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Symposium: Operations

Symposium: Mosquito Biology, Disease and Resistance

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Symposium: Public Policy and Administration

MVCAC Poster Session

Submitted Papers

William C. Reeves New Investigator Award

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

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

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

Revising alkali metals as a tool for mark-recapture studies to characterize patterns of mosquito (Diptera: Culicidae) dispersal and oviposition

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Introduction

During the long history of mosquito mark-recapture (MR) studies, several marking techniques have been developed, most of which have solely targeted the adult stage of the mosquito (Service 1997, Southwood and Henderson 2000). Alkali metals have been used repeatedly as mosquito markers in MR studies involving the release of adult females (2-6 days old) fed with blood enriched with rubidium (Rb) before release (Reiter et al. 1995, Honório et al. 2003, Liew and Curtis 2004, Maciel-de-Freitas et al. 2004). A major drawback of this and other marking methods for adult mosquitoes is that the marking and release process may modify the distribution, fitness and behavior compared with the unmarked field individuals.

In the present study, we revised the application of alkali metals as mosquito markers to increase both the purity and quantity of information collected during MR studies. We tested the efficacy of different concentrations of rubidium and cesium (Cs) in aqueous solutions used to hatch and rear *Aedes aegypti* immatures to develop a method which: 1) avoids any effects of handling by investigators during the marking process, thereby minimizing changes in behavior and fitness of the released individuals, and 2) allows the marking of females, males and F1 eggs which establishes the potential for characterizing oviposition patterns in MR studies.

Materials and Methods

Mosquito rearing

Aedes aegypti eggs from the Los Angeles, California, colony, were divided into batches of 150 eggs. RbCl and CsCl were added to water to obtain Rb and Cs concentrations ranging from 0 (control) to 1,000 mg/kg. These aqueous solutions were used to hatch eggs and rear larvae. Pupae collected from each tray during the first three days after observation of the first pupa were transferred into a 200-mL cup of de-ionized (DI) water that was placed into rearing cages. Emerging adults were fed a 10% sucrose solution *ad libitum*. Females selected for oviposition were placed in a different cage and offered defribinated sheep's blood (Hemostat, Dixon, CA) on two occasions, 6-8 and 12-14 days after eclosion. One day after each blood meal, plastic containers with DI water and seed germination paper were placed in each cage to supply the gravid females with an oviposition substrate. Females 6-8 and 12-14 days old as well as eggs laid by females when 9-11 (first gonotrophic cycle) and 15-17 days old (second gonotrophic cycle) were stored in 2 vials for spectrometric analysis. Egg, larva and adult mosquitoes were reared under controlled experimental conditions, at approximately 25.5 °C and 80.0 % relative humidity.

Effects of marking on fitness

The effects of different concentrations of Rb or Cs in larval water on *Ae. aegypti* were assessed using three measures of fitness: daily and cumulative pupation probability (E2P) as well as adult wing-length (herein WL). The right wing of 10 males and 10 females from each treatment concentration and the control (control had twice the number of individuals sampled) was measured under a dissecting microscope, from the alular notch to the apex of the wing, excluding the fringe (Packer and Corbet 1989). One-tailed Wilcoxon rank-sum tests with Holm adjustment were used to assess whether WL in the treated groups was shorter than in the control group. To further assess the effect of Rb and Cs on *Ae. aegypti* fitness, the survival of adults from the treatments with the lowest concentration of Cs and Rb (35 mg/kg) was assessed in a separate experiment. The number of dead adults was counted every day for 31 days from the day of first eclosion, and the daily cumulative mortality recorded.

Detection of rubidium and cesium

Concentrations of Rb and Cs were assessed through Inductively Coupled Plasma Mass Spectrometry which returned Rb and Cs counts (spectra) in each egg or adult female sample. One-tailed Wilcoxon rank sum tests with Holm correction were used to assess whether the log-transformed concentration of Rb and Cs of each analyzed sample was greater than that of the control.

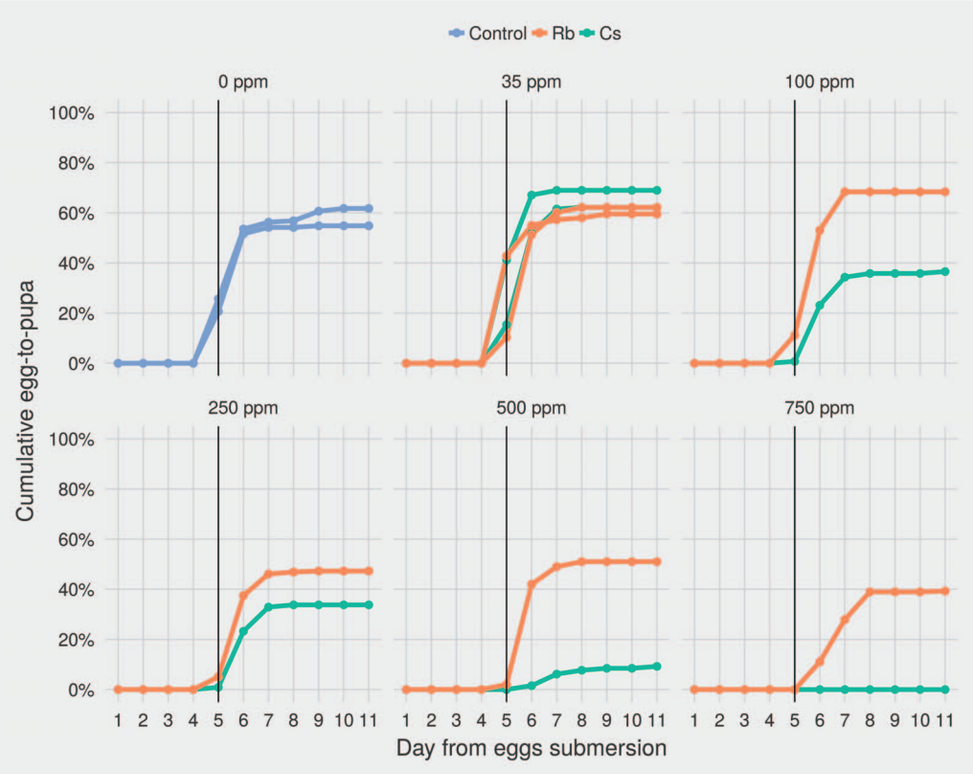

Figure 1.—Observed percentage of egg-to-pupa (a) and adult survival (b).

Results and Discussion

Results showed that a Rb concentration of 100 mg/kg or greater reduced the body size of adult mosquitoes, although an increase in immature mortality was not observed until concentrations reached 250 mg/kg (Figure 1). Overall, Cs was more toxic than Rb, having a small but negative effect on male wing length at 35 mg/kg, and on immature survival at 100 mg/kg (Figure 2), which is likely to be due to the stronger ionic charge of Cs (Relman 1956, Melnikov and Zanoni 2010). No effect was observed on immature and adult stages when larvae were reared at the 35 mg/kg Rb concentration. Therefore, adults and eggs from the Rb and Cs 35 mg/kg treatments were analyzed through spectrometry.

All adults and eggs contained Rb concentrations significantly ($P<0.001$) greater than the concentration of control samples. This pattern generally held for Cs samples, but there were two exceptions: a 15-17 day old female and an egg from the second gonotrophic cycle showed no significant difference in Cs accumulation compared to the respective controls. Moreover, the median Rb concentration in marked samples showed a greater difference from the control median compared to Cs (Figure 3).

Considering results from our fitness and survival experiments, together with results from spectrometric analysis, we conclude that Cs is not an optimal marker for *Ae. aegypti* MR studies. Even at very low concentrations (35 mg/kg), Cs had a negative effect on the mosquitoes and was not always detectable in exposed individuals.

In contrast, Rb at low concentrations (35 mg/kg) performed well as a marker. This element did not cause detectable effects on *Ae. aegypti* fitness and survival, and always accumulated at levels that exceeded natural levels in 2 week old females as well as in eggs through the second gonotrophic cycle. To the best of our knowledge, Rb is the only marker for mosquito MR experiments that has been shown to be transferred vertically from a female exposed as a larva to eggs in the next generation. This characteristic of Rb enables the acquisition of additional data during MR experiments, namely the spatial and temporal patterns of oviposition by dispersing adult females (Edman et al. 1998).

In recent years, ^{13}C and ^{15}N markers have dominated MR studies for container-inhabiting mosquitoes (e.g., Hamer et al. 2012, 2014, Opiyo et al. 2016, Medeiros et al. 2017). Despite their many positive characteristics, isotopes and isotopic analyses are costly, their effectiveness depends on the quantity of the most abundant isotope in the environment, and there is a relatively high risk of contamination. Moreover, artificially altered isotopic signatures are easily lost and thus undetectable in the next generation (eggs) due to the high physiological turn-over of C and N (Hamer et al. 2012). We have shown that Rb may

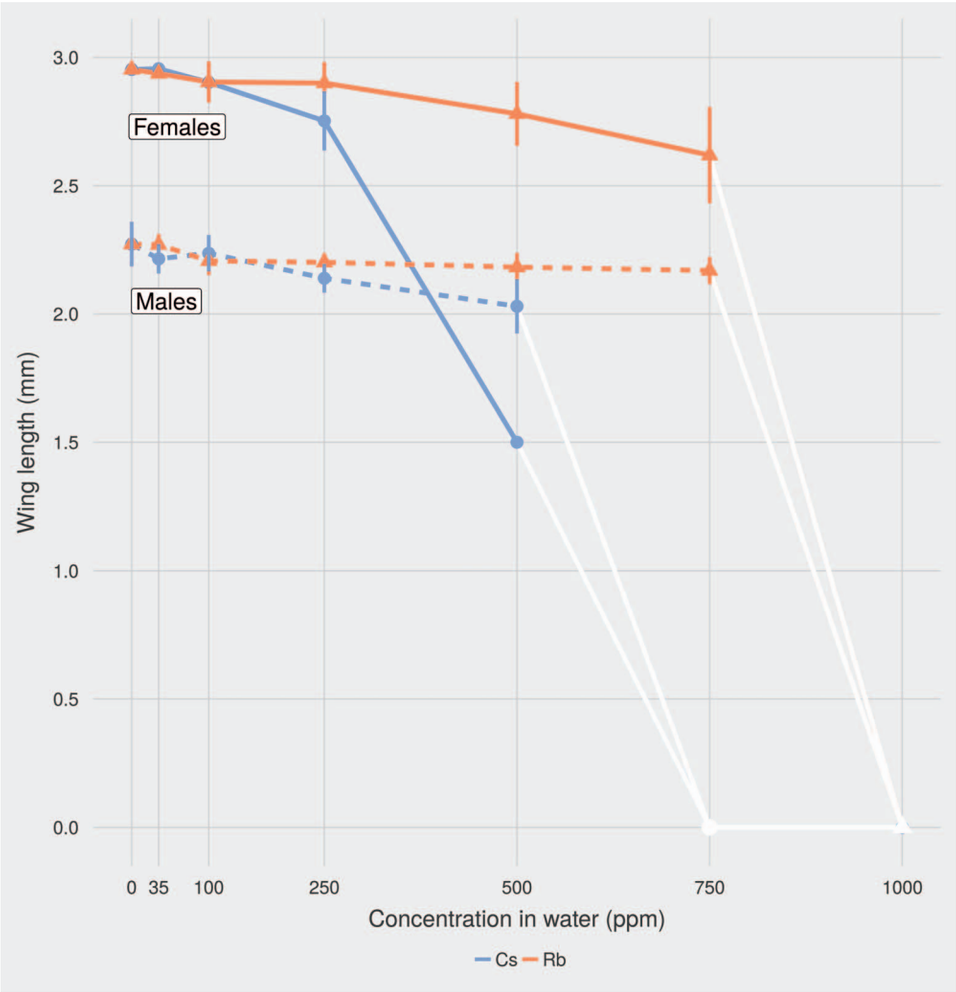

Figure 2.—Adult wing lengths for increasing concentrations of Cs (blue circles) and Rb (orange triangles). Point symbols indicate means for females (top) and males (bottom). Error bars indicate +/- 1 SD. White lines connect to treatments for which adults failed to emerge.

be a complementary and more practical mosquito marker compared to stable isotopes of C and N.

Conclusion

The importance of detailed information on the dispersal of mosquito vectors is increasing (Laith et al. 2008). To date, MR experiments for mosquitoes remain time-consuming, expensive, and complex to implement, and technological advancements to overcome these challenges have been slow to materialize. Maximizing the quality and quantity of data acquired during MR experiments is therefore critical.

The low cost of both Rb and ICP-MS analysis together with favorable environmental toxicity profiles of Rb make it a valuable marker for MR experiments with released or naturally occurring mosquitoes. On the contrary, Cs, even at low concentration, negatively affected *Ae. aegypti* fitness and did not consistently mark exposed individuals.

In conclusion, we recommend the use of Rb, alone or in combination with other markers, for mosquito MR experiments. We suggest that MR experiments with colonized or naturally occurring mosquitoes using stable isotopes of C and N could be enhanced by adding Rb as additional marker. In this way, data on the oviposition patterns would also be acquired and the number of possible marker combinations would increase from 3 to 6 (i.e., Rb+C, Rb+N, N, C, N+C, Rb), enabling marking of individuals from a higher number of larval habitats in the same MR experiment.

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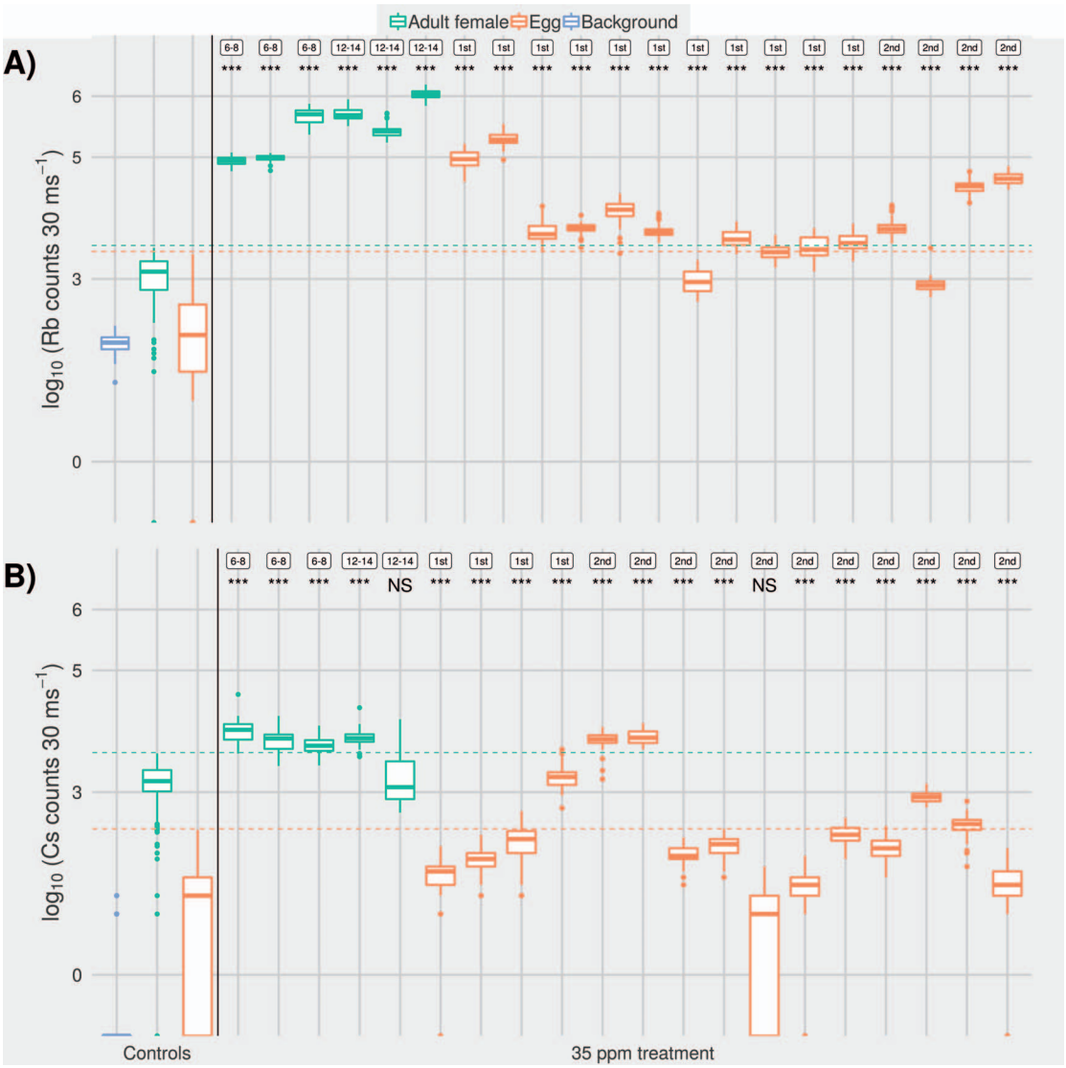

Figure 3.—Boxplots showing the concentration for control samples (in green) vs. each treatment (in orange; Rb, left or Cs, right) vs. control (green) samples. The Wilcoxon test significance symbol for each pairwise comparison is reported at the top of each pair of boxplots. All comparisons of treated vs. control samples were significant ($P<0.001$).

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Microbial ecology of *Aedes albopictus*: The impact of bacterial microbiota on mosquito development

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Abstract

Aedes albopictus (Skuse) is an important vector of dengue, Zika and chikungunya viruses. It is highly invasive and an aggressive biter that has spread to many California communitiesits introduction in the state since 2001. Mosquito control is currently the only effective method for suppressing the transmission of these mosquito-borne viruses. This study examined the hypothesis that the bacterial microbiota of aquatic habitats is key to the survival of larval *Ae. albopictus* mosquitoes. Life table studies of *Ae. albopictus* have found that reducing the bacterial load in natural aquatic habitats through water filtering and treatment with antibiotics significantly reduced the larva-to-adult survivorship, suggesting that bacteria play a crucial role in larval development. Mosquito gut bacteria microbiota was affected by bacteria in the environment and by endosymbiont *Wolbachia*. Elimination of *Wolbachia* from *Ae. albopictus* through the use of antibiotics and transfection with an exogenous *Wolbachia* strain reshaped the composition of bacterial microbiota and significantly increased gut bacterial diversity. The ability of *Ae. albopictus* larvae to utilize diverse aquatic habitats may be due to its capacity to assimilate live bacteria from aquatic habitats. These findings provide new insights into the role of bacteria in mosquito ecology.

INTRODUCTION

Aedes albopictus (Skuse) is an important vector of dengue, Zika, chikungunya and other viruses (Gratz 2004a, Liu et al. 2017). Due to its high physiological and ecological plasticity and ability to utilize container breeding sites, *Ae. albopictus* has rapidly spread globally, and it is now considered as the most invasive mosquito species in the world (Benedict et al. 2007, Bonizzoni et al. 2013). In California, it has been found in 67 cities and census-designated communities in five counties since its localized introduction during 2001 (Metzger et al. 2017, CDPH 2019). The establishment of *Ae. albopictus* in California has increased the risk of autochthonous disease outbreaks. Currently, mosquito control is the only effective method for suppressing these mosquito-borne viruses.

Interactions between bacterial microbiota and mosquitoes play an important role in the capacity of mosquito to transmit pathogens. However, the effects of environmental microbiota on mosquito development and reproduction are not clear (Minard et al. 2013, Dickson et al. 2017). The objective of the present study was to determine the impact of bacterial microbiota on larval development and survival of *Ae. albopictus*. We addressed two major questions: 1) would bacterial clearance or abundance reduction using antibiotics in larval habitats and through filtering of water of natural larval habitats inhibit or negatively affect larval mosquito development and survival; 2) would mosquito gut and body bacterial microbiota be altered by endosymbiont *Wolbachia,* and would such modifications of mosquito microbiota change mosquito immune response to arboviruses? Answers to these questions will provide important information on the role of environmental microbiota on mosquito larval ecology. Such information may be valuable to the development of new vector control tools.

METHODS

Study sites

The study was conducted in Guangzhou (113°20′ E, 23°10′ N), Guangdong province, China, and in Nabang (97°32′ E, 24°45′ N), Yingjiang County, Yunnan Province, China, on the China-Myanmar border area. The two sites have been experiencing major dengue outbreaks in the past three decades and *Ae. albopictus* is the major vector (Gratz 2004b, Wu et al. 2010). The annual average temperature in these two sites during the experimental period (June to August) was comparable, ranging from 27.8 to 29.9° C, within the optimal temperature for mosquito development.

Study design

Life table analysis under semi-natural conditions. To examine the impact of habitat bacteria on *Ae. albopictus* larval development and survival, life table studies were conducted using microcosms under semi-natural conditions in Guangzhou in 2015 and Nabang in 2016. The

microcosms were made of sterilized metal bowls (15 cm dia. x 5 cm deep) and 200 ml of water. In Guangzhou, we designed three treatments: 1) water from natural flower pots; 2) natural flower pot water filtered by 0.22 μm Millipore filter which is capable of filtering >95% bacteria (Wang et al. 2007); and 3) ampicillin added to water from natural flower pots, with a final antibiotic concentration of 100 μg/ml. Ampicillin is able to penetrate Gram-positive and some Gram-negative bacteria. We used ampicillin as it is a broad-spectrum antibiotic and has been used in several studies on mosquito microbiota (Coon et al. 2014). Fifty newly hatched larvae of the *Ae. albopictus* Foshan strain were added to each microcosm, and the number of surviving larvae was recorded daily until all larvae pupated or died. The Foshan *Ae. albopictus* strain originated in Foshan city, Guangdong Province and has been maintained in the laboratory since 1981. There were 6 replicates for each treatment, or 18 microcosms in total. To determine whether food limitation contributed to low larval survivorship in natural habitats and to test the effect of bacterial depletion in larval habitats on larval survival in the context of larval food enrichment, we set up 18 additional microcosms by adding ~ 1g per 250 ml water of autoclaved mosquito larval food (Brewer's yeast *Saccharomyces cerevisiae* cells) to each treatment. Overall, a total of 36 microcosms were used. In 2016, we conducted another life table study in Yunnan province with water from discarded tires and the same Foshan strain of *Ae. albopictus* to determine the generality of the findings. There were 2 treatments: 1) water from natural discarded tires, and 2) natural discarded tire water filtered by 0.22 μm Millipore® filter. There were 10 replicates per treatment. Similar to the Guangzhou experiment, additional equal number of microcosms were set up to test the effect of bacterial depletion on larval survival when larval food was not limited. In 2016, we used a total of 40 microcosms.

To verify whether filtration and antibiotics eliminated bacteria from larval habitats, on days 1, 5 and 10 since the initiation of the life-table study, 50 ml of water from the surface microlayer was collected from each microcosm using sterile plastic syringes and membrane filtration. Membranes were preserved in 100% ethanol in a -20° C freezer for subsequent quantification of total bacteria by qPCR.

Impacts of elimination and exogenous transfection of *Wolbachia* on gut microbiota. To examine the impact of elimination and transfection with exogenous *Wolbachia* on the gut microbiota of *Ae. albopictus* mosquitoes, three mosquito strains were used: GUA strain is a laboratory colony from Guangzhou and naturally contains *Wolbachia* (both *w*AlbA and *w*AlbB); GT strain was isolated from the Guangzhou colony, treated with tetracycline and has no *Wolbachia*; and HC strain isolated from the Guangzhou colony and then transfected with the *w*Pip strain of *Wolbachia* from *Culex pipens* (Zhang et al. 2015a, Zhang et al. 2015b, Zhang et al. 2016). For each strain, eggs, third-instar larvae, and adult midgut samples were collected (days 1 and 21), rinsed in double distilled water and 100% ethanol three times, and then preserved in 100% ethanol in -20°C freezer. Three biological replicates were used for each developmental stage and at both time points in the adult population.

Bacterial 16S rRNA gene library construction and pyrosequencing. For microbiota analysis, genomic DNA was extracted from pooled water samples and pooled mosquito specimens (10 larvae or adults per pool for each development stage or habitat type), using the ZR Fungal/Bacterial DNA MicroPrep™ kit (Zymo Research, CA). The 16S rRNA gene V4 region for microbiota was used in the analysis because the V4 region has been shown to be a sensitive marker for bacterial phylogenetic analysis and is employed widely (Caporaso et al. 2011, Kozich et al. 2013, Yang et al. 2016). Sequencing of amplicons was conducted by Laragen (Culver City, CA) using Illumina MiSeq platform with MiSeq 2×250 kit, following the instructions of the manufacturer.

Statistics

Life table data analysis. The emergence rate was calculated as the proportion of first-instar larvae developed into adults. The average emergence rate for each treatment was calculated. To determine the effects of bacterial removal by filtration and by the use of antibiotics, *t*-tests were conducted to compare the emergence rate difference between treatments. Similarly, to determine the impact of larval food addition on the mosquito emergence rate, a two-way analysis of variance (ANOVA) was conducted with water treatments and food addition as the factors. $P<0.05$ was regarded as significant difference.

Pyrosequencing data processing. All analyses were conducted in Mothur v.1.38.0, a software package that combines a variety of tools designed to process 16S rRNA gene sequence data (Kozich et al. 2013). Using the distance matrix and the furthest neighbor algorithm, all pre-processed sequences were clustered to detect operational taxonomic units (OTUs) at 0.03 distance levels (Schloss and Handelsman 2004). All the OTUs were filtered with 1) represented reads >5; 2) percentage of reads in library >0.005%, for further analysis. Permutational multivariate analysis of variance (PERMANOVA) was done using the adonis function in vegan package in R (v.3.2.0) to determine whether microbiota assemblage varied among the three mosquito strains (Chen et al. 2016).

RESULTS

Impact of bacteria on larval survivorship and development in life table study

Aedes albopictus larvae reared in the water from natural flower pots in 2015 in Guangzhou exhibited a 10% emergence rate, significantly higher than those in waters with depleted bacteria through 0.22 μm filtering (2%) ($t_{(3)} = 2.94$, $P < 0.05$) (Table 1). In habitats treated with ampicillin, no *Ae. albopictus* larvae successfully developed into pupae, suggesting that bacteria depletion by antibiotics significantly inhibited the development of mosquito larvae.

Table 1.—Pupation time, emergence rates and wing size of *Aedes albopictus* in larval life table study in 2015 and 2016. In 2015, the study used microcosms made of water from natural flower pots, water with bacteria removed by filtering and treated by ampicillin. In 2016, microcosms made of water from natural discarded tires and bacteria removed by filtering were used. NA = value not calculable due to lack of sufficient number of mosquito successfully developed to pupae or adults.

				Wing length (mm)	
Year	Treatment	Pupation time (day)	Emergence rate	Female	Male
2015	Natural Water	20.47±3.89	0.10±0.02	2.34±0.17	2.14±0.15
	0.22μm Filtered	19.27±4.65	0.02±0.03	NA	NA
	Antibiotics Treated	NA	0	NA	NA
	Natural Water with Food Supplement	11.74±3.86	0.57±0.18	2.37±0.22	2.03±0.14
	0.22μm Filtered with Food Supplement	9.13±2.50	0.46±0.22	2.71±0.15	2.27±0.14
	Antibiotics Treated with Food Supplement	11.64±3.71	0.73±0.08	2.60±0.23	2.13±0.15
2016	Natural Water	20.00±6.51	0.82±0.11	2.49±0.15	2.06±0.17
	0.22μm Filtered	33.55±5.49	0.03±0.03	2.56±0.27	2.20±0.20
	Natural Water with Food Supplement	7.50±1.15	0.91±0.07	2.62±0.23	2.31±0.21
	0.22μm Filtered with Food Supplement	6.90±0.77	0.91±0.07	2.64±0.24	2.24±0.20

The inhibition of larval development was likely caused by the lack of critical nutrients in the microcosms or the inability to digest nutrients because when autoclaved, larval food was added to the rearing condition, and the emergence rate of the larvae was increased to 57 – 73% (*t* test, $P < 0.001$) (Table 1). A non-significant difference in the emergence rate of larvae among the three treatments supplemented with food (ANOVA, $F_{2,6} = 1.32$, $P = 0.34$) suggests that the antibiotics itself in the rearing water was not detrimental to the development of mosquito larvae when nutrients were not limiting.

In 2016, we conducted similar life-table studies in Yunnan using water from natural discarded tires and found that the emergence rate of *Ae. albopictus* larvae reared in microcosms with bacteria-depleted waters through filtering by 0.22 μm filters was 3%. This emergence rate was significantly lower than that in the microcosms with water from natural discarded tires without bacterial depletion (82%; $t_{(8)} = 15.64$, $P < 0.0001$) (Table 1). When autoclaved larval food was added to microcosms of filtered water, emergence rate of larvae was significantly elevated to a level similar to the unfiltered natural discarded tire water (91%, $t_{(8)} = 24.76$, $P < 0.0001$). The body size of resulting adults is shown in Table 1. Overall, females were larger than males, and the mosquitoes were larger than treatment with food supplement than those without food supplement.

The qPCR analysis found that in the 2015 study, filtering of natural flowerpot water with 0.22μm filters reduced the total bacterial load by 3.9-fold ($P < 0.01$) and antibiotics treatment by 14.1-fold on day 1 ($P < 0.001$). The total bacteria load was significantly reduced by more than 90% on days 5 and 10 because mosquito larvae were introduced to the microcosms; this phenomenon was consistent among the three microcosm types (natural water, filtered water and water treated by antibiotics). In the 2016 study with discarded tire water, filtering with 0.22μm filters reduced the total bacterial load of larval microcosms by 2.4-fold ($P < 0.01$), paralleling the dramatic reduction in larva-to-adult emergence rate in the filtered microcosms from 82% to 3%. The addition of autoclaved larval food to the microcosms with filtered water increased the total bacterial load by 6.6-fold ($P < 0.0001$), similar to the large increase in the larva-to-adult emergence rate (3% to 91%). The total bacteria loads decreased over time, and this was consistent for all treatments regardless of food supplementation. Overall, these results suggest live bacteria from aquatic habitats played an important role in *Ae. albopictus* larval development and survival in natural habitats where nutrients for mosquito larvae were limiting.

Impact of *Wolbachia* elimination and exogenous transfection on the composition and diversity of bacterial microbiota

For GUA, GT and HC strains, higher bacterial microbiota diversity was found in both eggs and larvae compared to adults. Adult mosquito bacterial diversity decreased as mosquitoes aged (*t*-test, $P<0.05$). Elimination and exogenous transfection of *Wolbachia* increased microbiota diversity of *Ae. albopictus* eggs (*t*-test, $P<0.05$).

Composition of bacterial microbiota differed significantly among the three strains of *Ae. albopictus* within the same developmental stage. For example, in the egg stage α-*Proteobacteria* were the main bacteria in wild GUA strain at class level, whereas λ-*Proteobacteria* were the main bacteria in novel GT and HC strains (PERMANOVA; $F = 23.06$, $P = 0.0003$) (Fig. 1). A significant difference was also found at the larval stage, but the main bacteria were *Actinobacteria* (PERMANOVA; $F = 5.72$, $P = 0.025$). In adult mosquitoes, different bacterial compositions were found among these three strains, and the among-strain difference increased with mosquito age. Compared to the wild strain, both GT and HC strains showed different bacterial communities in egg and adult stages (PERMANOVA; all $P < 0.05$).

At the genus level, *Wolbachia* and *Paraburkholderia* were most abundant in GUA and HC strains, and *Elizabethkingia* and *Aeromonas* in *Wolbachia*-free GT strain in eggs. Elimination of *Wolbachia* by tetracycline increased the abundance of *Elizabethkingia* in adult guts. Exogenous transfection with the exogenous *Wolbachia*

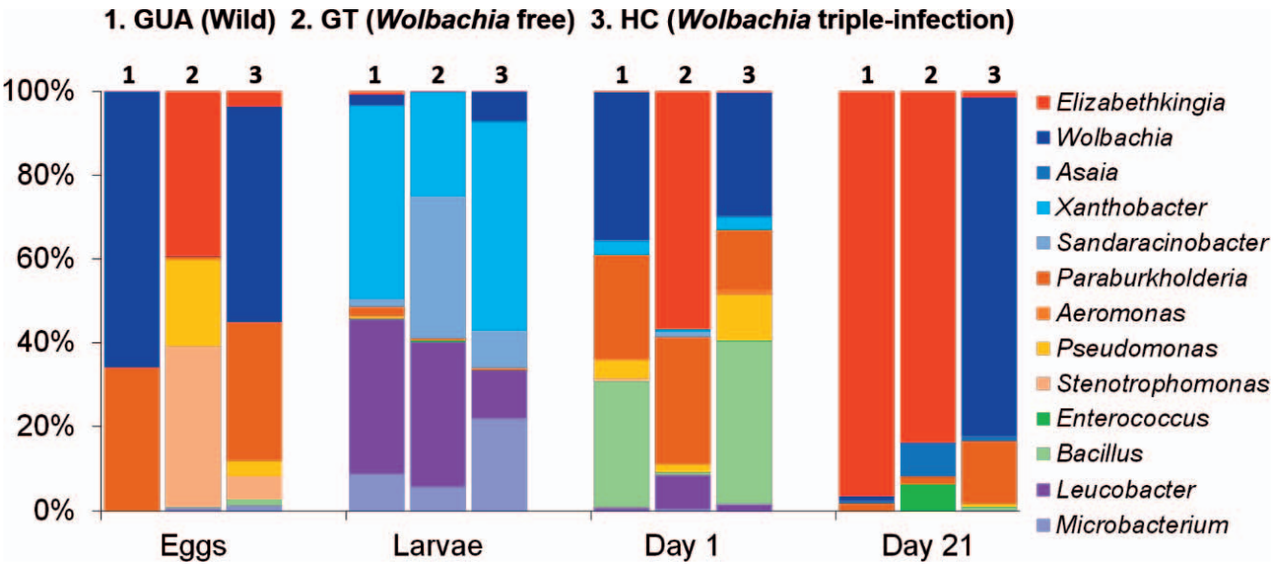

Fig. 1.—Composition of bacterial microbiota of three strains of *Aedes albopictus* eggs, larvae and adults (days 1 and 21). GUA: wild strain; GT: *Wolbachia* free strain; HC: *Wolbachia* triple-infection strain. Numbers at top of the figures: 1. GUA strain; 2. GT strain, and 3. HC strain.

strain significantly increased *Wolbachia* abundance in the gut whereas the wild type had low *Wolbachia* abundance (Fig. 1). Overall, elimination of *Wolbachia* from *Ae. albopictus* by the use of antibiotics and transfection of exogenous *Wolbachia* strain reshaped the composition of bacterial microbiota and significantly increased diversity.

DISCUSSION

Here we demonstrated that live bacteria in aquatic habitats are critical to the development and survivorship of *Ae. albopictus* mosquito larvae under natural conditions. The life table study found a significant reduction in emergence when bacterial content was dramatically reduced by water filtering or. ampicillin antibiotics. Our results were consistent with an earlier study on *Anopheles stephensi,* which reported that the pupation time of mosquito larvae reared in rifampicin-treated water was delayed by more than 4 days and that the pupation rate was reduced by 88% (Chouaia et al. 2012a). We observed a zero-pupation rate in our experiments with *Ae. albopictus* in habitats with ampicillin-treated water and an 11% pupation rate in the control (natural water). The difference in pupation rate between our experiments and those reported [around 5%-40%, Chouaia et al. (2012b)] was due to different experimental conditions and different mosquito species used by these two studies: We used water from natural flowerpots under semi-natural microcosm conditions, whereas Chouaia et al. (2012) used an insectary setting with minced commercial mouse food as larval food. Together with the findings of Coon et al. (2014) and other earlier literature (Kaufman et al. 2001, Kaufman et al. 2006), we conclude that live bacteria are critical to the larval development of *Ae. albopictus* mosquitoes in nature when nutrients for mosquito larvae are generally limited.

We found that *Wolbachia* was extremely common in adult *Ae. albopictus* mosquitoes, constituting >50% bacterial abundance. One field survey (Zhang et al. 2014) showed that the infection rates of *Wolbachia* in wild *Ae. albopictus* females and males in Guangzhou, China were up to 83% and 46%, respectively. *Wolbachia* can cause cytoplasmic incompatibility to *Ae. albopictus*, reduce mosquito lifespan, and alter vector competence to viruses (Blagrove et al. 2012, van Tol and Dimopoulos 2016, Fraser et al. 2017). Thus, *Wolbachia* have been proposed as a biopesticide for population suppression (O'Connor et al. 2012), and as a potential candidate to drive pathogen-blocking genes into natural mosquito populations (Caragata et al. 2013). Elimination or transfection with an exogenous *Wolbachia* strain has been reported to have no significant effect on mosquito fitness, such as egg hatch, pupation, emergence or body size of mosquitoes (Zhang et al. 2015b). Our current results demonstrated that, elimination of *Wolbachia* increased gut microbial diversity. The GT (*Wolbachia*-free) strain was isolated from wild colony via feeding antibiotics and has been stably reproduced in the laboratory for more than 20 generations. Herein, the observed microbial structure shift in this strain should not be from the impact of direct antibiotic exposure. Similarly, transfection with exogenous *Wolbachia* also changed the microbial community of all stages of *Ae. albopictus*. Further research is needed to explore the relationships between mosquito vector capacity and bacterial change.

In summary, the current study demonstrated bacteria's important role in *Ae. albopictus* larvae development and survivorship. *Aedes albopictus* larvae survived well in natural aquatic habitats when bacteria in the larval habitats were abundant. This species ability to use diverse bacteria for its larval development and survival has contributed to its successful utilization of containers as larval habitats and

facilitated its global expansion. *Wolbachia* was the predominant bacteria species in adult mosquitoes, and removal or introduction of new *Wolbachia* strains significantly affected the structure of bacterial microbiota, which may be an important mechanism for altered vector competence in *Ae. albopictus* mosquitoes transfected with new *Wolbachia* strains.

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Surveillance for mosquito-borne encephalitis virus activity in California, 2018

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Abstract

In 2018, 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 41 out of 58 counties in California, and St. Louis encephalitis virus (SLEV) activity was reported from 10 counties. A total of 243 human WNV infections were reported, and enzootic WNV activity was detected among horses, dead birds, mosquitoes, and sentinel chickens. Five human cases of SLEV disease were identified, and enzootic SLEV activity was detected in mosquitoes and sentinel chickens.

Introduction

The California Arbovirus Surveillance program is a cooperative effort between the California Department of Public Health (CDPH), the University of California Davis Arbovirus Research and Training 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 2018, the surveillance program included the following:

(1) Diagnostic testing of specimens from human patients who exhibited symptoms compatible with WNV disease, as well as blood bank and organ donor screening for WNV infection.
(2) Monitoring mosquito abundance and testing mosquitoes for the presence of 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 41 (71%) out of 58 counties in California (Table 1), while SLEV activity was reported from 10 (17%) 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), 11 county public health laboratories, and over 40 commercial laboratories. Local county laboratories tested for WNV using an IgM enzyme immunoassay (EIA) and/or an IgM immunofluorescence assay (IFA). Specimens with inconclusive results, or from counties with environmental SLEV activity, were forwarded to the VRDL for further testing with a plaque reduction neutralization test (PRNT). Additional WNV infections were identified through screening performed by blood and organ donation centers.

In 2018, a total of 217 symptomatic and 26 asymptomatic infections with WNV were identified, a 57.8% decrease in the number of infections compared to 2017 (Table 3). Of the 217 symptomatic cases, 154 (71%) were classified as West Nile neuroinvasive disease (WNND) (e.g. encephalitis, meningitis, acute flaccid paralysis, or other neurologic dysfunction) and 63 (29%) were classified as non-neuroinvasive disease. Case-patients were residents of 31 (53%) counties and 140 (64%) were male. In 2018, WNV incidence in California was 0.55 cases per 100,000 persons. Incidence was highest (7.0 cases per 100,000 persons) in Glenn County, although Los Angeles County reported the most cases (43, 20%) (Figure 1, Table 3). The median age of those with WNND was 63 years (range, 4 to 91 years), and among cases with non-neuroinvasive disease

Table 1.—West Nile virus activity in California, by county, 2018. Includes asymptomatic infections detected through blood bank and organ donor screening. NT = None tested

County	Humans	Horses	Dead Birds	Mosquito Pools	Sentinel Chickens
Alameda	0	0	20	15	0
Alpine	0	0	NT	NT	NT
Amador	1	1	0	NT	NT
Butte	18	0	4	48	37
Calaveras	0	0	NT	NT	0
Colusa	0	0	0	NT	6
Contra Costa	5	0	13	17	16
Del Norte	0	0	0	NT	NT
El Dorado	0	0	2	NT	NT
Fresno	15	0	0	119	NT
Glenn	2	0	NT	4	0
Humboldt	1	0	1	NT	NT
Imperial	0	0	NT	1	NT
Inyo	0	0	NT	0	NT
Kern	14	1	1	48	NT
Kings	0	0	0	22	NT
Lake	1	0	0	4	1
Lassen	0	0	NT	NT	NT
Los Angeles	47	0	19	75	30
Madera	4	0	0	55	NT
Marin	0	0	0	0	NT
Mariposa	0	0	NT	NT	NT
Mendocino	0	0	NT	NT	NT
Merced	2	2	0	12	16
Modoc	0	0	NT	NT	NT
Mono	0	0	NT	NT	NT
Monterey	1	0	0	NT	NT
Napa	1	0	0	0	0
Nevada	1	0	0	NT	0
Orange	12	0	18	96	NT
Placer	9	1	44	230	4
Plumas	0	0	NT	NT	NT
Riverside	16	0	6	36	NT
Sacramento	17	3	241	300	5
San Benito	0	0	0	0	1
San Bernardino	10	0	0	12	NT
San Diego	2	0	1	3	NT
San Francisco	0	0	0	0	NT
San Joaquin	15	1	16	533	NT
San Luis Obispo	0	0	0	0	NT
San Mateo	0	0	5	2	0
Santa Barbara	0	0	0	0	0
Santa Clara	1	0	56	5	8
Santa Cruz	0	0	2	0	0
Shasta	1	1	5	8	4
Sierra	0	0	NT	NT	NT
Siskiyou	0	0	NT	NT	NT
Solano	0	0	0	3	7
Sonoma	0	0	0	1	NT
Stanislaus	18	1	1	111	NT
Sutter	1	0	4	28	20
Tehama	3	0	0	NT	1
Trinity	0	0	NT	NT	NT
Tulare	8	0	0	77	NT
Tuolumne	1	0	1	NT	NT
Ventura	2	0	0	0	0
Yolo	12	0	41	90	5
Yuba	2	0	0	8	2
State Totals	**243**	**11**	**501**	**1,963**	**163**

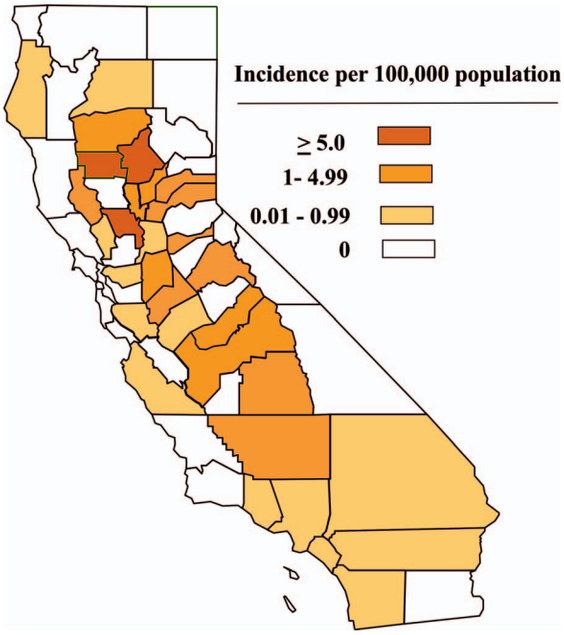

Figure 1.—Incidence of human cases of West Nile virus in California, 2018.

the median age was 53 years (range, 1 to 90 years). The median age of the 11 WNV-associated fatalities was 71 years (range, 53 to 87 years). Dates of symptom onset ranged from May 6 to December 20, with the peak occurring in week 35 (August 26 – September 1), when 28 (13%) symptomatic infections were reported.

Five symptomatic cases of SLEV infection were also identified in 2018. Four cases (80%) presented with neuroinvasive disease, and no fatalities were reported. Case-patients were residents of four counties (Table 2) and two (40%) were male. The median age was 63 years and

Table 2.—St. Louis encephalitis virus activity in California, by county, 2018. NT = None tested

County	Humans	Mosquito Pools[1]	Sentinel Chickens
Fresno	1	56	NT
Imperial	0	3	NT
Kern	1	65	NT
Kings	0	30	NT
Los Angeles	2	1	0
Madera	0	14	NT
Merced	0	0	1
Riverside	0	56	NT
Stanislaus	1	0	NT
Tulare	0	162	NT
Totals	**5**	**387**	**1**

[1]Positive mosquito pools included *Cx. quinquefasciatus* (263 pools), *Cx. tarsalis* (102 pools), *Cx. pipiens* (15 pools), *Cx. stigmatosoma* (6 pools), and *Cx. erythrothorax* (1 pool)

Table 3.—Reported West Nile virus human cases by county of residence, and year, California, 2009 – 2018.

