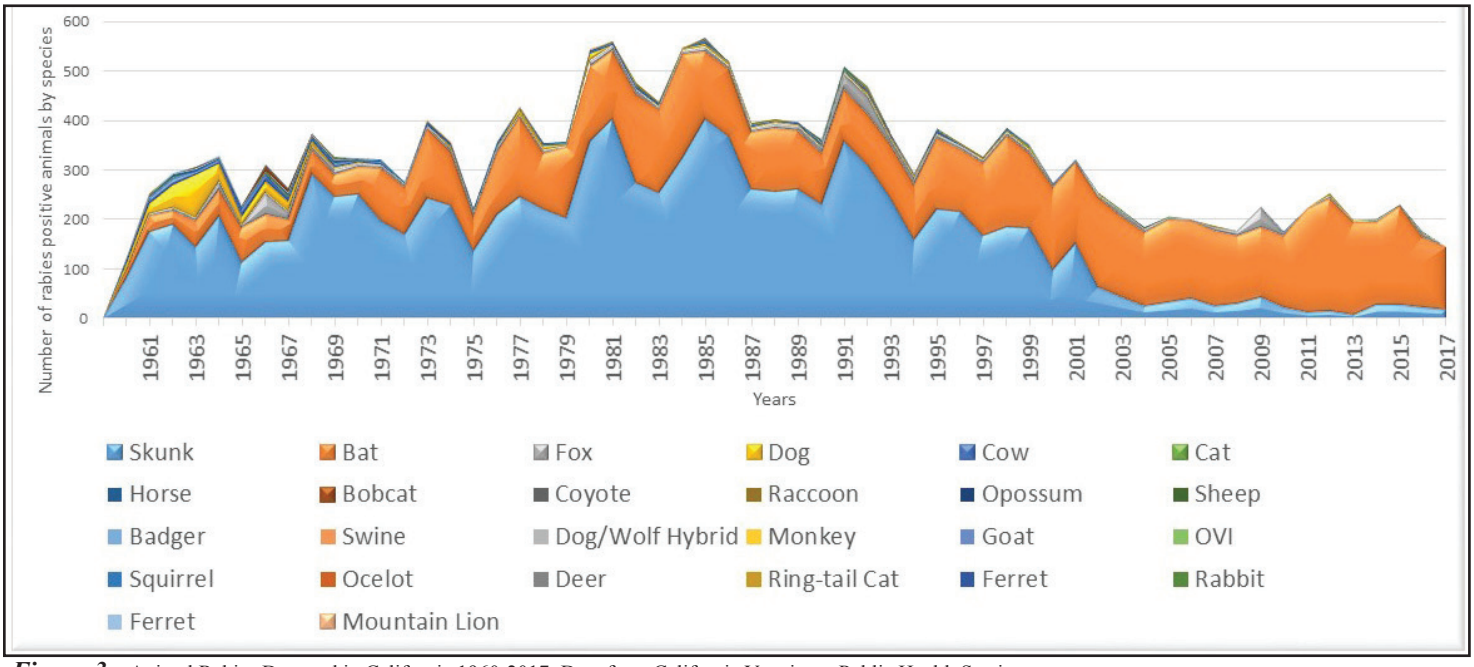

Figure 3 Animal Rabies Detected in California 1960-2017: Data from California Veterinary Public Health Section

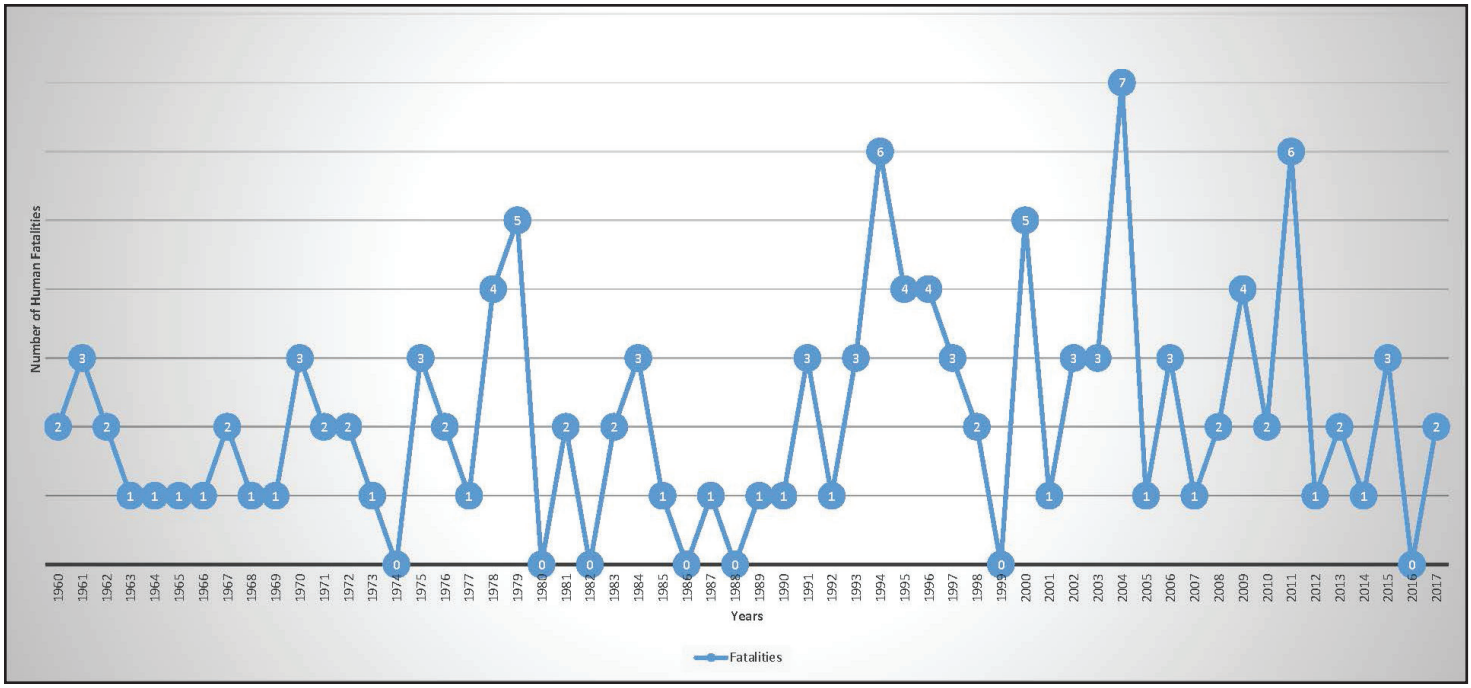

Figure 4 Human Rabies Fatalities in the U.S. from 1960-2017

bat species is broad: 22/32 bat species tested positive for rabies (Birhane et al., 2017). Five fatalities were due to skunk exposure, three to fox, two to cat, one to raccoon, and one to bobcat.

ACKNOWLEDGEMENTS

All of our staff at Alameda County Vector Control Services District are involved in the various aspects of the rabies control program, and deserve a sincere acknowledgement for all the hours invested serving Alameda County residents. Alameda County Public Health Laboratory and California Veterinary Public Health Section need recognition for providing much of the data used for this poster.

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