County	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2018 incidence per 100,000 person-years	Ten-year incidence per 100,000 person-years
Alameda	0	1	0	2	0	1	0	0	1	0	0.00	0.03
Alpine	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Amador	0	0	1	0	0	0	0	1	0	1	2.63	0.79
Butte	2	1	3	10	24	24	53	21	3	12	5.27	6.77
Calaveras	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Colusa	0	0	0	3	2	3	1	2	0	0	0.00	4.98
Contra Costa	5	4	3	4	5	5	1	4	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	0	1	0	1	0	0	1	0	0	0.00	0.21
Fresno	13	23	9	24	8	43	8	14	10	14	1.39	1.68
Glenn	0	2	1	7	9	10	19	6	0	2	6.95	19.45
Humboldt	0	0	0	0	0	0	0	0	0	1	0.74	0.07
Imperial	0	0	0	1	0	1	1	0	3	0	0.00	0.31
Inyo	0	0	0	0	0	0	0	0	4	0	0.00	2.15
Kern	18	15	18	25	25	11	11	17	29	13	1.44	2.02
Kings	3	1	1	3	1	4	0	8	4	0	0.00	1.71
Lake	0	0	0	1	0	1	2	1	0	1	1.54	0.92
Lassen	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Los Angeles	20	4	58	163	151	253	286	151	274	43	0.42	1.37
Madera	1	7	2	3	3	3	4	6	2	4	2.52	2.20
Marin	0	0	0	0	2	0	1	0	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	1	2	0	0	0	0.00	0.34
Merced	4	1	1	13	0	1	1	0	8	2	0.71	1.18
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	1	0	0	1	0	0	0	1	0	1	0.23	0.09
Napa	0	0	0	0	1	0	0	0	0	1	0.71	0.14
Nevada	0	0	0	0	0	0	2	0	0	1	1.01	0.30
Orange	4	1	10	42	10	263	92	32	35	9	0.28	1.54
Placer	0	3	1	12	6	7	0	7	0	9	2.31	1.16
Plumas	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Riverside	3	0	7	19	35	14	127	11	28	15	0.62	1.09
Sacramento	0	12	4	29	11	10	4	25	6	15	0.98	0.76
San Benito	0	0	0	0	0	0	0	0	0	0	0.00	0.00
San Bernardino	2	5	4	33	13	21	54	8	54	9	0.41	0.97
San Diego	4	0	0	1	0	11	42	20	2	2	0.06	0.25
San Francisco	0	1	0	1	1	0	0	0	1	0	0.00	0.05
San Joaquin	10	6	5	13	8	9	2	13	14	14	1.85	1.24
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	0	0	1	0	1	0	0	0	0	0	0.00	0.04
Santa Clara	0	0	1	0	2	10	8	1	0	1	0.05	0.12
Santa Cruz	0	0	1	0	0	0	0	0	0	0	0.00	0.04
Shasta	0	0	0	1	1	2	3	1	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	1	0	0	0	0.00	0.22
Solano	0	0	0	2	1	5	1	4	1	0	0.00	0.32
Sonoma	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Stanislaus	14	12	11	26	17	33	13	26	28	15	2.70	3.51
Sutter	0	0	0	8	10	8	2	12	2	1	1.03	4.52
Tehama	0	0	1	4	5	4	5	5	2	2	3.12	4.37
Trinity	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Tulare	4	12	11	7	5	21	13	10	12	8	1.68	2.16
Tuolumne	0	0	0	0	0	0	0	0	0	1	1.83	0.18
Ventura	0	0	0	7	2	1	6	7	1	2	0.23	0.30
Yolo	2	0	0	10	6	15	8	16	6	11	4.97	3.34
Yuba	1	0	3	4	13	6	10	11	1	2	2.68	6.82
Total WNV Cases	**112**	**111**	**158**	**479**	**379**	**801**	**783**	**442**	**536**	**217**	**0.55**	**1.01**
Asymptomatic Infections	17	20	18	48	54	91	77	41	41	26		
Total WNV infections	**129**	**131**	**176**	**527**	**433**	**892**	**860**	**483**	**577**	**243**	**0.61**	**1.12**

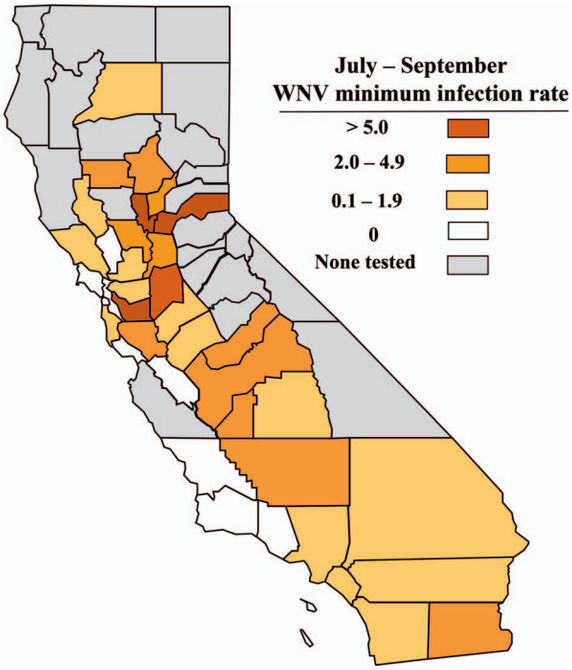

Figure 2.—West Nile virus minimum infection rate of *Culex* mosquitoes, by county, California, July – September, 2018. Minimum infection rate defined as the minimum number of infected female mosquitoes per 1,000 tested.

dates of symptom onset ranged from August 23 to October 28.

Mosquito Surveillance

In 2018, mosquito testing was performed at DART and 12 local mosquito and vector control agencies. A total of 1,220,033 mosquitoes (43,874 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 1,963 mosquito pools from 29 counties (Tables 1 and 4), and SLEV was detected in 387 mosquito pools from 8 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 1.6. During California's peak transmission period (July – September) the statewide MIR in *Culex* mosquitoes was 2.7 and four counties reported MIRs greater than 5.0, the epidemic threshold value (Figures 2 and 5) (California Department of Public Health).

West Nile virus was identified from five different *Culex* species (*Cx. erythrothorax*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. stigmatosoma*, and *Cx. tarsalis)* (Table 5); positive pools were collected from April 25 – November 20, with the peak occurring in week 32 (August 5 – August 11). St. Louis encephalitis virus was also identified from the same five *Culex* species, collected from June 5 – October 10.

Chicken Serosurveillance

In 2018, 27 local mosquito and vector control agencies in 25 counties maintained 110 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.

Of 10,124 chicken blood samples tested, 163 seroconversions to WNV were detected among 43 flocks in 16 counties (Tables 1 and 4). Seroconversions to WNV occurred between July 2 and November 3, with the peak occurring in weeks 32-33 (August 5 – August 18). In addition, one SLEV seroconversion was detected in a flock located in Merced County on August 17 (Table 2).

Dead Bird Surveillance

In 2018, the WNV Dead Bird Hotline and website received a total of 8,216 dead bird reports from the public in 53 counties (Table 6). Oral swabs or tissue samples from dead bird carcasses were tested at DART or at one of 12 local agencies by RT-qPCR. Of the 2,286 bird carcasses that were deemed suitable for testing, WNV was detected in 501 (22%) carcasses from 21 counties (Tables 1 and 6). Thirty-five different bird species tested positive for WNV: 46% were American crows, 21% were California scrub-jays, 8% were other corvids, and 25% were non-corvid species. Positive birds were detected from January 3 – December 19, with the peak occurring in week 30 (July 2 – July 28).

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 2018, WNV infection was confirmed in 11 horses from eight counties (Table 1). Six (55%) of the horses died or were euthanized as a result of their infection.

Discussion

In 2018, 217 WNV human cases were reported from 31 counties, which was the lowest number of cases reported since 2011 (Figure 3, Table 2). In 2018, the proportion of WNND cases among reported cases in California was 71%, which suggests that several thousand non-neuroinvasive cases also occurred but were not identified or reported, as these infections are less likely to be identified, laboratory tested, and reported (Figure 3) (Centers for Disease Control and Prevention, 2010). Non-human WNV activity was reported from 37 counties (Table 1), but the frequency of

Table 4.—Results of mosquito and sentinel chicken testing for West Nile virus, California, 2018.

County	No. mosquitoes tested	No. mosquito pools tested	WNV + pools	No. flocks	No. chickens	No. WNV positive flocks	WNV + sera
Alameda	1,267	147	15	2	10	0	0
Butte	18,927	405	48	7	44	7	37
Calaveras	0			1	10	0	0
Colusa	0			1	10	1	6
Contra Costa	23,142	689	17	5	50	3	16
Fresno	49,441	1,731	119	0			
Glenn	1,549	36	4	1	10	0	0
Imperial	1,659	58	1	0			
Inyo	443	11	0	0			
Kern	31,475	764	48	0			
Kings	8,648	292	22	0			
Lake	11,610	575	4	2	12	1	1
Los Angeles	122,328	3,390	75	28	206	6	30
Madera	16,589	398	55	0			
Marin	2,977	143	0	0			
Merced	12,679	539	12	8	48	5	16
Napa	2,295	129	0	1	11	0	0
Nevada	0			4	24	0	0
Orange	125,173	4,586	96	0			
Placer	42,823	2,484	230	2	12	1	4
Riverside	166,498	4,969	36	0			
Sacramento	94,701	4,994	300	2	10	2	5
San Benito	242	33	0	1	10	1	1
San Bernardino	49,507	2,712	12	0			
San Diego	8,858	1,081	3	0			
San Francisco	410	14	0	0			
San Joaquin	121,092	3,422	533	0			
San Luis Obispo	1,601	39	0	0			
San Mateo	1,296	131	2	2	14	0	0
Santa Barbara	1,969	57	0	5	35	0	0
Santa Clara	1,938	195	5	8	57	3	8
Santa Cruz	3,084	231	0	2	20	0	0
Shasta	18,906	700	8	7	51	2	4
Solano	12,162	378	3	3	36	3	7
Sonoma	13,232	506	1	0			
Stanislaus	76,761	1,900	111	0			
Sutter	7,370	202	28	5	35	4	20
Tehama	0			3	30	1	1
Tulare	118,600	3,888	77	0			
Ventura	2,079	46	0	5	51	0	0
Yolo	40,903	1,839	90	3	24	2	5
Yuba	5,799	160	8	2	14	1	2
Total	**1,220,033**	**43,874**	**1,963**	**110**	**834**	**43**	**163**

detections in birds, mosquitoes, and sentinel chickens were amongst the lowest in the last decade (Figures 4, 5, and 6). Although ecological surveillance data documented WNV activity throughout the year, the vast majority of detections occurred from June through October, with peak activity occurring in August.

For the fourth consecutive year, SLEV was also detected in California. Outreach to local health departments was conducted in areas after environmental detections of SLEV and medical providers were encouraged to include SLEV testing for suspect WNV cases. This resulted in the identification of five human SLEV disease cases from four counties (Table 2); none were fatal. This represented the most SLEV cases identified in California since SLEV reemerged in 2015. Enzootic activity was detected in nine counties via mosquito and sentinel chicken testing. The number of SLEV positive mosquito pools more than doubled compared to data reported from 2016 and 2017, and notably the SLEV MIR in five counties (Imperial, Kern, Kings, Riverside, and Tulare) was higher than the WNV MIR, suggesting that humans may have been at greater risk for acquiring SLEV than WNV infection in

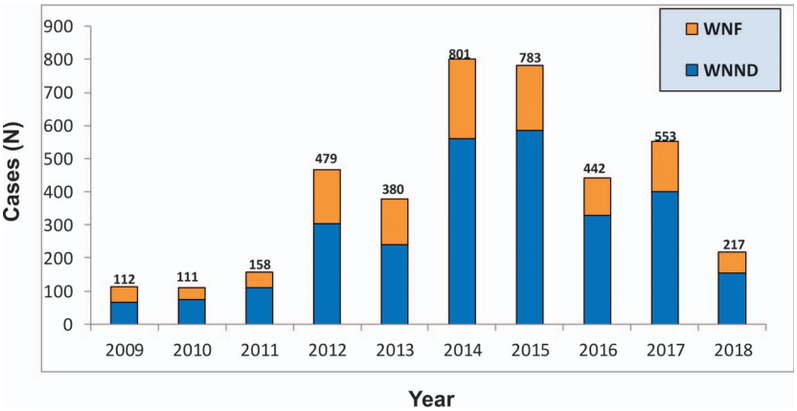

Figure 3.—Human cases of West Nile virus in California, by year, 2009 – 2018.

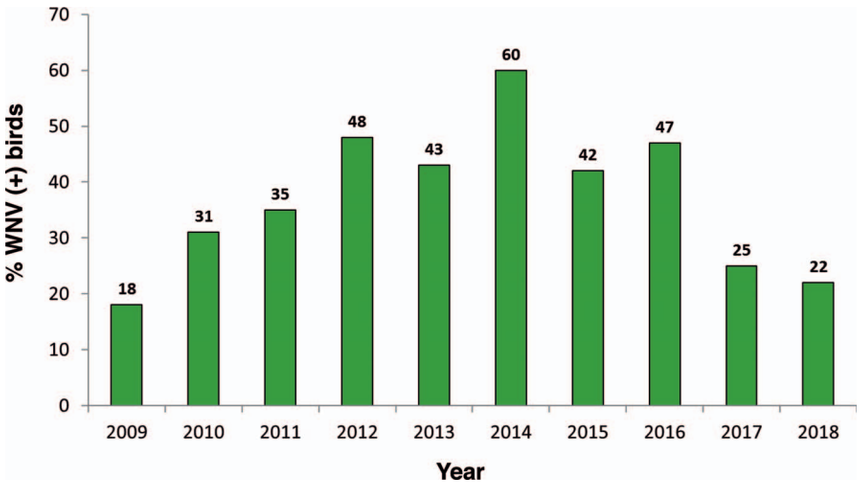

Figure 4.—Percentage of West Nile virus positive dead birds in California, 2009 – 2018.

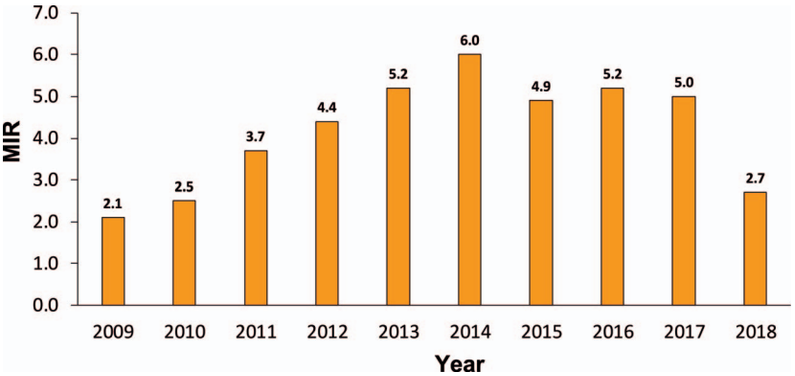

Figure 5.—Minimum infection rate of West Nile virus in *Culex* mosquitoes in California, July – September, 2009–2018.

Table 5.—Mosquito species tested for West Nile virus, California, 2018.

***Culex* species**	**No. Pools**	**No. mosquitoes**	**WNV +**	**MIR**
Cx. erythrothorax	1,281	44,477	3	0.1
Cx. pipiens	8,636	194,798	485	2.5
Cx. quinquefasciatus	16,949	496,372	419	0.8
Cx. stigmatosoma	956	11,552	9	0.8
Cx. tarsalis	14,975	463,707	1,047	2.3
Cx. thriambus	115	300	0	0.0
Culex species	33	255	0	0.0
All *Culex*	**42,945**	**1,211,461**	**1,963**	**1.6**

***Anopheles* species**	**Pools**	**No. mosquitoes**	**WNV +**	**MIR**
An. franciscanus	8	32	0	0.0
An. freeborni	6	71	0	0.0
An. hermsi	19	170	0	0.0
All *Anopheles*	**33**	**273**	**0**	**0.0**

***Aedes* species**	**Pools**	**No. mosquitoes**	**WNV +**	**MIR**
Ae. aegypti	491	2,631	0	0.0
Ae. albopictus	1	11	0	0.0
Ae. bicristatus	1	6	0	0.0
Ae. melanimon	2	2	0	0.0
Ae. notoscriptus	1	1	0	0.0
Ae. sierrensis	3	31	0	0.0
Ae. squamiger	6	64	0	0.0
Ae. taeniorhynchus	1	1	0	0.0
Ae. vexans	17	465	0	0.0
Ae. washinoi	18	701	0	0.0
All *Aedes*	**541**	**3,913**	**0**	**0.0**

Other species	**Pools**	**No. mosquitoes**	**WNV +**	**MIR**
Culiseta incidens	264	3,111	0	0.0
Culiseta inornata	64	389	0	0.0
Culiseta particeps	18	669	0	0.0
Psorophora columbiae	1	6	0	0.0
Unknown	8	211	0	0.0
All other	**355**	**4,386**	**0**	**0.0**

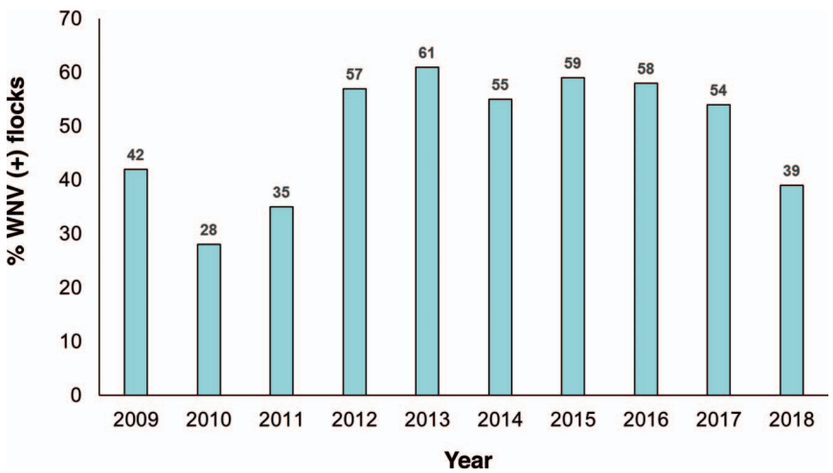

Figure 6.—Percentage of West Nile virus positive sentinel chicken flocks in California, 2009–2018.

Table 6.—Dead birds reported, tested, and positive for West Nile virus, California, 2018.

County	Reported	Tested	Positive	(%)
Alameda	344	80	20	(25.0)
Alpine	0			
Amador	16	1	0	(0)
Butte	102	30	4	(13.3)
Calaveras	8	0		
Colusa	7	1	0	(0)
Contra Costa	711	45	13	(28.9)
Del Norte	3	1	0	(0)
El Dorado	94	22	2	(9.1)
Fresno	162	4	0	(0)
Glenn	6	0		
Humboldt	24	5	1	(20.0)
Imperial	1	0		
Inyo	2	0		
Kern	43	3	1	(33.3)
Kings	14	2	0	(0)
Lake	21	7	0	(0)
Lassen	0			
Los Angeles	839	92	19	(20.7)
Madera	13	2	0	(0)
Marin	59	1	0	(0)
Mariposa	0			
Mendocino	10	0		
Merced	55	1	0	(0)
Modoc	0			
Mono	0			
Monterey	34	4	0	(0)
Napa	19	4	0	(0)
Nevada	26	5	0	(0)
Orange	518	389	18	(4.6)
Placer	465	214	44	(20.6)
Plumas	2	0		
Riverside	166	32	6	(18.8)
Sacramento	1,542	554	241	(43.5)
San Benito	9	1	0	(0)
San Bernardino	154	20	0	(0)
San Diego	186	90	1	(1.1)
San Francisco	56	3	0	(0)
San Joaquin	208	42	16	(38.1)
San Luis Obispo	23	6	0	(0)
San Mateo	409	127	5	(3.9)
Santa Barbara	34	6	0	(0)
Santa Clara	700	237	56	(23.6)
Santa Cruz	132	46	2	(4.3)
Shasta	37	9	5	(55.6)
Sierra	1	0		
Siskiyou	1	0		
Solano	134	15	0	(0)
Sonoma	113	13	0	(0)
Stanislaus	151	8	1	(12.5)
Sutter	76	24	4	(16.7)
Tehama	18	4	0	(0)
Trinity	2	0		
Tulare	61	10	0	(0)
Tuolumne	13	2	1	(50.0)
Ventura	97	15	0	(0)
Yolo	253	92	41	(44.6)
Yuba	42	17	0	(0)
Totals	**8,216**	**2,286**	**501**	**(21.9)**

these counties. Only one sentinel chicken tested positive for SLEV infection, but sentinel flocks were absent from all but one county where SLEV was detected in mosquitoes (Table 2). Of note, the SLEV positive chicken was the only indicator of SLEV activity in Merced County, highlighting the importance of utilizing multiple surveillance methods.

Conclusions

Statewide, WNV activity was much lower in 2018 compared to the last several years. In contrast, SLEV activity increased in some areas, especially in the south San Joaquin region. In most areas, environmental detections of both viruses preceded the incidence of human cases, highlighting the value of environmental surveillance to direct mosquito control efforts and decrease the risk arboviral diseases in California.

Acknowledgements

The authors gratefully acknowledge the cooperation and assistance of the local mosquito and vector control agencies in the collection and submission of samples for testing and their financial support to the testing laboratories and WNV Dead Bird Hotline; the local public health and commercial laboratories which tested clinical samples; the many physicians and veterinarians who submitted specimens from suspect cases of arboviral disease, and the valuable contributions of the staff of MVCAC, DART (especially Sandra Garcia), the California Animal Health and Food Safety Laboratory, and the CDFA Animal Health Branch. From CDPH, we thank the VRDL (especially Shoruq Alhajmohammad, Theresa Brown, Teal Bullick, Lyndsey Chaille, Giorgio Cosentino, Jill Hacker, Kim Hansard, Kristina Hsieh, Maria Liu, Ruth Lopez, Meghana Madala, Oliver Oyler, Leo Oceguera, Peter Patiris, Chris Preas, Maria Salas, Diana Singh, and Pat Stoll), the Veterinary Public Health Section (especially Curtis Fritz), the Infectious Diseases Branch (especially Claudia Erickson and Allyx Nicolici), and VBDS (especially Ervic Aquino, Margaret Kerrigan, Mary Joyce Pakingan, Robert Payne, and the WNV Hotline staff).

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Pacific Southwest Center of Excellence in Vector-Borne Diseases

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The Pacific Southwest Center of Excellence in Vector-Borne Diseases (PacVec) was funded by the Centers of Disease Control and Prevention (CDC) in September 2017 and is located jointly at UC Davis and UC Riverside. PacVec is one of five Centers of Excellence (CoEs) funded through 2021 and serves a region that incorporates Health and Human Services Region 9 and beyond. PacVec initially served California, Arizona, Nevada, Hawaii and the US-affiliated Pacific Islands. In the second year of the program, the geographic area served by PacVec expanded to include Utah. PacVec also funds a study of mosquito populations on the West Coast including Oregon and Washington.

The five Centers of Excellence have three primary objectives: (1) conduct applied research to develop and validate (i) effective prevention and control tools and (ii) methods to anticipate and respond to disease outbreaks; (2) train vector biologists, entomologists, and physicians in the knowledge and skills required to address vector-borne disease concerns; and (3) strengthen and expand collaboration between researchers and public health organizations for surveillance, prevention, and response. An important group of collaborators within PacVec includes academic institutions, the California Department of Public Health and member organizations of the Mosquito and Vector Control Association of California (MVCAC).

PacVec currently supports 27 research and development projects at 10 universities in our region examining new and existing ways to detect, characterize and control threats from mosquito- and tick-borne diseases. The CoE supports research projects of 16 principal investigators at UC Davis and UC Riverside, as well as research at other academic institutions through an annual training grant program. These research projects include collaborations with the MVCAC, the California Department of Public Health, 15 individual vector control districts, and other county and state health departments within the region served by PacVec.

PacVec maintains a website (https://pacvec.us/) that contains information on the CoE and its research activities as well as links to other resources. Some of the resources include a training grant program (https://pacvec.us/training-grants/) and a monthly seminar series (https://pacvec.us/seminars/). The seminars are transmitted by webcast via Zoom and occur on the first or second Tuesday of each month during the academic year (October through June). Presentations rotate between UC Davis and UC Riverside. An archive of recorded presentations also is available. Other resources include training videos and related supplementary material as well as links to publications by CoE members.

The current symposium will highlight research being carried out by PacVec investigators in collaboration with MVCAC member districts. Researchers from UC Riverside, UC Davis and other California academic institutions will discuss their findings on a variety of topics related to the deposition and degradation of public health pesticides, mosquito and tick biology/ecology and control, and pathogen emergence/surveillance.

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Disentangling the host cues used by female *Aedes aegypti* mosquitoes during close-range orientation.

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Introduction

Aedes aegypti, the yellow fever mosquito, has become established in California. Because it is diurnal, it may be thought of as using visual cues rather than odor cues to locate blood meal hosts (van Breugel et al. 2015). DeMasi-Sumner and Cardé found that female *Ae. aegypti* mosquitoes landed on a small, visually indistinct patch of glass beads covered with human odor. Given a choice, inexperienced mosquitoes also preferred to land on a source with visually indistinguishable skin odor rather than nearby sources with visual, heat, or heated visual cues. *Aedes aegypti* prefer to feed on humans. Skin odor, unlike heat and CO_2, is specific to the animal in question.

Methods

Protocol

Three to 9 days after emerging as adults, 5 female *Ae. aegypti* were transferred to clear cylindrical release cages three hours before each experiment. The experiments were conducted 4 hours into the photophase (light period) of the mosquitoes. The light intensity was 14 lux. The mosquitoes were allowed one minute to adjust to the wind tunnel, released from the cage, and then allowed to fly freely for 6 minutes. Mosquitoes were continuously video recorded (Lacey et al. 2014).

Data Collection

The videos were observed manually, and behaviors were recorded with BORIS (Friard et al., 2016). Landings were scored when a mosquito stopped on a cue presentation area. Infrequent landings outside of either 55 mm diameter cue presentation areas were not scored. A few mosquitoes did interact with the glass CO_2 release tube, but they were not scored.

Results

Mosquitoes landed on skin odor more frequently than any other stimulus. However, when the skin odor was not present the number of landings on the heated visual cue increased. The unheated visual cue always elicited the lowest number of landings.

Conclusions

Aedes aegypti, a diurnal/crepuscular mosquito, landed on visually indistinct human skin odor. The mosquitoes even landed on the visually indistinct odor when there were heat and visual cues available. Surveillance of *Ae. aegypti* should use human odor or a mimic to lure host-seeking females into traps.

Acknowledgements

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Bromeliads as *Aedes* breeding habitat in the San Gabriel Valley

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Introduction

Tank bromeliads, a group of plants that can trap standing water and thus serve as breeding habitat for mosquitoes including invasive *Aedes* spp. (Frank 1983), are popular ornamental plants in southern California. Although San Gabriel Valley vector control workers regularly detect and treat *Aedes* spp. and *Culex* spp. larvae in garden bromeliads, the role of these plants in local *Aedes* outbreaks is poorly understood. The goals of the current study were to 1) learn how invasive *Aedes* spp. use bromeliad habitat over a season, and 2) compare these activity patterns with those of adult invasive *Aedes* spp. detected nearby by means of standard adult trap collections.

Methods

Ten *Guzmania* "Allura" plants with a phytotelmata volume of 152 ± 39 mL (mean ± SD) were placed at each of ten sites across the cities of Alhambra, El Monte, and Pasadena, in which both *Aedes aegypti* and *Aedes albopictus* had been collected in 2017. Weekly between May-Oct. 2018 we triple-rinsed each plant, collected visible larvae from rinse water, and saved the rinse water of four plants per site per week to rear unseen larvae and/or eggs in the laboratory. Plants were then refilled with deionized water. In each city, we operated one BioGents BG-Sentinel (BG) trap baited with BG-Lure for 24 h each week.

Results and Discussion

Only *Ae. aegypti* and *Ae. albopictus* were collected from bromeliads, while BG traps detected *Aedes aegypti*, *Ae. albopictus*, *Culex quinquefasciatus*, *Cx. tarsalis*, and *Culiseta incidens*. *Aedes albopictus* was collected from bromeliads at all ten sites, with 5.8 ± 3.3 (mean ± SD) plants per site producing *Ae. albopictus* at least once during the study. *Aedes aegypti* was collected from seven plants at two sites. *Aedes albopictus* larvae were first found in bromeliads 8.2 ± 4.3 weeks later (mean ± SD) and last found 4.0 ± 4.4 weeks earlier than adults were collected in BG traps in the same cities.

Conclusions

Small sentinel bromeliads were colonized only by invasive *Aedes* spp. despite the presence of other mosquitoes. Approximately 60% of these plants were colonized by *Ae. albopictus* at least once during the season. *Aedes albopictus* adults were collected at BG trapsbefore and after larvae were found at most bromeliad sites.

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Population genomics of *Aedes aegypti* in California

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Abstract

In the summer of 2013, *Aedes aegypti (*L.) was detected in central California (CA) and and in subsequent years has become established and spread to multiple locations in central and southern CA. A number of published reports suggest that the current established CA populations are derived from individuals introduced from multiple different locations. The current project is the first to conduct a population genomics analyses based on whole genome re-sequencing data of individual, field-collected *Ae. aegypti*. The genomes of 132 *Ae. aegypti* were analyzed to establish genetic relationships among populations from sites in CA, Florida and Mexico. We identified 3 major genetic clusters within CA; one that includes all sample sites in the southern part of the state plus the town of Exeter in central CA and two additional clusters in central CA. Hybridization between the two central CA clusters appears to be occurring. Our study implicates that southern CA *Ae. aegypti* populations likely originated from Mexico. *Ae. aegypti* collected in 2014 from Exeter are genetically closest to the Vero Beach, Florida population. The population origin of the central CA populations remains unknown. Rare single nucleotide polymorphism (SNP) distribution in geographic space indicates that within 2-4 years, *Ae. aegypti* could move about ~270 km in both southern and central CA populations. Genomic changes that occurred in the Clovis population within 3 years may suggest adaptive processes in the *Ae. aegypti* genome after being introduced into a new area. Our dataset provides a foundation to investigate some outstanding questions on the population biology of *Ae. aeygpti*.

Introduction

Aedes aegypti (Linnaeus) is presumed to have become established in the southeastern United States between the fifteenth and eighteenth century (Tabachnick 1991). Its spread across the US between 1795 and 1905 precipitated major epidemics of yellow fever throughout the east coast and southern states (Crosby 2006). This mosquito has now been recognized as the vector of three additional human viruses, dengue, chikungunya and Zika, which pose a major threat to global public health (Huang et al. 2019).

The state of California (CA) had remained free of established overwintering *Ae. aegypti* populations (Gloria-Soria et al. 2014), until it was simultaneously collected in Fresno and Madera counties and later in San Mateo in 2013. Further expansion of its distribution (Lee et al. 2019) proved that this mosquito species is now capable of surviving through the winter in CA and that suitable habitats for its survival are present across the state. The invasion of *Ae. aegypti* in CA is ongoing and is significant both as a threat to the health and well-being of citizens of the state and as a useful biological system for the study of invasive species in general.

Successful vector control can benefit from population genetics and genomics analyses which can provide information on the potential population origin and point of introduction (Gloria-Soria et al. 2014, Evans et al. 2015, Pless et al. 2017), population movement (Lukindu et al. 2018), and identify the genetic basis of phenotypes such as insecticide resistance (Main et al. 2015) and host preference (Main et al. 2016). Hybridization between diverged vector populations may serve as a source of new genetic material including alleles that mediate adaptations to facilitate range expansion (Vicente et al. 2017) or that promote the evolution of resistance to insecticides (Norris et al. 2015).

Here, we have applied a population genomics approach to study invasive *Ae. aegypti* populations in CA. We conducted analyses based on genome sequences of 132 individual *Ae. aegypti*, collected from 35 locations in CA, and, for comparison, four specimens from Florida and three from Mexico. The genomic analyses presented here should serve as a step toward expanded population genomics studies aimed at understanding how invasive mosquito

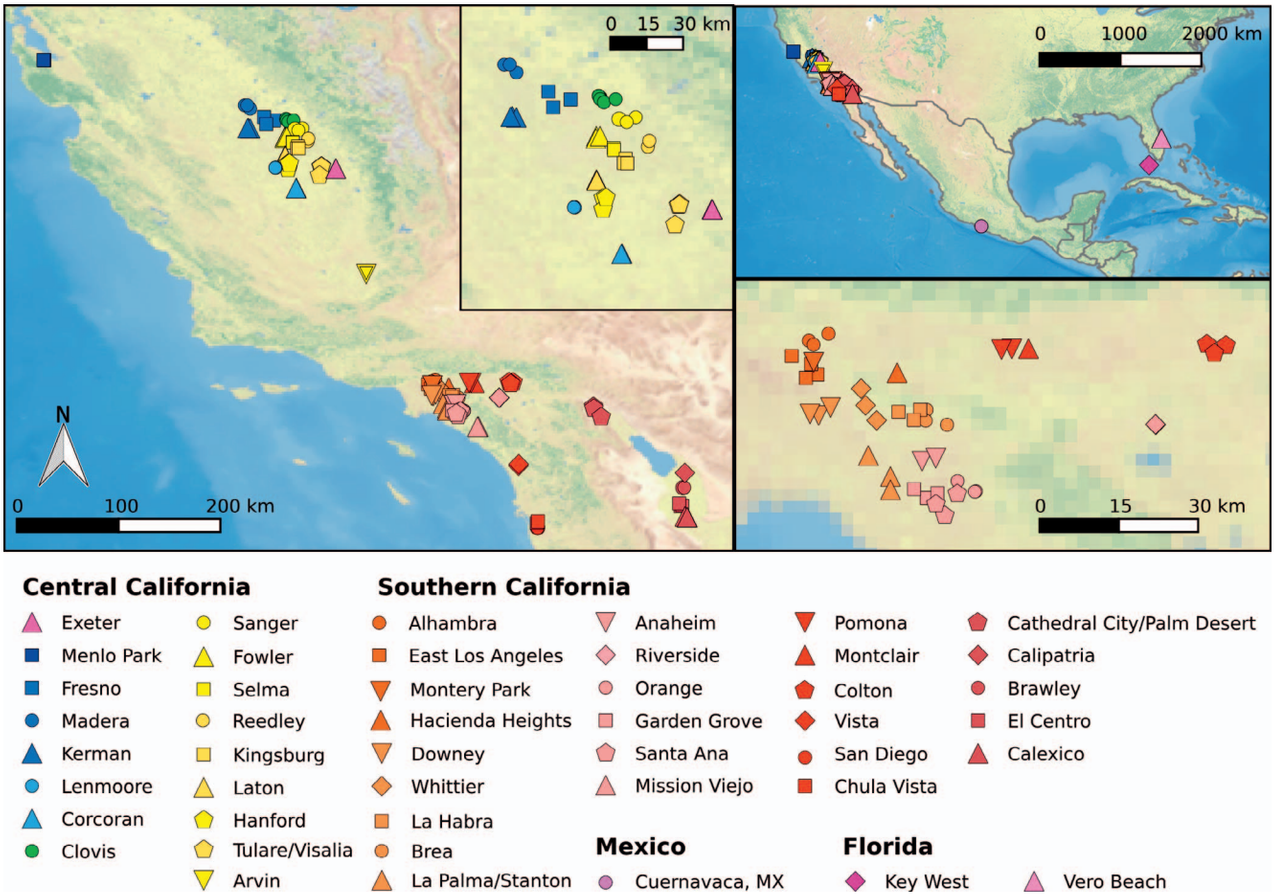

Figure 1.—Origin of *Ae. aegypti* samples. Left map shows location of all samples from California with the inset enlarging the Fresno/Clovis area. Top right shows a world map, and bottom right a map of Greater Los Angeles and neighboring locations. CleanTOPO2 basemap (Patterson 2008) was used as background.

species become established in new locations and how they subsequently interact genetically in their new environment.

Methods

Sample origin

Adult female *Ae. aegypti* were collected from 35 cities by personnel from Mosquito Abatement Districts in Coachella, Consolidated, Fresno, Greater Los Angeles, Imperial, Kern, Kings, Madera, Northwest, Orange, San Bernardino, San Diego, San Gabriel Valley, San Mateo, Tulare, and Tulare Delta (Figure 1). Mosquitoes were collected using BG Sentinel traps baited with CO_2 or collected as larvae from ovicups and individually stored in 80% ethanol prior to DNA extraction. All collections on private properties were conducted with permission from residents and/or owners.

Whole genome sequencing

Genomic DNA was extracted using established protocols (Nieman et al. 2015, Yamasaki et al. 2016). DNA concentrations for each sample were measured using the Qubit dsDNA HS Assay Kit (Life Technologies) on a Qubit instrument (Life Technologies). A genomic DNA library was constructed for each individual mosquito using 20 ng DNA following an established protocol (Yamasaki et al. 2016). Qiaseq FX 96 (Qiagen, Valencia, CA) or KAPA Hyperplus kit (Roche) was used for library construction. Ampure SPRI beads (Beckman) were used for library purification. Library concentrations were measured using Qubit (Life Technologies) as described above. Libraries were sequenced as 150 bp paired-end reads using a HiSeq 3000 or 4000 instrument (Illumina) at the UC Davis DNA Technologies Core.

Sequence Analysis

Raw reads were trimmed using Trimmomatic (Bolger et al. 2014) version 0.36 and first mapped to the *Ae13-CLOV028MT* mitogenome using BWA-MEM (Li 2013) version 0.7.15 to filter out mitochondrial reads that could potentially bind to nuclear pseudogenes (Schmidt et al. 2018). The unmapped and "mate-is-unmapped" reads were extracted from the resulting bam files using sambamba (Tarasov et al. 2015) version 0.6.7. Those unmapped reads were mapped to the AaegL5 reference genome (Matthews et al. 2018) using BWA-MEM (Li 2013). Mapping statistics were calculated using Qualimap version 2.2

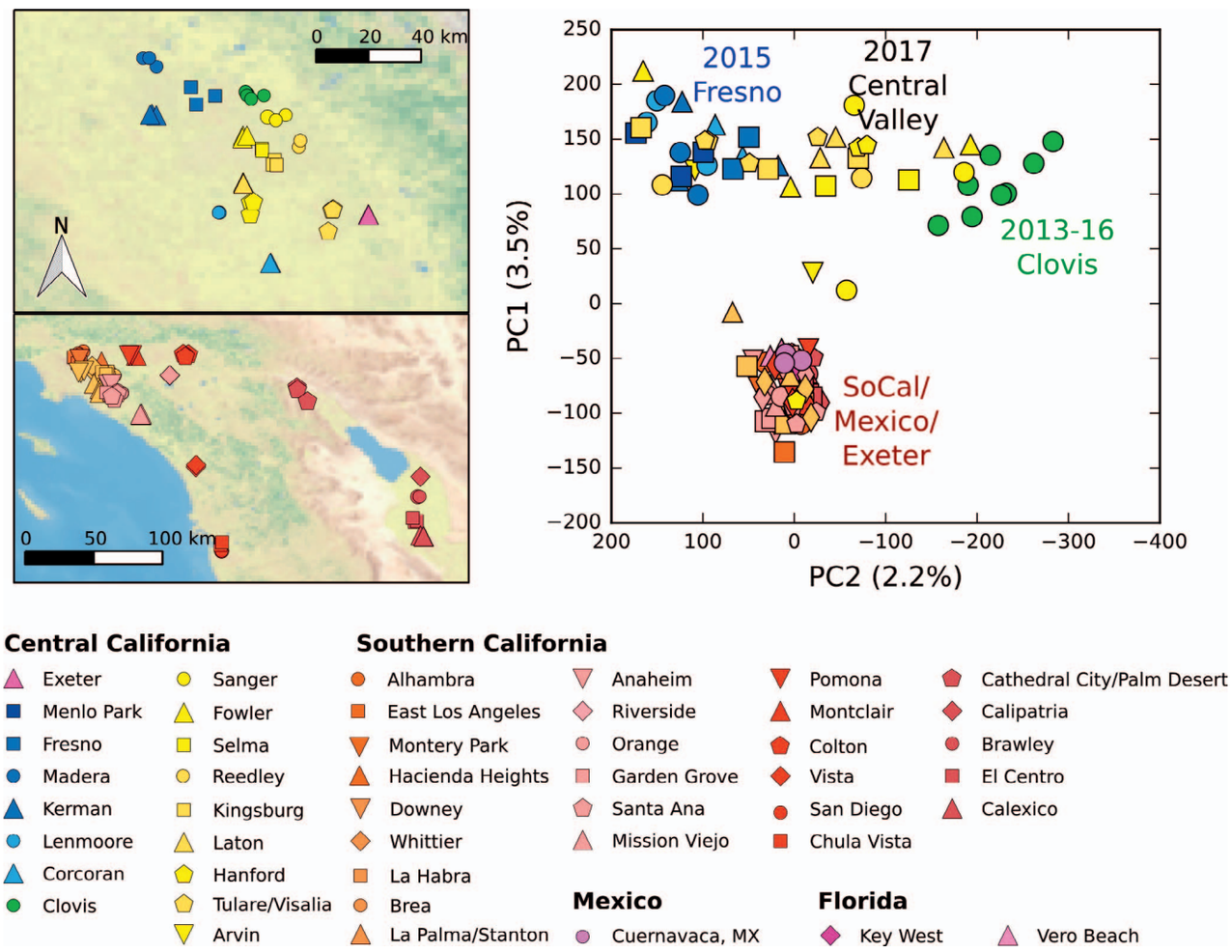

Figure 2.—Genetic Clusters of *Ae. aegypti* based on PCA analysis components 1 and 2. Principal Component Analysis based on the SNP data. Principal component 1 (PC1) separates southern CA population from central CA populations. PC2 further separates the Central California populations into two groups (Clovis and Fresno). Florida and Mexico populations cluster together with southern CA populations.

(Okonechnikov et al. 2016) to check the depth of coverage. Samples with insufficient coverage (<6X) were excluded from analysis. Joint variant calling using all samples was done using Freebayes (Garrison and Marth 2012) version 1.0.1 with standard filters and population priors option disabled. We tested minimum depths of 6-8X to call variants for each individual and found no qualitative difference. Results are, therefore, presented using the biallelic SNP set with minimum depth of 6X here. SNPs in the "soft-masked" regions were excluded from genome analysis. A missing data threshold of 10% was used to filter SNPs. Hudson F_{ST} (Hudson et al. 1992), nucleotide diversity (π) and Principal Component Analysis (PCA) was done in Python version 3.6.6 using the scikit-allel module version 1.2.0 (Miles and Harding 2018).

Data Visualization

QGIS version 2.18 was used to create maps. The CleanTOPO2 basemap (Patterson 2008) was used as background. Python matplotlib version 3.0.2 (https://matplotlib.org/) was used for generating plots. Inkscape (https://inkscape.org/) version 0.92 was used to edit images.

Results and Discussion

We sequenced the genomes of 132 specimens of *Ae. aegypti* from California (N=125), Florida (N=4) and Mexico (N=3) with median genome coverage of 9.6X per sample. Filtering for biallelic SNPs and a minimum depth of 6 with at most 10% missing data yielded 12,745,300 SNPs.

Principal Components Analysis based on the genotypes of these SNPs revealed three distinct genetic clusters consistent with previous reporting (Lee et al. 2019) as well as populations containing hybrids (marked in yellow symbols, Figure 2). Overall, the three genetic clusters containing CA *Ae. aegypti* populations have a nearly

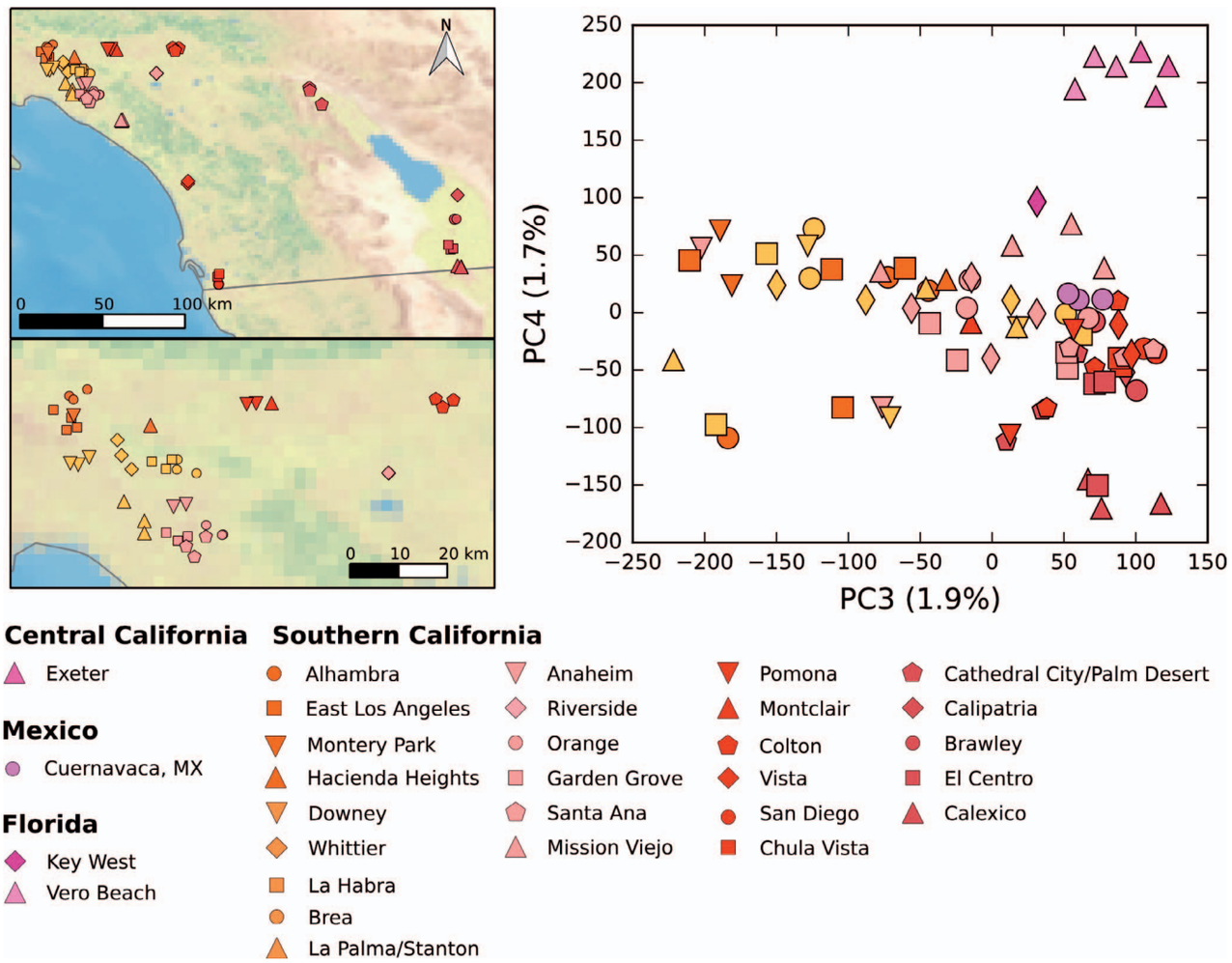

Figure 3.—Genetic Clusters of *Ae. aegypti* based on PCA analysis components 1 and 2. Shown is a PCA plot based on the principal components 3 and 4. Principal component 3 (PC3) structures southern CA populations. PC4 mainly separates Key West (FL) from Mexico populations and from a cluster with Exeter and Vero Beach (FL) samples.

parapatric distribution with the three groups potentially converging in the Central Valley. The populations at Fowler, Hanford, Kingsburg, Laton, Reedley, Sanger, Selma, Tulare, Visalia, and Arvin appear to have multiple genetic clusters occurring in sympatry. Although previous reports (Lee et al. 2019) suggested small amounts of hybridization, this phenomenon actually appears to be extensive in central CA. Subordinated principal components indicated additional population structure in southern CA, Florida and Mexico samples (Figure 3). Populations from San Diego area as well as inland locations - Pomona, Montclair, Colton, Vista, San Diego, Chula Vista, Catherial City, Palm Desert, Calipatria, Brawley, El Centro, and Calexico – appear to be more homogeneous in their genetic makeup (Figure 3). The 2014 Exeter population appears to be closely related to Vero Beach, Florida but not as close to Key West, Florida. The population from Cuernavaca, Mexico is indistinguishable from San Diego, Imperial and some inland populations. This is consistent with a previous report suggesting highly structured populations within southern CA and a Mexican or southwestern USA origin of those populations (Pless et al. 2017). Overall, our data supports multiple introductions into CA from genetically distinct source populations as the most plausible history of this invasion.

The degree of genetic differentiation found in the Clovis population between the years 2013 and 2016 (Figure 4) indicates that the population is undergoing rapid changes in its genome, potentially reflecting local adaptation, or less likely, drift. We compared the genomes of samples obtained in Clovis in 2013 with those from samples collected in 2016. Overall genome divergence is negligible ($F_{ST} = -0.025 \pm 0.002$) consistent with our PCA results showing very similar genetic makeup. However, a whole genome scan using 1 Mbp windows for F_{ST} values indicated several genomic regions with markedly elevated F_{ST} values (>0.1) (Figure 4). Further, a comparison between samples from 2013 and 2016 showed an increase over time of nucleotide diversity in chromosome 1 and 2 (mean π_{2016}- π_{2013} value of 7.22×10^{-5} and 2.32×10^{-5},

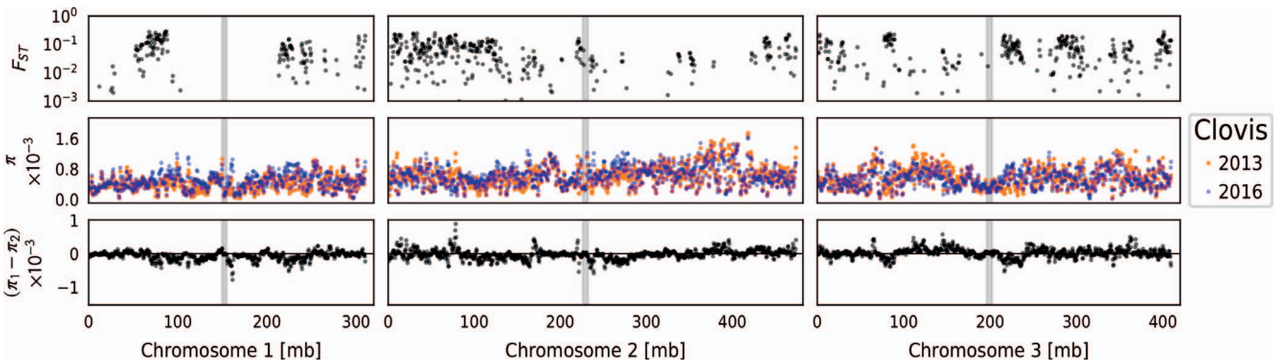

Figure 4.—Genome-wide comparison of 2013 and 2016 Clovis *Ae. aegypti* populations. The top subpanel reports Hudson F_{ST} estimator between groups, the middle subpanel shows nucleotide diversity (π) within each group, and the bottom subpanel shows the difference in nucleotide diversity ($=\pi_1 - \pi_2$). Values were calculated using 1 Mbp windows with 500 Kbp steps. Vertical gray bars indicate the location of centromeres.

respectively) but a decrease on chromosome 3 (π_{2016}- π_{2013} value of -1.70x10^{-5}). However, regions with a relatively large change in nucleotide diversity between 2016 and 2013 are visible on all three chromosomes, some of which also coincide with highly differentiated (F_{ST} >0.1) regions. These highly differentiated regions with a relatively large nucleotide diversity change may indicate genomic regions under selection, presumably as the founding population adapts to local environmental conditions (Main et al. 2015).

For rare allele analysis, we selected SNPs with the alternative allele occurring 2-4 times in our dataset and measured the geographic distance between a pair of samples sharing the same SNP. This filtering resulted in 158,094 SNPs in southern CA samples and 255,545 SNPs in central CA samples. Exeter and Florida samples were excluded from this analysis. We assumed those pairs of samples sharing the same rare SNP to likely being descendants from a recent common ancestor and a single, unique mutation event therein; similar to the assumptions made in *Raddle* program (Novembre and Slatkin 2009). Thus, the geographic distances between rare SNPs may reflect recent migration events. Our results indicated that 95% of rare SNPs occur within approximately 270 km in both southern and central CA (Figure 5). As our data encompassed four years (2013-2017), this result indicated that *Ae. aegypti* in CA could move about 270 km over the course of 4 years, corresponding to an average of ~70 km/year.

There are some questions remaining that could be addressed in upcoming studies. For instance, the initial introduction in Exeter in 2014 indicates the samples likely originated from the southeastern USA (e.g. Florida). The Exeter population was thought to be eliminated thereafter; however, some *Ae. aegypti* were again detected in 2018. Given the genetic differences we observed in the region, further genomic analysis could inform if the Exeter population suffered severe bottleneck and recovered (2014 and 2018 Exeter population would cluster together) or if it

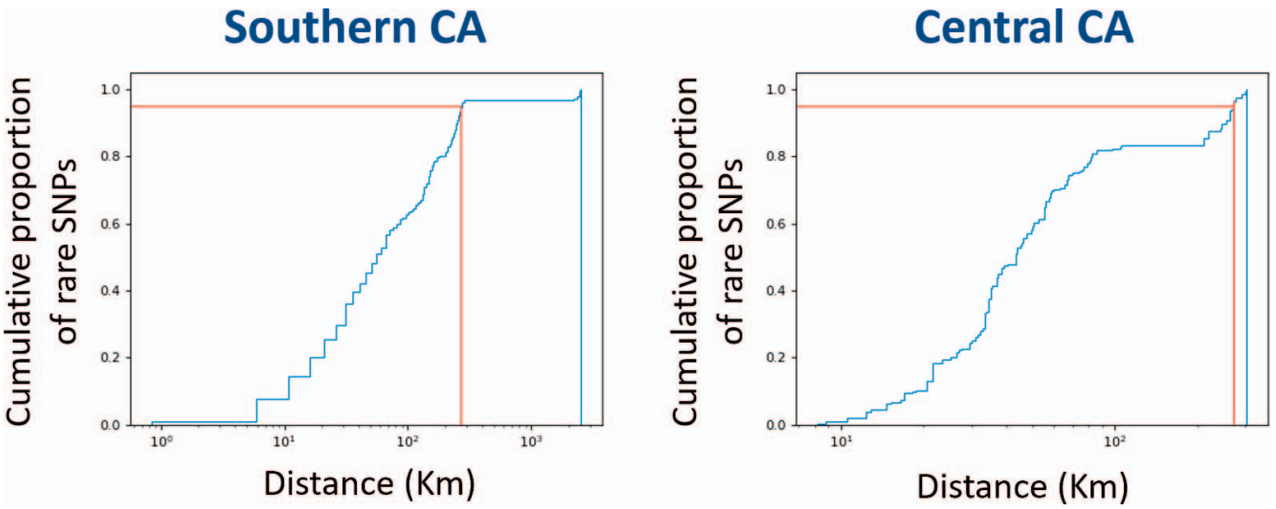

Figure 5.—Cumulative distribution of geographic distances between two individuals sharing a rare SNP. Horizontal red lines indicate 95% of rare SNPs and vertical red lines indicate the geographic distance where 95% of rare SNPs occur within. Distances are in log scale.

was locally eliminated from Exeter but reintroduced from a neighboring towns (2018 Exeter would cluster together with Clovis and/or Fresno). Investigation on the genes corresponding to the genomic regions of high differentiation and large nucleotide diversity changes (e.g. 2013 vs 2016 Clovis) could inform potential mechanisms facilitating the adaptation of *Ae. aegypti* to survive the dry and hot summers and also the much cooler winter conditions in central CA. Variations in the known and potential insecticide resistance genes (e.g. *voltage-gated sodium channel* or P450 genes) detected in our dataset may facilitate analysis of the other novel mutations contributing to the insecticide resistance of CA *Ae. aegypti* (Cornel et al. 2016). Further genome investigation on other populations such as Lousiana may also inform if the central CA population was originated from elsewhere in the USA.

Conclusion

Our data together with previous reports strongly support multiple introductions of *Ae. aegypti* into California. The most likely scenario includes four independent introductions: (i) Clovis area, probably in 2013; (ii) Madera area, probably in 2013; (iii) southern CA, probably in 2014; and (iv) Exeter, probably in 2014 introduced from someplace in the southeastern USA such as Florida. The years of first detection are based on surveillance reports from the California vector control districts. From our data the geographic origins of the introductions and the means by which they were introduced remain uncertain with only the Exeter population showing signs of presumable derivation from the southeast USA. Our dataset serves an important step toward future studies aimed at understanding population divergence, gene-environment interactions, and dispersal of this invasive species.

Abbreviations

CA: California
SNP: single nucleotide polymorphism
PCA: Principal Component Analysis

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Movement of St. Louis encephalitis virus in the Western United States, 2014-2018

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Introduction

St. Louis encephalitis (SLEV) is an encephalitic arbovirus that has re-emerged in California after 11 years of not being detected. Although epidemiological and mosquito surveillance data indicate that the virus is spreading in California and the Western US, the specific pattern of expansion remains unclear. In this study, phylogeographic methods were used to identify the routes of SLEV spread and to help identify environmental features that may have promoted or inhibited viral movement.

Methods

Twenty-eight SLEV genomes were sequenced from mosquito pools collected during routine arbovirus surveillance activities in Arizona, Nevada, Idaho, Oregon, Texas and multiple counties throughout California. Maximum likelihood and Bayesian phylogeographic approaches were applied to map the patterns of SLEV expansion.

Results and Discussion

Three independent routes of SLEV expansion were identified in the Western US: Arizona to Southern California, Arizona to Central California and Arizona to all locations east of the Sierra Nevada Mountains. Each route appears to have been influenced by the mountain ranges surrounding California (the Sierra Nevadas, the Cascades and the Transverse Ranges), which are known to restrict the movement of host birds and the distribution of vector mosquito species.

Conclusions

The identification of mountains as natural barriers capable of influencing SLEV dispersal enhances our understanding of arbovirus ecology in the Western US and may also support regional public health agencies in implementing more effective strategies for protecting their communities.

Acknowledgements

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Estimates of aerial adulticide efficacy based on 12 years of mosquito surveillance in Sacramento-Yolo MVCD

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Introduction

Aerial adulticide treatments are used during periods of epidemic risk to rapidly reduce the abundance of infectious mosquitoes in proximity to humans (Rose 2001, CDPH 2017). A common assessment of the efficacy of each treatment is to compare pre- to post-treatment trap counts inside the treatment zone versus changes for the same period in an adjacent unsprayed control area (Reisen 2010). However, reported estimates vary widely (Elnaiem et al. 2008, Macedo et al. 2010) with some studies indicating mixed results and even occasional increases in trap counts following spray events (Lothrop et al. 2007), in part because pre- vs. post-spray methods do not account for weather that may cause night-to-night volatility in trap counts. Reliable estimates of the efficacy of aerial treatments are imperative for appropriate implementation of control measures and allocation of resources. In this study, we aim to overcome the limitations of shorter-term studies by using 12 years of surveillance and control records to estimate the magnitude and duration of the effect of aerial adulticides on adult populations of *Culex tarsalis* and *Culex pipiens* in Sacramento and Yolo counties, CA.

Methods

We used collection records for female *Cx. tarsalis* and *Cx. pipiens* from CO_2-baited EVS traps ($n = 24{,}343$) and aerial spray records ($n = 930$) for 2006-2017 from Sacramento-Yolo Mosquito and Vector Control District (SYMVCD). We developed generalized additive models to relate nightly trap counts of female mosquitoes to aerial treatments during the previous 5-week period, adjusted for variation in trap counts due to season, location, year, land use, 2-week average temperature, and nightly deviations in average temperature during trapping. Spatial coverage of each trap was quantified as the percent of the area within 5 km of the trap, the area from which a trap collects mosquitoes, that was covered by aerial treatment zones. Traps outside treatment zones were also modeled to capture baseline spatio-temporal mosquito dynamics in the absence of aerial treatments. All treatments within five weeks prior to trapping were included to capture effects of repeated spray events. We used the percent deviation from the baseline abundance under a hypothetical no-spray scenario as the measure of effect for aerial treatments.

Results and Discussion

Aerial treatments resulted in decreased abundance of both *Cx. tarsalis* and *Cx. pipiens* across the range of spatial coverage and temporal sequences of treatments during the five weeks preceding a trapping event, with the pattern and magnitude of reduction differing between the species. For most sequences of treatments during the five weeks prior to trapping, *Cx. pipiens* were reduced compared to the no-spray baseline. The effect increased with greater spatial coverage of the trapping area (5 km buffer area surrounding each trap), with 53% reduction estimated for trapping areas fully within the spray zone of treatments one week prior (and no other sprays within the prior five weeks). Notably, *Cx. pipiens* populations immediately adjacent to aerial treatment zones ($\leq$ 50% spatial coverage) were also reduced. For *Cx. tarsalis*, 31% reduction was estimated for trapping areas fully within aerial spray zones during only the previous week, although traps along the margins of aerial treatment zones ($\leq$ 50% spatial coverage) showed no reduction. Different habitat utilization and dispersal of these species may partially explain this difference in results. 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 or suburban areas so a treatment in these areas will impact a large portion of the total population and leave fewer left to repopulate the area from outside the treatment zone. In contrast, *Cx. tarsalis* typically emerge in large numbers from agricultural habitats and may immigrate into urban areas (Reisen and Reeves 1990), potentially rapidly replacing adults killed by aerial adulticide treatments in both urban and agricultural areas.

Conclusions

Aerial adulticides effectively reduced the abundance of the primary West Nile virus vectors, *Cx. tarsalis* and *Cx.*

pipiens, in Sacramento and Yolo counties. Estimates of efficacy of aerial spray events using trap count data need to take into account modifying factors, such as temperature and mosquito habitat, that contribute to stochastic variation in nightly trap counts. Further work will expand upon these methods to estimate the change in WNV transmission potential and resulting human infections.

Acknowledgements

We would like to thank Ruben Rosas, Marcia Reed, and Gary Goodman from Sacramento-Yolo Mosquito & Vector Control District for providing trapping and aerial treatment data used in this study. KMH acknowledges funding support from the Floyd & Mary Schwall Fellowship in Medical Research at UC Davis, and KMH and CMB acknowledge support from the Pacific Southwest Center of Excellence in Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516).

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Pyrethroid resistance in *Culex tarsalis*

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Introduction

Culex tarsalis is found throughout the western United States and is one of the most abundant vectors of encephalitis viruses such as West Nile virus (WNV) and St. Louis encephalitis virus (SLEV). Insecticides play a significant role in the reduction of vector-borne diseases, but the effectiveness of vector control is threatened by the increase of insecticide resistance. The goal of the current study is to determine the prevalence of pyrethroid resistance in several *Cx. tarsalis* populations across Northern California.

Methods

Resistance was assessed using bottle bioassays with permethrin and permethrin + piperonyl butoxide (PBO). Activity of detoxifying enzymes was determined using colorimetric microplate assays modified from CDC protocols.

Results and Discussion

Bottle bioassays were completed for 16 populations across Lake, Placer, San Joaquin, Sacramento, and Yolo Counties during the summer of 2018. Resistance to permethrin varied greatly among populations, with mortality ranging from 8.6 to 96.2% after 2 hours of exposure to permethrin. In all but one population, mortality rose to over 90% when PBO was added to the assay; that is, when mosquitoes were exposed simultaneously to both permethrin and PBO, mortality increased. These data indicate that detoxifying oxidases may be playing a role in resistance in some of the tested populations. Preliminary results from enzymes assays supported this idea, as oxidase levels were higher in some field populations than in the susceptible *Cx. tarsalis* colony.

Conclusions

The current results are preliminary, but there is evidence of pyrethroid resistance in *Cx. tarsalis* in several counties in Northern California. Work on this project is ongoing and will continue to evaluate the role of oxidases, as well as esterases and glutathione-s-transferases, as mechanisms of pyrethroid resistance. Additionally, populations will be tested for the prevalence of *kdr*, a DNA mutation that is associated with permethrin resistance in other mosquito species.

Acknowledgements

We would like thank San Joaquin MVCD, Sacramento-Yolo MVCD, Placer MVCD, and Lake County VCD for collaborating on this project and providing funding. We also acknowledge funding support from the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516)."

Preparing for the unknown – the threat from emerging vector-borne pathogens

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Introduction

Vector-borne emerging diseases, such as Zika virus, will continue to appear, often with unexpected consequences. By their nature emerging pathogens are poorly understood, unexpected, and unlikely to be preventable with vaccines. Reducing their impact on the American population will be a major challenge.

Methods

This presentation was based on analyses of 100 years of data in the literature, reports of notifiable diseases to the CDC, a recent report on vector control preparedness by National Association of County and City Health Officials (NAACHO 2017), and the presenter's opinions.

Results and Discussion

Vector-borne diseases have been dramatically increasing in the U.S. (Rosenberg 2018). The incidence of tickborne bacteria, such as Lyme borrelioses and Rocky Mountain spotted fever, have been increasing steadily as vector range expands and human contact increases. Mosquito-borne RNA viruses, which have high rates of mutation, tend to produce explosive epidemics. There are more than 90 known arboviruses pathogenic to humans and another 100 known so the potential for emergence is high, especially because most could be transmitted by indigenous species (Rosenberg et al. 2013). Although the best defense is sensitive surveillance and rapid response, a NAACHO study commissioned by CDC found nearly all public vector-control operations self-reported deficiencies in at least one of five core competencies.

Conclusions

The Country's only hope to mitigate the threat from endemic and emerging vector-borne diseases is vector control. It is impractical to rely on the rapid development of new vaccines. This will be the public health mandate for all the Country's vector control personnel.

Acknowledgements

The MVCAC for the invitation and support, and the CDC for allowing me to accept.

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Using Google Forms to learn about our constituency.

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Introduction

It may be challenging for small mosquito and vector control agencies to conduct outreach and elicit feedback from their constituency due to limited staff and limited budgets. Mosquito and vector control agencies distribute educational materials as part of an integrated pest management program (IPM), yet knowing the impact of such outreach and the needs of the agency's constituency can be hard to track, although equally important. Santa Cruz County Mosquito and Vector Control (SCCMVC) explored using Google Forms, a survey tool, in various formats to trace public knowledge of our services and the influence our outreach has on the community.

Methods

SCCMVCs measurement of outreach impacts used various experimental and exploratory Google Forms methods. First, a survey was created in Google Forms for the 2018 Santa Cruz County Fair that asked basic questions such as, "Do you live in the county?", "Have you heard of our agency or services before?", "Have you used our services before?". It was administered during staffed hours at our booth for an approximate total of 15 hours. During that time, SCCMVC employees used smartphones with the Google Forms program to record visitors' answers. We tried a variety of methods of recording responses on the smartphone: one-on-one (employee records each response in Google Forms while speaking to a visitor in real-time), teams of two (one employee asks the visitor questions, other employee records responses in Google Forms in real-time), and a clipboard tally (employee tallies responses in real-time, but enters responses in Google Forms at the end of the shift). A similar survey was administered at the 2018 SCCMVC Open House. Lastly, Google Forms was used to create a survey for requesting feedback after a provided service (i.e. Mosquito Inspection, Rodent Inspection, Tick Identification) and was distributed by email. The recipient was asked to evaluate response time, service quality, and whether their issue was resolved.

The following is a link to a tutorial video that presents our experimental use of the program in a way that might be helpful to agencies using this for the first time.

(https://prezi.com/m98pjppvguis/?token=8fba8751a10415067aaf195285f5594f2b1c913d40557c43a662f70358465151&utm_campaign=share&utm_medium=copy).

Results and Discussion

Overall, we found Google Forms to be both an intuitive survey building tool and an easy way to look at survey results as a whole. The program can display pie charts, graphs, etc. (depending on type of question asked) in real-time and unlike other free survey tools, Google Forms will continue tallying and summarizing results using the same URL link even if the survey is edited or updated. For the 2018 County Fair survey, we received 90 responses, and the 2018 Open House survey had a 20% response rate (18 responses, 90 attendees). At both events, we found that the team of two method yielded the most responses and was the easiest to administer. If one employee was available for a shift, the clipboard method was preferred over one-on-one with a phone, as the clipboard was a more acceptable distraction from the interview. SCCMVC is still experimenting with the program itself and the methods of administering the surveys. With the flexibility of Google Forms, we can add or edit questions for upcoming events (perhaps add demographic measurements to help inform the direction of outreach funds) and explore other uses such as door-to-door surveys or autonomous tablet stations.

Conclusions

Google Forms is a free and low-maintenance survey tool that can help an agency learn more about their constituency by tracking responses and performing basic analytics. The goal is to continue using Google Forms at upcoming events and for post-service follow-up to better understand the impact of our outreach and learn more about the community we serve.

Acknowledgements

Special thanks to all staff at SCCMVC, and to Deborah Bass (formerly Contra Costa Mosquito and Vector Control) for suggesting Google Forms as a free and low-maintenance feedback tool.

Homeless encampments and the rise (and fall) of the Norway Rat (Breaking the cycle of unsheltered encampments sanitation and vermin problems in Oakland, CA)

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INTRODUCTION

In Alameda County, the Alameda County Vector Control Services District (ACVCSD) thought we were winning the war against the Norway rat (*Rattus norvegicus*). Most control activities relating to Norway rats were limited to the "sewer baiting" program designed to keep rat populations inhabiting our aging sewer system in check. Above ground Norway rat infestations in the past have been localized and small in scale.

During the last few years dozens of homeless encampments have sprung up throughout the city of Oakland, with a total homeless population of 2,761 (ARS 2017). Along with the encampments have come an overabundance of food, brought into areas where people have not previously lived, including freeway easements and overpasses, rail corridors, and semi-industrial/business areas. Many of these encampments do not have formal addresses and even though they look terrible, rodent complaints have not come from these enclaves, leaving the City of Oakland with the dilemma of how to handle this social and public health problem. Initial efforts centered around periodic site cleanups, with a 72-hour posting of pending "clean and clear", and subsequent removal of whatever was left on the property. Legal actions/lawsuits arising from conditions surrounding these encampments have been numerous. Plaintiffs have included Americans with Disabilities Act (ADA) lawsuits due to the city allowing the blocking of sidewalks and forcing pedestrians into the street, homeless activists (such as American Civil Liberties Union) legal actions due to violation of the unsheltered persons property rights, businesses that have been affected by nearby encampments, and resident groups that see the city deteriorating. ACVCSD involvement started a few years ago, after being invited to attend a Caltrans and the City of Oakland working group that coordinated cleanup of encampments, encampment closures and movement. The major problems from District perspective were sanitation; i.e., garbage, rubbish, no toilets and hand washing stations. There was reluctance to sanctify the encampments by placing these public health tools at encampment sites. Eventually encampment site planning was developed for a few pilot sites with structure enough to include these sanitation tools, that provided adequate health and safety for the residents and community. After receiving an invitation to attend the "Encampment Management Team" meetings 18-months ago, we began to work closely with the City of Oakland towards positive results, including garbage containerization and weekly removal, vector control, and portable toilet and hand washing station installations.

METHODS

The Veterans Administration Medical Outpatient Center (VAMOC) in Oakland vowed to relocate, if the conditions surrounding their facility were not improved. Campers had blocked their sidewalks, garbage and rubbish were everywhere and pervasive injectable drug syringes were discarded in public areas (Fig. 1). To reopen the sidewalks around the VAMOC required the movement of the unsheltered living, but the location selected to relocate (across the street) had a significant Norway rat infestation, because it had been an illegal dumping site for a couple of years, with irregular cleanup. The District reduced the rat problem by baiting the rat burrows with Ditrac™ rodenticide tracking powder and greater sanitation was promised, including the installation of portable toilets, a

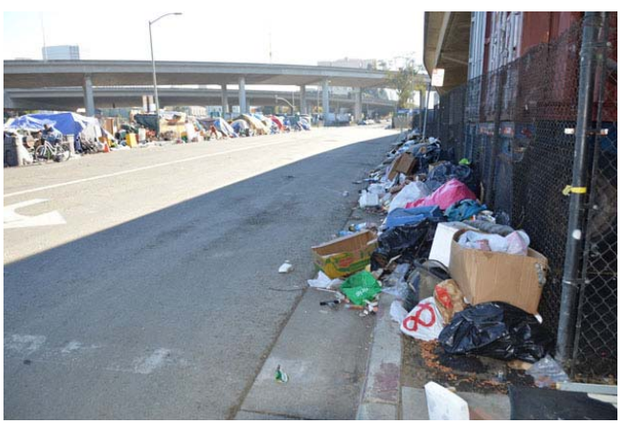

Figure 1.—Conditions surrounding the future site of Castro Street Community Cabins Navigation Center

Figure 2.—Staff trapping Norway Rats near future site of the Castro Street Community Cabins Navigation Center

hand washing station, and garbage containers for refuse. This accomplished, smooth relocation occurred.

The next undertaking involved an emergency intervention designed to provide a temporary bridge from 'sidewalk to services and housing', with an organized navigation center prototype constructed on leased property at 6th and Castro Streets in Oakland, christened, the Castro Street Community Cabins Navigation Center (CSCCNC). The surrounding five-block area was heavily infested with Norway rats, due to the ongoing dumping of food items, and many of the rats were infested with oriental rat fleas, *Xenopsylla cheopis*. The District expended considerable effort controlling the Norway rats by trapping with wire cage live traps and burrow baiting with Ditrac™ tracking powder and Contrac™ rodenticide pellets, as well as dusting the burrows with Delta Dust™ to control ectoparasites (Fig. 2).. The city erected a navigation center comprised of 20 residential Tuff Sheds™ as double occupancy living quarters. They created a secure compound with a site manager 24/7, portable toilets, hand washing stations, garbage dumpsters with weekly service, mobile bathing units, pest control, and intensive social worker interaction geared towards finding the residents permanent housing, employment, and medical, as well as behavioral health care. The maximum duration of the residents stay at the navigation center was limited to 6 months. The city eliminated camping on the surrounding streets and began the process of getting the unsheltered off the streets and helping them to resurrect their lives that had been hindered by their lack of shelter.

RESULTS ABD DISCUSSION

This intervention process has shown success and has now been duplicated, with enhancements in two other high-density homeless encampment areas; the Northgate Community Cabins (opened May 7, 2018), and the Lake Merritt Community Cabins (LMCC) which opened on October 8, 2018 (Berton and Boyd 2018) . The high population of Norway rats at LMCC created an initial problem during meal times, when the rats seemed to think they were also being served. The ACVCSD helped with control using Fastrac™ rodenticide pellets and are working with Oakland's pest control company to help protect the LMCC from vermin during the period of operation. Public scrutiny was focused on this site due to its prominent location, next to Lake Merritt and Laney College, with the ambitious intention of resettling all the unsheltered residing at the Lake Merritt Park in the following months. The main goal is rapid housing placement to allow the progressive clean up through the park with the ending of all camping. The closure of Lake Merritt Park to camping is now in force.

Figure 3.—Castro Street Community Cabins Navigation Center after area cleanup and abatement of encampment

The city is continuing this approach, with the opening of the Miller Avenue Navigation Center on January 21, 2019. Three more navigation centers as well as four managed RV/safe parking locations are slated to be completed in 2019. The city also purchased a renovated residential hotel, the Holland House, that can house 99 individuals. These are all part of an overarching plan that has the potential to help many of the unsheltered in Oakland, as well as addressing the sanitation and vermin problems related to homeless encampments.

The successful Castro Street Navigation Center (Fig. 3) closed January 2019, having accomplished its purpose with an overall rehousing success rate of 68%. The surrounding streets (closure areas) are back to normal, with sanitation and rat issues resolved. There are some unsheltered that refuse services, and when an encampment closure occurs, they move away to another area, rather than be confined to housing with rules, and help to create new rat infestations with their unregulated food refuse in their new location. This is an unfortunate aspect of this social problem, though as time passes those who refuse help will become more isolated. The initial drive is to help those who accept help.

ACKNOWLEDGEMENTS

All our staff at Alameda County Vector Control have been involved with our response to the unprecedented conditions at the homeless encampments throughout Oakland and deserve a sincere acknowledgment for all the hours invested helping to resolve the vermin and sanitation problems at the encampments. The City of Oakland employees, and officials deserve recognition for developing a successful plan that addresses the homeless encampments poor sanitation conditions, and a humane approach to breaking the cycle of irregular cleanup of encampments with their managed approach.

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Large media for less: Leveraging relationships to increase public awareness on a limited budget

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Abstract

Establishing relationships with key city officials and personnel has provided opportunities for newer and more innovative public awareness methods for the Orange County Mosquito and Vector Control District (OCMVCD). This analysis compares past outreach and partnerships to the most recent 2018 campaign. The 2018 "Make Your Home Mosquito-Free" campaign was a multi-pronged media approach that empowered residents to engage through a variety of different outlets and provided cities and organizations with ready-to-use resources to spread the message of mosquito control to their residents. By leveraging relationships formed in previous years, OCMVCD's 2018 media campaign resulted in a more effective, high-quality public outreach program with over $100,000 in perceived savings to the District, a wider opportunity for engagement, and increased public awareness.

INTRODUCTION

The Orange County Mosquito and Vector Control District (OCMVCD/District) is a public health agency formed in 1947 with the mission to protect the residents of Orange County from vectors and vector-borne illnesses. OCMVCD's service area consists of more than 800 square miles, encompassing all 34 Orange County cities and unincorporated areas of the county.

In an effort to fully engage all municipal partners, the OCMVCD significantly expanded its public outreach, public engagement, and advertising program in 2018.

INCREASING OUTREACH

Outreach and education are an integral part of the District's mission and goals. In 2018, the OCMVCD launched a large summer media campaign entitled "Make Your Home Mosquito-Free" (Figure 1). In developing the campaign, OCMVCD staff set the following goals:

- Engage cities to share the message with their constituents
- Increase the awareness of mosquitoes and breeding sources around the home
- Showcase both *Culex* and *Aedes* breeding sites
- Encourage the partnership with cities and residents, so the task was not solely the responsibility of the District – this is a community effort and the residents must do their share

Though the campaign was released in June 2018, preliminary work was started in December 2017 with goals to accomplish leading up to the launch date. These included developing the campaign, securing vendors for media buys, confirming city partners, conducting a photo and video shoot, finalizing the campaign, and finally the launch in time for National Mosquito Control Awareness Week in June.

OCMVCD and its stakeholders wanted to increase awareness through a multi-media campaign, however, due to budget constraints, the District had less than $40,000 allocated towards the campaign. Due to previous relationships established in past programs and partnerships, OCMVCD staff was able to work collaboratively with cities to leverage their media streams and public outreach outlets. To increase participation, District staff contacted cities at least three months prior to launching the campaign to develop partnerships on billboards and other media placement. The District pre-launched all campaign materials to city contacts two weeks prior to the official campaign launch date, providing adequate time for cities to implement OCMVCD's media message into their outreach calendars. All items were provided to city contacts via Dropbox.

The most extensive and significant media placement for the 2018 media campaign included fixed-route transit stops in multiple languages. In 2017, the District collaborated on 16 fixed-route transit stops with cities; in 2018 that more than doubled to 35 placements. This expanded outreach to an additional 3 cities, and through collaboration, the District only paid production cost ranging from $0 - $300 per unit. (See Figure 2)

Additionally, the District purchased more than 30 exterior bus tails from the Orange County Transportation Agency (OCTA). These placements were selected due to the nature and size of the buses involved, as well as visibility in routes through high-risk areas for West Nile virus transmission.

Figure 1.—Image used for the 2018 large summer media campaign "Make Your Home Mosquito-Free." This was on bus shelters, billboards, exterior bus tails, magnets, and all over social media during the campaign run from June 2018 – September 2018.

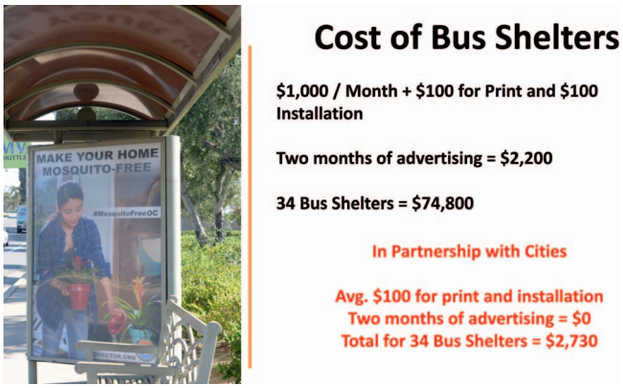

Figure 2.—The average cost of a bus shelter (fixed-route transit stop) is $2,200 per month. With city partnership, the District only paid production cost ranging from $0 - $300 per unit.

Large billboards owned by cities were also used at no cost, increasing from three billboards in 2017 to eleven billboards in 2018.

Resident engagement was another critical component of the "Make Your Home Mosquito-Free" campaign. With an aggressive social media campaign consisting of Twitter, Facebook, Nextdoor and Instagram, the District was able to encourage residents to become more familiar with the services offered by the OCMVCD as well as help them take the necessary actions to prevent mosquito-borne illnesses. The expanded social media coverage resulted in increased engagement and, on average, Nextdoor posts received over 115,000 impressions per post. Platforms such as Facebook also saw an increase in participation and positive engagement from residents. Active engagement was obtained by engaging users through trivia questions, polling, and posting of multilingual material. Through actively engaging social media users, the District had an increase in followers, and a 150% increase in engagement on social media platforms such as Facebook when compared to the previous year.

Actively engaging stakeholders provided cities not only the opportunities to assist with traditional campaign mediums, but also the ability to suggest non-traditional ways to increase public awareness of mosquitoes. When presented with the campaign materials, many cities positively responded to the opportunity and presented additional ideas to the District. Some of these additional media included: customized web pages for vector control information on websites, featured content and campaign material in city newsletters, and placement of magnetic signage on city vehicles. In addition, highlighting the Public Service Announcements at Movie-in-the-Park nights, on local cable access TV, community events, and spotlighting campaign messaging at City Hall and other city sites. In total, 19 of Orange County's 35 cities participated in one, or more, non-traditional outreach methods to disseminate the District's "Make Your Home Mosquito-free" message to their residents.

The total cost of the 2018 large summer media campaign, excluding staff salary cost, was approximately $27,000. With city partnerships and program discounts, the perceived total cost of this campaign was approximately $152,000. This amounts to an overall savings of $125,000. (See Figure 3)

CHALLENGES AND LIMITATIONS

Although there have been many partnership opportunities with some cities, there also have been some challenges. Smaller cities do not have the media platforms to accommodate partnership requests. These cities do not have a dedicated communications staff and do not have a social media presence to share or publish OCMVCD's messages. Other cities have protocols to not share or retweet content from other agencies or organizations.

Limitations were also set for the number of free or discounted media platforms such as the fixed-route transit stops or billboards. Although the campaign officially launched in the month of June, some of the transit stops or billboards were not posted until the month of July based on availability and some signage remained in place for months after the campaign ended.

CONCLUSION

The 2018 campaign was designed and launched by the OCMVCD, yet its overall success came from a multi-pronged media approach that empowered residents to engage on a variety of different levels, leveraged relationships with city officials, and ready-to-use resources provided to implement the "Make Your Home Mosquito-free" campaign. The campaign provided multiple opportunities for cities within the District's jurisdiction to promote education and awareness, utilize city resources to spread the message to residents, and use of new non-traditional media to increase awareness.

The relationships formed in previous years led to a very positive and collaborative relationship between the District and cities, and will lead to future opportunities to spread the message of vector control.

Campaign Summary: *Quantifiable*

Medium	2018	Ad Cost	Perceived Total Cost	Actual Cost
Bus Shelters	35	$2,200	$74,800	**$2,730**
Billboards/Readerboards	11	$2,500	$27,000	**$6,000**
Bus Tailgates	34	$125	$4,250	**$3,000**
City Work Vehicles	58	$125	$7,250	**$754**
OCMVCD Work Vehicles	70	$125	$8,750	**$910**
Broadcast Advertisements	2	$10,250	N/A	**$10,250**
Social Media Posts		$0	N/A	**$0**
Photography + actors	1	$3,000	N/A	**$3,000**
Videography	1	$1,000	N/A	**$1,000**
PR Firm	0	$0	$30,000	**$0**
		TOTAL	**$152,050**	**$27,020**

Figure 3.—With city partnerships and program discounts, the perceived total cost of the 2019 large summer campaign amounted to a grand total savings of $125,000.

ACKOWLEDGEMENTS

The author would like to thank Rick Howard, Lora Young, and active members of the OCMVCD Board of Trustees for their support of this project. Members of the OCMVCD Communications Team are also essential, for providing professional and thoughtful assistance year-round. I also gratefully acknowledge our city contacts (Trustee Members, Public Information Officers, Deputy City Managers, and Public Works Staff) who have graciously shared their programs and resources to help obtain discounted pricing for OCMVCD. A big thanks to the following cities for their assistance and participation in the campaign: Anaheim, Brea, Costa Mesa, Fountain Valley, Fullerton, Garden Grove, Huntington Beach, Irvine, La Habra, La Palma, Laguna Woods, Mission Viejo, Newport Beach, Placentia, Rancho Santa Margarita, Tustin, Villa Park, Westminster, and Yorba Linda.

Targeted and real time community outreach in response to elevated West Nile virus activity

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Abstract

A key element in the Sacramento-Yolo Mosquito and Vector Control District's (District) Public Information and Communication's program is education and outreach at local community events. During the year we attended approximately 35 large events throughout both counties to educate and provide information about the District's services and programs. This year, as a result of the intense and escalated West Nile virus (WNV) activity being detected in specific communities, we took the approach of responding in real time and by attending events in the neighborhoods where virus activity was at elevated levels. This allowed us to provide information and repellent directly to residents living in the communities that were being targeted with both ground and aerial mosquito control treatments.

Targeting communities in real time required new strategies in order to effectively get messaging out to the public. These strategies included coordination with local elected officials to disseminate information at sponsored events, coordination of media stories directly in affected communities, setting up information booths at small community events to directly reach residents in affected areas, targeted social media ads and collaboration with city agencies to provide a uniform message to residents. By targeting smaller community events in this manner the District has been able to more effectively get information and resources to the residents that are at higher risk of contracting WNV.

Effectiveness of a community clean-up event for the control of *Aedes aegypti*

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INTRODUCTION

Control efforts for *Aedes aegypti* have been more time-consuming than work done controlling *Culex* mosquitoes (Metzger et al. 2017). *Ae. aegypti* rarely fly far from the larval source, and control of larvae has been conducted through inspections and treatments of yards. Within those yards, larvae can be found in a variety of containers and items that can hold water, meaning that careful systematic inspections are needed to find the source. To improve our inspections and to reduce the number of potential habitats within yards, we partnered with the Southern Coachella Valley Community Services District to conduct a Fight the Bite! Block Party and Community Clean-up Event on one of the days that their waste management site (hereafter, TOM site) would be open. In choosing this day, we planned to assist residents with removing unwanted items from their yards, providing fewer potential mosquito sources and reducing the population of mosquitoes.

Conducting physical control can take more time and labor than chemical control. We completed inspections before and after the clean-up event to measure changes in the number of potential and active breeding sources where mosquitoes were found.

MATERIALS AND METHODS

Three weeks prior to the clean-up event, 1,000 postcards were sent by mail to inform the Mecca community about this event. The postcards encouraged residents to participate in the clean-up by dropping off small items at the block party site and large items at the TOM site or by scheduling a curbside pick-up (free of charge). Pre-event surveys and inspections were completed between 24 Apr and 11 May 2018. During pre-event inspections, postcards were distributed in-person by vector control technicians to residents who reported that they did not receive a postcard via mail. Vector control technicians also scheduled curbside pick-ups for residents.

On 12 May 2018 the Fight the Bite Block Party and Community Clean-up was held at the Mecca Family and Farmworker's Service Center from 0800 to 1200 h. The Fight the Bite Block Party offered interactive games, educational displays, prizes, face painting and a free Italian ice to residents that participated in the event. District employees were at the TOM site to assist residents with the removal of items from their vehicles. District employees also picked up items from the above referenced residences, noting what kinds of items were removed.

Follow-up inspections and surveys were completed from 2 Jul to 24 July 2018. The residences selected for post-event inspections and surveys were based on participation in the event, more than 10 potential breeding sources seen at the initial inspection, or mosquitoes found at the initial inspection.

Throughout the season, adult mosquitoes were collected from 6 locations using BG-Sentinel 2 traps baited with dry ice and the lure. Mosquitoes were identified to species, sex, physiological state (females), and enumerated.

RESULTS

Of the 187 initial residential inspections, 7 were removed from potential re-inspection, because the addresses listed on the form did not match what was recorded in the District's electronic system. Of these 180 inspections, mosquitoes were found in 22 sources at 16 residences. The number of sources with larvae ranged from 0 at 164 residences to 3 at 2 residences. There were 1,487 potential breeding sources, ranging from 0 to 43 per residence.

A total of 104 residents participated in the Fight the Bite Block Party and Community Clean Up event. Thirty residents participated in a scheduled curbside pickup and 34 residents dropped items off at the TOM site (Figure 1). We had approximately 100 people attend the Block Party, but it was not clear how many also participated in the pickup or drop-off.

After the event, inspections were conducted at 43 of the 180 original residences. Mosquito breeding was found at 13 residences before the event and 8 after the event. Two of the latter positive residences had mosquito breeding during the pre-event survey, while 6 residences were new mosquito breeding locations. A total of 562 items were detected as potential breeding sources before the event. After the event, 747 potential breeding sources were found, ranging from as few as 2 sources and up to 40 items per residence.

The number of adult *Ae. aegypti* collected per trap decreased slightly following the event, but then increased again shortly after, with a large increase in adults observed in July (Figure 2).

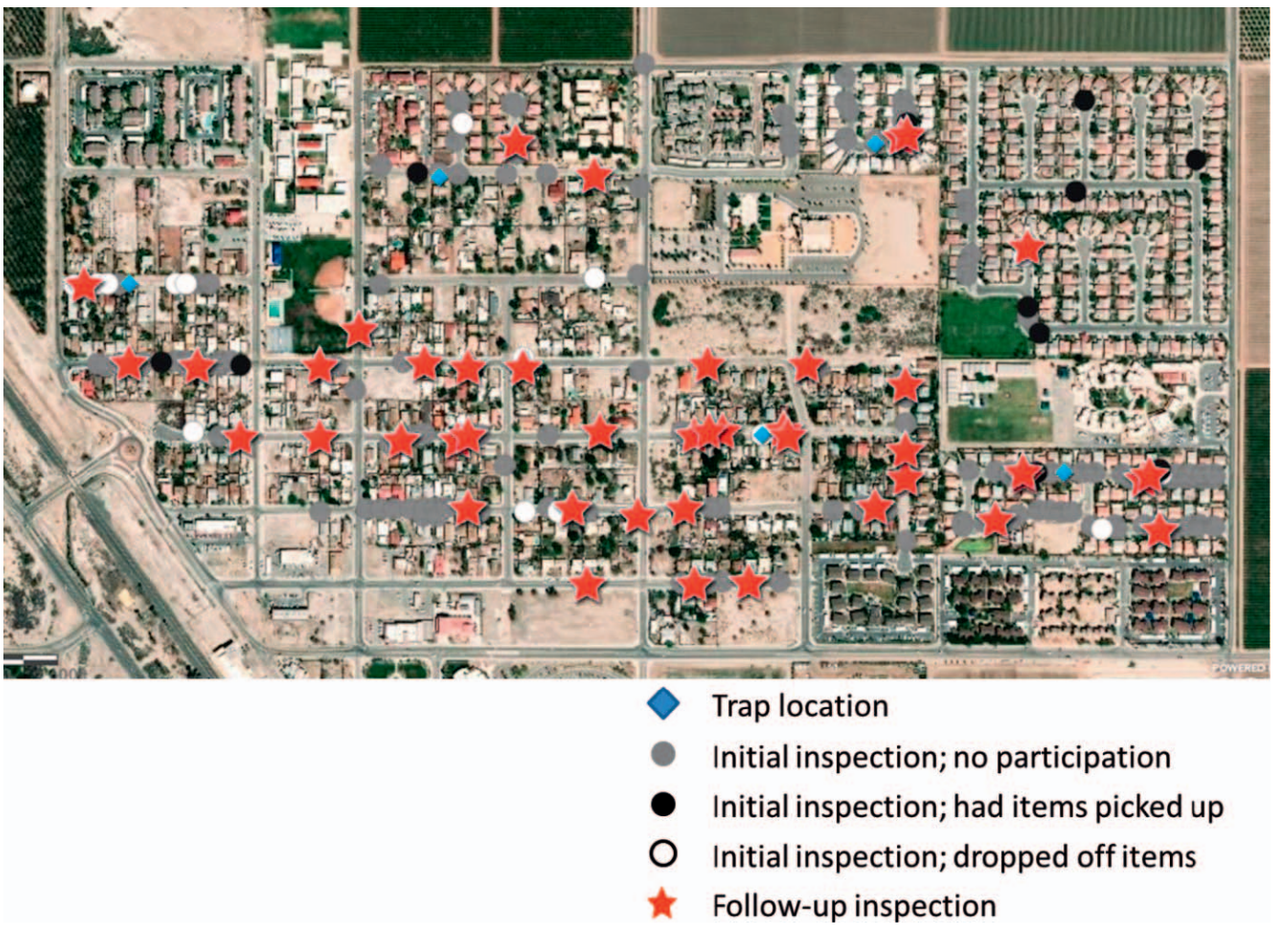

Figure 1.—Map of Mecca. Locations of traps are indicated by blue diamonds. Residences where initial inspections were conducted but no further participation occurred are shown with gray circles; residences where initial inspections and items were picked up were shown in black circles and follow-up inspections were shown by red stars.

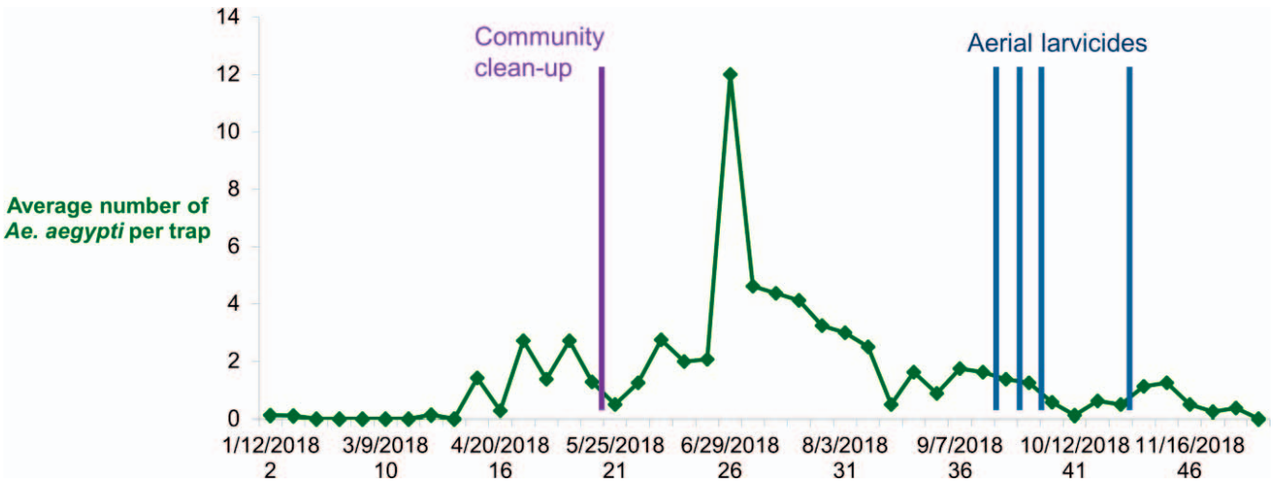

Figure 2.—Adult *Ae. aegypti* collected in 2018 in Mecca. Average number of adults collected per trap plotted as a function of date. Date of the Block Party and Community Clean-up indicated. In the fall, aerial larvicide applications were made on the dates indicated.

DISCUSSION

Conducting campaigns to educate communities about mosquito habitats is important to reduce mosquitoes and potential mosquito breeding sources. During follow-up inspections, we learned that some residents received different messages about the goals of this event. One resident reported that her spouse removed all potted plants from their yard, because he believed all potted plants presented a mosquito breeding hazard in their residence. Another resident reported that he believed that the clean-up was a monthly event conducted by the District, so he placed unwanted items in his yard to prepare for the next event, increasing the number of potential mosquito breeding sources from 10 to 16. For future events, we should consider surveying residents during pre-inspections about their knowledge about mosquito breeding habitats and about the goals of the event to avoid miscommunication.

Post-event inspections of 43 residences indicated that the number of potential breeding sources increased; however, the number of residences with positive mosquito breeding sources decreased at residences where active breeding was found before the event. Only two residences out of the 43 re-inspected residences had reoccurring mosquito breeding, and these residences did not participate in the clean-up event. The residences that were identified as new mosquito breeding locations also did not participate in the event. Presumably residents at positive mosquito breeding locations were unaware of mosquito breeding habitats, because they did not participate in the event. For future events, we may want to focus on targeting residences with reoccurring mosquito breeding and a high number of potential breeding sources to be able to record the impact of a clean-up event.

The date selected for clean-up was a holiday weekend and therefore not ideal for all residents. To increase the number of residences that participate in clean-up activities, we should target non-holiday weekends.

Our overall adult mosquito population did dip slightly during the time that we were actively working in the community of Mecca. However, it rebounded strongly at the end of June. The numbers collected were sufficient to initiate aerial larvicide applications (Figure 2). Although our traps were spread throughout Mecca (Figure 1), those residents who participated in the event were not evenly distributed. Focusing our efforts on residents with known mosquito breeding and large numbers of potential sources may lead to better outcomes in the future. Working to ensure a higher percentage of residences actively participate is also necessary to reduce the population for a longer period of time.

ACKNOWLEDGEMENTS

We appreciate the cooperation of the residents of Mecca in ridding the community of these invasive mosquitoes. The District worked with AmeriCorps and Burrtec Waste Industries to host the Block Party and to conduct the clean-up. EAC, Paul Bustamante, Geneva Ginn, Jonathan Herrera, and Diana Reyes worked at the Block Party. Salvador Becerra, Fernando Gutierrez, Trinidad Haro, Linda Petersen, Charles Rodriguez, Rosendo Ruiz, Vincent Valenzuela, and Jonathan Zamaniego conducted the pick-ups from residents. MM, Greg Alvarado, Oldembour Avalos, Jess Lucia, Jeff Rushing, Victor Scrima, and Miguel Vargas worked at the TOM site with Southern Coachella Valley Community Services District to assist residents dropping off materials. Antonio Molina printed signs for the events. Melissa Snelling provided mosquitoes for displays at the Block Party. Diana Reyes and Abelina Torres scheduled curbside pick-ups and re-inspections of the residences. Jonathan Herrera and Gonzalo Valadez trained HVA to conduct follow-up inspections. Gerald Chuzel, Barbara Gerardo, Abelina Torres, and Diana Reyes assisted with the follow-up inspections and surveys.

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Constructed wetlands still produce mosquitoes...

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Abstract

There is renewed interest to incorporate constructed wetland technology as part of watershed water management strategies for supplemental water storage, flood control and water quality improvement. Increased variation in precipitation presumably associated with global climate change has contributed to recent multi-year droughts as well as unprecedented rainfall events. The addition of hardscape associated with human development has exacerbated flooding in urban and suburban areas. Contamination of irrigation waters and crops with pathogens associated with fecal matter has caused outbreaks of foodborne illness. Whereas constructed wetlands can lengthen conveyance times of overland water flow to enhance storage in lentic and palustrine habitats as well as facilitate infiltration of groundwater reservoirs, create multi-use habitat capable of absorbing decennial or centennial floods, and provide a cost-effective technology for improving water quality, recent publications promoting the use of constructed wetlands have failed to account for the public health consequences associated with the production of nuisance and vector mosquitoes by man-made wetlands. The importance of design and maintenance protocols for constructed wetlands to lessen the production of mosquitoes that reduce the quality of life and pose tangible threats for the transmission of mosquito-borne pathogens are reviewed.

INTRODUCTION

A desire to reduce the economic losses and loss of human life caused by flooding associated with recent storms and hurricanes has created renewed interest in constructed wetlands as a component of "un-engineering" strategies to provide ecological security for urban areas worldwide (Gies 2018). Rapid urbanization during the last several decades has converted natural areas capable of absorbing stormwater into urban hardscapes and gray infrastructure that often exacerbates the flooding caused by ever stronger storms. Recent disasters in Houston, New Orleans and Beijing are grim reminders of the consequences of the oxymoron that is "urban planning". Intensive urbanization also has contributed to water shortages as areas providing recharge to subterranean aquifers have been reduced. For example, in China, unbridled use of groundwater supplies has lowered water tables and caused subsidence, while 62% of cities flooded between 2011 and 2014 resulting in $100 billion in economic losses (Gies 2018).

Retrofitting cities for greater ecological security operates on a wide range of scale from localized projects to city-wide and watershed-level projects intended to reduce flooding and promote water storage and infiltration. Known as low-impact development in the U.S., green infrastructure in Europe and Australia, or sponge cities in China, approaches include restoration and preservation of floodplains, restoration of lotic habitats, and the creation of landscape features to provide retention and treatment of rainwater runoff such as bioswales, retention ponds, sunken parks and permeable parking lots (Gies 2018). To allow natural hydrologic and ecologic processes to fulfill a greater role in water management strategies, standing water that is conducive to mosquito production can occur as part of some technologies.

Renewed interest in constructed wetlands is not limited to applications in cities. Constructed and restored wetlands are proposed to filter and improve water quality of agricultural runoff that contains a wide range of contaminants (O'Geen et al. 2010, O'Geen and Bianchi 2015). Recent outbreaks of foodborne illness caused by contamination of lettuce and spinach grown in California with pathogens found in fecal matter has raised concerns about inadequately treated agricultural wastewater and contamination of irrigation water and crops by wildlife (Beretti and Stuart 2008, Dowd et al. 2008). Whereas construction and restoration of many wetlands in California was supported by the USDA-NRCS through the Environmental Quality Incentives Program (EQIP) and the Wetland Reserve Program (WRP) to mitigate the loss of wetlands and improve wildlife habitat, fulfilling the dual goals of agricultural water quality improvement and wildlife habitat enhancement has proven to be difficult (O'Geen and Bianchi 2015).

Besides water quality improvement and crucial wetland habitat, the projected benefits of multipurpose constructed treatment wetlands are numerous, including amenities for nearby housing developments, wildlife conservation, education and recreation (Cole 1998). The inability of man-made wetlands to fulfill multiple diverse goals is not uncommon (USEPA 2000, NRC 2001). Even if the focus of engineered wetland systems is limited to one or two key objectives (Carey 2013), the outcome of ecological

Figure 1.—The brush cutter (left panel) used reduce emergent vegetation surrounding experimental exclosures and the wetland showing inundated cut vegetation (right panel). This practice should be discouraged because it greatly enhances mosquito production.

processes such as succession is by no means certain and repeatable even in adjacent replicate wetlands. Meeting the conservation needs of diverse fauna and flora can be problematic. For example, man-made riverine wetlands can serve the needs of riparian and wetland birds (Zembel et al. 2003) and mammals, but are habitat sinks for native fishes and amphibians because of predation and competition from non-native fauna that proliferates in lentic conditions (Why et al. 2014).

The theme of this symposium "Don't Reinvent the Wheel: Past Vector Control Research and Practices Relevant Today" is an appropriate venue for my topic. It is perplexing that despite a wealth of peer-reviewed literature on the topic of mosquito production from man-made wetlands (e.g., Russell 1999; Walton 2002, 2003, 2012; Knight et al. 2003; Metzger 2004; Lawler and Lanzaro 2005; Rey et al. 2012), many of the aforementioned recent publications promoting the use of constructed wetlands fail to mention the potential role of man-made wetlands and agricultural operations for producing mosquito populations that affect public health. Here, I revisit the evidence supporting the importance of design and maintenance protocols for constructed treatment wetlands to lessen the production of mosquitoes that reduce the quality of life and pose tangible threats for the transmission of mosquito-borne pathogens.

METHODS

The Prado Wetlands consist of 47 interconnected marshes/ponds (surface area = 186 ha) located 7 km northwest of Corona, California (33.9°N, 117.9°W) and are managed by the Orange County Water District (OCWD). The area of wetlands complex encompassed by shallow wetlands conducive to mosquito production is approximately 142 ha. The wetland complex receives approximately one-half of the flow (1.7 - 2.3 $m^{3\cdot} s^{-1}$) of the Santa Ana River. Data from basin-wide mosquito surveys collected using 350-mL dippers and 0.25 m^2 screen emergence traps by Keiper et al. (1999, 2003) were used to estimate the basin-wide production of female mosquitoes between July and September. Mosquito emergence rates were corrected for the proportion (~0.55) of the cohort collected by the emergence trap design during the trapping period (Walton 2009).

Unpublished data from immature mosquito surveys using 350-mL dippers in a 1-ha wetland (see Fig. 1 in Why et al. 2014) that had undergone vegetation management were used to estimate mosquito production. Mosquito production following cutting of emergent vegetation with a rotating brush cutter and leaving inundated cuttings (Figure 1) was estimated during a 6-week period. Treatment of the wetland with bacterial larvicides was required (Why et al. 2014); however, the decaying vegetation in the wetland attracted egg-laying mosquitoes for about 6 weeks.

Because this paper is primarily a review of previously published material, the relevant methods for the research cited herein can be found in the accompanying references.

RESULTS AND DISCUSSION

Multipurpose constructed treatment wetlands provide important ecological services and benefits; however, production of pestiferous and pathogen-transmitting mosquitoes is a potential drawback. Mosquito production typically increases as water quality declines and coverage by inundated vegetation increases (Walton 2012). Problems related to mosquito production can be acute in the arid southwestern U.S. where rapid human development, a susceptible populace unaccustomed to the presence of mosquitoes, endemic activity of arboviruses, and the presence of competent mosquito vectors of the causative agents of human diseases combine to create public health concerns (Walton 2002).

The design and management of a constructed wetland can influence mosquito production. Ideally, one should

incorporate as many design features that reduce mosquito production as is feasible to still achieve the goal(s) of the wetland system. "Design" mosquitoes out of the wetland as much as possible. Design features that have the potential for source reduction include basin design and topographic configurations, hydrological control, vegetation management and planting design for emergent vegetation and structures that limit allochthonous subsidies (Walton 2003, 2012). Fluctuating water levels can draw immature mosquitoes out of emergent vegetation into open water where mortality is higher or strand mosquito larvae on the substrate undergoing drying (Walton et al. 2016), but flood-pulse wetlands perform comparatively poorly for improving water quality in some agricultural applications (O'Geen and Bianchi 2015). The number of wetland cells, redundancy of flow paths, configuration of the basin sides, slope of the bottom, hydroperiod, and features that hold isolated pools of water affect mosquito production (Knight et al. 2003).

What you put into the wetland matters. The abundance of mosquitoes is influenced by loading rates of organic matter and nutrients (Walton 2012). Under high loading of organic matter and nutrients ($>$ 50 mg TN liter^{-1}) such as that found in barnwash and municipal effluent that has undergone only primary treatment (i.e., removal of solids by screens), mosquito production is nearly continuous, reflecting the seasonal activity of adult mosquitoes. Immature mosquito abundance often exceeds 50 to 100 larvae per dipper sample when constructed wetlands or dairy wastewater ponds receive high levels of nitrogen in municipal or agricultural wastewater; counts may routinely exceed 500 larvae per dipper sample (O'Meara et al. 2010).

Mosquito production also may be continuous during the annual period of host-seeking activity at constructed wetlands receiving loading rates of organic matter and nutrients (e.g., secondary-treated municipal effluent: 10-30 mg TN liter^{-1}) that are comparatively lower than for dairy wastewater and sewage lagoons. Immature mosquito abundance during the summer might range between 20 and 40 larvae per dipper sample (Walton 2012).

Under low nutrient levels ($<$ 10 mg TN liter^{-1}) such as those found in tertiary-treated municipal effluent or flood irrigated rice fields, immature mosquito abundance in constructed wetlands is typically $<$ 1 larva per dipper sample (Keipter et al. 1999, 2003; Walton 2012). Predators are often capable of exerting significant mortality on immature mosquito populations in low nutrient conditions (Walton 2012). It is not uncommon to observe vernal and autumnal peaks in mosquito populations, albeit at comparatively low levels of adult production. Despite low levels of adult mosquito production per unit area, large expanses of inundated palustrine or riverine wetlands are capable of producing significant numbers of mosquitoes.

What you put around the wetland matters. The area circumscribed by a constructed wetland does not restrict the area over which adult female mosquitoes are searching for hosts. Mosquitoes are searching for blood meals both at the wetland and in the surrounding landscape. The birds living in and around constructed wetlands, and associated with surrounding regions of human habitation, may be reservoirs for arboviral pathogens transmitted by vector mosquitoes (Knight et al. 2003). Depending on the mosquito species and local environmental conditions, host seeking mosquitoes move from $<$ 1 km to tens of kilometers from wetland developmental sites.

The emergent vegetation that is planted in the wetland influences mosquito production. In general, vegetation density is directly correlated to mosquito production. Dense stands of large (height 1.5 - 3 m) emergent macrophytes planted in constructed wetlands can (1) reduce wetland performance for improving water quality during natural senescence and by channeling or short-circuiting water flow, (2) increase mosquito production by providing harborage and reducing mortality of immature mosquitoes, (3) reduce effectiveness of abatement measures by blocking control agents from contacting the target stage of the mosquito life cycle, and (4) increase the costs of wetland management (Knight et al. 2003; Walton 2003, 2012). Cutting and removal of cut plant biomass typically is expensive ($>$ \$10,000 ha^{-1}). Mosquito abatement at constructed wetlands receiving secondary-treated municipal effluent that contain dense stands of emergent vegetation is expensive (cost of mosquitocides and application in 2016 dollars: \$5,000 to \$10,000 ha^{-1} yr^{-1}) and is often ineffective. Weekly, even semi-weekly, application of larvicides and adulticides can be required (Walton 2002).

The distribution of emergent vegetation in the constructed wetland matters. Incorporating open water zones with water depths that inhibit colonization and growth of emergent vegetation will reduce mosquito production by increasing immature mosquito mortality (Thullen et al. 2002). The shallow open water present after wetland construction eventually will be colonized by emergent vegetation and mosquito production is likely to increase concomitantly (Walton et al. 2007). The distribution of emergent vegetation can influence water quality treatment (O'Geen and Bianchi 2015).

How you manage the wetland matters. Based on basin-wide surveys from May until October, the mean areal production of mosquitoes (males and females) from emergent vegetation at the Prado Wetlands ranged from 0.070 mosquitoes m^{-2} d^{-1} (Keiper et al. 1999) to 0.093 mosquitoes m^{-2} d^{-1} (Keiper et al. 2003). The total estimated production of female mosquitoes (corrected for trap efficiency) from 142 ha of palustrine wetlands ranged between 7,600,000 and 10,120,000 female (*Culex* spp. and *Anopheles hermsi*) mosquitoes between July and September. Whereas this production is low per unit area and water quality is high (tertiary-treated municipal effluent), the large area of the shallow marshes creates significant levels of adult mosquito production.

However, poor management practices can increase mosquito production $>$ 100-fold in a very small area of the wetland. Following vegetation management in a 1-ha wetland, mosquito abundance in the decaying vegetation

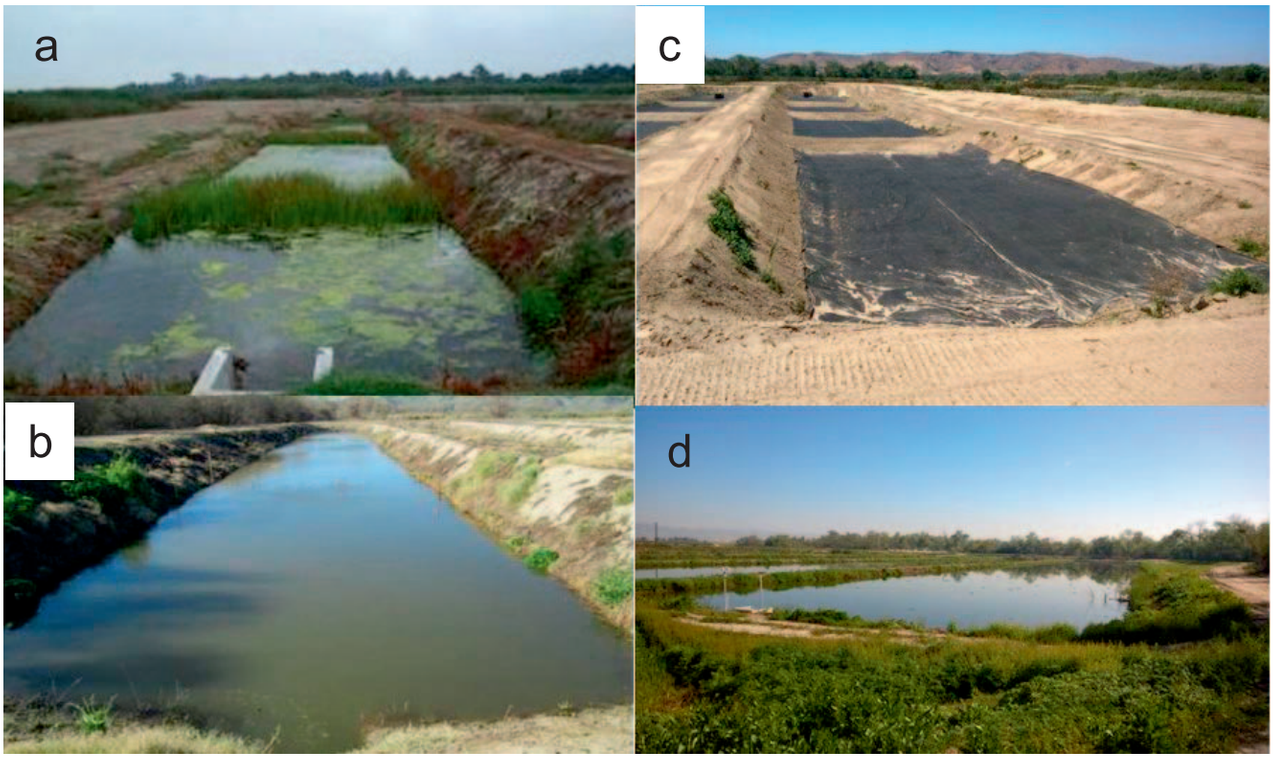

Figure 2.—A wetland with emergent vegetation (*Schoenoplectus maritimus*) restricted to narrow zones in October (a). The same wetland the following spring (March) after the emergent vegetation above the substrate died back naturally during late autumn and before regrowth (b). A wetland with raised planting beds and shade cloth to limit the distribution of emergent vegetation (c). A wetland that maintains open water by utilizing periphyton communities to improve water quality (d).

was enhanced significantly. Based on dipper samples, the emergence rate of mosquitoes was estimated at 50,128 mosquitoes m^{-2} wk^{-1}. Total mosquito production was 500,000,000 mosquitoes wk^{-1} from the 1 ha wetland. During the 6-week period following vegetation management (without mosquito abatement using bacterial larvicides), the production of female *Culex* would have been approximately 1.5 billion female mosquitoes.

Design features and operational procedures that help to limit mosquito production include the following approaches (Knight et al. 2003; Walton 2003, 2012). Create open water zones by restricting emergent vegetation to islands or raised planting beds (Figure 2). Incorporate water quality treatment processes that reduce organic matter and nutrient loading before placing water into wetlands containing emergent vegetation such as open-water settling ponds or forebays which promote settling of particulates and volatilization of nitrogen. Maintain the lowest coverage of emergent vegetation necessary to achieve the performance goals for the wetland. Recent approaches for treatment wetlands are moving away from emergent vegetation to periphyton-based systems for water quality improvement (Figure 2). Incorporate design features and management practices that enhance populations of mosquito predators, including the addition of larvivorous fishes. Compartmentalize the wetland so that source reduction for mosquitoes can be carried out in a subset of wetland cells without requiring that the entire wetland be taken offline and mosquito control agents can be applied effectively to emergent vegetation when needed. Build dikes that can accommodate mosquito control vehicles. Maintain an excellent working relationship among agencies.

CONCLUSIONS

The addition of wetlands and other landscape features to urban areas has great promise to increase ecological security by reducing the impact of flooding from ever intensified storms associated with global climate change and from the hardscape architecture that dominates cities. Green infrastructure also can address the chronic water shortages in urban areas by enhancing infiltration of water into groundwater reservoirs. In rural areas, constructed wetlands can reduce pollution and contamination from agricultural operations. Nevertheless, the failure to address the potential production of mosquitoes in standing water on the landscape while promoting green solutions to current problems does a disservice to communities by potentially decreasing quality of life from mosquito bites and increasing the prevalence of vector-borne diseases in humans, companion and domesticated animals, and wildlife.

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Don't let your operational evaluations be out of control[1]

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Introduction

Both research programs and operational quality control evaluations fall into three main categories: 1) treatment of caged sentinels of colony mosquitoes of known susceptibility and/or field mosquitoes of unknown susceptibility to estimate resistance as well as operational parameters such as droplet size, dispersal and swath width, 2) measurement of abundance of the target population before and after spray, and 3) measurement of abundance before and after spray in treated and untreated or control areas. The last approach provides the best indication of percent control of the target population, but is based on the important assumptions that 1) abundance at each trap is an independent measure [hard to determine], 2) ratio of abundance among traps is consistent over time, and 3) change in this ratio is due to treatment or spray effects. Mulla's formula provides a useful method for calculating percent control from the data collected in type 3 evaluations, where:

Control = 1 − [(C1/T1)*(T2/C2)] and C is abundance measured in the unsprayed or control area and T is abundance within the sprayed or treated area before (time = 1) and after (time = 2) spray (Mulla et al. 1971).

The present paper describes the application of this formula to adulticide applications to estimate control in experimental ground applications in Coachella Valley (Lothrop et al. 2007) and in emergency aerial applications in Sacramento (Elnaiem et al. 2008) and Davis (Nielsen et al. 2007).

Methods

Using a 'cross-hair' trap deployment scheme in Coachella Valley (Reisen et al. 1984), control was estimated experimentally for three successive applications of Aqua-Reslin, with control traps placed 1 and 2 miles outside the 1 square mile treated spray area. We also estimated control during emergency applications of Pyrethrin with PBO applied to Sacramento on 3 successive nights in 2005 and to Davis in 2006 using data from WNV surveillance.

Results

In Coachella Valley, wind shadows, direction and speed produced marked variation in sentinel mortality over time and space, ranging from 10 to 100%. Relative abundance at central core traps was progressively less well correlated with traps placed a greater distances, ranging from 0.942 for traps in the adjacent mid-zone to 0.495 for control traps at 2 mi distance. However, despite this variation, control after each of the three successive applications ranged from 75 – 85% based on abundance in the core or central traps (Lothrop et al. 2007).

During emergency aerial applications in Sacramento, sentinel mortality ranged from 0 to 100% at cages positioned in habits ranging from under canopy to open areas, respectively. Comparing epidemiological metrics collected from the sprayed and unsprayed areas before and after aerial ULV applications, percent control was estimated to be 76% for host-seeking *Culex* abundance, 93% for the WNV minimum infection rate, 66% for the number of reported dead birds, and 76% for the number of WNV human cases [based on estimated date of onset] (Elnaiem et al. 2008). These results were confounded to some extent by the application occurring late in the transmission season; however, this temporal change was accommodated by using Mulla's formula and indicated WNV transmission activity was interrupted by the application.

Similar results were observed during the emergency spray of Davis in Aug 2006. Again sentinel mortality varied from 0 – 100% for sentinel cages placed under canopy or in the open, respectively. Percent control from abundance measured by dry ice baited traps for *Cx. tarsalis* and by gravid traps for *Cx. pipiens* was 25.6 and 73.1%, respectively (Nielsen et al. 2007). However, the epidemic immediately subsided after spray and few dead birds or positive mosquito pools were observed for the remainder of the summer, indicating that the application interrupted transmission.

Conclusions

Intervention evaluations are difficult because of mosquito population dynamics and multiple intervening factors. Although mortality of sentinels in cages was

[1] Taken from a previous presentation at a 2007 Symposium on Vector Biology, Ecology and Control in Celebration of Professor Mir Mulla's 50 Years at UC Riverside (Reisen 2008). Paper was cancelled and not given at the 2019 conference.

always highly variable, percent control of the target field population was acceptable and typically greater than 65%. Mulla's formula was useful in calculating these estimates, because it accounted for changes in mosquito population abundance and other epidemiololgical parameters in both sprayed and unsprayed areas, pre and post spray.

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The importance of targeting adult *Culex* control in time and space

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Introduction

Weather factors including wind and thermal inversion as well as physical aspects of the landscape including vegetation canopy and buildings effect the distribution of adulticide droplets. Often these factors lessen the impact of adulticide applications. Effective adult *Culex* control requires directed timing and placement of ultra-low volume (ULV) fog that coincides with the behavior of the target species. Knowledge of mosquito flight periods and location within the landscape is critical to optimize suppression of mosquito abundance and interrupt disease transmission.

Methods

Carbon-dioxide baited time segregated traps were used to determine host-seeking flight of *Culex* mosquitoes in rural habitats in the San Joaquin and Coachella valleys of California (Reisen et al. 1997). Broader features of vegetation in the landscape were initially sampled using carbon-dioxide CDC style traps (Lothrop and Reisen 2001) , but these traps largely described only distribution of the host seeking portion of the population. Distribution of the entire population of flying *Culex* subsequently was sampled using 8-inch diameter unbaited suction traps deployed in rural landscapes in the Coachella valley (Lothrop et al. 2002). Unbaited traps allowed a more discrete sampling of the landscape because mosquitoes were not being drawn to the trap along a scent plume, but rather were collected while traversing the landscape along flight paths.

Results

Culex host-seeking flight was generally highest within the first 2 hours after sunset, usually peaking within the first hour. During periods of higher temperature and lower humidity, host-seeking flight was delayed and distributed throughout the night. Flight abundance was greatest at 1 m above the ground along ecotones of elevated vegetation. These ecotones appeared to be corridors of movement in the landscape.

Conclusions

These data of temporal and spatial patterns of abundance can be used to focus the timing and placement of ULV applications. Aerial applications are less focused than ground applications, but still need to coincide with mosquito flight to maximize effectiveness. Regional differences in both temporal and spatial distribution of flying mosquitoes should be calibrated by similar methods.

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Review: Evaluation of ixodid tick control approaches in California

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Abstract

Ixodes pacificus, *Dermacentor occidentalis*, and *Dermacentor variabilis* are the ixodid ticks on the West Coast that most commonly bite people and are the species most often implicated in the transmission of tick-borne pathogens in California. Although these ticks present an ongoing health risk to California residents, there has been little focus on methods of tick control as applied to West Coast tick species and their environments. The current paper presents an overview of findings from California-focused ixodid tick control research, including personal protection, environmentally-focused methods, and acaricide applications. Results of these evaluations were mixed, indicating that while some tick control techniques that were used successfully in some regions can be effective in California others are less effective in local environments.

Introduction

Several of the most significant human diseases caused by zoonotic pathogens in California are transmitted by "hard" ticks of the family ixodidae. *Ixodes pacificus*, *Dermacentor occidentalis* and *Dermacentor variabilis* are the species on the West Coast that most commonly bite people and are most often implicated with the transmission of tick-borne pathogens in California (CDPH 2015). The tick of greatest public health concern is *I. pacificus* which is the West Coast vector of *Borrelia burgdorferi*, the causative agent of Lyme disease. This tick can also transmit *Anaplasma phagocytophilum*, the agent of anaplasmosis, and a number of other pathogens. Ixodid ticks in the genus *Dermacentor* are not considered to be vectors of *B. burgdorferi*, but have been known to transmit Rocky Mountain spotted fever, tularemia, Pacific Coast tick fever, and other pathogens, some of which have only recently been characterized (Padgett et al. 2016, CDPH 2015).

Although many tick-borne diseases such as Lyme disease and Anaplasmosis are more prevalent in other regions of the continental United States than California, the risk of local transmission for tick-borne pathogens is not wholly insignificant or spatially limited to only a few areas. Confirmed cases of Lyme disease have been diagnosed in residents from over 50 California counties over the past decade (CDPH 2018). The species and stages of vectors, hosts, ecosystems and climatic conditions vary significantly in different parts of the U.S. and within California. As a result, risk factors and successful approaches to mitigate tick-borne disease should not be over-generalized.

Although these ticks present an ongoing health risk to California residents, there has been little focus on local methods of control. A number of very good recent reviews of the substantial body of tick control research are available (Eisen and Dolan 2016, TBDWG 2018, White and Gaff 2018). However, few studies targeting California ticks have been included. This current review paper presents an overview of findings from California-focused ixodid tick control research.

Personal Protective Measures: Spray-on Repellents

Two studies conducted in the 1980s demonstrated that permethrin is an effective protective measure against *I. pacificus* and *D. occidentalis* when applied to clothing before entering tick habitat (Lane and Anderson 1984, Lane 1989). In laboratory and field trials, permethrin did not deter either species from climbing onto clothing, but ticks subsequently dropped off sooner (preventing attachment) and died or became morbid after even brief contact with the treated material. Results from these field trials are summarized in Table 1.

Environmentally-Based Tick Control Methods

Published research on tick suppression methods that have focused on manipulation of tick habitat in California fall into three categories. One study evaluated whether mowing of trailside vegetation affected the presence of questing ticks (Nakano 2009). An additional two studies examined the effects of fire on tick abundance and density (Padgett et al. 2009, MacDonald et al. 2018). The experimental removal of lizards from a tick habitat in northern California represents a third type of environmental control method tested (Swei et al. 2011). A limited summary of results from all methods of environmental-based control is presented in Table 2.

Table 1.—Morbidity/Mortality of ticks exposed to permethrin-treated clothing (overalls treated with Permanone spray (0.5% permethrin)) in California experiments. Ticks found on clothing during field sampling were removed immediately upon observation and held for evaluation. Ticks placed on clothing were allowed to stay on clothing up to 15 minutes or until they fell off.

Ticks climbed on clothes in field or placed on clothes	Time between treatment of clothes and tick exposure	Tick species	Stage	Sex	Morbidity or mortality 1h post-exposure	Morbidity or mortality 24h post-exposure	Reference
Field	< 1 day	*I. pacificus*	adults	mixed	15/15	13/15	Lane 1989
Field	< 1 day	*I. pacificus*	adults	mixed	58/58	30/58	Lane 1989
Field	15 days	*I. pacificus*	adults	mixed	0/8	2/8	Lane 1989
Placed	< 1 day	*I. pacificus*	adults	male	20/20	20/20	Lane 1989
Placed	< 1 day	*I. pacificus*	adults	female	20/20	20/20	Lane 1989
Placed	< 1 day	*I. pacificus*	larvae, nymphs	mixed	50/50	50/50	Lane 1989
Placed	5 days	*I. pacificus*	adults	male	20/20	20/20	Lane 1989
Placed	5 days	*I. pacificus*	adults	female	0/20	15/20	Lane 1989
Placed	5 days	*I. pacificus*	larvae, nymphs	mixed	50/50	50/50	Lane 1989
Field	< 1 day	*D. occidentalis*	adults	mixed	Not evaluated	60%	Lane & Anderson 1984
Placed	< 1 day	*D. occidentalis*	adults	mixed	19/20	Not evaluated	Lane & Anderson 1984

Trailside mowing

A single study conducted in San Mateo County in 2006 examined whether trailside areas mowed for the primary purpose of fire suppression created conditions that would decrease questing tick density in these areas (Nakano 2009). The abundance of *D. occidentalis* was unaffected by mowing, but *D. variabilis* was unexpectedly collected in higher numbers in mowed areas compared to control plots. Additionally, no significant correlation was found between grass height and the density of either species of *Dermacentor*, irrespective of whether areas had been mowed or unmowed. No studies were found that evaluated the effect of mowing vegetation on *I. pacificus*.

Fire in tick habitats

Research conducted on tick species located in other regions of the United States support the general conclusion that burning habitat for control of ticks does not provide long-term benefits and that any reduction in tick abundance does not last more than two years after the fire (White and Gaff 2018). In California, two studies that evaluated the effects from very different types of fires in tick habitat suggested these findings should not be over-generalized. Padgett et al. (2009) found that prescribed fire in a chaparral environment reduced the density of rodents capable of hosting immature *I. pacificus* and *D. occidentalis* ticks in subsequent months. However, the remaining rodents had higher tick burdens in burned areas compared to those in control areas. Additionally, ticks experimentally placed under shallow substrate were able to survive the fire, suggesting that the risk of encountering ticks may persist even in recently burned areas.

A more recent study examining the effect of an unplanned wildfire in oak woodland and savannah habitat in Santa Barbara County found an increase in the abundance of questing *I. pacificus* approximately one year post-fire, but a decrease in abundance in the subsequent two years (MacDonald et al. 2018). The fire did not seem to affect the abundance of western fence lizards (*Sceloporus occidentalis*) and deer mice (*Peromyscus maniculatus*) one year post-fire, but did significantly decrease dusky-footed woodrats (*Neotoma fuscipes*), with no evidence of any woodrats returning to post-burn areas until the third year after the fire. All three of these vertebrate species are important hosts for immature ixodid ticks in California (Swei et al. 2011). Tick loads on *S. occidentalis* and *P. maniculatus* were evaluated only during the first year after the fire, and there was only a minor decrease in ticks per host in burned compared to unburned areas. MacDonald et al. (2018) suggest that the failure of many questing ticks to find suitable hosts post-fire could account for the decline in questing ticks in the second and third years after the fire.

Removal of vertebrate hosts (lizards)

A major differentiating factor between eastern and western United States tick ecology is the involvement of lizards as larval and nymphal hosts on the West Coast. Although it has been established that the blood of western fence lizards (*S. occidentalis*) have a borreliacidal effect on infected ticks, the role of these lizards in tick-borne disease ecology is complicated (Lane and Quistad 1998). In an experiment conducted in Marin County, California, all western fence lizards were removed from a tick endemic area in 2008 to evaluate the effect on tick abundance and

Table 2.—Changes in tick abundance in response to different environmental intervention methods or events

Type of intervention	Timing of intervention	Setting	Timing of evaluation after intervention	Tick Species	Stage	Change in abundance of questing ticks after intervention compared to control sites	Change in density of ticks on vertebrate hosts after intervention compared to control sites	Reference
Mowing	June	trailside	< 1 to 3 wk	*D. occidentalis*	adults	No sig. difference	Not evaluated	Nakano 2009
Mowing	June	trailside	< 1 to 3 wk	*D. variabilis*	adults	More ticks in mowed areas	Not evaluated	Nakano 2009
Controlled Burn	June	chaparral	1 to 13 mo	*I. pacificus*	adults	No sig. difference	Not evaluated	Padgett et al. 2009
Controlled Burn	June	chaparral	1 to 13 mo	*D. occidentalis*	adults	No sig. difference	Not evaluated	Padgett et al. 2009
Controlled Burn	June	chaparral	1 to 13 mo	*I. pacificus*	larvae, nymphs	Not evaluated	More ticks in burned areas	Padgett et al. 2009
Wildfire	May	oak woodland/ savannah	1 to 3 yr	*I. pacificus*	adults	Increase in yr 1; decrease yr 2-3	Not evaluated	MacDonald et al. 2018
Wildfire	May	oak woodland/ savannah	1 to 3 yr	*I. pacificus*	nymphs	Increase in yr 1; decrease yr 2-3	[a] mixed results	MacDonald et al. 2018
Wildfire	May	oak woodland/ savannah	1 to 3 yr	*I. pacificus*	larvae	Decrease in burned areas	[a] mixed results	MacDonald et al. 2018
Lizard removal	Mar & Apr	Mixed evergreen forest	<1 to 2 mo	*I. pacificus*	larvae	Increased	[b] mixed results	Swei et al. 2011
Lizard removal	Mar & Apr	Mixed evergreen forest	1 yr	*I. pacificus*	nymphs	Decreased	Not evaluated	Swei et al. 2011

[a] Immature tick burdens decreased for *N. fuscipes* but were not affected for *P. maniculatus* or *S. occidentalis* in burned areas. Rodents were only assessed in the first year following the fire.

[b] Larval tick burdens on female *N. fuscipes* increased in lizard removal areas, but decreased for male woodrats. There was no difference in tick burdens in lizard removal and non-removal plots for *P. maniculatus*.

disease risk (Swei et al. 2011). Removal of lizards led to a significant decline in nymphs in the subsequent season, suggesting that *S. occidentalis* is a critical blood source in this region for the maintenance of larval *I. pacificus* populations. Although some larval ticks likely "host switched" to rodents when *S. occidentalis* became unavailable, the *B. burgdorferi* prevalence did not significantly increase in nymphs the year after the lizard removal. Swei et al. (2011) ultimately concluded that the amplifying effect of lizards on ticks may counteract the diluting effect they have on tick infection prevalence.

Application of Acaricides

Personal correspondence with California vector control professionals indicates that application of acaricides is an uncommon practice. Although no published studies could be found on the efficacy of acaricides used on residential properties, a handful of experiments have investigated the efficacy of two categories of acaricide applications: treatment of the environment and host-targeted methods. Two studies evaluated the direct application of acaricides to vegetation in tick habitat (Monsen et al. 1999, Rory and Peavey 2007). Three published studies and one additional (unpublished) experiment compared approaches to controlling ticks by application of various acariciding techniques to their rodent hosts (Leprince and Lane 1996, Lane et al. 1998, Slowik et al. 2001). Selected results from these evaluations are presented in Tables 3 and 4.

Direct application to tick habitat (trailside vegetation)

The efficacy of three different acaricides applied to vegetation in tick habitat on ixodid ticks in California has been evaluated. Chlorpyrifos, an organophosphate insecticide, and carbaryl, a carbamate insecticide, were both effective in reducing the abundance of questing *I. pacificus* when used to treat plots of vegetation located in outdoor recreational areas in Butte County (Monsen et al. 1999). Carbaryl, chlorpyrifos, and the organophosphate diazinon were initially evaluated in a laboratory setting to assess the susceptibility of *I. pacificus* ticks to these active ingredients. Diazinon was ultimately excluded from field trials due to these bioassay results (Monsen et al. 1999). In a separate study conducted by the San Mateo County Mosquito Abatement District in 2006, deltamethrin, a pyrthroid insecticide, provided complete control of adult questing *D. occidentalis* ticks in treated trailside vegetation for at least 7 weeks after application (Rory and Peavey 2007). Percent reduction in ticks after field acaricide applications are summarized in Table 3. It should be noted that chlorpyrifos, carbaryl, and diazinon are no longer commonly used as vector control materials in California.

Delivery of acaricides via rodent hosts

Host-targeted acaricide applications, both on deer and rodents, have been examined extensively in environments in the northeastern U.S. (Eisen and Dolan 2016). Three studies conducted at the University of California Hopland Research and Extension Center (HREC) in Mendocino County make up the entire body of published research on

Table 3.—Control of adult ticks following a single field application of an acaricide to trailside vegetation. Percent control is the reduction in tick abundance in treated areas from pre-treatment to post-treatment sampling, relative to reduction in control sites. This was calculated using Henderson's method as modified by Mount et al. (1976).

		% Control at time intervals post-treatment								
Material	**Tick Species**	**24h**	**1 wk**	**2 wks**	**3 wks**	**4 wks**	**5 wks**	**6 wks**	**7 wks**	**Reference**
carbaryl	*I. pacificus*	95%	100%	96%	96%	75%	92%	81%	66%	Monsen et al. 1999
chlorpyrifos	*I. pacificus*	100%	100%	100%	100%	100%	94%	95%	96%	Monsen et al. 1999
deltamethrin	*D. occidentalis*	98%*	100%	100%	100%	100%	100%	100%	100%	Rory & Peavey 2007

* Rory and Peavey conducted the first post-treatment sampling at 72h after treatment.

host-targeted tick control techniques on the West Coast (Leprince and Lane 1996, Lane et al. 1998, Slowik et al. 2001). All three of these studies tested variations of rodent-targeted methods. Although laboratory experiments demonstrated that *N. fuscipes* would readily gather cotton for nesting material when presented to them in metal tubes, permethrin-impregnated cotton distributed to wild woodrats using this treatment method was not effective in lowering tick burdens on these rodents. This method did, however, effectively reduce the number of fleas collected from woodrats compared to those in untreated areas (Leprince and Lane 1996).

A related technique was subsequently developed: applying a liquid permethrin formulation to a ring of carpet lining the ends of a plastic tube baited with a non-toxic bait block (Lane et al. 1998). Each tube was placed next to a woodrat nest, and the woodrats were allowed to self-treat with this topical acaricide as they entered and exited the tube. This technique was highly effective at reducing populations of both ticks and fleas on treated woodrats; however, this formulation of liquid permethrin is no longer commercially available. The application of this technique to trailside areas was recently tested by the San Mateo County Mosquito and Vector Control District using deltamethrin powder (Delta Dust Insecticide, Bayer CropScience LP, Research Triangle Park, NC) (Shelton, unpub. data). These tubes, baited with non-toxic bait blocks, were deployed from April through July 2016 and 2017 along hiking trails in two parks in San Mateo County. This experiment found rodents in treated areas had lower flea and tick loads than in control areas, a result consistent with Lane et al. (1998). However, the density of questing ticks on trails in treatment areas did not differ from untreated areas.

In 1998, a third rodent-targeted acaricide technique was evaluated at the HREC (Slowik et al. 2001). Non-toxic bait cubes were treated with fluazuron, an arthropod growth inhibitor that is commonly used to suppress ectoparasites on pets and livestock. Woodrats brought the bait cubes into their nests and ingested the treatment over time, an ideal

Table 4.—Percent reduction post-intervention in abundance of ticks from applications of rodent-targeted acaricides

Acaricide delivery system	**Acaricide**	**Timing of treatment**	**Rodents targeted**	**Tick species**	**Stage**	**Timing of evaluation after start of intervention**	**Abundance of questing ticks in treated areas compared to control sites**	**Density of ticks on vertebrate hosts in treated areas compared to control sites**	**Reference**
Treated cotton balls	permethrin	Dec - Sept	*N. fuscipes*	*I. pacificus*	larvae	<1 to 9 mo	Not evaluated / none collected	Fewer ticks in treatment area in spring & summer	Leprince & Lane 1996
Treated cotton balls	permethrin	Dec - Sept	*N. fuscipes*	multiple*	larvae, nymphs, adults	<1 to 9 mo	too few collected to evaluate	No significant difference	Leprince & Lane 1996
Bait tube with treated lining	permethrin, pyrethrins, PBO	May - Aug; then Apr - June	*N. fuscipes*	*I. pacificus*	larvae, nymphs	1 to 9 mo	Not evaluated	Fewer ticks in treatment area	Lane et al. 1998
Bait tube with treated lining	permethrin, pyrethrins, PBO	May - Aug; then Apr - June	*N. fuscipes*	*D. occidentalis*	larvae, nymphs	1 to 9 mo	Not evaluated	Fewer ticks in treatment area	Lane et al. 1998
Bait tube with treated lining	deltamethrin	Apr - July, in 2 consec. years	multiple	*I. pacificus*	larvae, nymphs	2 to 4 mo, in 2 consec. years	No significant difference	Fewer ticks in treatment area	(Shelton, unpub.)
Treated bait cubes	fluazuron	Mar - Nov; then Feb - Mar	*N. fuscipes*	*I pacificus*		3 to 16 mo	Not evaluated	No significant difference	Slowik, Lane & Davis 2001
Treated bait cubes	fluazuron	Mar - Nov; then Feb - Mar	*N. fuscipes*	*D. occidentalis*		3 to 16 mo	Not evaluated	No significant difference	Slowik, Lane & Davis 2001

* Tick species identified on woodrats in Leprince and Lane (1996) were*: I. woodi, I. neotomae, I. pacificus,* and *D. occidentalis*. Ticks flagged from environment were *D. occidentalis* and *I. pacificus*.

application technique for this type of systemic acaricide. Similar to the earlier experiment with permethrin-treated cotton, this treatment was effective on reducing fleas but not on ticks.

Conclusions

Research to evaluate tick control methods in California has been pursued only sporadically over the past 35 years and has been conducted by a very limited number of laboratories and vector control agencies. Some technologies for tick abatement that are in wide use in other parts of the United States have not been practical to use or not effective in California environments and on ixodid tick species of local concern. Many avenues of tick control, from biological controls, to robotic devices, to integrated approaches, are currently being evaluated in other areas of the country (Eisen and Dolan 2016). Their potential as future public health interventions in California deserves to be examined.

As a final note, three of the studies included in this review were published only in conference proceedings or have yet to be published. Future researchers should endeavor to publish results in widely available formats to enable broad access to results.

Acknowledgements

Special thanks go to Theresa Shelton (SMCMVCD) for sharing unpublished rodent bait tube data, Tara Roth and Chindi Peavey (SMCMVCD) for research support, Dennis Candito (ADAPCO) for technical assistance, and Kerry Padgett (CDPH) for ongoing enthusiastic support of tick control research in California.

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Program overview including current projects and future ideas for culturing high yields of *Gambusia affinis*

Tony Hedley, Samer Elkashef, Sarah Wheeler

Sacramento-Yolo Mosquito and Vector Control District

Abstract

The Sacramento-Yolo Mosquito and Vector Control District (District) annually plants approximately 3500 pounds of *Gambusia affinis* (mosquitofish) in a wide variety of natural and manmade habitats. This undertaking requires optimal water quality and efficient seining to ensure that high yields of mosquitofish are achieved. To this end the District is investigating several methods to increase fish yield and decrease labor costs to achieve these goals. Current projects range from evaluating traditional pond fertilization methods to the use of pond dye as a means of reducing nuisance filamentous algae levels to decrease seine times and eliminate the need to sort fish. Another project is the use of solar powered pond aeration to achieve desired dissolved oxygen levels throughout the nighttime hours when oxygen levels typically drop. Aeration would prevent ponds from crashing resulting in higher a yield of quality fish for use in treating a multitude of sources. Concurrent with these projects water quality parameters including temperature, ammonia, pH and invertebrate levels are monitored to ensure maximum production capabilities at all steps in the aquaculture process.

Evaluation of Sumilarv 0.5G in catch basins in Sacramento and Yolo Counties

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Introduction

In parts of the Sacramento-Yolo Mosquito and Vector Control District (District) service area long term larval control in catch basins is difficult due to periodic flushing, high organic matter content and mosquito resistance issues. In an effort to improve control of urban *Culex pipiens* populations, the District performed an evaluation of the new McLaughlin Gormley King Company (MGK) product Sumilarv 0.5G. The active ingredient, pyriproxyfen, provides two advantages to controlling mosquitoes in catch basins; 1) it has a new active ingredient that mosquitoes have yet to be exposed to, providing a potentially powerful rotational tool and 2) MGK claims that this product can bind to both the cement walls of catch basins as well as the organic content found within, potentially overcoming flushing issues.

Methods

The District selected 60 catch basins representative of the types found within District boundaries. A third of these basins were in Elk Grove, a third in downtown Sacramento and a third in Woodland. These catch basins were initially sampled for larvae and then treated with either 35g or 70g of Sumilarv 0.5G. On a weekly basis, 10-20 pupae were collected from each basin and brought back to the District in water from the basins, placed into emergence jars, and emergence inhibition observed over a 7 day period.

Results and Discussion

Catch basins treated with Sumilarv 0.5G in all the cities showed an emergence inhibition level of at least 95% over the course on a 24 week period after a single application of 70g of material (Figure 1). Higher emergence inhibition levels were found in basins with higher organic content, such as the basins found in Woodland and downtown Sacramento. This correlates with manufacturer claims that pyriproxyfen can bind to the organic matter that accumulates in catch basins. Also of note was that during disease weeks 19 and 20, the District received approximately 0.6 inches of rain in Elk Grove which did not disrupt the emergence inhibition (Figure 2). This observation provides evidence that pyriproxyfen can bind to cement and can flow back into a basin after the water being held in a basin is flushed out of it and refilled with new water.

During the course of the study the District compared two application rates, the manufacturer recommended 70g and a dose of 35g. The results between these two application rates throughout the duration of the season were similar (Figure 2). However, during the last two weeks of the sampling period the average emergence inhibition of the 35g dose dropped to 85%. This observation could point to the start of the end of this products viability to control mosquitoes in this environment at the lower dose.

Conclusions

The District found that Sumilarv 0.5G was a good rotational product to be used for mosquito control in the catch basin environment. Moving forward, the District is planning to evaluate this material in dairy sumps. Dairy sumps are another difficult to control source for *Culex pipiens* that have high organic matter content.

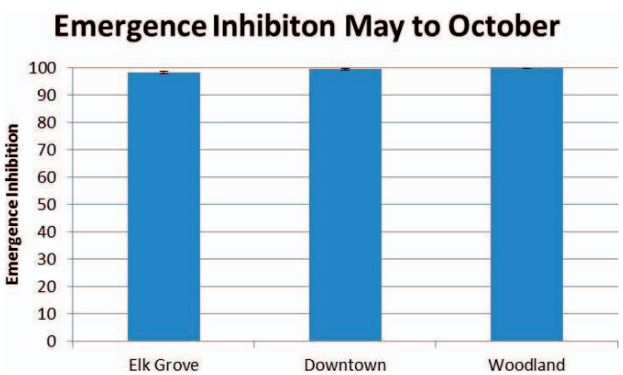

Figure 1.—Emergence inhibition of *Culex pipiens* collected from catch basins treated with Sumilarv 0.5G. Emergence inhibition was averaged over the 24 week period for each municipality where it was tested in. Bars denote standard error.

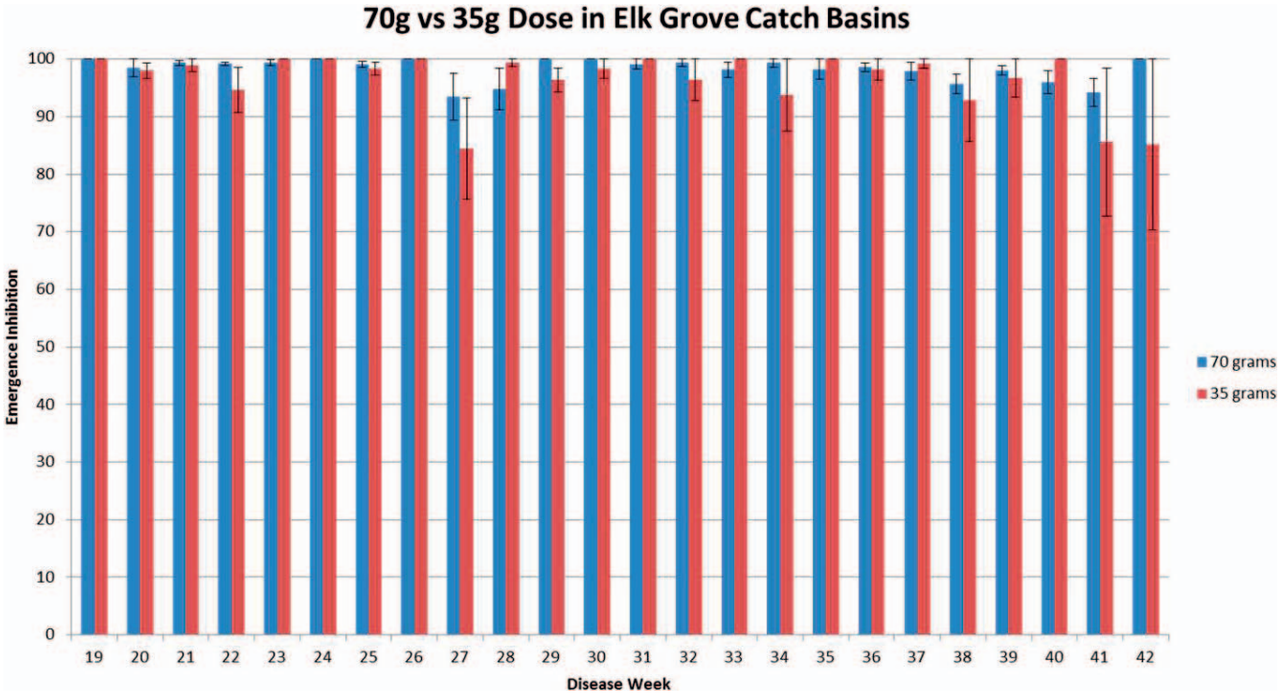

Figure 2.—Emergence inhibition of *Culex pipiens* collected from catch basins treated with Sumilarv 0.5G in the City of Elk Grove. Emergence inhibition was averaged over catch basins per disease week and error bars denote standard error. Blue bars represent basins treated with a dose of 70g while red bars a dose of 35g.

Triumphs and tribulations: year three of Delta Vector Control District's expanded mosquito surveillance program

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Introduction

During the 2018 mosquito season, Delta Vector Control District (DVCD) continued to operate its expanded West Nile Virus (WNV) surveillance program and implemented a new *Aedes aegypti* surveillance program. The 2018 WNV surveillance program allowed abundance and infection rate values from gravid and EVS traps to be compared to data collected in both 2016 and 2017, and BGS trap data to be collected this year for future comparisons. The addition of the BGS trapping program increased the number and variety of mosquitoes found, not just the invasive *Ae. aegypti*, but native species as well, allowing more information on mosquito abundance and virus infection.

Methods

We used a spatially uniform sampling format. Four gravid traps were dispersed evenly within each square mile of urban habitat, based on sites used in previous years and the public land survey system (PLSS) (USGS 2018), resulting in a total of 172 gravid traps deployed weekly to determine female mosquito abundance and viral infection. To monitor invasive *Aedes* populations (CDPH 2018), one BGS trap, baited with sugar-yeast-water to create CO_2 and a BG lure, was located within each square mile of populated area, based on vegetation density, known infestation areas, other risk factors (Tushar et al. 2017) and the PLSS, resulting in 60 BGS traps operated weekly to determine abundance and viral infection. To cover gaps in surveillance, an additional 20 EVS traps for rural areas and 20 strategic BGS traps for urban areas were assigned to surveillance staff each week and were operated in non-fixed locations as determined by vector control technician input, historical breeding sources, and previous WNV or *Aedes* activity (Tushar et al. 2017). Mosquitoes were counted and identified to species, daily, with *Cx. quinquefasciatus*, *Cx. tarsalis*, *Cx. stigmatosoma*, and *Cx. erythrothorax* being placed into pools of 10-50 female mosquitoes per trap site and virus testing being done for WNV, Saint Louis encephalitis virus (SLEV), and Western equine cncephalitis virus using RT-PCR, with results being available within the next work day to staff and the public.

Results and Discussion

During the 2018 surveillance season, 4,399 gravid, 393 EVS, 1,224 fixed BGS and 398 strategic BGS traps were set. WNV infection rates were lower than previous years, with 2,332 mosquito pools with a minimum infection rate (MIR) of 0.4 per 1,000 females from gravid traps, 712 mosquito pools with a MIR of 1.3 per 1,000 from EVS traps, and 840 mosquito pools with a MIR of 0.2 per 1,000 from BGS traps. Our sampling program attained our planned schedule that included 172 gravid, 20 EVS and 60 BGS traps set per week; however, the strategic BGS trapping program did not, mostly due to an increase in service requests related to *Ae. aegypti*. Additionally, the BGS traps used for the *Aedes aegypti* surveillance program collected substantial populations of native blood meal seeking *Aedes*, *Culex*, *Anopheles*, and *Culiseta* populations within urban and suburban areas, contributing new information that gravid traps and EVS traps were unable to provide, as well as allowing more information on virus infection to be gathered. Success of the program is measured not only in the consistency of data gathered, but also in being able to potentially reduce domestic mosquito populations, which compared to the three-year average seems to have happened in 2018. (Figure 1)

Conclusions

The expanded surveillance program allowed Delta VCD to continue to monitor WNV while also allowing the tracking of the expansion of *Ae. aegypti,* and will continue to be used for the foreseeable future. The main goal of the program will be to keep our community safe with prompt knowledge of abundance and viral activity and immediate action, and the secondary goal of establishing a robust baseline of finite special data of mosquito abundance and virial activity. Looking forward Delta VCD hopes to create district standardizations, such as quantifying our infusion and lures, to better acquire consistency across trap sets.

Acknowledgements

The authors would like to thank Delta VCD Manager, Michael Alburn, Superintendent, Paul Jobe, Systems Administrator, Mark Dynge, and previous Scientific

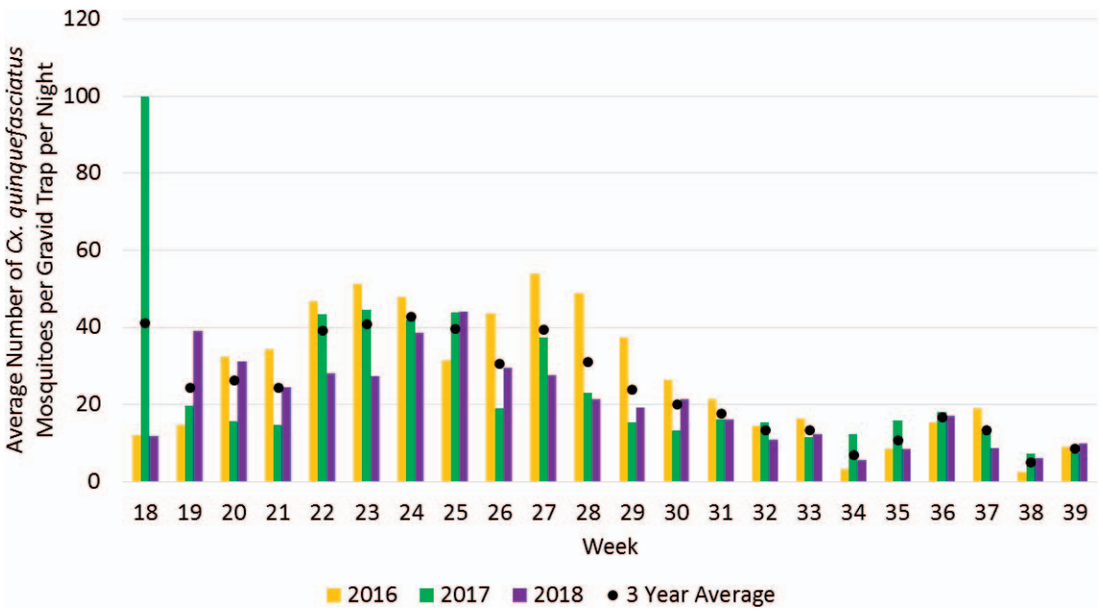

Figure 1.—Average number of *Culex quinquefasciatus* mosquitoes collected per gravid trap per night within the Delta VCD during 2016 - 2018, with the three-year average included.

Program Manager, Taylor Tushar, for all of their assistance and support for the enhanced surveillance program.

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Stormwater treatment control devices as significant mosquito breeding sources

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Abstract

Stormwater treatment control devices have been linked to mosquito production. Requirements for municipal development to comply with the MS4 permit in San Bernardino County has created new mosquito sources that previously did not exist before development. In response, the West Valley Mosquito and Vector Control District created a BMP program that inspects and treats all types of BMP's whether they are above ground or below ground. A study of these devices during the 2018 season showed mosquito breeding to be 2 to 3 times more likely in these devices than other District sources combined. This study examined three types of stormwater devices: infiltration basins, media filters, and hydrodynamic separators. In the underground hydrodynamic separators and media filters, *Culex quinquefasciatus* was the dominant mosquito collected and our primary vector for West Nile virus. With hundreds of treatment control devices installed underground and designed to hold water within our District, our efforts to control mosquitoes is challenging. With 528 known treatment control devices within the 210 square mile District, it will require working with the Water Board and San Bernardino County to eliminate underground treatment control devices that are designed to hold water.

"Tip this House"

A multi-agency mosquito borne disease outbreak preparedness exercise under Incident Command System

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Abstract

On June 9, 2018 volunteers from the Los Angeles County Medical Reserve Corps and various public health emergency volunteer (PHEV) network partners, in cooperation with and under the leadership of two Los Angeles County vector control agencies and County Health Department Emergency Preparedness and Response Division staff, participated in a mosquito-borne disease outbreak preparedness exercise. These public health volunteers had been trained to assist with information dissemination to residents as well as with door to door yard inspection and sanitation efforts to supplement staff during a potential outbreak. Because any vector-borne disease emergency response will have to be a multi-agency effort of undetermined size, using the Incident Command System (ICS) would be the best way to ensure that all participants operated within a common organizational structure. This article describes the organization, execution and outcome of the 2018 preparedness exercise.

Introduction

With the rediscovery of *Aedes albopictus* populations in the San Gabriel Valley during the fall of 2011, 10 years after their prior introduction in shipments of lucky bamboo (Madon et al. 2002, Metzger et al. 2017), the storyline for invasive *Aedes* populations in Los Angeles County has been rewritten. Not only is it not too dry (Madon et al. 2003) for these invasive species to survive in Southern California, but they have spread and thrived. The 2011 detection of *Ae. albopictus* was followed by the discovery of *Aedes aegypti* in the nearby City of Commerce in 2014 (Metzger et al. 2017). Both invasive species have since increased in abundance as well as distribution, so that during the following 4 years the entire San Gabriel Valley was infested with *Ae albopictus,* and *Ae aegypti* has spread throughout most of Los Angeles County (Metzger et al. 2017). (Figure 1).

After the emergence of Zika virus in Brazil and its rapid spread throughout the Americas (Zhang et al. 2017), the CDC identified Los Angeles County, along with Florida and Texas, as an area at high risk for a potential outbreak of the disease and the County Health Department was awarded Local Laboratory Capacity (LLC) funding earmarked for Zika virus prevention and mosquito control. County health officials from Acute Communicable Disease Control (ACDC), the Emergency Preparedness and Response Program (EPRP) and local vector control agencies came together to prepare a Zika Virus Emergency Response Plan. During this process and by observing the events during the Zika virus outbreak in Florida (Likos et al. 2016), it became apparent that there would be the need for serious force multiplication to contain an outbreak of Zika in Los Angeles County and EPRP staff suggested the use of the LA County Medical Reserve Corps (MRC) as well as other PHEV network partners. In the spring of 2016 county public health and vector control staff from San Gabriel Valley and Greater Los Angeles County Vector Control Districts began the process of training volunteers to assist with information dissemination to residents as well as with door to door property inspection and sanitation efforts. Vector control field staff, in turn, were trained on how to work with the volunteers under the organizational structure of the Incident Command System (ICS).

Los Angeles County Operational Area Health and Medical Zika Virus Readiness, Response and Recovery Plan

The Los Angeles County (LAC) response plan aligns with the California Zika Response Activities and Resources document (CDPH Division of Communicable Disease Control. 2016; https://www.cdph.ca.gov/Programs/CID/DCDC/CDPHDocumentLibrary/CAZikaResponseActivitiesResources.pdf) and the CDC Zika Interim Response Plan (CDC 2017; https://www.cdc.gov/zika/pdfs/zika-draft-interim-conus-plan.pdf). The document describes the actions that will be taken in the LAC Operational Area (OA) in response to any invasive *Aedes* mosquito-borne virus, such as chikungunya, dengue, Zika or yellow fever,

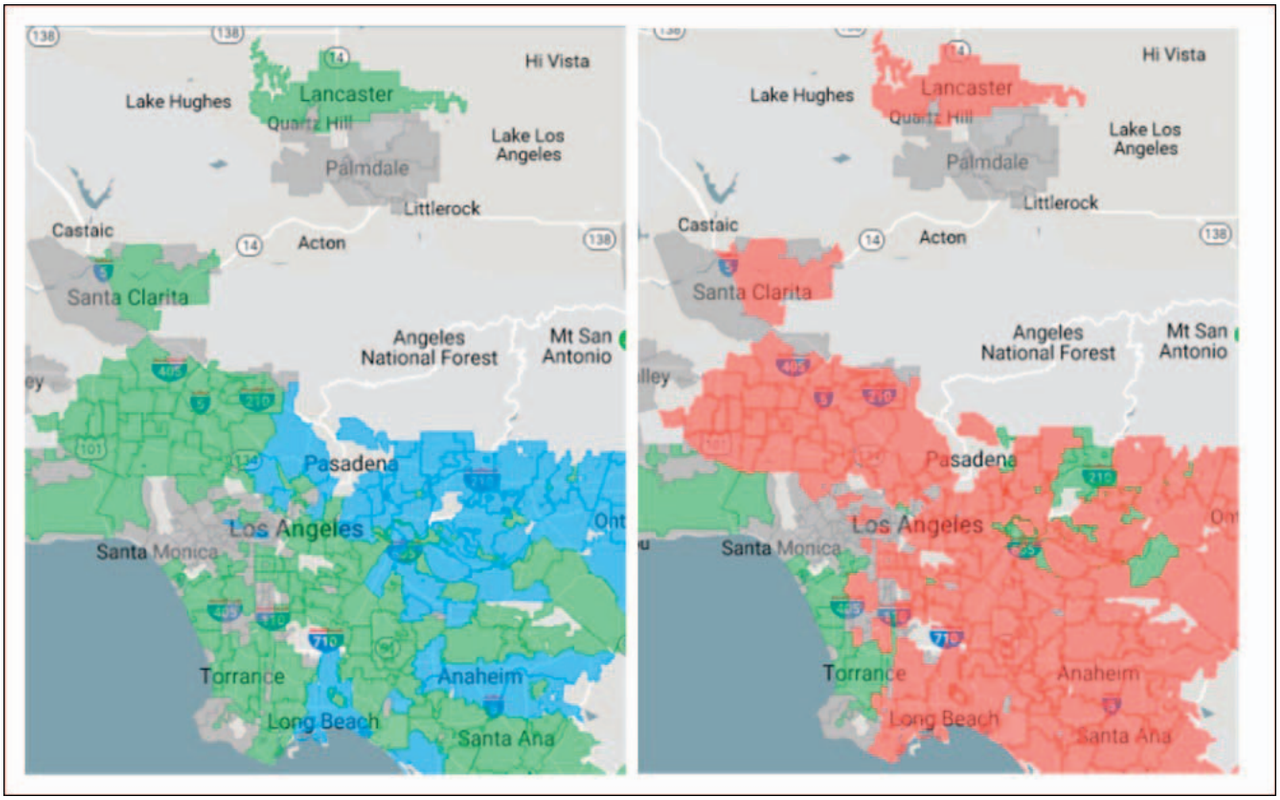


Figure 1.—2018 *Aedes albopictus* ■ and *Aedes aegypti* ■ distribution in Los Angeles County

following detection of local transmission. The plan also includes Pre-Incident readiness activities to address planning and coordination required for an effective response.

In any such response, the County Health Department's ACDC section will have to conduct the case surveillance and epidemiological investigation, while local vector control districts implement door to door property inspection campaigns for mosquito source elimination and adult control activities as information on case locations is obtained. The CDC recommends sanitation and control efforts be conducted in a 150 meter (200 yard) radius around confirmed case residences and additional potential exposure sites, such as place of work or recreational activity. Given the average property size in much of LAC, this area equates to approximately 200 homes and back yards. LAC has a robust MRC and PHEV partner network able to augment vector control staff and act as force multipliers during a potential *Aedes* transmitted virus outbreak. Volunteers have been trained to help with resident education and property inspection thereby extending ground-based control efforts to case residence and potential exposure locations. Once case numbers and OA exceed staff resource availability for a ground control approach, aerial applications of both larvicide and adulticide are prescribed to be conducted over the outbreak area.

The cooperative response between vector control agencies and County Health Department staff, will require involved agencies to work under a unified command structure. The best available command structure for multi-agency incident response is known as Incident Command System (ICS).

Incident Command System (ICS)

The Federal Emergency Management Agency (FEMA ICS300; https://training.fema.gov> emiweb>assets) describes ICS as a "standardized approach to the command, control, and coordination of on-scene incident management that provides a common hierarchy within which personnel from multiple organizations can be effective. ICS specifies an organizational structure for incident management that integrates and coordinates a combination of procedures, personnel, equipment, facilities, and communications." This organizational structure prescribes five major functional areas that can be staffed as needed depending on the scale of the incident: Command, Operations, Planning, Logistics, and Finance/Administration. (Figure 2).

The simplicity of the structure makes it easily adaptable to any required response level or incident magnitude. Due to the simple chain of command communication approach under ICS, staff from different agencies can work together seamlessly without having to be familiar with each other or each other's home agencies' organizational structures. Staff can be assigned any necessary position within the incident response according to mission need and skill set, but

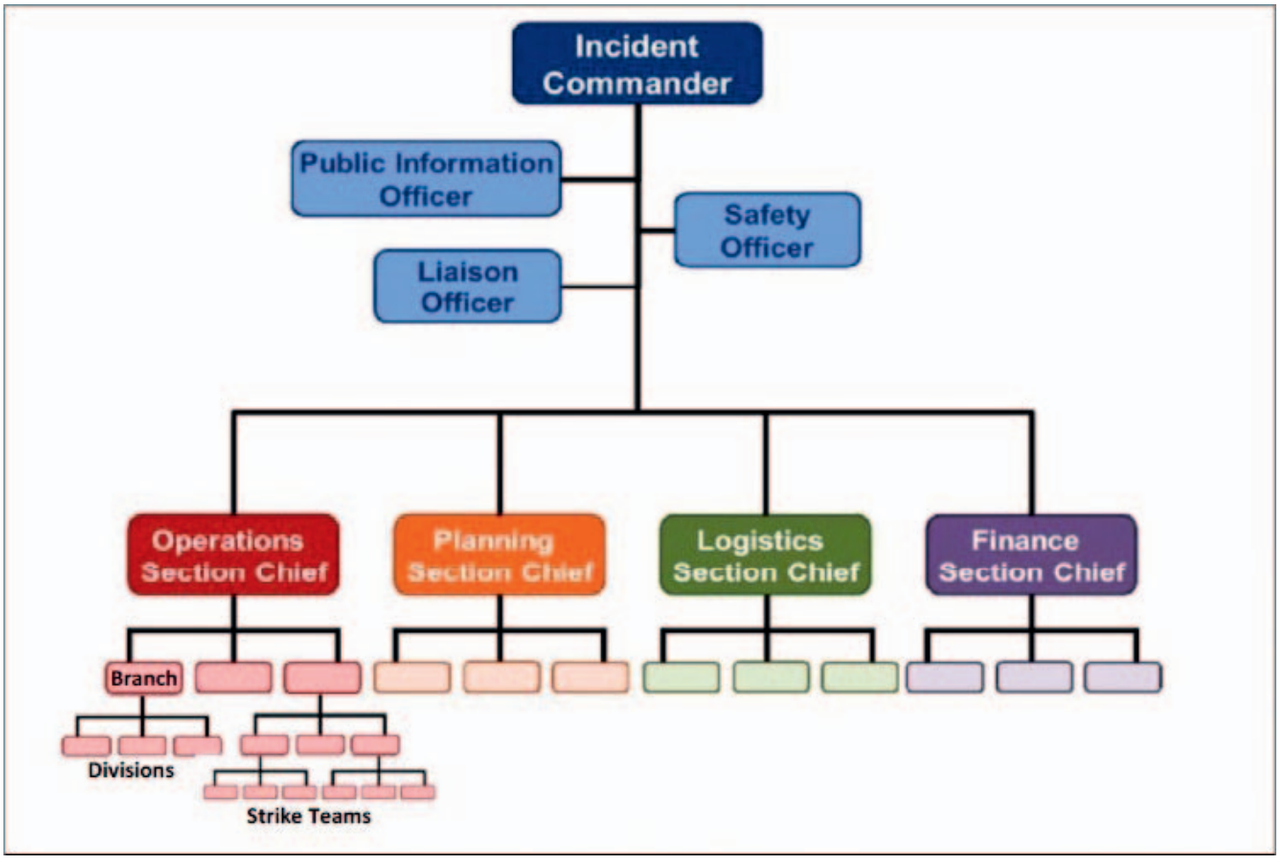


Figure 2.—Incident Command System organizational structure

regardless of the rank they hold in the agency from which they are deployed.

Incident Command defines the goals and operational period objectives that need to be accomplished and command staff usually includes the Public Information Officer, Safety Officer, Liaison Officer, and other advisory positions required. 'Sections' are the organizational level with responsibility for a major functional area of incident management (e.g., Operations, Planning, Logistics, and Finance/Administration). Depending on the magnitude of the incident the Section is organizationally situated between the 'Branch' and the Incident Command or the 'Division' and Command. Branches have geographical responsibility for major aspects of incident operations and can contain multiple Divisions that are responsible for operations within a defined geographic area and can, in turn, contain multiple Strike Teams.

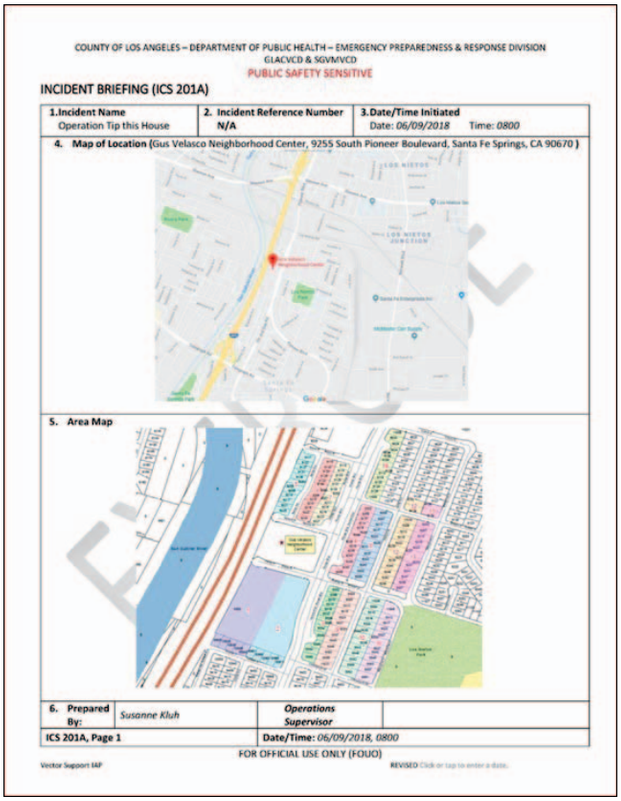
Operations section staff are the 'Doers' in charge of accomplishing the objectives set for the operational period. They are supported by Logistics section, the 'gofers', charged with providing Operations with all the necessary supplies and equipment. The Planning section collects situation and resources status information, evaluates it, and processes the information for use in developing the incident wide action plans. These plans can be shared in general briefings or in the Incident Action Plan (IAP).

The IAP is the central document for any operational period under ICS, also referred to as ICS form 201, or in our case, the Exercise Action Plan (EAP). This document provides team leaders with incident as well as staging area information and detailed operation area maps. It includes a description of the incident at hand in the Situation Summary and spells out responder safety concerns in the Health and Safety Summary as well as operation period objectives (Figure 3). In addition it contains a diagram of the Organizational Structure. Due to the limited scope of the response to a single human case simulated in the "Tip this House" exercise, the organizational structure was relatively simple. (Figure 4)

Outbreak Control Exercise

In preparation for "Tip this House", the third exercise since the inception of vector control staff and volunteer training efforts, a day was spent training San Gabriel Valley and Greater Los Angeles County Vector Control District staff on working with public health volunteers under ICS. During an additional training day vector control staff and volunteers had a chance to meet and work through exercise protocols together.

To fully simulate possible outbreak events, the week of the exercise began with a mock notification email from LA



COUNTY OF LOS ANGELES – DEPARTMENT OF PUBLIC HEALTH – EMERGENCY PREPAREDNESS & RESPONSE DIVISION
GLACVCD & SGVMVCD
PUBLIC SAFETY SENSITIVE

INCIDENT BRIEFING (ICS 201A)

1.Incident Name	2. Incident Reference Number	3.Date/Time Initiated
Operation Tip this House	N/A	Date: 06/09/2018 Time: 0800

4. Map of Location (Gus Velasco Neighborhood Center, 9255 South Pioneer Boulevard, Santa Fe Springs, CA 90670)

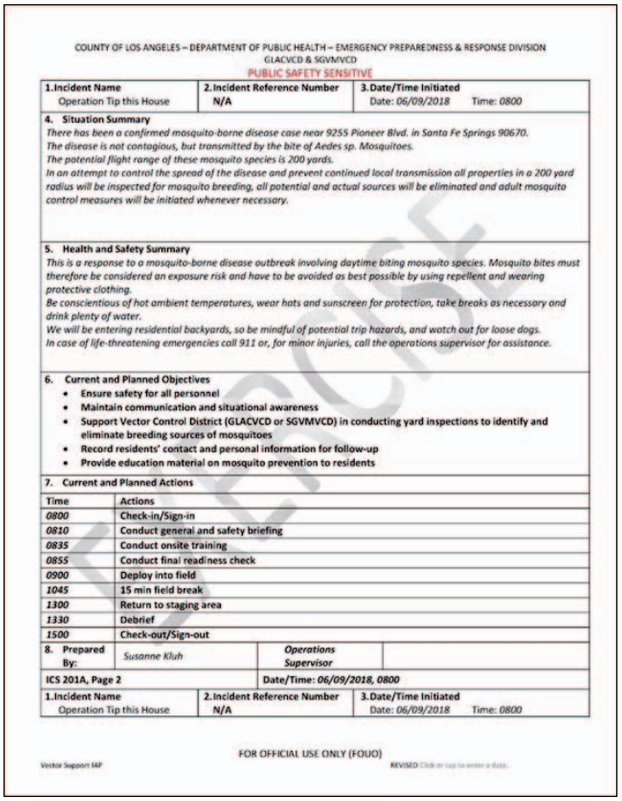
5. Area Map

6. Prepared By:	Susanne Kluh	Operations Supervisor	
ICS 201A, Page 1		Date/Time: 06/09/2018, 0800	

FOR OFFICIAL USE ONLY (FOUO)

Vector Support IAP REVISED Click or tap to enter a date.

Figure 3.—Incident Action Plan, ICS 201 – location information



COUNTY OF LOS ANGELES – DEPARTMENT OF PUBLIC HEALTH – EMERGENCY PREPAREDNESS & RESPONSE DIVISION
GLACVCD & SGVMVCD
PUBLIC SAFETY SENSITIVE

1.Incident Name	2.Incident Reference Number	3.Date/Time Initiated
Operation Tip this House	N/A	Date: 06/09/2018 Time: 0800

4. Situation Summary
There has been a confirmed mosquito-borne disease case near 9255 Pioneer Blvd. in Santa Fe Springs 90670.
The disease is not contagious, but transmitted by the bite of Aedes sp. Mosquitoes.
The potential flight range of these mosquito species is 200 yards.
In an attempt to control the spread of the disease and prevent continued local transmission all properties in a 200 yard radius will be inspected for mosquito breeding, all potential and actual sources will be eliminated and adult mosquito control measures will be initiated whenever necessary.

5. Health and Safety Summary
This is a response to a mosquito-borne disease outbreak involving daytime biting mosquito species. Mosquito bites must therefore be considered an exposure risk and have to be avoided as best possible by using repellent and wearing protective clothing.
Be conscientious of hot ambient temperatures, wear hats and sunscreen for protection, take breaks as necessary and drink plenty of water.
We will be entering residential backyards, so be mindful of potential trip hazards, and watch out for loose dogs.
In case of life-threatening emergencies call 911 or, for minor injuries, call the operations supervisor for assistance.

6. Current and Planned Objectives
- Ensure safety for all personnel
- Maintain communication and situational awareness
- Support Vector Control District (GLACVCD or SGVMVCD) in conducting yard inspections to identify and eliminate breeding sources of mosquitoes
- Record residents' contact and personal information for follow-up
- Provide education material on mosquito prevention to residents

7. Current and Planned Actions

Time	Actions
0800	Check-in/Sign-in
0810	Conduct general and safety briefing
0835	Conduct onsite training
0855	Conduct final readiness check
0900	Deploy into field
1045	15 min field break
1300	Return to staging area
1330	Debrief
1500	Check-out/Sign-out

8. Prepared By:	Susanne Kluh	Operations Supervisor	
ICS 201A, Page 2		Date/Time: 06/09/2018, 0800	

1.Incident Name	2.Incident Reference Number	3.Date/Time Initiated
Operation Tip this House	N/A	Date: 06/09/2018 Time: 0800

FOR OFFICIAL USE ONLY (FOUO)

Vector Support IAP REVISED Click or tap to enter a date.

Figure 4.—Incident Action Plan, ICS 201 – Situation, Health &Safety, Objectives

Co Public Health to vector control reporting the occurrence of an *Aedes* transmissible disease case without travel history. In response to this notification, vector control immediately requested the assistance of the MRC and PHEV network partners, who require 72 hrs. to be ready to deploy. In the following days the IAP was prepared and the affected neighborhood was posted with the announcement of the door to door property inspection campaign for Saturday June 9, 2018 (Figure 5). Preauthorization forms were given to residents to display at their front door or gate to allow access for inspection in absence of the homeowner (Figure 6). A press release was issued announcing the exercise and its importance for disease control preparedness.

On June 9, 2018 a total of 63 response team members reported to the exercise staging area:

27 vector staff (including incident command), 4 LA County DPH staff and 40 volunteers from the Los Angeles County MRC and PHEV Network Partners signed in and received their team assignments. The general and safety briefing were conducted (Figure 7) and team leaders collected the team IAP and field supply kits from logistics. Field supply kits contain informational handout materials to be distributed to residents as well as first aid supplies, sunscreen and insect repellent for response team members. After an additional team briefing, team leaders announced their team readiness and deployed into the field to the operations section chief. Each team was assigned a section of properties within which to contact residents, provide informational materials and conduct property inspections and necessary mosquito larval habitat removal. Vector control team leaders could request adult mosquito control measures, if *Aedes* activity was observed and a logistics team would be deployed to conduct the treatment.

Most teams deployed around 0900h and returned to the staging area by 1130h for debriefing (Figure 8) that provided

Figure 5.—Neighborhood postings

MOSQUITO INSPECTION AUTHORIZATION

EMERGENCY RESPONSE TRAINING EXERCISE

Teams of Certified Vector Control Specialists and Health Department Volunteers will be inspecting and treating properties (if needed) for mosquitoes in this neighborhood on:

Saturday, June 9th
Between 9:00AM and 1:00PM

This is a critical training exercise and we need your cooperation. If you will not be home, your signature below will authorize District representatives to enter, inspect, and treat your yard (at no cost) for mosquitoes.

(Signature)

Please sign and:
- Attach this notice to your door or side gate by *7:00 AM* on the inspection date
- Leave gate(s) *unlocked*
- Keep dogs locked up during this time (staff will not inspect yards with dogs)

Inspectors will be looking for containers or drains holding standing water capable of breeding mosquitoes. Adult mosquitoes and standing water sources may be removed/treated to eliminate this public health threat. An inspection report will be left noting any problems found and corrections taken.

For additional information, please contact the Greater Los Angeles County Vector Control District at 562.944-9656 or visit www.GLACVCD.org

AUTORIZACIÓN DE INSPECCIÓN DE MOSQUITOS

SIMULACIÓN Y ENTRENAMIENTO A REPUESTA DE EMERGENCIA

Personal Certificado de Control de Vectores y Voluntarios del Departamento de Salud inspeccionaran y controlaran mosquitos en su propiedad (si es necesario) en el día:

Sábado, 9 de Junio
Entre las 9:00AM y 1:00PM

Este es una simulación de entrenamiento crítico y necesitamos su cooperación. Si no estará en casa, su firma autorizará a los representantes del Distrito a entrar, inspeccionar, y controlar mosquitos en su patio sin algún costo.

(Firma)

Por favor firme y:
- Adhiera este aviso en la puerta o en el porton antes de las *7:00 AM* el dia de la inspección.
- Deje el porton sin *llave*
- Mantenga los perros encerrados durante este tiempo (personal no inspeccionara patios con perros)

Inspectores estarán buscando contenedores o desagües con agua estancada capaz de criar mosquitos. Mosquitos adultos y fuentes con agua estancada seran eliminadas/controladas para evitar riesgo a la salud pública. Se dejará un informe de la inspección notando los problemas encontrados y las correcciones que fueron tomadas.

Para más información, por favor llame a Greater Los Angeles County Vector Control District at 562.944-9656 o visite www.GLACVCD.org

Figure 6.—Yard inspection authorization form

information on what was accomplished, as well on what went well with the exercise and which areas are in need for improvement. In 2.5 hrs. 204 residences were visited, of which 144 were inspected. Of these 10 had sources positive for *Ae. aegypti* and 2 were treated with mosquito adulticide after adult activity was observed. A total of 31 residents had signed and posted inspection authorization forms to facilitate property inspections in their absence.

Conclusion

To respond fast and efficiently to locally acquired cases of *Aedes* mosquito transmitted viruses such as dengue,

Figure 7.—Pre-exercise briefing

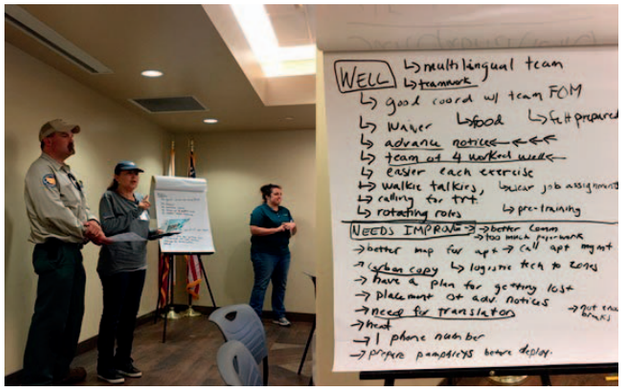


Figure 8.—Post-exercise debriefing

chikungunya, Zika or yellow fever, it is important to have a response plan. Because a disease emergency would certainly involve multiple local public health agencies, the response to a disease outbreak would best be conducted under ICS to ensure seamless integration of all available staff and equipment resources. This requires vector control staff to be trained on ICS concepts and procedures.

The use of public health volunteer groups can serve as a force multiplier for outreach and vector control purposes. These volunteer teams are most effective when adequate training is provided and full scale exercises are conducted for everyone to practice their respective roles and responsibilities. During the exercise on June 9, 2018, the response to one locally transmitted case of mosquito-borne disease was practiced. Due to the labor intensive property inspection and sanitation efforts, this response was estimated to have required approximately 200 staff-hours for vector control, but with help from the volunteers this time was reduced to only 37 vector staff-hours, allowing 5 times the number of on the ground case-location investigations to be accomplished with the same vector control staff resources.

Posting the neighborhood surrounding the locally transmitted disease case residence with notification signs of the impending inspection campaign and asking residents to be home or post yard inspection authorization forms has increased yard access from 35% from an exercise the year prior when no such signs were posted or authorization forms distributed to 70% in the current exercise. Staff also found that residents had done quite a lot of yard clean-up work in anticipation of the inspections, thus reducing mosquito abundance in advance and limiting necessary staff effort during the inspections.

Continued training and repeated exercises will be necessary to ensure deployment readiness. This coming year's exercise will include volunteer teams from neighboring southern California counties to further increase regional volunteer availability and potentially form relationships between those organizations and their local vector control agencies in the attempt to be as prepared as possible for a local *Aedes* borne disease transmission event.

Acknowledgements

The author would like to thank all collaborators from the Emergency Preparedness and Response Division at the Los Angeles County Department of Public Health, but especially the Medical Reserve Corps Director Dr. Jee Kim and MRC Coordinator Joseph Kim, as well as staff at GLACVCD and MRC and Public Health Emergency Network Partner volunteers.

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Vector Control Operations around Oakland's Homeless Encampments

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Abstract

Homeless encampments in Oakland are increasing in number and can create a public health risk of vector borne disease transmission. Alameda County Vector Control Services District collected data on and performed suppression of vector species around these encampments. This presentation discussed the history, control methods, and operational hazards. Techniques used for monitoring populations rodents, ectoparasites and pathogens within homeless encampments over large areas were described. Solutions provided by the city of Oakland as they affect our operations were detailed.

WNV Resource Allocation in the Age of *Aedes*: An Operations Perspective

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Abstract

West Nile virus has driven the need for effective mosquito control and outreach for our District over the past 15 years. Our program was built to address the many challenges presented by the transmission cycle among WNv, *Culex quinquefasciatus*, birds and humans. In 2011, we had our first detection of *Aedes albopictus* in a residence, followed by *Aedes aegypti* and *Aedes notoscriptus*. Over the past seven years, *Aedes* have spread throughout the District and increased the burden on our limited resources. WNV is endemic and presents the biggest risk of disease and death from a vector borne illness in Los Angeles County. This paper will discuss the shift of resource allocation between early season WNV activity and late season *Aedes* control efforts. Intense WNV control activity begins after the rains have finished, typically from March to June. As larval sources dry down and swimming pool lists are cleared, resources then can be shifted to the *Aedes* program which begins around June and continues into November.

Operational control activities targeting invasive *Aedes aegypti* in the Coachella Valley

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Introduction

Aedes aegypti (Linnaeus) was first detected by Coachella Valley Mosquito and Vector Control District (CVMVCD) staff on 9 May 2016 in the City of Coachella (CVMVCD, 2016). Since that discovery through end of 2017, the CVMVCD worked on an intensive door-to-door inspection and treatment campaign with the objective of eradicating the invasive mosquitoes from the Coachella Valley (Henke 2017). The objective for the 2018 season was to focus on inspections and sanitation of properties and on treatments limited to those properties that were infested or had potential to be infested. Despite extensive efforts and a large expenditure of resources, by the end of 2018 *Ae. aegypti* had spread into all cities in the Coachella Valley, with the exception of Desert Hot Springs.

Response to the first detection of *Ae. aegypti* in Coachella Valley through the end of 2017 was modeled after the California Department of Public Health's (CDPH), "Guidance for Surveillance and Response to Invasive *Aedes* Mosquitos and Locally Acquired Exotic Mosquito-borne Infections Transmitted by These Mosquitoes in California" plan. This strategy focused on the intense canvasing and treatment of all properties within a 450 foot radius from the initial detection property. Teams of 5 to 7 technicians would be assigned to areas where *Ae. aegypti* had been detected. These teams were rotated throughout the infested areas until all houses were inspected and treated. The staff also was expected to complete their routine work controlling *Culex* mosquitoes and Red Imported Fire ants (*Solenopsis invicta)*. The CDPH 450 foot radius strategy proved too labor intensive and unsustainable, especially with staff conducting other control activities. In 2018, the 450 foot intensive control efforts were limited to areas with properties that were found to have larval and or adult *Ae. aegypti* present. Also technicians were allowed the discretion of treating properties only when there was evidence of breeding or harborage, thus improving the efficiency and judicious use of CVMVCD resources while still serving the residents of the Coachella Valley.

Materials and Methods

Two full-time technicians and five seasonal staff were assigned to the invasive *Aedes* program and overseen by a Field Supervisor. Inspections and treatments were conducted at properties within a 450 foot radius only if the index property had the detection of *Ae. aegypti*. This new program consisted of inspecting properties where *Ae. aegypti* was detected as well as inspecting the surrounding 8 properties in what we referred to as "the rule of 9." This strategy improved response time to new detected properties and service requests by residents.

Surveillance for *Ae. aegypti* involved trapping adults using a BioGents (BG) Sentinel trap, CDC Autocidal Gravid Ovi-traps (AGO), and Ovi Cups attached to CO_2 trap stands. The detection of *Ae. aegypti* in these traps placed at specific locations directed where the field technicians should conduct the inspections and treatments. Detection of an *Ae. aegypti* location generated a point of interest (POI) in our computer system database displayed on the GIS ArcMap program. The POI's were categorized either as a 450 foot radius or "the rule of nine" by the program's Field Supervisor.

The properties inspected for *Ae. aegypti* were searched for items that had the potential for holding water and therefore serve as possible mosquito breeding sites. These items included buckets, plant saucers, tires, neglected fountains, containers holding plants, and many other features that may be found in yards. In addition, staff educated residents on invasive *Ae. aegypti* and the importance of dumping and draining water sources. Inspections and education focused on sanitation and source reduction encouraging property owners to get rid of unused containers or items that could hold water. Larvicides and adulticides were applied on properties if residents were receiving bites and/or had the presence of adult mosquitoes, or if there was an abundance of potential larval sources or resting areas for this mosquito. Sampling mosquitoes during inspections was conducted using dippers, concentrators, and turkey basters for larval mosquitoes, and an aspirator for flying adult mosquitoes.

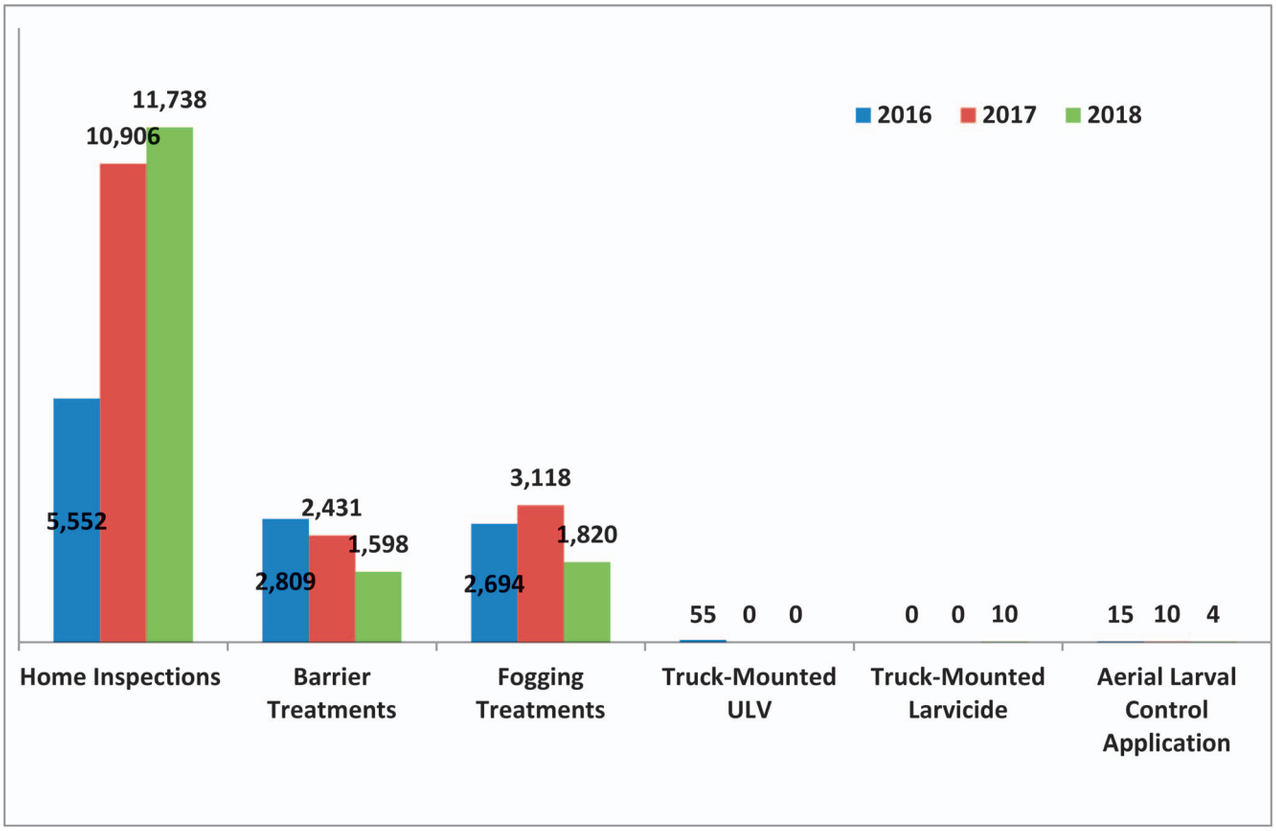

Figure 1.—Total number of annual home inspections, barrier and fogging treatmentsas well as truck-mounted larvicide and adulticide treatments, and aerial larval treatments against *Ae. aegypti* in 2016 through 2018 in the Coachella Valley.

Treatments conducted against *Ae. aegypti* were backpack barrier applications, ground Ultra-Low Volume (ULV) applications and larvicide treatments. The barrier applications were conducted to reduce the infestation of adult mosquitoes resting in the vegetation. The products used for barrier applications consisted of VectoBac WDG mixed with Demand CS using STIHL, and/or Maruyama backpacks. The mix was applied onto vegetation where adult mosquitoes rest and onto containers with potential to hold water for *Ae. aegypti* breeding. Ground ULV applications were conducted using a Longray backpack cold fogger (American Longray, Hayward, CA, USA) with Aqua Reslin applied at 0.007 pounds of permethrin per acre mixed in water at the ratio of 1:15. The applications assumed a 50 feet swath width.

Truck-mounted and aerial larvicide applications were performed in the month of September through October 2018 to reduce populations of *Ae. aegypti*. Four aerial applications were conducted in the City of Mecca during 03 to 0500 h on 15, 22 and 29 September, and 10 October 2018 using VectoBac WDG at a rate of 0.5 pounds per acre covering 1,233 acres. Ground applications were conducted in the City of Palm Springs using two types of spray equipment: the Dyna Fog LV-8 (Curtis Dyna Fog, Westfield, IN, USA) and A-1 Super Duty (A1 Mist Sprayers, Ponca, NE, USA). Two routes were utilized, one route for each spray equipment. Each route was done by two Field Technicians, one driving the pilot truck and the other the spray truck. VectoBac WDG was sprayed at 10 mph between 02 and 0700 h on 26 September and 3, 10 and 24 October 2018 at the rate of 0.5 pounds per acre covering an area of 1,059 acres assuming a swath width of 300 feet.

Results and Discussion

A total of 11,738 properties were inspected for *Ae. aegy*pti in 2018 , about 830 more than in 2017 and 5,000 more than 2016 (Figure 1). This increase in the number of inspections was due to staff focus only on properties with potential invasive *Aedes* and a reduction in unnecessary treatments. In 2018 barrier treatments and ground fogging (ULV) decreased compared to the two previous years. Although no truck-mounted adulticide applications were conducted in 2017 and 2018, a total of 55 such truck-mounted applications were done in 2016. In 2018 ten truck-mounted larvicide applications of Vectobac WDG were conducted compared to no such applications in 2016 and 2017 (Figure 1). In 2018, four aerial larval control applications using Vectobac WDG were conducted compared to ten such applications in

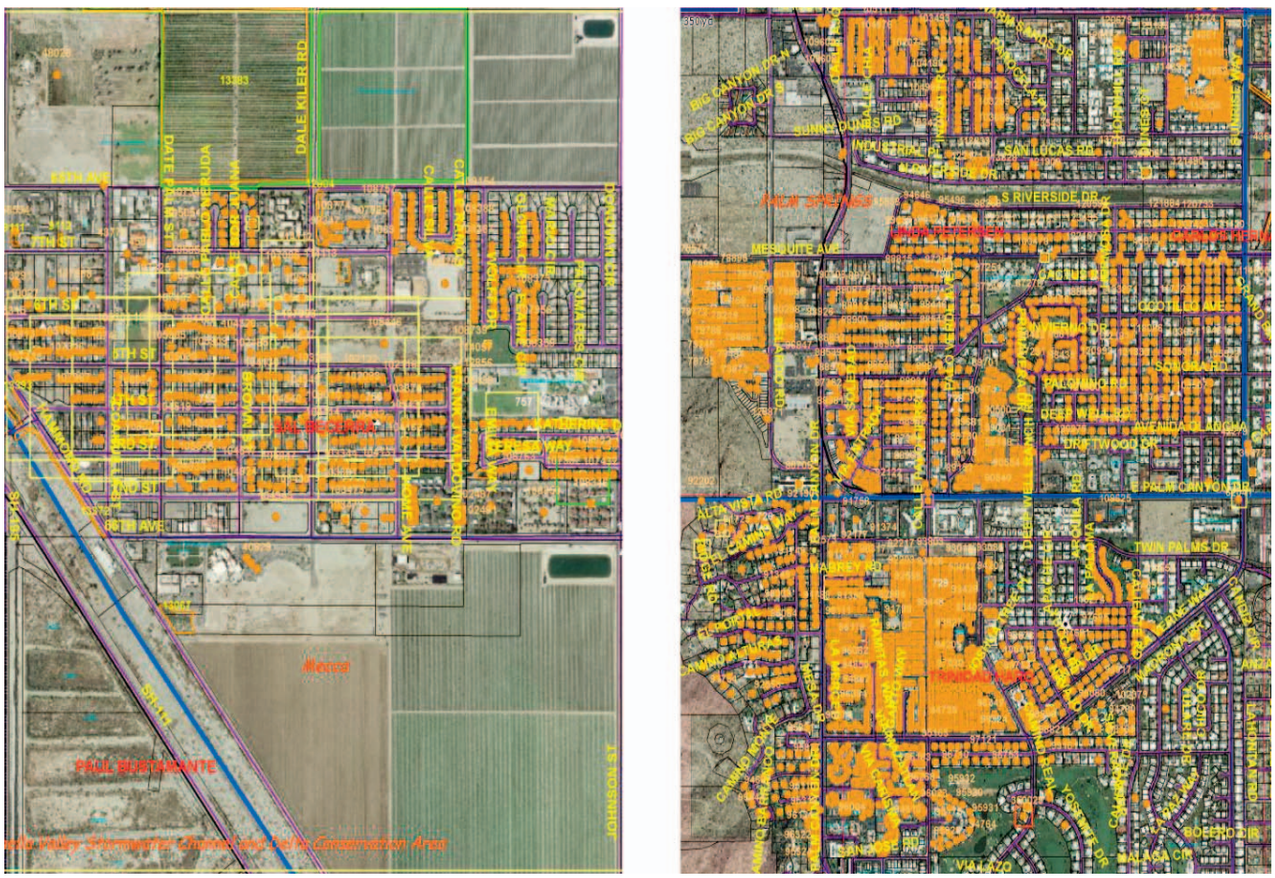

Figure 2.—Aerial and truck-mounted applications of VectoBac WDG in the cities of Mecca and Palm Springs in the fall of 2018. a (Left panel): aerial treatment ofMecca with 673 positive sites, and b (Right panel): truck-mounted treatment of Palm Springs with 1,979 positive. The yellow dots indicate individual parcels (properties) that have been inspected and/or treated and are in the computer system for history and reference.

2017 and fifteen applications in 2016 (Figure 1, 2). These applications were reflective of the number of mosquitoes collected in traps and findings by the field technicians of *Ae. aegypti* eggs, larvae, pupae and adults in inspection areas. The home inspections and public education conducted by field staff and focused messaging by public outreach may have helped residents to understand their responsibility in mosquito control and change their habits by managing small containers with the potential to hold water. Some examples of such activities included residents turning over containers , containers that had the potential to hold water being relocated away from irrigation run off or rain water, and large items such as water fountains or other water features not filled when not in use, and unwanted items discarded during or prior to inspections.

Conclusion

The invasive *Ae. aegypti* operations program in 2018 increased the staff's ability to respond efficiently to an increasing abundance of this mosquito throughout the Coachella Valley. The number of home inspections increased due to the operations team no longer following the California Department of Public Health's (CDPH), "Guidance for Surveillance and Response to Invasive *Aedes* Mosquitos and Locally Acquired Exotic Mosquito-borne Infections Transmitted by These Mosquitos in California" plan as there were no transmitted diseases. Previously we treated all properties regardless of breeding or harborage and this new program focused on positive inspections treating properties only when necessary. These changes improved the overall performance of field technicians and increased efficiency in controlling *Ae. aegypti* in the Coachella Valley.

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equipment, Kim Hung for mosquito identification and biology, Jennifer Henke for help planning the wide-area treatments using truck- and helicopter-mounted spray equipment, and Jill Oviatt for facilitating community outreach.

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Focal control efforts against West Nile and St. Louis encephalitis virus positive mosquitoes is key to limiting human cases in Coachella Valley

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Abstract

The detection of West Nile (WNV) and St. Louis encephalitis (SLEV) viruses in mosquito populations indicates a public health risk but does not guarantee infection and illness among human residents. Proactive steps taken by vector control involving all tools available lowers the risk of spillover transmission to humans. Herein, we outline mosquito control activities routinely utilized by the Coachella Valley Mosquito and Vector Control District (District) to mitigate or limit exposure of residents to virus-infected mosquitoes within communities once virus detection is confirmed. We present operational activities conducted during the 2018 mosquito season that includes the District's operational plan, communication between the District and affected residents, execution of the control plan, and its evaluation to improve future vector control operations.

Introduction

The Coachella Valley Mosquito and Vector Control District utilizes multiple approaches to control mosquito-borne viruses. Mosquito traps are deployed to estimate abundance, and samples tested to determine the presence of mosquito-borne arboviruses. Once virus is detected by the laboratory, operations initiate enhanced surveillance. A team of technicians conducts inspections and treatments of all larval production sites up to a 1-mile radius from the positive trap. In addition, a determination is made to conduct an aerial or ground ULV application based on the level of virus activity, mosquito abundance, and location. Rural areas, specifically along the Salton Sea shoreline, have limited access for ground ULV applications, so aerial applications provide a more effective control option (Lothrop et al. 2008). A residential/urban area provides road access to conduct ground ULV applications (Lothrop et al. 2007).

In addition to internal collaboration, a press release is sent to inform residents of virus activity and that control activities are being done in the area. Signs are posted informing residents of timeline and control activities within the area around the positive trap sites. Notifications are sent to the County Agricultural Commissioner informing of planned timeline, area to be treated, and products being used.

Materials and Methods

The detection of WNV/SLEV-infected mosquitoes by the laboratory spatially focuses the response across the District to eliminating the presence of positive mosquitoes. Surveillance activities are enhanced, where Operations Department supervisory staff ensures that field technicians inspect and treat all known mosquito sources within a mile radius of virus-positive trap locations. The full season for mosquito trapping is March 1 through November 30, with reduced trapping in the winter months. The location and timing of the positive pools are part of the Risk Model that the District uses to focus its operations and public outreach campaigns.

The area for aerial and ground ultra-low volume (ULV) applications are delineated using the ArcMap 2017 (ESRI, Redlands, CA) excluding environmentally sensitive areas that are not treated. A polygon is drawn in ArcMap around the target areas defining the specific aerial application area providing acreage, amount of product to be used, and total time needed to complete the treatment. Similarly, a polygon showing the planned ground ULV application route is drawn, showing the total length of the treatment area thereby allowing the estimation of time required completing the application, amount of product, and number of trucks with sprayers required to complete the assignment. The timing of applications is dependent on the mosquito activity, the weather, particularly the wind, and

Table 1.—Truck-mounted adulticide applications in the cities of Indian Wells, Mecca, and Palm Desert showing product used and total acreage treated per city in 2018 mosquito season[A]

Location	Adulticide Used	No. of Applications	Total Acres
Indian Wells, Palm Desert	Aqua-Reslin	6	1,980.08
	Evergreen 5-25	6	1,581.19
Mecca	Aqua-Reslin	12	2,514.17
		24	**6,075.44**

[A] The cities of Indian Wells and Palm Desert are contiguous and were treated on same routes.

the lowest amount of outdoor activity by residents in the area. Treatments are conducted either after sunset to midnight or from 2:00 to 6:00 am. Aerial applications were conducted by helicopter equipped with Micronair electric atomizers and the ground ULV applications were conducted by the London 18-20 and the Guardian 190 sprayers as previously demonstrated by Lothrop and others (2008).

Results and Discussion

For *Culex* mosquito surveillance, the District set CO_2 traps at 60 locations in the eastern rural valley every two weeks. The 53 locations in the urban areas were trapped weekly with CO_2 and gravid traps. Of the 352,184 mosquitoes captured, 140,529 were tested for arboviruses in 4,337 pooled samples using the RT-PCR methods described in Brault and others (2015). Samples were tested for WNV, SLEV and western equine encephalomyelitis virus (WEEV). In 2018, 24 samples were positive for WNV and 56 were positive for SLEV (6 were positive for both viruses). The areas within the district in 2018 where WNV/SLEV-positive mosquitoes were detected were in the cities of Indian Wells and Palm Desert, the town of Mecca, and surveillance sites along the shoreline of the Salton Sea. Truck-mounted ULV applications were conducted within the cities of Indian Wells, Palm Desert, and Mecca between 0200 to 0600 h using two teams, each with a pilot truck and spray truck on three consecutive nights. The cities of Indian Wells and Palm Desert each received a total of twelve truck-mounted applications: six of them were done using Aqua-Reslin (™); the other six were completed using Evergreen 5-25. A total of twelve truck-mounted applications were conducted in the town of Mecca using Aqua-Reslin (Table 1). Twelve aerial adulticide applications were conducted along the Salton Sea shoreline from 2100 to 2300 h, six using Aqua-Reslin, three using Scourge 18-54, and three using Evergreen 5-25 (Table 2).

Table 2.—Aerial adulticide applications on the Salton Sea shoreline showing product used and total acreage treated in 2018 mosquito season

Adulticide Used	No. of Applications	Total Acres
Aqua-Reslin	6	10,200
Scourge 18-54	3	6,900
Evergreen 5-25	3	5,100
Grand Total	**12**	**22,200**

Prompt response after detection of a positive sample impacts virus activity, and application of adulticide within a few days of such findings suppressed subsequent virus detection from two to six WNV-positive mosquito pools to none in as seen in the cities of Indian Wells, Mecca and Palm Desert (Figure 1 and 2). WNV-positive mosquito samples were first detected in the cities of Indian Wells and Palm Desert during week 23, persisted into week 24 and 25, and prompted truck-mounted applications using Evergreen 25-5. The applications were conducted on three consecutive nights of week 25. Following these applications, there was a dip in the number of WNV-positive samples in week 26, but virus activity rebounded during week 27, prompting another three consecutive nights of truck-mounted application of Aqua-Reslin (Figure 1a, Table 1). Four SLEV-positive mosquito samples were detected in the town of Mecca on week 30, triggering three consecutive nights of truck-mounted application using Aqua-Reslin. The following mosquito trapping detected only one SLEV-positive mosquito sample which was followed by three consecutive night truck-mounted treatments with the same product. Mosquito samples collected after the second round of treatments in Mecca showed no evidence of SLEV (Table 1, Figure 1b).

Utilizing the same approach to all locations where WNV/SLEV-positive mosquitoes were detected showed that the prompt focusing of adulticiding in locales with positive mosquitoes immediately yielded the intended results. Figure 1 illustrated this point by showing that an area of slightly more than 6,000 acres treated in twelve applications in the town of Mecca, twelve applications in the cities of Indian Wells, and Palm Desert with a combination of Aqua Reslin and Evergreen 25-5 using two teams each night eliminated WNV/SLEV-positive mosquitoes (Table 1, Figure 1).

The shoreline area to the north and west of the Salton Sea presented with several WNV/SLEV-positive mosquito pools cumulatively shown in Figure 2 and triggered immediate adulticide activities. The truck-mounted activities and the limited distribution of WNV in mosquitoes in 2018 showed an immediate interruption of transmission after initial adulticide activities during week 29 and 31. Aerial applications that followed in week 35 and 39 were able to eliminate WNV/SLEV-positive mosquitoes throughout the valley (Table 2, Figure 2). Prompt intervention changed the trajectory of virus activity and

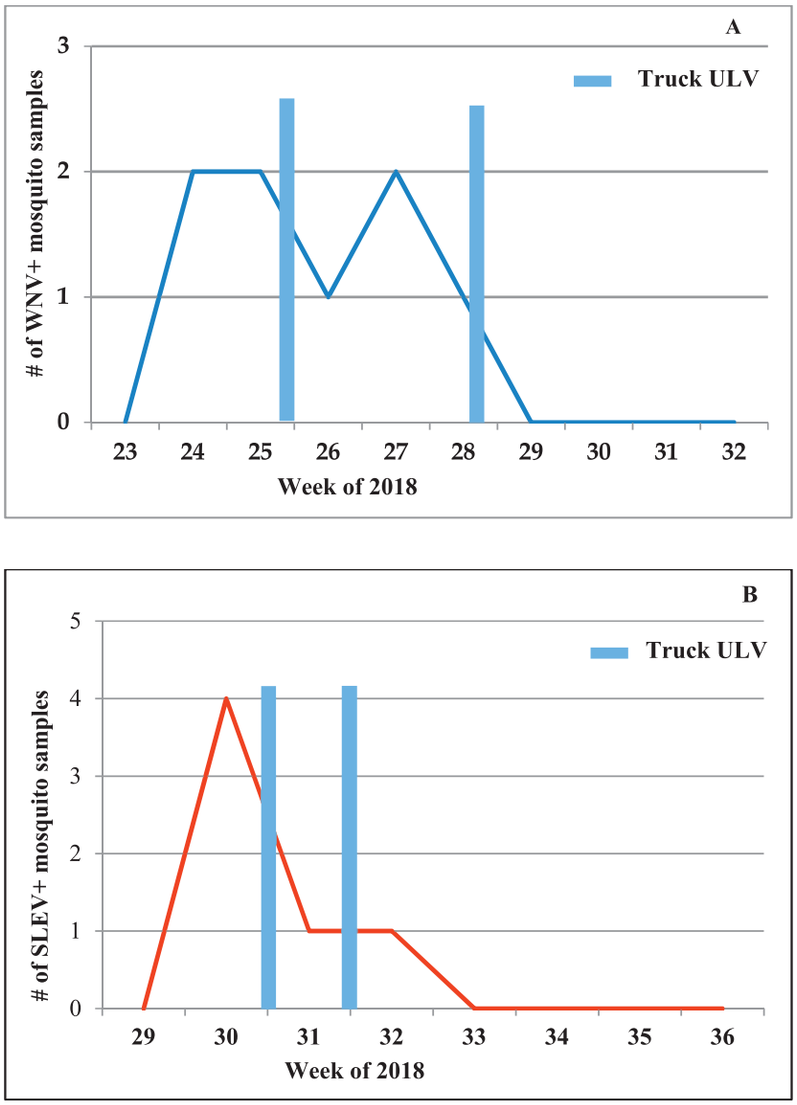

Figure 1.—a. Number of WNV-positive mosquito pools detected after two rounds of truck-mounted adulticide applications of Evergreen 5-25 and Aqua-Reslin in the cities of Indian Wells and Palm Desert; b. Number of SLEV-positive mosquito pools detected after two rounds of truck-mounted adulticide applications of Aqua-Reslin in the town of Mecca.

enhanced the mission of the District by minimizing the occurrence of human cases, with zero cases in the 2018 season.

Conclusion

Prompt response to WNV/SLEV-infected mosquitoes by conducting ultra-low volume adulticide applications, weather permitting, limiting the amplification of both. Such sustained control efforts coupled with intensive surveillance for infected mosquitoes limited human exposure to infection.

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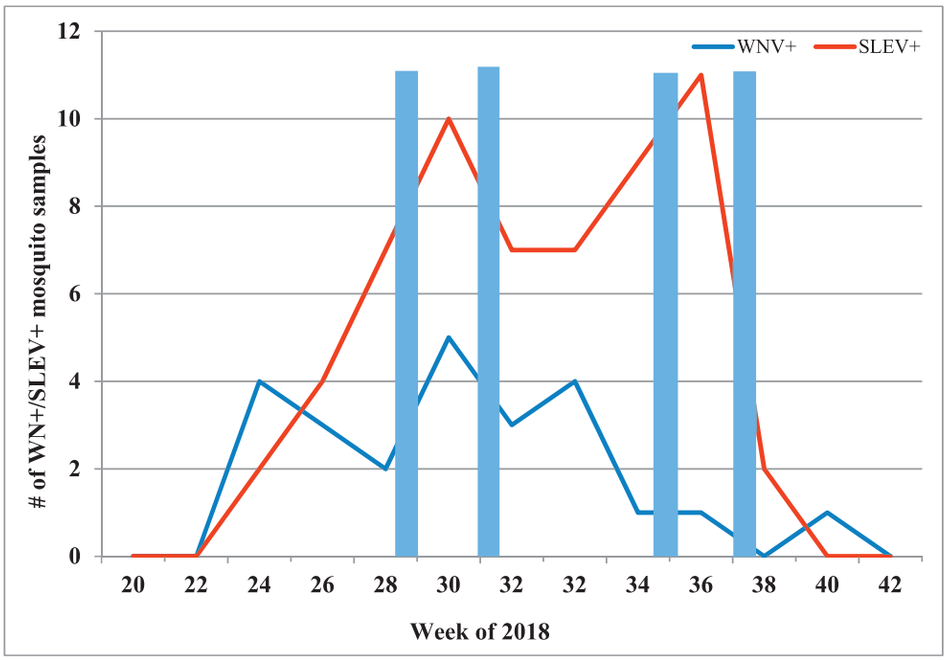

Figure 2.—Aerial application of Aqua-Reslin, Scourge 18-54, and Evergreen 5-25 applied to rural areas along the Salton Sea in 2018 and number of WNV- and SLEV-positive mosquito samples detected.

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Effectiveness of a wide area larviciding in the Coachella Valley

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Abstract

Aedes aegypti initially were detected in the Coachella Valley in May 2016. Since then, the Coachella Valley Mosquito and Vector Control District has conducted several missions of wide area larviciding for the control of mosquitoes in residential yards. In 2018 we made larvicide applications using truck-mounted A1 Super Duty and a Curtis DynaFog LV-8 spray equipment in Palm Springs. We examined the ability of droplets to reach the front and back yards upwind and downwind of the application. We found no significant difference in the mortality in bioassay cups based on the equipment used or the locations of the cups. We also monitored the adult population in Palm Springs and in Cathedral City where we did not make area-wide applications. We did find a reduction in the *Ae. aegypti* population during the third week of application which was maintained through the end of the trapping season, but not in the *Culex quinquefasciatus* population.

INTRODUCTION

Many districts in California have conducted *Aedes aegypti* control at the individual properties where they were found by inspecting thoroughly and making applicationsas needed. In 2016, the Coachella Valley Mosquito and Vector Control District (District) conducted repeated aerial applications of larvicide to one infested area, garnering good results in reducing the adult mosquito population (Henke 2017). The work was repeated in 2017 in three cities. Although there were few complaints of the use of pesticides, there were some residents who disliked the amount of noise that helicopter applications produced. The District also was interested in the ability to make applications to smaller areas than what was economically feasible using a helicopter and chose to explore area-wide truck larviciding applications.

The benefits of area-wide applications include coverage of a particular area, leading to a sustained reduction in the population. By conducting ground-based applications instead of aerial applications, District staff could allow some residents to 'opt out', if they could demonstrate that they were not permitting mosquitoes to breed on their property. Ground-based applications also permit more flexibility with start and stop times if weather conditions were not ideal. Based on work examining the swath width of different truck-based equipment in June 2018, we expected that the noise would similar or reduced as trucks would be making a single pass on each road.

Truck-based larviciding is not a commonly used technique in California. Anecdotal discussions indicate that most US based mosquito control operations had not applied larvicides to a wide-area prior to the local transmission of Zika virus in Miami (Peter DeChant, personal comunication). Due to its novelty, agencies were unsure of the performance of different equipment. In the current project, we compared adult mosquito abundance in an area treated by trucks equipped with either the A1 Super Duty or the Curtis DynaFog LV-8 over a 5 week periodto a similarly-sized area that was not treated by truck-based equipment.

MATERIALS AND METHODS

Two routes were selected in Palm Springs covering 1,059.44 acres (route 1: 536.78 acres; route 2: 522.66 acres). Applications of VectoBac WDG were made by trucks with either the A1 Super Duty or the Curtis DynaFog LV-8 fogging equipment at a rate of 0.5 lbs. per acre assuming a 200 ft. swath width. Applications were made on September 26, October 3, 10, and 24 between 04 and 0600h.

To examine the movement of the droplets, bioassay cups were placed at 28 residences (13 were downwind and 15 were upwind of the truck route; Figure 1). At each residence, one cup was placed in the following location the day before the application: front yard unobstructed; front yard obstructed (up to 50%); back yard unobstructed; and back yard obstructed. Cups were kept in place for at least one hour following the application. Cups were then retrieved, capped, and returned to the District. Cups were frozen over the weekend. They were allowed to thaw for 30 min before water and *Cx. quinquefasciatus (*Bakersfield –

Figure 1.—Example of locations where bioassay cups were deployed. Winds were consistently from the northwest during all applications. Houses were categorized as downwind or upwind. Cups were categorized as front open, front cover, back open, or back cover.

CQ1) larvae were added as described in Henke (2017). Mortality was checked at 4, 24, 48, 72, and 96 hours after water and larvae were added. Reports presented here are counts at 96 hours. Additional cups were placed outside of the application area and treated similarly to those in the treatment area as untreated controls. No mortality was seen in those cups. ANOVA was conducted using SYSTAT 13.

Ten BG Sentinel 2 traps each were set weekly in Palm Springs (area-wide treatment) and Cathedral City (no area-wide treatment). Traps were baited with dry ice and the BG lure and operated from 1400 to 1000 h the following morning. Mosquitoes were identified to species, sex, physiological state (females), and enumerated.

RESULTS

Although there was variability in mortality among the bioassay cups, there was not a significant difference based on the type of equipment used ($p = 0.164$), upwind or downwind ($p = 0.099$), or location within a yard ($p = 0.159$)

Table 1.—Average percent mortality for *Cx. quinquefasciatus* 15 larvae in cup bioassays by factor. No significant differences were found for equipment type ($p = 0.164$), the side of the street ($p = 0.099$), or location of the cup within a yard ($p = 0.159$)

Factor	Average Percent Mortality
Equipment: A1 Super Duty	93.7
Equipment: DynaFog LV-8	95.1
Upwind	93.9
Downwind	95.4
Location: front open	93.5
Location: front cover	95.3
Location: back open	91.0
Location: back cover	92.3

(Table 1). Droplets from both pieces of equipment were able to reach obstructed cups in backyards, causing mortality in the bioassay cups.

More *Ae. aegypti* were captured in Palm Springs than in Cathedral City until the third weekly application (Figure 2). The number of *Cx. quinquefasciatus* was similar in both locations throughout the application period (Figure 3). The number of *Cx. quinquefasciatus* collected per trap was greater than the number of *Ae. aegypti* .

DISCUSSION

Both the A1 Super Duty and the Curtis DynaFog LV-8 performed well in the Coachella Valley under dry conditions. Droplets moved over fences and houses into back yards, with most cups receiving enough droplets to produce

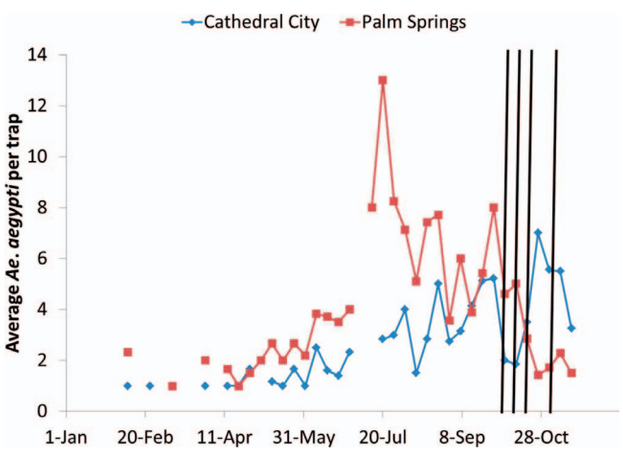

Figure 2.—Average number of *Ae. aegypti* per trap in Palm Springs (treated) and Cathedral City (untreated). Vertical black lines indicate the treatment dates.

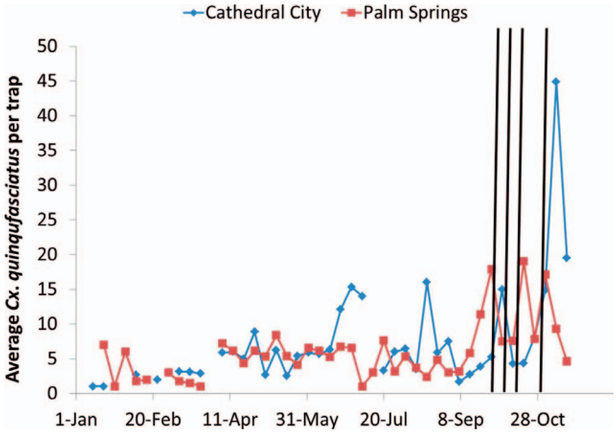

Figure 3.—Average number of *Cx. quinquefasciatus* per trap in Palm Springs (treated) and Cathedral City (untreated). Vertical black lines indicate the treatment dates.

mortality in mosquito larvae during bioassays. Although we expected to find lower mortality in the bioassay cups in the front yards of the upwind houses, mortality here was not significantly different from mortality in bioassay cups placed in other locations. This could be due to set-up of the equipment. The A1 Super Duty uses a powerful motor to spray droplets at an elevated pitch, potentially leading to carry of the application through the yards. The Curtis DynaFog LV-8 has two arrays with 4 nozzles each, and product may be pushed into the wind at low wind speeds, allowing for good coverage both upwind and downwind.

Reductions in adult *Ae. aegypti* mosquitoes were not seen until 3 weekly applications were made, indicating the delay in the reduction of adults when larval control products are applied. Counts were reduced to 2 per trap, well below the District threshold for area-wide treatments.. Although not significantly different, we believe that our applications led to a quicker decline in the population in Palm Springs than in the untreated area in Cathedral City. Similar reductions were not observed in the *Cx. quinquefasciatus* population; perhaps due to to the additional control measures used for *Culex* mosquitoes in the area – namely the application of long-term residual pesticides in the storm water system (such as Natular XRT and Altosid XR briquettes). Because these applications were continuous throughout the period of area-wide truck larviciding, no change in the population was expected.

Area-wide truck larviciding did provide some benefits to the residents compared with other control methods. Some residents reported less noise compared with applications using a helicopter made to the same area in 2017. The change to truck larviciding also permitted residents to choose to not receive treatment provided that they passed an inspection by District staff before the application ensuring that no mosquitoes or standing water was present. Only one resident requested and was granted this request; others rescinded their request upon learning more about the pesticide and the reason for the applications.

Truck larviciding was approximately as costly as aerial application of larvicides in this case. In 2017, approximately 78% of the area was treated by helicopter (the known area of activity was expanded in 2018) in 5.5 hours. With mixing and treatment, we completed the applications in 8 hours. Using District staff to complete early morning applications did mean that we incurred overtime costs and lost opportunities for staff to conduct other control activities, but we were able to be more flexible and responsive to our residents' needs. We plan to explore our future thresholds for applications to make use of both application methods.

ACKNOWLEDGEMENTS

A project of this size is impossible without a large amount of teamwork. We are grateful to the residents of Palm Springs who allowed us into their yards frequently to assess whether our treatments were effective. In addition to the authors, Jessica Arellano, Salvador Becerra, Paul Bustamante, Michael Esparza, Geneva Ginn, Oscar Guerrero, Fernando Gutierrez, Marc Kensington, Jess Lucia, Richard Ortiz, Jeff Rushing, Vincent Valenzuela, and Miguel Vargas, made applications, assisted with cup assays, and trapped mosquitoes for this study. Jill Oviatt coordinated the notifications for these applications, ensuring the residents were informed ahead of time. We had additional assistance from San Gabriel Valley Mosquito and Vector Control District who loaned us the Curtis DynaFog LV-8 and Adapco who loaned us the A1 Super Duty. The project benefited greatly from discussions and earlier evaluations with Peter DeChant from Valent BioSciences.

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Optimization of aerial larvicide applications: Two vs four rotary atomizers

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Introduction

In 2017 Placer Mosquito and Vector Control District evaluated both aerial characterizations and aerial applications of Vectobac 12AS®(*Bacillus thuringiensis israelensis* 11.61%, Valent Biosciences, Libertyville, IL) at 8 fl.oz./acre. Applications were made with a fixed wing Air Tractor 402, equipped with two rotary atomizers. During these evaluations, we documented large variations in the droplet density and bioassay cup mortality, with 'peaks and valleys' within a single swath width. The valleys in both data were particularly noted as being under the belly of the aircraft. These results indicated that the efficacy of our aerial larvicide applications could be increased by increasing the consistency of the applications along each swath width.

Based on our 2017 findings, the objectives of the current study were to determine if four rotary atomizers, instead of two, would, within a single swath width:, 1) produce a more consistent droplet spectrum and droplet size, 2) decrease the variation in droplet density, and 3) increase mortality in bioassay cups.

Methods

An Air Tractor 402 was equipped with two, and then four, Micronair AU5000® rotary atomizers (Micron Group, Herefordshire, U.K., Figure 1). Rotary atomizers are used for ultra low volume (ULV) or low volume (LV) pesticide applications. These particular rotary atomizers are wind driven by fan blades, which spin within a wire woven mesh producing the droplets as the material is pumped through it. This system was calibrated to disperse the target application rate of Vectobac 12AS ® at 8.0 fl.oz./acre. The calibration was based on an assumed swath width of 180 feet, an application speed of 135 mph, and application height of 30-35 feet.

Efficacy stations were placed every 10 feet in a straight line and marked as 0 through 250 feet. Each station had a Kromekote® card (CTI Paper, USA) mounted on a plastic compact disc case and a plastic bioassay cup with approximately 200 ml of water (Figure 2). The cards and cups were collected after each pass and replaced with new cards and cups. The aircraft flew perpendicular to the efficacy stations and into the wind. The aircraft made two passes with two atomizers, and two passes with four atomizers.

The Kromekote® cards were collected, scanned with a portable scanning system for evaluation of spray deposit distribution, and later analyzed using a USDA program called DepositScan. This program was used to measure the droplet spectrum and deposition density.

Spray deposition also was determined by bioassay. Deposition within the cups was first evaluated by introducing 20 laboratory-reared 3rd or early 4th instar pesticide-susceptible *Culex quinquefasciatus (CQ1)* mosquitoes to the 200 ml of water exposed during application and counting mortality after 24 h. Initial mortality was

Figure 1.—Rotary Atomizer (Micronair AU5000®) showing the mesh cage and fan blades

Figure 2.—Kromekote® card mounted a CD case and a bioassay cup filled with 200 ml of water.

100% in most of the cups. To further investigate possible variation among the bioassay cups, the contents were diluted to 50% by removing 100 ml of water from the initial collection and then adding 100 ml of clean deionized water to the cup.

Results and Discussion

A visual assessment of the DV50, as well as calculations of the relative droplet spectrum suggested that four rotary atomizers produced a more consistent droplet size and density than two rotary atomizers (Figure 3, 4, Table 1). The relative span describes the overall droplet size variation within a spray cloud.

The initial testing of the bioassay cups provided 100% mortality in almost all of the test cups for both the two and four atomizer applications (data not shown). When the water in the bioassay cups was diluted 50%, larval mortality from passes with two atomizers ranged from 0% to 100%, with an average mortality of 39%. Due to unforeseen complications, only one set of the two atomizers bioassay cups were tested. The first four atomizer application (pass A) produced mortality that ranged from 15% to 100%, with an average mortality of 89%, whereas the second four atomizer application (pass B) produced mortality that ranged from 0% to 100%, with an average mortality of 73%) (Figure 5, Table 2).

The relative droplet span as indicated in Table 1 showed that the four atomizers produced a more consistent droplet spectrum and droplet distribution than two atomizers. With a consistent droplet spectrum other variables may be adjusted such as the swath width, application height, and application rate to decrease or increase droplet deposition. Environmental conditions play a key role when character-

Table 1.—This chart shows the average DV10, DV50, DV90, and droplet density for each swath width (Pass). The chart also displays the average bioassay cup mortality

	Pass	DV10 (µm)	DV50 (µm)	DV90 (µm)	Droplet Density (drops/cm^2)	Avg. Mortality (50% dilution)*
Two Rototary Atomizers	2 (A)	151	337	471	3	39%
	2 (B)	120	209	308	3	–
Four Rotoary Atomizers	4 (A)	138	225	309	3	89%
	4 (B)	124	215	286	2	73%

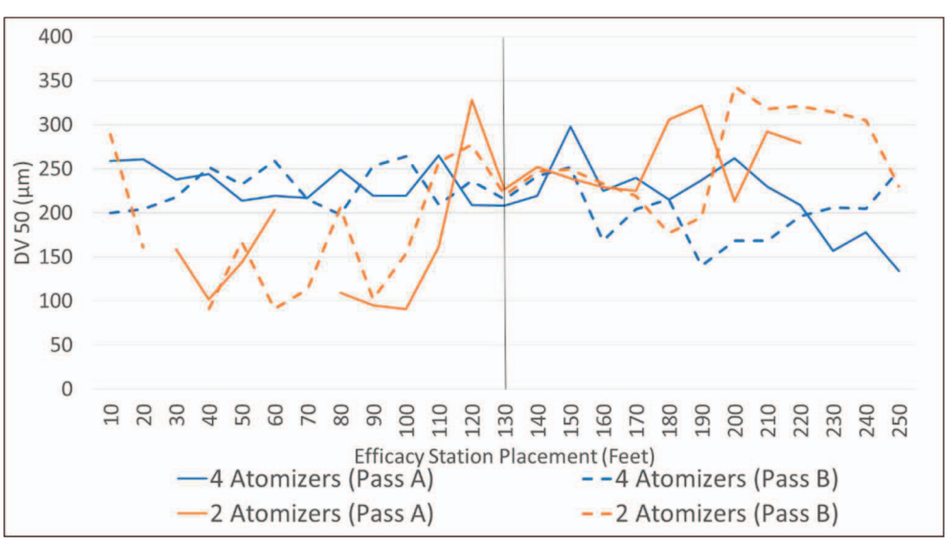

Figure 3.—Results for an April 17, 2018 aerial larvicide characterization of Vectobac 12AS®. This figure indicates the DV50 results for the swath width of four separate single passes, two of the passes were made with two rotatory atomizers and two passes were made with four rotary atomizers. The vertical black line indicates where the center of the aircraft passed over for each pass.

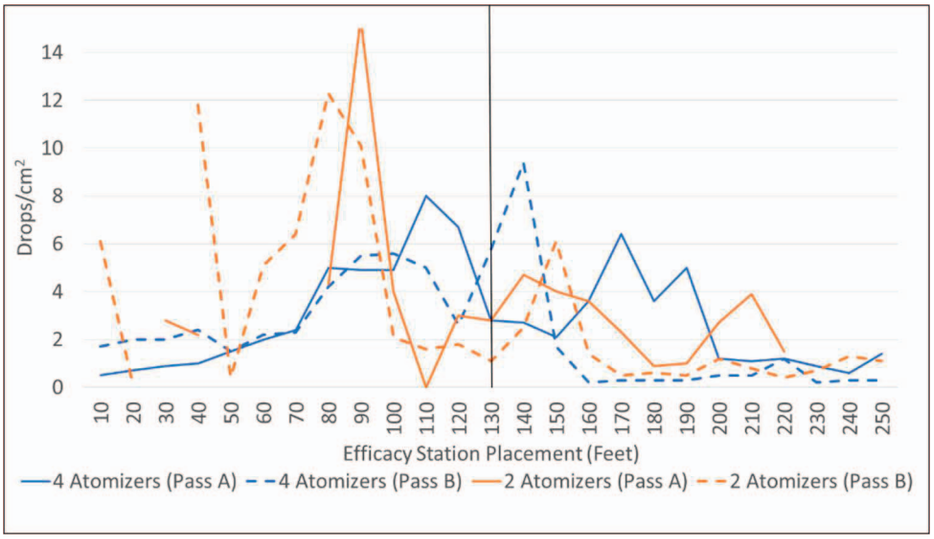

Figure 4.—Results for the April 17, 2018 aerial larvicide characterization of Vectobac 12AS®. This figure indicates the droplet density results for the swath width of four separate single passes, two of the passes were made with two rotatory atomizers and two passes were made with four rotary atomizers. The vertical black line indicates where the center of the aircraft passed over for each pass.

izing aircraft liquid larviciding. Some of these variables include wind direction, wind speed, and temperature. The day of this characterization, the wind direction and speed was moderately inconsistent which may have influenced droplet deposition and/or the droplet size spectrum among applications. Due to time, cost, and aircraft availability, the number of replicate passes per rotary atomizer setup was limited to two passes each. In the future, multiple passes on different days and environmental conditions should be completed to create a more robust data set.

Although this project only focused on the characterization and calibration data, we continually monitor the effects of how adding two additional atomizers (4 total) to the aircraft relate to mortality during field applications. Initial tests (data not shown) have demonstrated similar results to our characterization data, in that we were able to produce a more consistent droplet spectrum and deposition, but we are continuing to monitor the overall effects on larval mortality.

Conclusions

The April 17, 2018 characterization provided insight into how well our aerial larviciding applications were working. Although the results were not clear-cut, we believe that

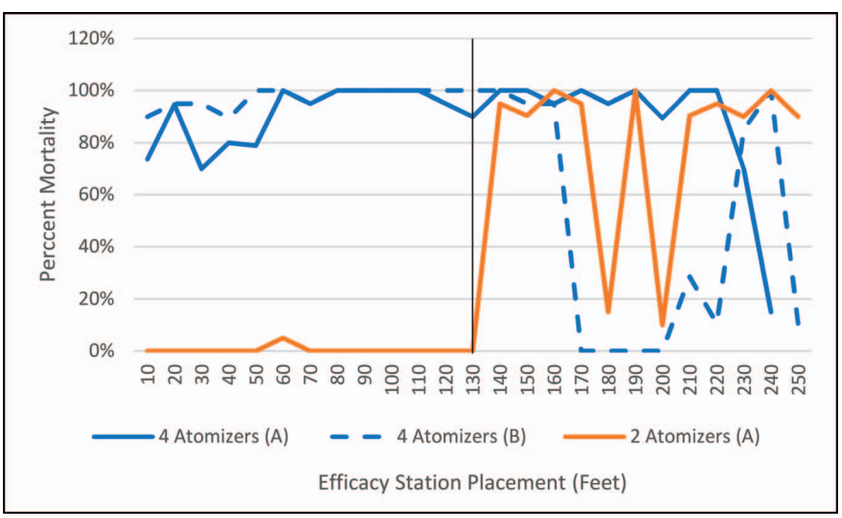

Figure 5.—Mortality results for the April 17, 2018 aerial larvicide characterization of Vectobac 12AS®. This figure indicates mosquito mortality (CQ1) for the swath width of three separate single passes, two of the passes were made with two rotatory atomizers and two passes were made with four rotary atomizers. The vertical black line indicates where the center of the aircraft passed over for each pass. The control cups for this data showed a percent mortality of 0% for the CQ1.

Table 2.—The relative span (RS) was calculated ((DV90-DV10)/DV50) for each card. This graph shows the overall difference between the smallest relative span and the largest relative span that was calculated for each pass. The difference shows that two rotary atomizers produced a wider spectrum of droplet sizes during the characterization than the four rotary atomizers produced.

	Pass	Min RS (Per Swath)	Max RS (Per Swath)	Average RS	Difference of Min RS & Max RS
Two Rototary Atomizers	2 (A)	0.6	2.3	0.87	1.7
	2 (B)	0.7	2	0.94	1.3
Four Rotarry Atomizers	4 (A)	0.6	1.3	0.77	0.7
	4 (B)	0.7	1.4	0.77	0.7

four rotatory atomizers may be optimized to reduce droplet spectrum variance and variation in comparison with two rotary atomizers. Part of this optimization may include future work with adjusting swath width and application rates. With a consistent droplet spectrum other variables may be adjusted such as the swath width, application height, and application rate to decrease or increase droplet deposition within a single application swath.

A new mosquito control product for California: A first look at DeltaGard efficacy in a rice growing habitat of Sacramento County

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Introduction

The Department of Pesticide Regulation has recently approved DeltaGard (Bayer, Pittsburg, PA), containing the active ingredient deltamethrin, for truck-mounted ultra-low volume (ULV) applications in California. A trial was performed in Sacramento County rice fields over the course of two nights to compare product efficacy and swath width between DeltaGard and Pyronyl Oil Concentrate #525 (Central Life Sciences, Schaumburg, IL; 5% pyrethrins, 25% Piperonyl Butoxide). Pyronyl #525 is a product routinely used by Sacramento-Yolo Mosquito and Vector Control District to control high adult mosquito abundance and in response to West Nile virus activity.

Material and Methods

A two-night trial was conducted in conventional rice fields north of Natomas, CA. A total of four transects, perpendicular to the spray path were established. The transects were grouped in tandem so that two transects could be treated without product drift to the other two transects. The products were applied in a cross-over design where on the first night one set of transects was treated with DeltaGard and the other set was treated with Pyronyl #525, and on the second night products were rotated to the opposite transects; therefore, no transect received the same treatment twice. Each transect was set with sentinel cages containing 25 mosquitoes per cage and droplet impingers at 50, 100, 150, 200, and 300 foot intervals from the spray path. The sentinel cages contained both *Culex pipiens* complex pyrethrin/pyrethroid susceptible (CQ1 colony) and resistant (WCP, recent colony started in 2017 from mosquitoes collected in Woodland, CA) populations, and *Culex tarsalis* susceptible (KNWR colony) and resistant (District 108 field-collected) populations. Products were applied using truck-mounted ULV Foggers (London Foggers, Minneapolis, MN; model #XKE), at a rate of 0.8 fl oz/acre for Pyronyl #525 and 0.67 fl oz/acre for DeltaGard. Trucks were driven at 10 mph.

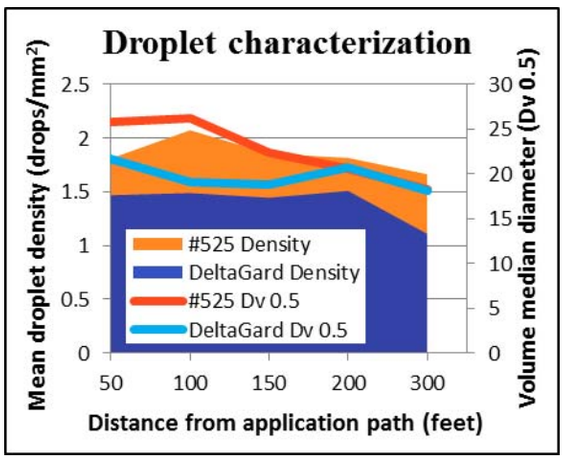

Figure 1.—Droplet size and density were calculated by DropVision (LeadingEdge, Fletcher, NC).

Results and Discussion

The mean droplet size and density (Fig. 1) was within label specification for both products at all transect intervals. Overall, DeltaGard and Pyronyl #525 efficacy against susceptible mosquitoes (CQ1 and KNWR) was similar resulting in nearly complete mortality at all sampling points (Figure 2). However, DeltaGard appeared to outperform Pyronyl #525 in resistant populations (Figure 2), indicating that this product may serve as an important rotational product in the battle against pyrethrin and pyrethroid resistance. One observational note regarding the application of undiluted DeltaGard was marked clogging of the ULV fogger filter. In an attempt to avoid clogging, all ULV foggers that apply DeltaGard will be equipped with flushing systems. Additionally, work is planned to evaluate whether a larger micron filter and/or dilution of the product will improve the clogging.

Conclusion

DeltaGard became available for truck-mounted ULV mosquito control operations in 2018. The product performed well against wild caught and colony mosquitoes

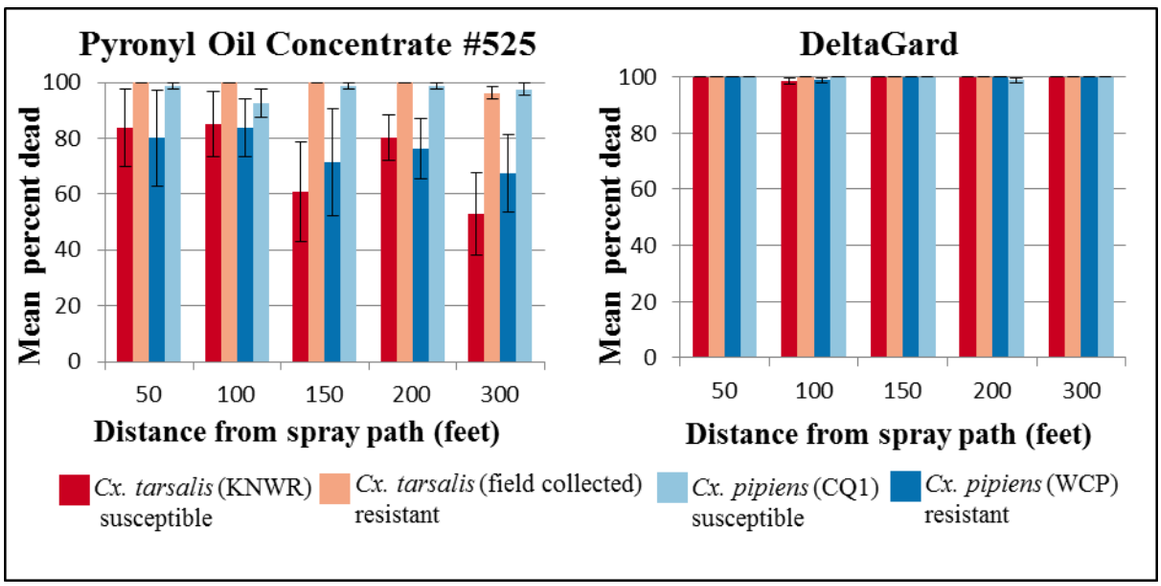

Figure 2.—Sentinel cage data from a rice field truck mounted ULV trial comparing Pyronyl Oil Concentrate #525 and DeltaGard. Presented is the mean percent mortality by species for both of the products tested, the error bars represent the standard error of the mean.

that exhibited pyrethroid resistance. This trial demonstrated that DeltaGard is a promising mosquito control product for California, where insecticide resistance in mosquitoes is a growing problem.

Acknowledgments

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Evaluation of Vectobac WDG use in Wide Area Larvicide Spraying for the control of *Culex pipiens* in urban environments

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Introduction

The Sacramento-Yolo Mosquito and Vector Control District (District) is in the process of evaluating the use Vectobac WDG as a control response to West Nile virus positive *Culex pipiens* pools through Wide Area Larvicide Spraying (WALS) in urban environments. Targeting areas that historically have high *Culex pipiens* populations with WALS may enable the treatment of numerous cryptic sources that might otherwise go unnoticed and untreated may have a significant impact on adult populations. WALS applications deliver Vectobac WDG in Low Volume (LV) droplets to normal and cryptic sources.

Methods and Materials

The District used two truck mounted fogging units, the A-1 Super Duty with AU 5000 atomizers and the Guardian 190 G4. Initial trials focused on optimizing application methodologies to treat cryptic sources with a dose high enough to achieve larval mortality. Weather stations (Kestrel 5500 weather meter) were used to monitor inversion and wind speeds in trial areas to determine the best time to use WALS. Spray routes were based on weather readings, utilizing wind direction to achieve the best coverage during application. Plastic cups were placed above ground under different cover (no, partial, and full

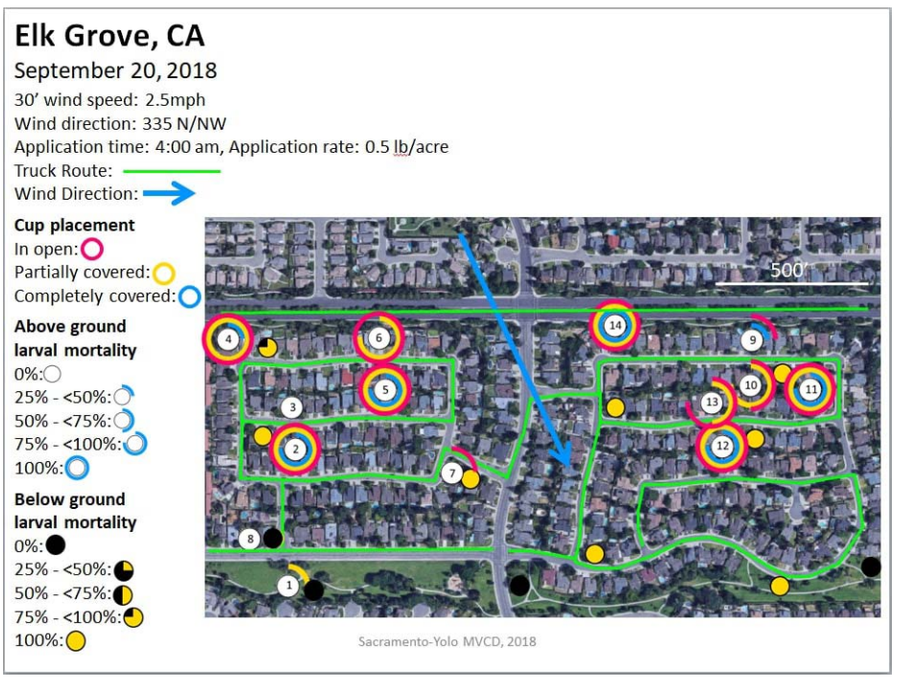

Figure 1.—WALS treatment of Elk Grove CA performed on 9/20/2018. Green lines indicate truck route, while the blue arrow indicates wind direction. Colored circles correspond to cup placement as open, partial or complete with the amount of the circle indicating mortality achieved. Below ground mortality indicated by black circles over layed with yellow, amount of the circle that is yellow corresponds to the percentage of mortality achieved.

cover) and underground in catch basins at various lengths upwind and downwind of the spray route to capture Vectobac WDG droplets that reached the cups. Routes were sprayed at a rate of 8 dry ounces of Vectobac WDG per acre mixed in ¼ gallon of water per acre. To determine larval mortality, field-exposed cups were filled with water and 20 CQ1 colony *Culex quinquefasciatus* mosquitoes added. Larval mortality was monitored for 48 hours.

Results and Discussion

Dispersal of Vectobac WDG through WALS application consistently attained at least a 300ft swath from the spray route, delivering a high enough dose to achieve almost 100% mortality in bioassays using susceptible CQ1 larvae. As seen in the Elk Grove treatment route (Figure 1) the target area received acceptable coverage, with half of the open and partial placement cups recording 100% mortality. Additionally, half of the completely covered cups recorded at least 25%-50% mortality. The Elk Grove treatment also demonstrated a high mortality rate in cups placed in catch basins, with over half of the test sites achieving 100% mortality. Treatment over large urban areas was fast, effective and achieved with minimal manpower, thereby allowing the District to treat many large and cryptic sources.

When coupled with predictive modeling to target historic areas of high adult populations, WALS may be used to proactively minimize adult mosquito populations in urban areas.

Conclusion

The District will continue to evaluate the potential use of WALS in urban areas. More data still needs to be collected to find the optimum rate of Vectobac WDG for these treatments. The District will be using heat maps to target areas of historically high adult abundance and utilize WALS applications to determine if there is a notable decline of adult mosquitoes in those communities.

Analyzing the movement of data with heat maps

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Introduction

The Sacramento-Yolo Mosquito and Vector Control District (District) has been utilizing geographic information systems (GIS) as a framework for managing and analyzing spatial and temporal data. The District has been using GIS to create heat maps that provide the ability to analyze the density, spatial distribution and movement of datasets such as West Nile virus (WNV) activity, mosquito abundance, dead birds, and service requests. Furthermore, these datasets can be superimposed with treated areas allowing the District to analyze and draw insights for its control efforts. By viewing, identifying and interpreting seasonal trends, models can be created to predict where WNV and mosquito abundance may originate and spread to throughout the season.

Methods

Using ArcGIS Desktop and ArcGIS Online, the District analyzed the timing, densities and spatial distributions of data sets to better understand patterns and trends. Heat map symbology was used to display spatial density while enabling time settings allowed the District to visualize the movement of data for certain time periods. Interactive heat maps were created to display current and historical data. This allowed the District to map data on weekly basis or cumulatively throughout the season.

Results and Discussion

By analyzing changes in the dispersion of female mosquito abundance and WNV detection, the District has identified areas of interest that had consistent patterns over the past three years. By identifying areas with historically high female abundance in addition to tracking specific mosquito species, the District can take a proactive approach and target these areas once activity is detected in an attempt to prevent the spread and surge of abundance.

Conclusion

Mapping and tracking changes in data has become a valuable tool at the District. By utilizing interactive heat maps, the District will have a better understanding of when and where activity occurs. Once an area of interest has been identified, the District will target these areas with Wide Area Larvicide Sprays (WALS™) in an effort to decrease the adult mosquito emergence.

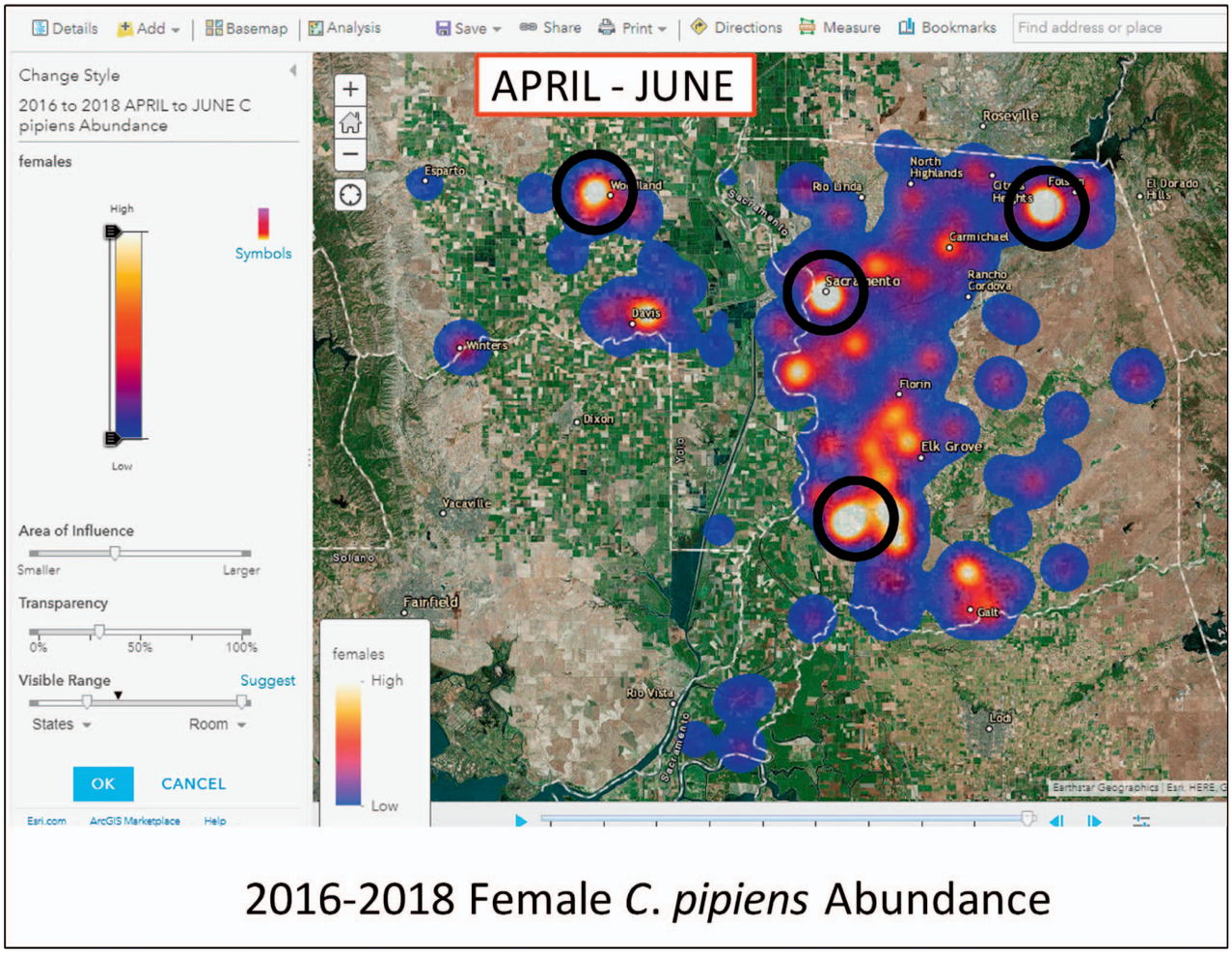

Figure.—Analysis of *Culex pipiens* female abundance from 2016 to 2018 from April to June. Four locations within the District have been identified as historically high abundance areas.

Using mammal tracking techniques to determine species identity and activity in bait stations.

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Abstract

Integrated Pest Management (IPM) is a key component of modern vector control that focuses on long-term prevention of pests or their damage through a combination of biological control methods, habitat manipulation, modification of cultural practices, and judicious application of pesticides. Successfully implementing an IPM strategy requires correctly identifying the pest and determining the best strategy to minimize non-target impacts. We have developed a modified mammal tracking technique for use in bait stations that may allow technicians to determine if stations are being utilized by the target species, how heavily the stations are being utilized, and whether bait avoidance is occurring. We also demonstrate potential research uses for the tracking technique including preliminary data on roof rat (*Rattus rattus*) behavior in creek baiting programs. This is a low cost technique that can be utilized by both pest control operations and public health agencies.

INTRODUCTION

Integrated Pest Management (IPM) is defined as "an ecosystem-based strategy that focuses on long-term prevention of pests or their damage through a combination of techniques such as biological control, habitat manipulation, modification of cultural practices, and use of resistant varieties (UCANR)." IPM also includes the judicious application of pesticides in a way intended to minimize risk to human health, non-target organisms, and the environment. This requires correctly identifying the range and distribution of the target, determining if there are non-target species present, and whether there is a risk for these non-targets to become exposed to the chemical control (Kogan 1998). Techniques to reduce non-target exposure to pesticides may include restricting application times to periods when only the target species is present, applying chemicals that have species specific effects, or restricting access to the chemical by placing it in an area that is only accessible to the target species.

Bait stations are utilized broadly throughout the world as a means to distribute rodenticides to manage rodent populations while limiting non-target access to the control method. Determining the activity level of a station is generally dependent on looking for teeth marks on the bait, or feces or urine marks in and around the bait station. While such markings are useful for determining activity, they do not provide sufficient detail to identify the species visiting the station. In addition, rodents may visit bait stations without sampling the bait which may give the false impression that there is no rodent activity in the area. While wildlife cameras are considered the gold standard for species identification, they may be cost-prohibitive or impractical to deploy. The inability to determine the species entering the station, or to definitively identify whether the station is being visited, may lead to inefficient or inappropriate distribution of rodenticides.

Animal tracks are frequently used by biologists as a means to determine the presence and identity of the animal community they study. The size of the print, number of toes, structure of the foot, and gait are often species or clade specific and have been well characterized in the scientific literature (Russell et al. 2009). We have modified these tracking techniques for use in bait stations in order to develop a technique to determine whether stations are appropriately placed, and whether the species utilizing the stations are the intended target.

MATERIALS AND METHODS

A template of the inside bottom of a Protecta LP bait station (Bell Laboratories, Madison, WI, 53704) was created out of heavy paper stock. This template was then traced onto white, opaque, self-adhesive paper (Con-Tact Brand, Pomona, CA, 91766) and cut to shape to form the track pad. The inside floor of the bait station was briefly wiped with a paper towel to remove soil build-up, the backing of the self-adhesive paper tracing was removed, and the tracing was applied to floor of the bait station. Approximately 4 ml (about 1 tsp) of a 1:1.5 solution of mineral oil to black marking chalk (Irwin Tools, Southington, CT, 06489) was placed on the first 3 cm of the self-adhesive paper at both entrances (see Figure 1). Stations were baited with Detex non-toxic monitoring bait cubes (Bell Laboratories, Madison, WI, 53704) and/or a rodent seed mix (Kaytee, Chilton, WI, 53014) where appropriate (specifics detailed below). Prints were identified using

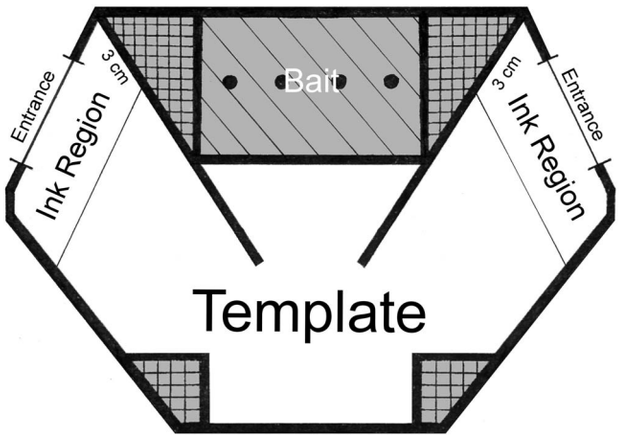

Figure 1.—Diagram showing the inside of the bait station with placement of the Con-tact paper and ink within.

Peterson Field Guide to Animal Tracks (Murie and Elbroch, 2005) and various online resources.

Determining specificity of the track pads

Bait stations loaded with track pads were deployed in three locations within San Mateo County: a dense vegetative area along the western border of the Cañada Cove Mobile Home Park in Half Moon Bay, CA (37°25′40″N, 122°25′37.1″W), an off-trail area in Water Dog Lake Park in Belmont, CA (37°30′31.59″N, 122°18′57.78″W), and inside a dry sanitary vault on San Mateo Ave and E 4th street in the City of San Mateo (37°33′49.98″N, 122°19′25.38″W). Deployment occurred on January 17 and April 20, 2018 at the Half Moon Bay site, February 15, 2018 at the Belmont site, and on February 13, 2018 at the City of San Mateo site. Each bait station was paired with a traditional tracking tube constructed of 4 inch PVC pipe outfitted with self-adhesive Con-tact paper and mineral oil/chalk mixture. A wildlife camera (Bushnell, Overland Park, KS, 66214) was deployed concurrently at the Half Moon Bay and Belmont sites but not at the City of San Mateo site. Bait stations and tracking tubes were loaded with Detex bait cubes and removed after 24 hours. Track pads were photographed and the tracks were identified to species. Results were compared to the camera footage in order to determine if the tracks matched the camera images.

Determining the sensitivity of the track pads

Starting May 31, 2018, bait stations were deployed along San Mateo creek (37°34′10.82″N, 122°18′58.19″W), Laurel creek (37°32′8.68″N, 122°17′48.81″W), and Leslie creek (37°33′21.53″N, 122°18′37.71″W), in San Mateo County, CA as part of a yearly rodent control program. The neighborhoods near these creeks historically report high levels of rodent activity and represent a high proportion of rodent complaints issued to the San Mateo County Mosquito and Vector Control District. All bait stations were stocked with four 28g blocks of Contrac All Weather BLOX (bromadiolone 0.005%) (Bell Laboratories, Madison, WI, 53704) which was refreshed monthly. A total of 150 bait stations were deployed; 62 stations at San Mateo creek, 47 at Laurel creek, and 41 at Leslie creek. Of these, 74 (25 at San Mateo and Laurel creek, and 24 at Leslie creek), were chosen by random selection and outfitted with a track pad. The track pads were checked each week for three weeks, replaced if damaged, and records were taken on the presence of prints, gnaw marks, urine and feces. If tracks were present on the pad, the pad was removed, covered with wax paper, attached to a foam board for transport back to the laboratory, and replaced with a fresh one. Positive track pads were brought back to the district where they were photographed and the tracks identified to species. A paired-T test was used to determine if track pads were identifying changes in recent activity significantly more often than looking at the combination of gnaw marks/urine/feces alone.

Application of track pad method – evaluating trends in rodent behavior

During the rodent control study outlined above, binary data (yes/no) was collected on ecological variables connected to each bait station's location that may influence the attractiveness of that station to commensal rodents (predominantly mice and roof rats (*Rattus rattus)*. These factors are listed in Table 1. We did not evaluate distance from the water edge as there was not enough variation between bait stations to be statistically evaluated. Binary data was recorded only once for each station and only during the first collection period. This information was then used to create a logistic regression model. The dependent variable was station activity. A station was considered active if rat and mouse prints were visible on the pads (Figure 2), and inactive if there were no prints or the prints were from non-target species (e.g. insects or reptiles). Variables were analyzed separately and those with a p-value of 0.2 or less and/or that were biologically plausible were included in an ANOVA Analysis of Deviance to determine the best fit model.

RESULTS

Specificity

At the Half Moon Bay site, mouse tracks were clearly visible on the tracking pad and mouse entry into the station was recorded by the wildlife camera. No other species were recorded entering the bait station and no other tracks were visible on the track pad. On the follow-up date, there were no tracks on the pad and the wildlife camera showed a mouse investigating the bait station but not entering. At the Belmont, CA site, the track pad had prints of both woodrats (*Neotoma fuscipes*) and mice (*Peromyscus* spp.) and the wildlife camera had pictures of both species. At the City of San Mateo site, the track pad inside the bait station did not show any evidence of activity but the tracking tube deployed in the same vault picked up a clear rat track. No bait was taken and no urine or feces were present.

Table 1.—List of ecological factors evaluated for impact on commensal rodent behavior in bait stations placed along three creeks in San Mateo County, CA, 2018.

Factor	Description of bait station location
Against Building	Set against the wall of a building or other structure (e.g. a shed).
10 feet from a Building	Set within 3 meters (10 feet) of a building or other structure.
Garbage	Set within 1.5 meters (5 feet) of a pile of garbage (including dumpsters).
Human Activity	Set in or adjacent to an area with frequent human activity (such as a garden, a hiking trail or a park).
Foliage patch	Set in a patch of foliage that is not connected to any other source of foliage cover.
Contiguous foliage	Set in a region with foliage that is contiguous over 6 or more horizontal meters (20 feet or more horizontal feet).
Ivy	The foliage surrounding the bait station is ivy.
Dense Grass	The foliage surrounding the bait station is dense grass.
Steep	Set on a steeply graded surface (30 degrees or greater).
Deep Shade	Set under cover such that the sun never directly shines on it (including dappled light).
Fruit trees	Set within 3 meters (10 feet) of fruit trees or other sources of cultivated foods.
Exposed	Set so completely exposed with little to no shade or coverage.

Sensitivity results

Track pads detected changes in activity more often than looking at the combination of gnaw marks/urine marks/feces presence, but these differences were not significant (Paired t-test: 95% confidence= -0.806, 2.58; p=0.261; df=8). In the most extreme case, track pads were able to detect activity in four additional bait stations compared to looking at gnaw marks/urine/feces alone (Table 2). Bait avoidance was detected four times during the three week period (one station at Leslie creek and three stations at Laurel creek), and activity after the bait had been consumed was detected in three stations (one station at San Mateo creek and two stations at Laurel creek). None of these events were detected by looking at gnaw marks/urine/feces presence alone.

Figure 2.—A comparison of mouse tracks (top) and rat tracks (bottom) recorded on track pads placed inside bait stations set along creeks in San Mateo County, CA in 2018

At San Mateo creek, 12 stations out of 25 (48%) were active within the study period. Of these, 11 out of the 12 (91.6%) had rat tracks (44% of the total stations monitored) and one had mouse tracks. At Laurel creek, 13 stations out of 25 (52%) were active. Of these, all 13 had rat tracks with 3 stations having both rat and mouse tracks. Three stations out of the 13 had no gnaw marks on the bait and no feces in the station. At Leslie creek, five stations out of 24 (20.8%) were active. Of these, one had rat tracks and four had mouse tracks. Rat activity was only detectable on the track pad; there was no sign of gnawing on the bait and no feces within the station. Three stations had tracks from non-target species including lizards and a California newt (*Taricha torosa*). Insects (predominantly European earwigs (*Forficula auricularia*) and common pillbugs (*Armadillidium vulgaris*) also left detectable tracks and were found most frequently in the stations at Leslie creek and least frequently at San Mateo creek. Slugs (Families Limacidae and Agriolimacidae, *Ariolimax californicus*) were found most frequently in bait stations at Laurel creek and least frequently at Leslie creek.

Table 2.—A comparison of the number of bait stations placed along three creeks in San Mateo County, CA, 2018, that were considered active using either track pads or a combination of urine/feces presence and gnaw markings.

Creek Name	Method of Determination	Activity Week 1	Activity Week 2	Activity Week 3
San Mateo	Gnaw/Urine/Feces	2	7	5
	Track Pad	3	8	5
Laurel	Gnaw/Urine/Feces	3	5	6
	Track Pad	5	7	8
Leslie	Gnaw/Urine/Feces	1	2	4
	Track Pad	1	2	8

Table 3.—ANOVA results for ecological variables that may impact commensal rodent behavior recorded for bait stations set along three creeks located in San Mateo County, CA, 2018

Factor	Estimate	Std. Error	P-value
Against Building	-15.15	1455.39	0.99
10 feet from a Building	-1.46	1.06	0.013*
Garbage	-1.25	1.12	0.26
Human Activity	-1.46	1.11	0.19*
Foliage patch	-0.27	0.9	0.76
Contiguous foliage	1.18	0.49	0.017*
Ivy	0.23	0.49	0.63
Grass	-0.69	1.18	0.557
Steep	0.17	0.804	0.835
Deep Shade	0.04	0.591	0.943
Fruit trees	-0.91	0.84	0.278
Exposed	-0.74	0.512	0.148*

* Indicates the variable had a p-value of 0.2 or less which may indicate a significant effect on rodent behavior.

Results of Rodent Behavioral Study – Application of Track Pad Method

Variables with a p-value of 0.2 or less included: 10 feet from a building, human activity, contiguous foliage, and exposed (see Table 3). The ANOVA determined that activity in the bait stations was best explained by the station being set within 10 feet of a building and within contiguous foliage (p=0.021, AIC=88.2).

DISCUSSION

The adoption of a robust IPM program is being increasingly emphasized due to the environmental impact the improper usage of rodenticides can have (Thomas et al. 2011; DuVall et al. 1989). While it may be difficult or impossible to completely avoid using rodenticides, emphasis should be placed on minimizing the risk to non-targets as much as possible. Track pads may be used as a tool to identify the species in a given area and determine the risk to non-targets before chemical controls are applied.

The track pads were highly effective at differentiating between different clades of rodents (e.g. rats and mice) but related clades (e.g. commensal rats and woodrats) were difficult to differentiate. In addition, high traffic into and out of stations may cause tracks to extensively overlap, obliterating diagnostic details which may make identification difficult. While track pads are effective, additional environmental clues (such as habitat, nests, burrows, chew marks, urine markings or feces) should be examined in order to accurately identify the species present in sensitive areas. Lizards were the most common non-targets detected within the bait stations, particularly at Leslie creek. It is possible the large number of insects within the stations may have attracted insectivorous species. Previous studies have shown that rodenticides may bioaccumulate in slugs (Alomar et al. 2018), however the population impact of secondary poisoning on insectivorous species feeding on contaminated arthropods has not been evaluated.

Track pads were highly sensitive to changes in rodent activity and this sensitivity may be increased through checking the track pads more frequently than the once per week schedule we used in this study. The track pads were able to detect multiple instances of bait avoidance, which was not detectable by looking at gnaw marks/feces/urine alone. In addition, multiple instances of continued activity after the bait had been consumed was also recorded. Highly active stations, even without the presence of bait, may indicate a large population of commensal rodents in the area, or it may indicate that sub-lethal doses are being consumed. In both of these cases, alterations to the control method should be considered.

Track pads showed potential for usage in characterizing commensal rodent behavior. Our preliminary data suggested that stations placed within 10 feet of a human dwelling and in a region with contiguous foliage were predictors for detecting activity on the track pad. These findings are consistent with previous literature which suggested that rats are behaviorally tied to human structures (Feng and Himsworth 2014) and that they prefer to remain under cover (Dutson 1974). These findings are preliminary, but the technique demonstrated may lead to further studies characterizing rodent behavior and improved methods in targeting commensal rodents. Additional work needs to be done to determine if our results are consistent between regions, seasons, and years.

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Direct mail, text messages, and an impossible goal - Resolving 2,000 unmaintained swimming pools in three months

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Abstract

Through a new program, the San Gabriel Valley Mosquito and Vector Control District was able to reduce the amount of staff and time needed to address unmaintained swimming pools. The program reduced the amount of physical interaction between residents and District staff by using a combination of direct mail and text message confirmation to motivate residents to fix issues on their own.

In June of 2017, the District received its first ever list of swimming pools collected by aerial surveillance. The list contained photos of 3,569 unmaintained swimming pools within the 246 square mile district. The District's eight vector control specialists addressed the pools in the traditional way, making visits to each property to determine appropriate action. Working through the list dominated time and resources during the most critical time of year. Ultimately, the specialists had to give up at the end of October with hundreds of properties left unchecked, and the status of the swimming pools left unresolved. Although the District resolved over a thousand mosquito breeding sources that year, it did not come close to reaching the full potential of aerial surveillance. The District needed a way to address these swimming pools much faster, using significantly less resources.

This presentation discussed the chronic nature of unmaintained swimming pools and the danger they pose. It briefly examined the unsuccessful and unsustainable methods used in the past. Finally, it introduced a new program developed by the District to prioritize data provided by aerial surveillance and ultimately ensure that the thousands of swimming pools identified annually do not become a habitat for mosquitoes. The District tested the program in 2018 and successfully processed over 2,000 swimming pools in under three months.

Successful population suppressions of wild *Aedes aegypti* by release of *Wolbachia*-infected males

Jacob E. Crawford

Abstract

Aedes aegypti presents an immense burden on human health and continues to expand its global distribution, including its recent expansion into California. Traditional mosquito abatement techniques have not proven sufficiently effective to control this invasive mosquito, underscoring the need for new, cost-effective technologies that can be deployed across large areas. The Sterile or Incompatible Insect technique has been used successfully in agricultural systems, but large-scale deployment of sterile/incompatible male mosquitoes at low cost remains a challenge. We have developed new automated processes to rear and release millions of competitive, male-only Wolbachia-infected mosquitoes and showed that these technologies can be used to suppress wild *Aedes aegypti* in three communities in Fresno County, CA. Daily releases began in mid-April and lasted until the end of the mosquito season. We monitored wild *Ae. aegypti* in the release areas as well as similar non-release neighborhoods and found that the number of female *Ae. aegypti* was greater than 90% lower in the release areas compared to non-release areas at peak mosquito season. In this experimental setting, stronger suppression appears limited by daily incursions of migrant mosquitoes from heavily infested surrounding areas. These results demonstrate that incompatible-male mosquito releases using our automated processes can successfully suppress *Ae. aegypti* populations in an open field setting.

New CalSurv Gateway maps to track arbovirus activity and *Aedes* spread

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Introduction

The CalSurv Gateway is the statewide repository for California's surveillance data for mosquitoes and arboviruses. For many years, these data have been visualized on public-facing maps, but until recently, the view of the data had been limited to the current year. Here we describe new features that allow users to visualize surveillance results since 2003, along with new graphs that show trends over time.

Methods

The CalSurv Maps (CalSurv Development Team 2019) rely on the data stored in the CalSurv Gateway. These data include information about mosquito and arbovirus detections, which are used to generate maps and charts that show spatial and temporal patterns in surveillance results. Technologies underlying the new visualizations include Mapbox (Mapbox 2019), Uber's open source React-Vis library (Uber 2019), and D3 (Bostock 2019) visualization tools.

Results and Discussion

As of March 2019, the CalSurv Maps (CalSurv Development Team 2019) include arbovirus detections, invasive *Aedes* distributions, and model-based risk estimates for Zika virus. All maps allow the user to filter the data using radio buttons or tabs, and user instructions are shown when each map loads. Arbovirus maps show locations of positive samples for West Nile, St. Louis encephalitis, and western equine encephalomyelitis viruses since 2003, with sites randomly shifted to obfuscate the specific site location but accurately represent the general spatial pattern. Invasive *Aedes* maps provide summaries of detections by city or neighborhood since 2011, and Zika virus risk maps show model-based risk estimates for local transmission if a local case has been detected.

Conclusions

The maps are meant to be informative and useful for mosquito control districts, public health officials, researchers, and the public. The latest maps have increased performance, clearer instructions, and a more intuitive user interface. Feedback for the maps, or ideas for what kinds of information would be helpful for mosquito control districts can be sent anytime to help@calsurv.org.

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Towards comparable estimates of urban *Aedes* abundance

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Introduction

Invasive urban *Aedes* species such as *Aedes albopictus* and *Ae. aegypti* are both present in multiple California counties (CDPH 2019). Their ecology and habitat suitability have been widely studied and modeled based on climate factors (e.g. temperature and rainfall) as well as factors that influence their ecology such as land cover, human demographics and movement, and survival and life cycle features (Kraemer et al. 2015, Brady et al. 2014, Monaghan et al. 2016, Vallela et al. 2015). The current project aims to develop a model that estimates abundance and describes its variation in space and time while accounting for trap types and trapping effort, which vary among vector control districts.

Methods

A generalized additive model (GAM) was used to estimate adult *Ae. aegypti* counts per trap night based on trap type and time of year. The use of a GAM allows for fitting flexible functional relationships between covariates and vector abundance that may be nonlinear. This is also amenable to future analysis that will incorporate additional lagged climate variables. The model was fit using surveillance data from five counties in the Central Valley region (Fresno, Kern, Kings, Madera, and Tulare counties). Initial analysis examined abundance curves over 2013-2016 for the county group and individual counties, overall and stratified by trap type (CO_2-baited CDC light traps or BG Sentinels) for those counties operating both types (Fresno, Kern, Kings, and Madera).

Results and Discussion

For counties utilizing both trap types, a greater number of *Ae. aegypti* adults per trap night were estimated for BG Sentinel traps both overall and for each individual county, with Madera having the highest overall counts. Abundance estimates were lowest for Tulare, followed by Fresno. Seasonal trends were relatively consistent for Fresno and Madera counties with peaks occurring in summer to early fall. For districts utilizing multiple trap types, using trap type in the model may help to parse components of the trapping scheme that are more likely to detect *Ae. aegypti*.

Conclusions

These findings are preliminary and have allowed us to develop a comparative modeling framework for estimating urban *Aedes* abundance. Future aims are to continue developing the model to include adjustment for environmental variables, including lagged effects, and to expand geographic coverage to more California vector control districts.

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Aedes aegypti blood- and sugar-feeding patterns in Los Angeles, California

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Introduction

Aedes aegypti is the vector most responsible for the recent Zika outbreak in the Americas, and is the primary vector of dengue virus globally (Halstead 1990, Petersen et al. 2016). *Ae. aegypti* have adapted to live in urban environments and feed frequently and primarily on humans, which makes *Ae. aegypti* uniquely an effective and important vector for the transmission of arboviruses in urban human-mosquito cycles(Scott et al. 1993, Richards et al. 2006, Barrera et al. 2012, Bowman et al. 2016). In the U.S., improved housing quality minimizes exposure to *Ae. aegypti* biting (Reiter et al. 2003, Ramos et al. 2008, Walker et al. 2011); however, no study has investigated how feeding varies with household characteristics and human behaviors at the home (e.g. window screens, air-conditioner usage, time spent outdoors, etc.). The rate at which mosquitoes feed on humans is an important driver of vectorial capacity and is highly variable in space and time. Female mosquitoes generally require at least one blood meal to enable ovarian development and periodic sugar meals in the form of nectar to synthesize energy reserves for flight and other activities (Clements 1992). Studies have shown that female *Ae. aegypti* rarely feed on sugar, and that feeding exclusively on blood results in reproductive advantages and brings them into more frequent contact with humans, thus increasing vectorial capacity (Edman et al. 1992, Scott et al. 1997, Harrington et al. 2001). In the current study, we aimed to improve the gold-standard anthrone and vanillin assays used to identify blood and sugar-feeding in female mosquitoes as a step toward quantifying the blood and sugar-feeding prevalence for *Ae. aegypti* in Los Angeles, California.

Methods

To determine the sugar and blood feeding prevalence of field-collected *Ae. aegypti*, we developed a modified protocol for the hot anthrone and vanillin assays. The hot anthrone assay and the vanillin assay are gold-standard tests that quantify fructose and glycogen and lipids respectively, which reflect the balance of metabolic activities and nutritional inputs and storage from feeding on blood and sugar (Kaufmann and Brown 2008, Stone et al. 2011). Our protocol reduces and scales the reagents required for the original anthrone test to fit within 96-well microplates which are used to read absorbance. We tested this protocol on five cohorts of laboratory mosquitoes that consisted of the following feeding regimes for 8 consecutive days: access to human blood every day with no sugar access, access to human blood on day one with sugar (sucrose on cotton wick) access the remaining days, access to human blood twice (on day one and day five) with sugar access between and after bloodmeals, access to sugar every day with no access to blood, and completely starved with no access to blood or sugar.

Results and Discussion

Preliminary results of this study indicate that our protocol can detect differences in fructose, glycogen, and lipid content among cohorts of adult *Ae. aegypti* exposed to different blood and sugar-feeding regimes. After running this assay on the laboratory cohorts, we found the following: individuals in the group fed only sugar had the highest quantities of fructose, followed by those given access to blood on day one with sugar after; those same individuals given the opportunity to blood feed once and with sugar access thereafter had the highest quantities of glycogen, followed by those in the group that were fed only sugar; and individuals that were fed only blood had the highest quantities of lipids followed by those that were given access to two blood meals. Results of this laboratory proof-of-principle experiment will provide a reference for comparative studies of the nutritional status of field-caught mosquitoes as a proxy for access to blood and sugar meals. A better understanding of mosquito access to blood meals in field settings, especially in relation to measurable social and demographic factors, will help vector control programs to target resources to communities at greatest risk for Zika virus and other urban *Aedes*-borne viruses.

Acknowledgements

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Extrinsic incubation period of Zika virus in *Aedes aegypti*

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Introduction

Aedes aegypti is the primary vector of Zika virus (ZIKV) worldwide (Ferreira-de-Brito et al. 2016, Guerbois et al. 2015, Hall-Mendelin et al. 2016), yet little is known about the interaction of ZIKV and *Ae. aegypti* outside of the narrow temperature range of 26-28°C (Costa-da-Silva et al. 2017, Chouin-Carneiro et al. 2016, Main et al. 2018, Pompon et al. 2017, Roundy et al. 2017). Arboviral extrinsic incubation periods (EIPs), and therefore transmission efficiency, are known to be affected strongly by temperature (Chan and Johansson 2012, Reisen et al. 2006, Tesla et al. 2018). To better understand the relationship between ZIKV EIPs and temperature, we evaluated the effect of adult mosquito exposure temperature on ZIKV infection, dissemination, and transmission in *Ae. aegypti* across the temperature range to which the mosquito is likely to be exposed in its current range.

Methods

Aedes aegypti mosquitoes from Clovis, California (F4) were exposed to viremic mice infected with a 2015 Puerto Rican ZIKV strain, and engorged mosquitoes were sorted into four temperature treatments (18, 21, 26, and 30°C) with 80% RH and constant access to 10% sucrose. ZIKV infection, dissemination, and transmission rates were assessed using RT-qPCR with a previously described ZIKV-specific assay (Lanciotti et al. 2008) from individual mosquito bodies, legs and wings, and saliva, respectively, at 3-5 time points per temperature from 3 to 31 days, based on expectations from other flavivirus EIPs. The median time from ZIKV ingestion to transmission (median EIP, EIP_{50}) at each temperature was estimated by fitting a generalized linear model for time post-feeding and temperature.

Results and Discussion

EIP_{50} ranged from 5.1 days at 30°C to 9.6 days at 26°C to 24.2 days at 21°C. At 18°C, only 15% of mosquitoes transmitted by day 31, so the EIP_{50} could not be estimated. Results from this study were compared with other published flavivirus EIPs, including DENV (Chan and Johansson 2012) and the one published study on ZIKV (Tesla et al. 2018). The two ZIKV curves are remarkably similar despite deviation of methods for determining ZIKV transmission. Comparison of the EIP vs. temperature curves indicated that the ZIKV EIP was shorter than that of DENV in *Ae aegypti*.

Conclusions

This is among the first studies to characterize the effects of temperature on ZIKV EIP in *Ae. aegypti*. This information is critical for modeling ZIKV transmission dynamics to understand the geographic and seasonal limits of ZIKV risk. It is especially relevant for determining risk in subtropical regions with established *Ae. aegypti* populations (e.g. California) as these regions experience cooler and more variable temperature ranges than tropical regions.

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Simultaneous detection of chikungunya, dengue and Zika viruses in mosquitoes using RT-PCR

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Abstract

Over the years, there have been endemics and epidemics of arthropod-borne diseases worldwide due to increased globalization and urbanization. Because infections with viruses such as chikungunya, dengue and Zika have become widespread, it is vital that accurate and efficient detection tools become available to better assess viral prevalence within mosquito populations. Here we describe a protocol for a triplex RT-PCR for simultaneous detection of chikungunya virus (CHIKV), dengue virus serotypes 1-4 (DENV1-4) and Zika virus (ZIKV) in mosquitoes. CHIKV, all four serotypes of DENV and ZIKV were detected individually or in combination using this triplex. When tested individually, CHIKV was detected to 1.98×10^3 pfu/ml, DENV1 was detected to 1.90×10^4 pfu/ml, DENV2 to 1.60×10^4 pfu/ml, DENV3 to 4.00×10^2 pfu/ml, DENV4 to 7.90×10^2 pfu/ml, and ZIKV to 3.10×10^3 pfu/ml. Mixing DENV serotypes did not compromise the detectable levels of individual serotypes when tested by the DENV1-4 mix alone. CHIKV, DENV1-4 mix or ZIKV tended to have lower detectable levels when tested in a full combination of six targets (CDZ mix) as compared with those tested alone, particularly for the DENV1-4 mix. This triplex consistently detected all six viral targets, individually and in combination, and did not cross react with an *Aedes aegypti* (L.) homogenate.

Incorporation of new technologies and methods to improve surveillance and control at Salt Lake City Mosquito Abatement District

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Abstract

Like invasive mosquito species, new technologies are constantly emerging. Some of these technologies can be utilized in the mosquito control field. At Salt Lake City Mosquito Abatement District new technologies and innovations were used to try and improve surveillance and control. 3-D printing methods are being used to aid in mosquito surveillance, replace hard to find parts, and design new equipment. We have found that 3-D printing technology can reduce costs of for some items and encourage staff members come up with ideas for useful parts and equipment to be printed. Additional trials were conducted looking at new batteries for traps, creating a granular dispenser for a sUAS and modifying resting boxes to increase the catch of resting mosquitoes for different studies. In addition, a study with the ultra-fast metagenomics analysis software, Taxonomer, was conducted on mosquitoes from the Salt Lake City area. These studies show ways in which mosquito control can benefit from the adoption of new technologies and methods.

Larval mosquito abundances in herbicide-treated water hyacinth

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Abstract

In the California Delta Region, invasive aquatic weeds are managed with herbicides in order to reduce their negative impacts on the water ways. Herbicides create a dynamic environment of living and decomposing plant matter which affects not only larval mosquitoes, but also other aquatic invertebrates, such as predators and competitors. We studied the effects of herbicide management of water hyacinth on larval mosquito abundances in replicated pond mesocosms. We sampled weekly for mosquitoes and other aquatic invertebrates in mesocosms with water hyacinth, water hyacinth treated with glyphosate and adjuvant (Round-up Custom and Agri-Dex), open water, and water treated with glyphosate and adjuvant. Early in the study, there was a trend for more larval mosquitoes to be present in open water tanks than those with water hyacinth. Addition of herbicide resulted in an immediate decrease of larval mosquitoes. As decay progressed, larval mosquitoes became most abundant in mesocosms with herbicide-treated hyacinth, and very few larval mosquitoes were recovered in the other habitat-treatments. The number of predatory and competitor insects found rarely varied within and between treatments. With better information regarding the effects of herbicide use for invasive weed management on larval mosquitoes, integrated management practices for both larval mosquitoes and invasive weeds may be improved.

Analysis of factors affecting patterns of daily mosquito activity using data from an automated mosquito counting trap

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Abstract

The efficacy of adulticide missions can be maximized by applying the control material when the maximum number of mosquitoes are active. The BG Counter automated mosquito trap (Biogents, Regensburg, Germany) provides a previously unattainable amount of data (counts every day, in 15 minute increments) on mosquito flight times. BG counter data (mosquito abundance, temperature, humidity, and ambient light data collected every 15 minutes) were used to create a linear model of timing of mosquito detection. Unfortunately, the model only explained 24% of the variation in mosquito abundance per 15 minute period, suggesting that possibly one or more unmeasured variables (environmental, physiological, or behavioral) could be important in determining mosquito abundance counts and timing.

Introduction

The efficacy of adulticide missions can be maximized by applying control materials when the most mosquitoes are actively flying. Many previous studies have attempted to determine when mosquitoes are most active (e.g. Yee and Foster 1992, Reisen et al. 1997, Lothrop and Reisen 2001, Veronesi et al 2011, Montarsi et al. 2015, Stough and Wallace 2016, Boisvert et al. 2018), but results have been conflicting and studies have utilized small data sets due to the challenges of manual collections or rotating traps usually employed for these studies. Because most mosquito traps use an attractant to lure host-seeking mosquitoes, many studies, including this one, describe the activity of the host-seeking portion of the mosquito population.

In 2013, a preliminary study (18 trap nights over 3 months) collected flight time information with the traditional rotating trap (Sorensen, unpublished data). Although sunset and sunrise were very important "cut offs" for mosquito (primarily *Culex tarsalis*) detection, temperature, humidity, and sun altitude were also statistically significant predictors of the number of mosquitoes captured. Together these variables explained less than 15% of the variation in timing of mosquito detections..

The BG Counter (Biogents, Regensburg, Germany) is a newly available technology for mosquito surveillance which uses infrared sensors to count mosquitoes entering the device (Figure 1a). Non-mosquito insects and objects are counted as "large" or "small". The Counter is meant to be used in combination with a BG Sentinel trap that can be baited with a chemical lure, CO_2, or both. The counters additionally contain sensors to detect and record temperature, humidity, and ambient light.

The goal of this project was to use the newly available BG Counter technology to analyze daily timing of mosquito detection at a resolution (every 15 minutes) and scale (nightly data for many weeks, at multiple sites) that were previously impractical. Because adult mosquito control treatments are thought to be primarily effective to control flying mosquitoes, results were examined to determine the implications of patterns of daily timing of mosquito activity for wide-area adult mosquito control treatments. The same trapping data presented herein was additionally used to look at peaks of mosquito counts in different environments, and to compare abundance at a single site across two years (Boisvert et al., in press).

Methods

Placer MVCD operated BG Counter traps inside of a custom welded cage (Figure 1b) for protection from theft and vandalism, and to hold the trap components, including a 20lb CO_2 tank, solar panel, and 12V battery. The welded cage with expanded metal mesh had openings approximately 2.5cm x 1cm, allowing mosquito access. With the Placer MVCD set-up, traps could run without maintenance for up to two weeks. Data were transmitted by cellular network to the Biogents company servers and then downloaded to the Placer MVCD database via an application programming interface. Counts also could be viewed on the BG Counter website with a registered account.

This study was conducted in the western part of Placer County, California, a mixed-agricultural region with a large amount of acreage devoted to growing both conventional and organic rice, and with a large population of mosquitoes dominated by *Cx. tarsalis*. West Nile virus is frequently detected in this area and wide-area adult mosquito control treatments are conducted several times per year by fixed-wing aircraft.

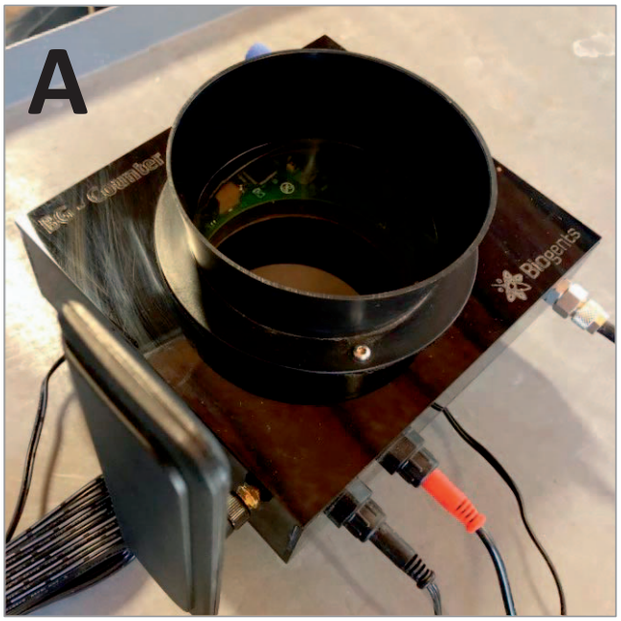

Figure 1.—a) BG Counter automated mosquito counting device. This device is designed to sit atop a BG Sentinel mosquito trap and count mosquitoes entering through the central opening using infrared sensors. Data are transmitted with a cellular data connection. b) A BG Counter deployed in the field, with BG Sentinel trap, battery, CO_2 supply, solar panel, and welded enclosure

In 2017, two BG Counter devices were deployed at three field sites (Figure 2) through the summer season. In 2018, six more BG Counters were purchased and deployed in western Placer County in various agricultural and industrial

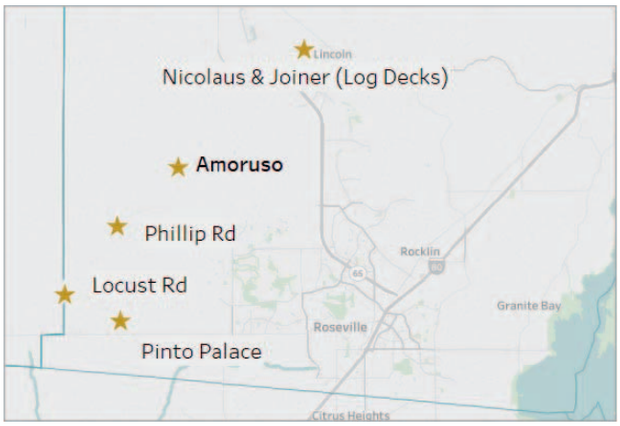

Figure 2.—This map shows the western Placer County locations of BG Counter traps in 2017 and 2018 which were included for analysis of patterns of daily timing of mosquito abundance per 15 minute period.

areas (Figure 2) near rice fields, pastures, a corn field, and a lumber yard. The dominant mosquito species at all sites was *Cx. tarsalis*, except for the lumber yard site where the dominant species was *Cx. pipiens*. Device failures and replacements led to some down time and gaps in data collection; nonetheless, a very large body of data was collected on the timing of mosquito detection. Data collected by the BG counters in 2017 and 2018 were used for the analysis of daily timing of mosquito capture activity.

Weekly Fay traps set nearby the BG Counters provided an approximation of the species captured, which were largely *Cx. tarsalis* for all sites discussed, except the Log Decks, where the main species was *Cx. pipiens*.

To determine trap accuracy (data not shown), error for the traps in 2018 was determined for 107 collections where manually counted mosquitoes in a catch bag were compared to automated counts from traps. Based on the baseline level of "extra" mosquito counts found in the preliminary data, trap nights with less than 100 total mosquitoes were excluded from analysis. Also excluded were collections where the fan or CO_2 was not running, trap problems were recorded, traps were under evaluation, aerial adulticide was scheduled in Placer County, or months other than July, August, or September.

Analysis and data visualization were performed with R, Excel, and Tableau software. Day and night collections were compared. Any collections with missing data were excluded from analysis. Forward stepwise linear regression was performed in R. Where predictors were correlated, the linear regression model was tried with each individual predictor or with both, and the model with the highest predictive value was retained. Standardized coefficients were generated using R package "QuantPsyc" to compare the relative importance of the predictor variables. Data visualizations were prepared for the most important predictor variables to show the effects of each variable or

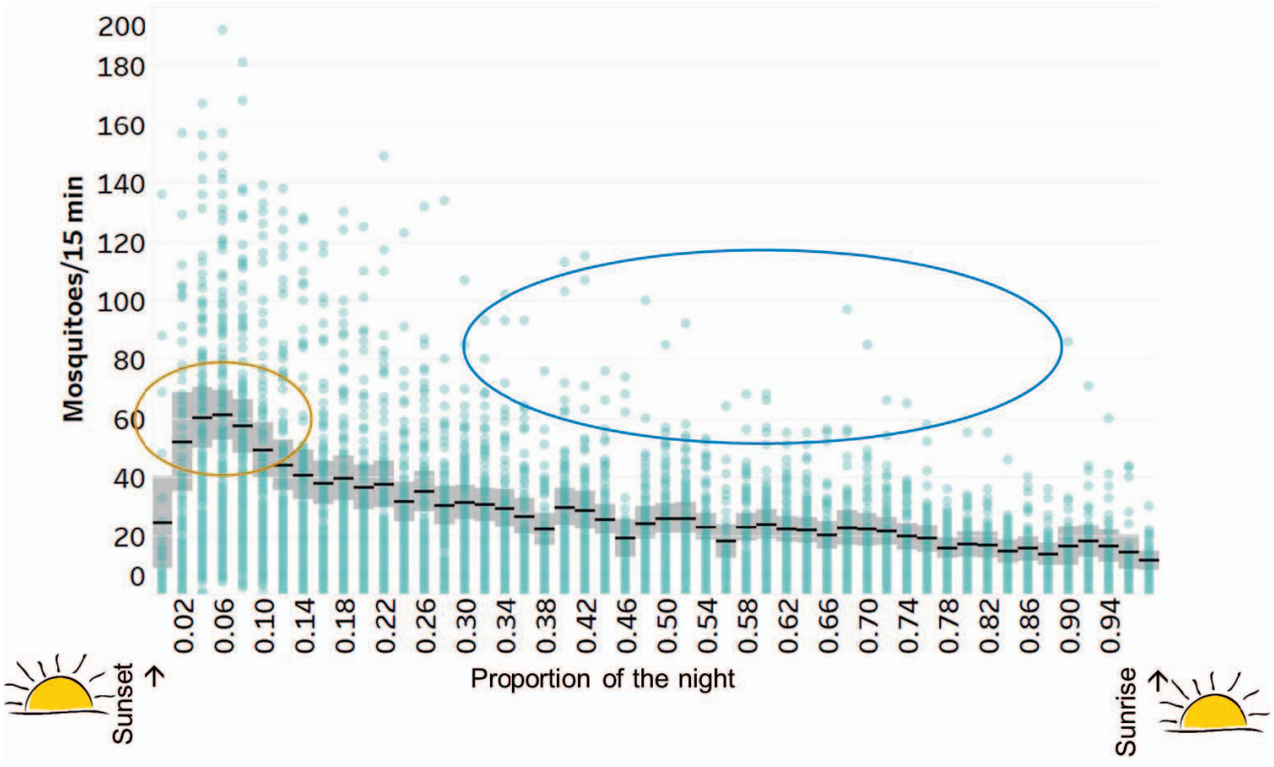

Figure 3.—Relationship of the variable "proportion of the night" (time after sunset in minutes/total minutes sunrise and sunset) to the number of mosquitoes counted per 15 minute interval by a BG Counter automated device. Time of highest average mosquito abundance is circled in yellow, observations with higher-than-average counts are circled in blue.

combination of variables on daily patterns of mosquito detection.

Results and Discussion

Over two years and 450 trap nights included in the analysis, 98% of all mosquitoes were collected during the night period (defined as between sunset and sunrise) as opposed to the day. For the remainder of the analysis, day collections were excluded.

Although the overall linear regression model and many of the predictor variables were found to be significant ($P<0.05$), the adjusted R^2 value for the final model was only 0.24. This R^2 value indicates that only 24% of the variation in mosquito count per 15 minute interval was explained by the combination of time after sunset, humidity, temperature, along with seasonality (day of year), year, and various interactions of those predictors.

The most important predictor variables (Table 1) were found to be time after sunset (analyzed as "proportion of the night" to standardize for changing length of the night period), relative humidity, the interaction of proportion of the night and humidity, and temperature. Also important were seasonality (day of the year), year, the interaction of proportion of the night and temperature, the three-way interaction of proportion of the night, temperature, and relative humidity, and the interaction of temperature and humidity (Table 1). Ambient light was non-significant and was removed from the model.

To further understand the relationship of each predictor variable to the number of mosquitoes counted per 15 minutes, these relationships were visualized as shown below.

The standardized coefficient (-2.73) for proportion of the night (time after sunset in minutes/total minutes between sunrise and sunset) had the largest absolute value, indicating that proportion of the night was the most important predictor variable in the model. The sign of the coefficient was negative, meaning that as proportion of the night increased, number of mosquitoes decreased. However, upon closer inspection the relationship of proportion of the night and mosquito abundance is clearly non-linear (Figure 3), with a peak of abundance per 15 minute period (Figure 3, yellow circle) after sunset and then a gradual decline in mosquito detection. Furthermore, the circled areas of higher-than-average abundance (Figure 3, blue circle) demonstrate that despite a later time of the night with lower average mosquito counts, many observations of high mosquito abundance were recorded, emphasizing that the predictor variable proportion of the night alone is not sufficient to accurately predict mosquito capture per 15 minute interval.

A similar relationship was seen with humidity (Figure 4), where the linear regression model coefficient was negative

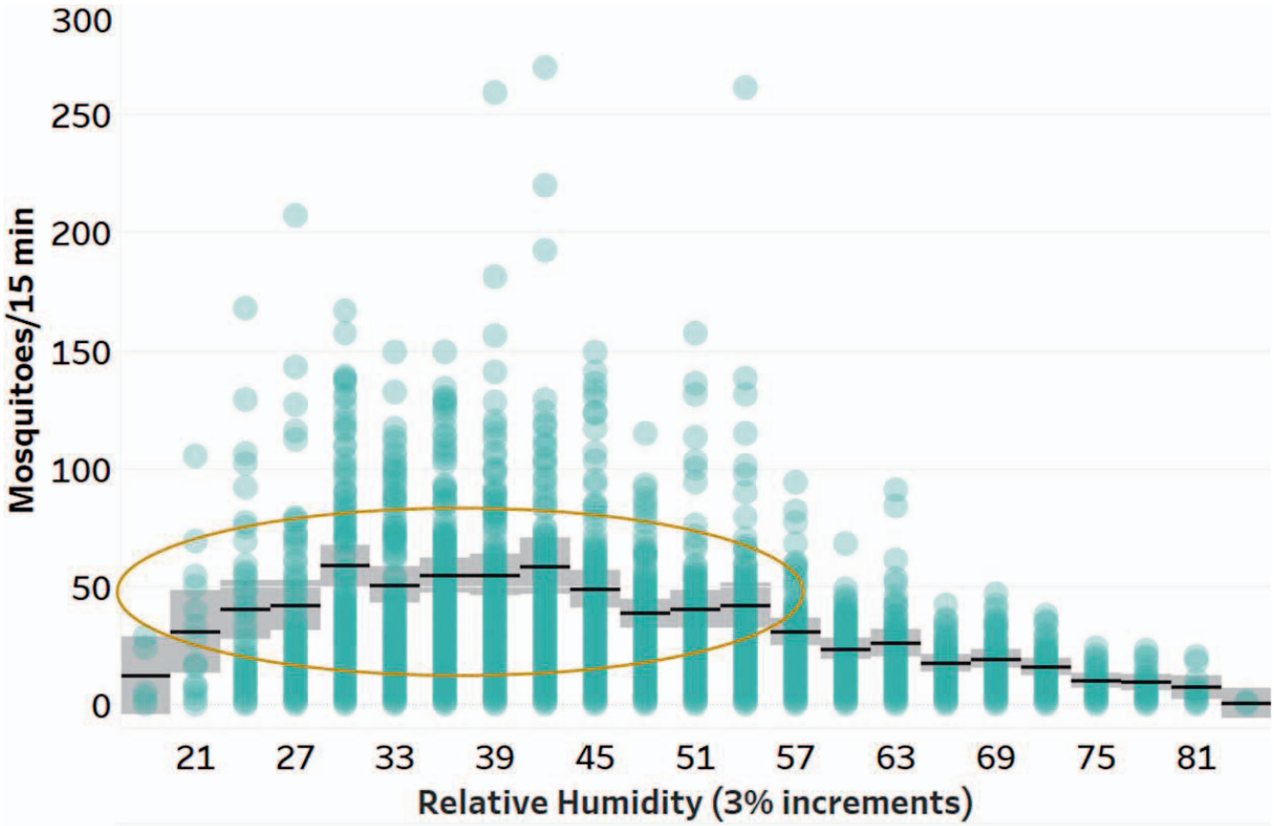

Figure 4.—Relationship of the humidity to the number of mosquitoes counted per 15 minute interval by a BG Counter automated device. The area circled in yellow indicates a range of conditions that seem to be favorable for increased mosquito numbers, despite many records of low mosquito counts within that range of humidity.

Table 1.—Results of linear regression of the effects of environmental variables on mosquito abundance per 15 minutes. Standardized coefficients (absolute values) reveal the relative importance of each predictor variable.

Linear regression results, mosquito flight time						
R2=0.24, F(9,15349)=533.8, P<0.001						
	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**		**Standardized Coefficient**
(Intercept)	2.82E+04	1.03E+03	27.367	< 2e-16	***	
PropNight	-2.52E+02	1.51E+01	-16.641	< 2e-16	***	-2.7328
RH	-1.39E+00	1.70E-01	-8.195	2.70E-16	***	-0.6226
PropNight:RH	3.66E+00	2.66E-01	13.759	< 2e-16	***	0.6087
Temp	-2.72E+00	3.44E-01	-7.901	2.96E-15	***	-0.4511
dayofyear	-2.83E-01	8.20E-03	-34.501	< 2e-16	***	-0.2748
Year	-1.39E+01	5.10E-01	-27.223	< 2e-16	***	-0.2418
PropNight:Temp	1.06E+01	6.79E-01	15.585	< 2e-16	***	0.1149
PropNight:Temp:RH	-1.65E-01	1.25E-02	-13.176	< 2e-16	***	-0.0740
Temp:RH	1.84E-02	7.19E-03	2.564	0.0104	*	0.0003

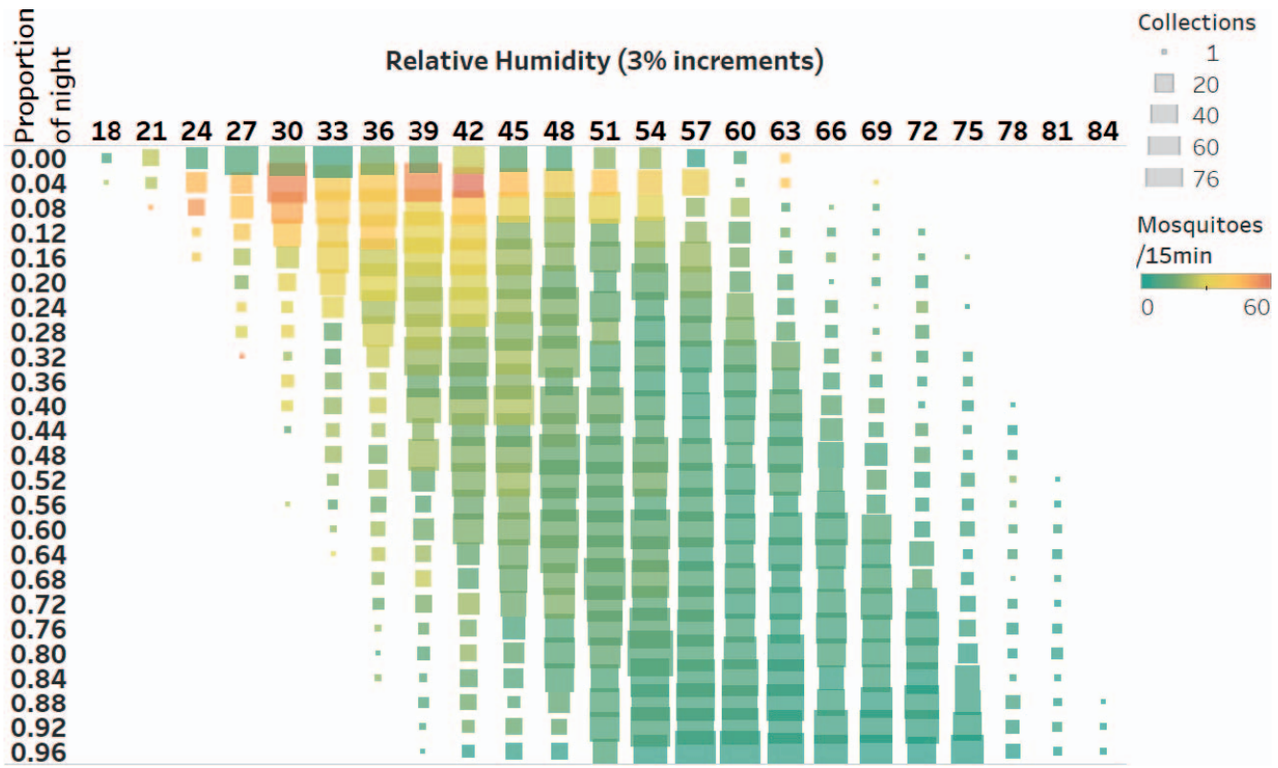

Figure 5.—Relationship of the interaction of time of night and humidity to the number of mosquitoes counted per 15 minute interval. Size of the squares indicates the total number of observations under that combination of conditions. Color indicates average mosquito count.

(-0.62), indicating that more humidity led to a decrease in mosquitoes counted per 15 minutes. However, upon closer inspection, the relationship between humidity and mosquito count was non-linear, with more mosquitoes active in the mid-range of humidity, between around 30 and 55%. Higher humidity is generally considered favorable for mosquitoes and for insects in general, making this result somewhat surprising. Additionally, higher humidity was more likely to occur later in the night, and a greater portion of the night was shown to be correlated with lower mosquito abundance (Figure 3).

The effect of the interaction of time of night and humidity on mosquito counts (Figure 5) was more complex to visualize. Figure 5 shows humidity on the x axis and proportion of the night on the y axes, with the size of the squares at each position indicating the total number of observations under that combination of conditions. The color of each square indicates mosquito abundance. From this visualization, it appears that mosquitoes are most abundant when humidity is in the mid-range of 30-57% and proportion of the night is about 4-8%. However, mosquitoes are also abundant (yellow color squares) further into the night, provided that humidity is less than about 45%.

Finally, temperature in the linear regression model (Figure 6) also had a negative coefficient (-0.45, Table 1), suggesting fewer mosquitoes were collected at higher temperatures. However, again the visualization suggests a more complex relationship where mean mosquito abundance increased with temperature up to 24°C (75°F), after which mosquito abundance per 15 min interval decreased.

Conclusions

The results of this study strongly indicated that not all the environmental variables determining mosquito abundance per 15 minute period were included in this dataset. For example, localized wind conditions may have been an important predictor variable for number of mosquitoes entering the traps, or there may have been some other unknown and unmeasured variables. Additionally, some aspect of the timing of mosquito counts may have been due to non-environmental causes such as different subsets of the mosquito population (genetic differences) or physiological status (age, reproduction, or feeding).

Using a different analytical technique to incorporate non-linear effects and better accommodate covariation among predictor variables may produce model that explains a greater percentage of the observed variation in mosquito detection times. For example, the existing model demonstrated that time, humidity, and temperature were correlated, but in the Sacramento area as time after sunset increases, humidity increases and temperature decreases, making teasing apart the effects of these environmental variables very challenging.

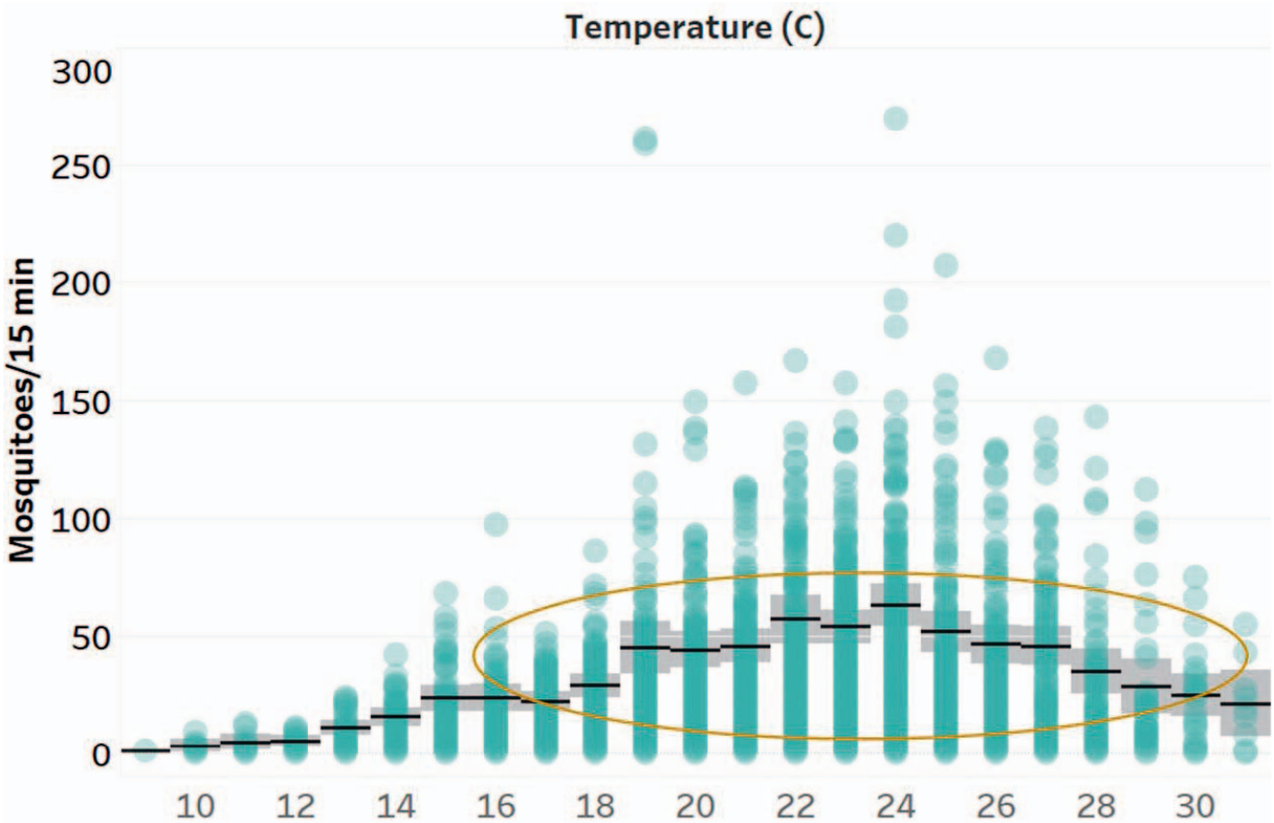

Figure 6.—Relationship of temperature to the number of mosquitoes counted per 15 minute interval. The area circled in yellow indicates a range of conditions that seem to be favorable for increased mosquito abundance, on average, despite many records of low mosquito counts within that range of temperature.

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Behavior of *Culex tarsalis* in different landscapes using BG-Counters

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Abstract

The BG-Counter (Biogents, Regensburg, Germany) is a relatively new auto-counting adult mosquito collection device that can be a very efficient tool to determine peaks of activity of mosquitoes and their abundance. The objectives of our study were to document peak(s) of activity of *Culex tarsalis* at five sites with different environments/landscapes using BG-Counters, and to compare data collected over two years at the Locust Road site. Three sites ('*Cx. tarsalis* sites') were surrounded by rice fields and one site by corn. The fifth site ('*Cx. pipiens* site') was included to compare data with the *Cx. tarsalis* sites. The abundance of mosquitoes varied greatly per site as did the pattern of host-seeking activity. Locust Road showed similar data (patterns, peaks) over the 2-year trial, even though during the second year the surrounding environment was very different. For Locust Road, Philip and Amoruso there were no significant differences in the number of minutes after sunset when the first peak of activity appeared (47.30 to 49.56 minutes). Pinto and Log decks were both significantly different from each other and the three other sites. When all the *Cx. tarsalis* sites were combined and compared to the *Cx. pipiens* site, there was a significant difference in the number of minutes after sunset for the first peak of activity (50.23 minutes versus 41.21 minutes). Data showed that within the same site, the time of the first peak (minutes after sunset) can change over the summer and vary among different sites. Analysis of other variables such as the age of the mosquitoes caught at different time of the day or the wind speed could also help better define and interpret the data.

Introduction

Time of host-seeking by *Culex tarsalis* has been described in California using an automatic time-segregated sampler as well as human landing counts (Nelson and Spadoni 1972, Reisen et al. 1997). In the last 20 years, new types of traps and new technologies have improved the way mosquito surveillance and the study of mosquito behaviors are performed. The BG-Counter (Biogents 2017) is a relatively new auto-counting adult mosquito collection device that is designed for use with a BG-Sentinel trap. Results of a recent field study showed that the BG-Counter can be a very efficient tool to determine peaks of activity of mosquitoes and their abundance (Boisvert et al. 2018). The objectives of our current study were 1) to document peak(s) of activity of *Cx. tarsalis* in different types of environments/landscapes using BG-Counters, 2) to compare data collected over two years at the Locust Road site, and 3) to compare five sites with different environments/landscapes. The same trap data also were used to analyze factors (temperature, relative humidity, sites, ambient light, etc.) affecting the daily timing of mosquito activity (Sorensen et al. 2019).

Materials and Methods

BG-Counters were used in combination with a BG-Sentinel trap (Biogents, Regensburg, Germany) and counted mosquitoes entering the trap at 15-minute increments using infra-red sensors. BG-Counters were deployed at five locations to determine the peak(s) of activity of *Cx. tarsalis* and *Cx. pipiens* in different environments. Three sites (Locust, Philip and Pinto) were surrounded by rice fields and one site (Amoruso) by corn. Those four sites were considered as '*Cx. tarsalis* sites' because that species accounted for more than 90% of the catch obtained by our regular surveillance program. The fifth site (Log Decks) was included in the study as a '*Cx. pipiens* site' to compare data with the *Cx. tarsalis* sites. Log decks was the property of the Sierra Pacific Industries sawmill where logs are piled up into decks that are sprayed with water to prevent them from cracking, generating a huge population of *Cx. pipiens* in the impounded run-off.

For our current study, BG-Counters were set to release CO_2 at a pre-adjusted rate of 450 ml/min and count mosquitoes for 24 hours a day, every day over a three month period (from July to September inclusively). The Locust Road site was the only site where data were collected the year before, thereby allowing us to compare data over two years.

Data from our regular surveillance program with Fay traps (set up close to our BG-Counters) provided us with the identification of the main species captured, confirming the aforementioned species at the respective locations.

Data from the BG-Counters over the three-month period were either averaged for 2-week periods (averages of data

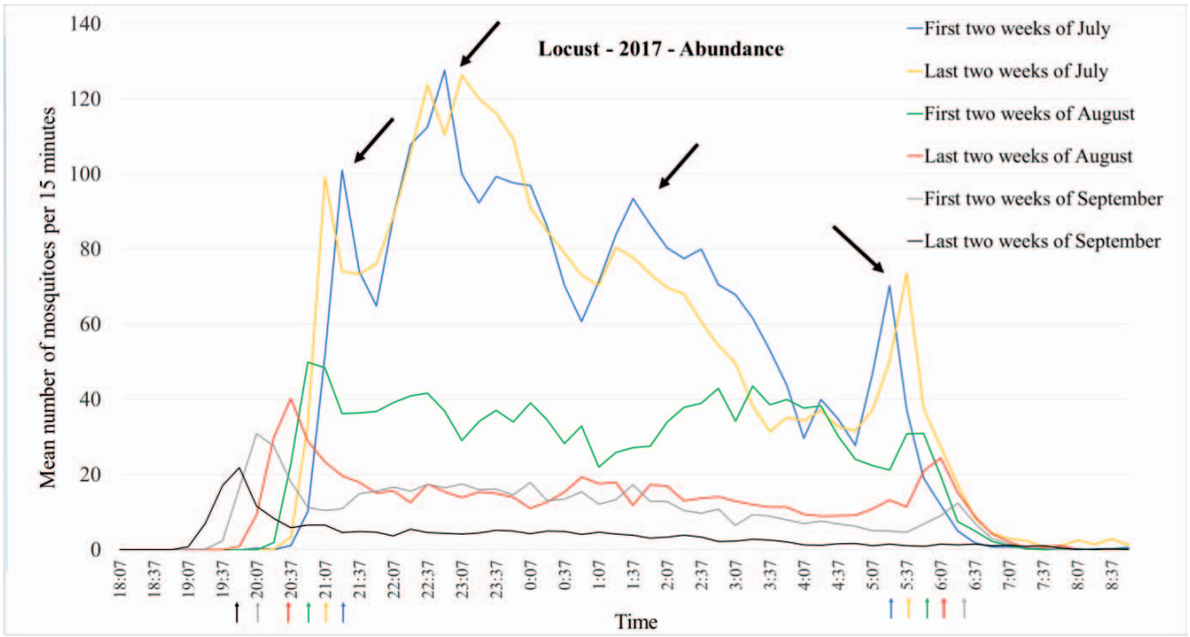

Figure 1.—Abundance of *Culex tarsalis* per 15 min period (averaged over 2-week periods) in a rice field over a 3-month period (July to September) in 2017. Black arrows indicate the four major peaks of activity in July between sunset and sunrise. Smaller colored arrows in the bottom of the graph indicate the time of peak of activity with respect to the time of the sunset and sunrise.

collected over 15 days) for a total of 6 curves per site or averaged to create only one curve for the entire sampling period. Comparisons between sites or patterns of activity dictated data aggregation (2-week period or the whole period).

Results and Discussion

Locust Road site: a 2-year comparison

In 2017, data from a BG-Counter positioned next to a rice field at the Locust Road site showed that peak of activity for *Cx. tarsalis* occurred before midnight and in the early morning during July and August (Figure 1). Four very distinct peaks were observed between sunset and sunrise during July. Peaks in the morning and the evening maintained a consistent time interval before sunrise and after sunset. Reisen and Aslamkhan (1978) observed that some culicine mosquitoes had distinctly bimodal biting rhythms, with well-defined early evening and predawn peaks. In species having a distinct bimodal curve, the amplitude of the predawn increase varied considerably from month to month. We also observed that decrease in the amplitude for the predawn peak over the three-month period (Figure 1). The overall abundance was quite high especially during the month of July when an average of up to 120 mosquitoes where caught per 15-minute period at the greatest peak of activity (Figure 1).

The trap located at the Locust Road site allowed us to compare abundance and activity data from 2017 and 2018. Overall abundance in 2018 (at July maximum) was on average about 50% of what was observed in 2017 (Figure 2). Although abundance declined, the timing of daily peaks of activity remained the same over the three month period for both years. The four main peaks of activity in July matched the ones observed during 2017 (Figure 2).

In 2017 there were many acres of rice planted next to the Locust trap, but in 2018, no rice was planted in the same area (Figure 3). This difference might explain (at least in part) the decrease in the abundance of mosquitoes at the Locust Road site in 2018 compared to 2017.

Abundance

The abundance of mosquitoes varied greatly among and within sites during the season (Figure 4). In 2018, the four *Cx. tarsalis* sites showed differences in abundance. A huge difference was also observed at the Locust Road site between 2017 and 2018 (Figure 4). Pinto showed the highest abundance during 2018, followed by Locust Road (even though there was no rice planted in 2018 near to the trap), Amoruso (corn field) and Philip. All sites showed a decrease in mosquito abundance from July to the end of September, except for Amoruso. A seasonal decrease of abundance also was noticed at the Log decks site (*Cx. pipiens* site).

We did not observe the "expected" bell-shaped curve characterizing the abundance of mosquitoes over the course of the season, because we started to collect data at the peak of abundance of the *Cx. tarsalis* and *Cx. pipiens* in July. Amoruso is the only site showing the expected bell-shaped curve. Although Amoruso was the only *Cx. tarsalis* site surrounded by corn instead or rice, other reasons (temperature, relative humidity, etc.) than just the sur-

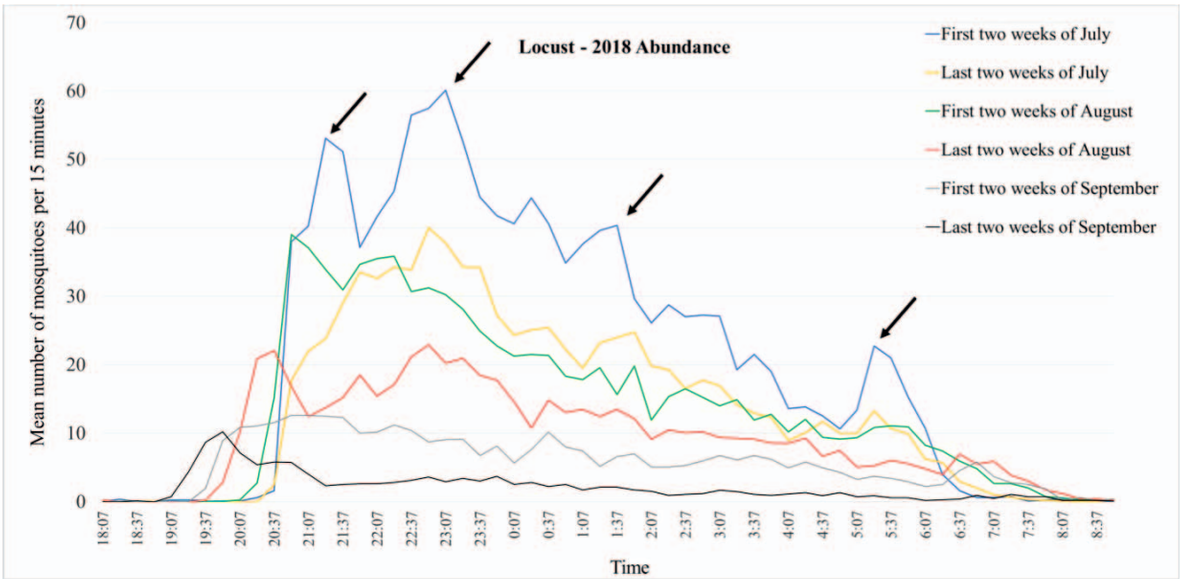

Figure 2.—Abundance of *Culex tarsalis* per 15 min period (averaged over 2-week periods) in a rice field over a 3-month period (July to September) in 2018. Black arrows indicate the four major peaks of activity in July between sunset and sunrise.

rounding landscape also should be considered to explain the different abundance pattern at Amoruso.

Our results showed that mosquito abundance can vary for multiple reasons (seasonally and/or spatially), including:

- Ecological characteristics of the location sampled
- Larvicide treatments (rice fields are treated many times during the course of the summer)
- Productivity of the surrounding sites (food, predation, etc.)
- Movement of mosquito populations
- Temperature, relative humidity, wind, etc.

Patterns of activity

Locust Road was the only site exhibiting four very distinct peaks of activity during July, and this was repeated

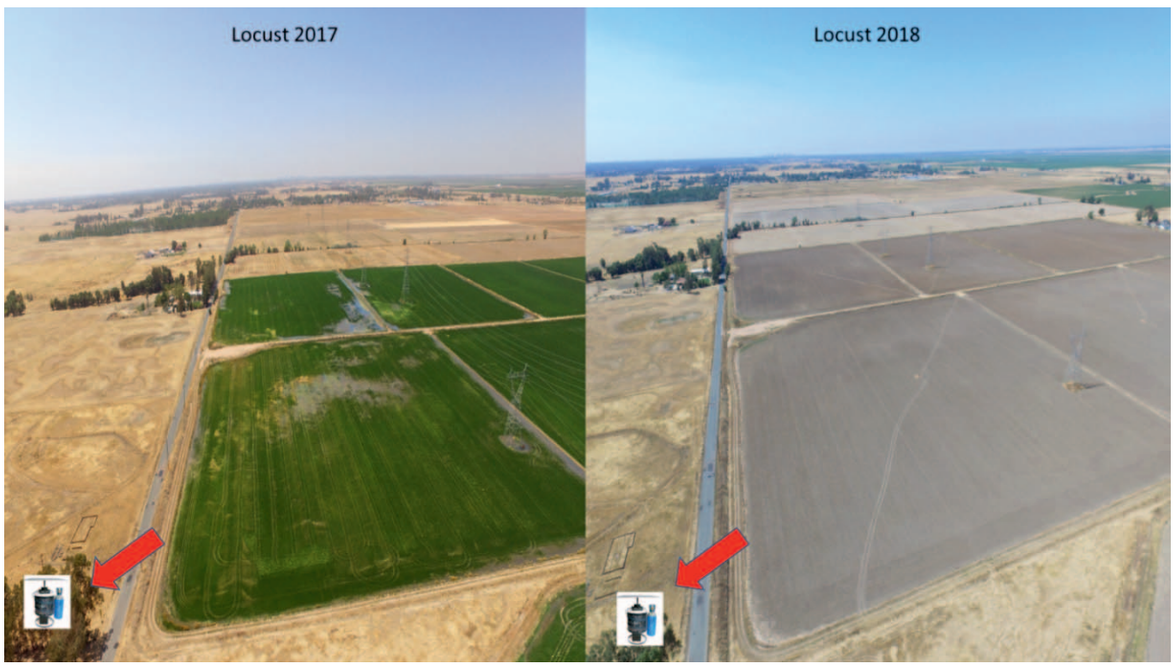

Figure 3.—The immediate environment (presence of rice or not) next to the BG-Counter at the Locust Road site in 2017 compared to 2018. Red arrows indicate the location of the BG-Counter.

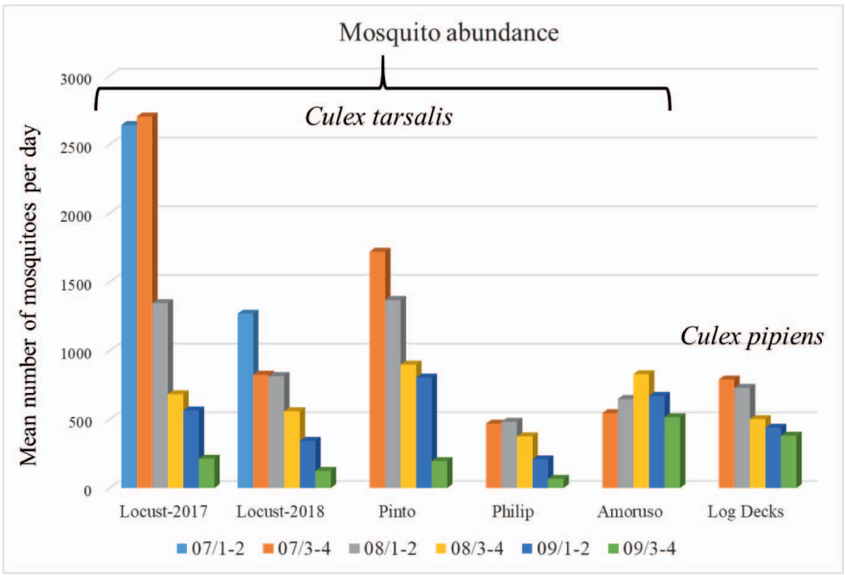

Figure 4.—Abundance of mosquitoes for six 2-week time-periods over three months for five locations (including 2 years at Locust Road).

during both 2017 and 2018 (Figures 1 and 2). When data were combined for the whole season (one curve – average of annual data), Locust Road was also the only site where "sustained" activity (plateau) was observed for many hours after sunset (Figure 5 – red rectangle) compared to the four other sites. Those four other sites were characterized by a major peak of activity after sunset, followed by decreasing activity until sunrise (Figure 5 – black rectangle).

Intuitively, the peak of activity was anticipated after sunset, but that is not always the case. Sometimes that initial

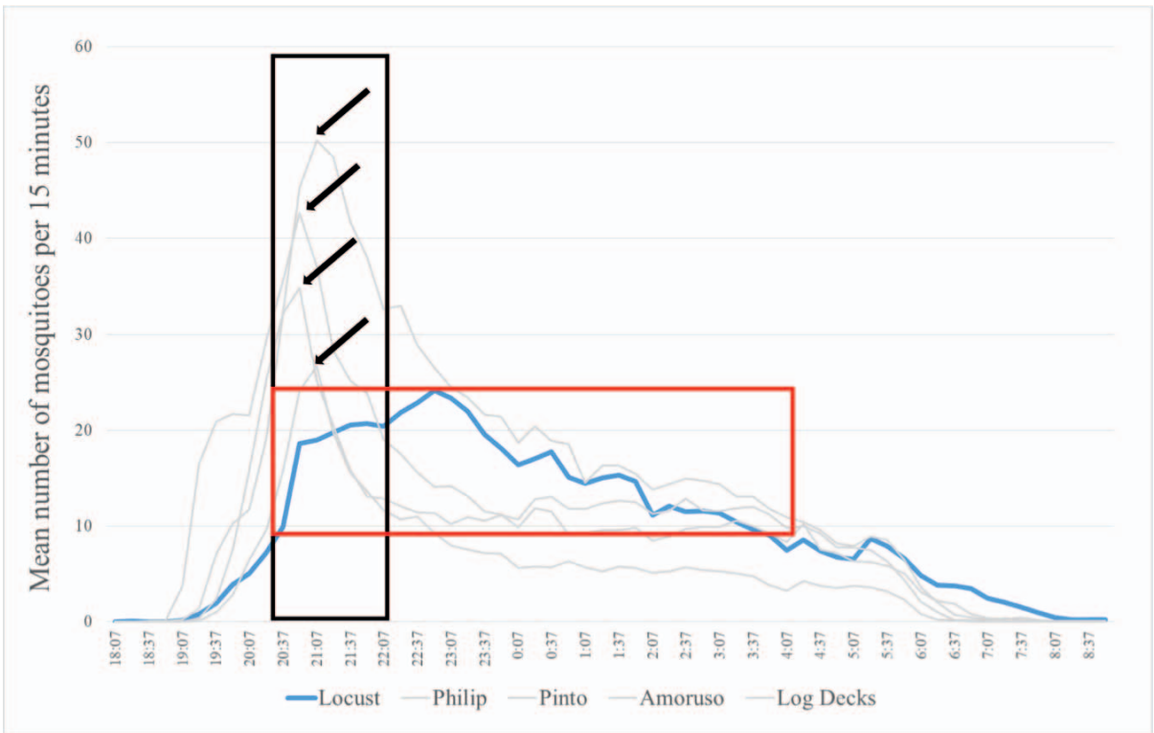

Figure 5.—Average number of mosquitoes per 15-minute increment over a three-month period for five different sites. The black rectangle and arrows indicate the main peaks of activity for four sites (Philip, Pinto, Amoruso and Log decks). The red rectangle indicates the more sustained activity at the Locust Road site between sunset and sunrise.

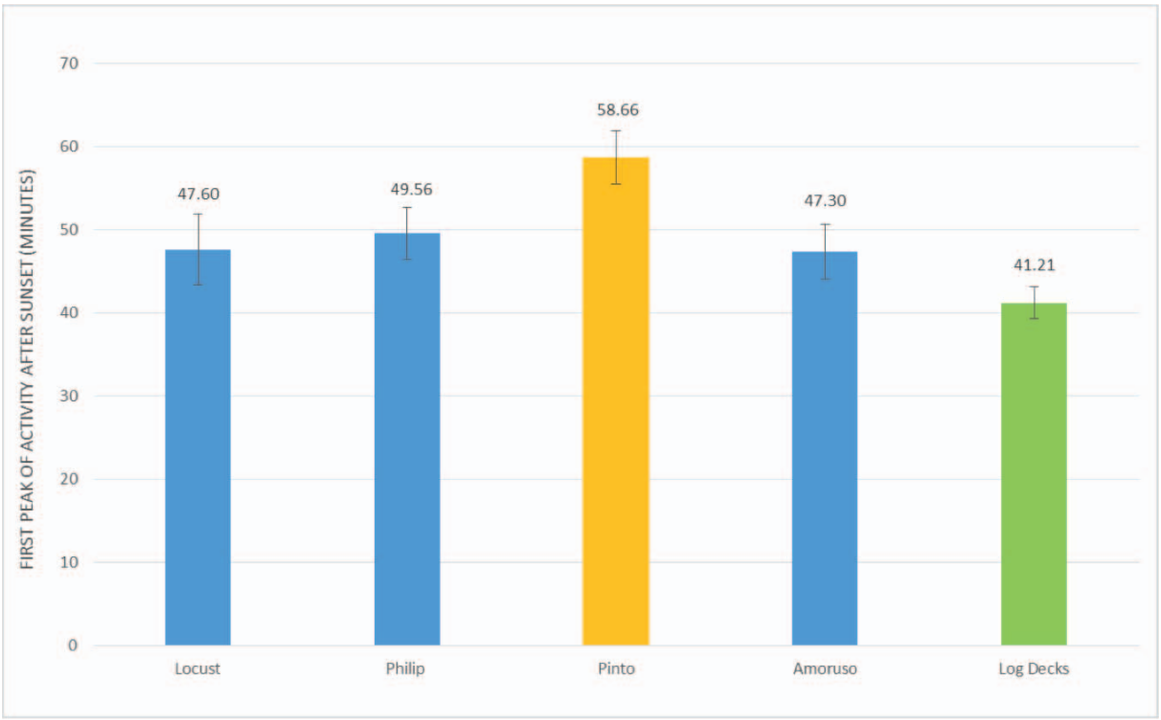

Figure 6.—Time (number of minutes) of the first peak of activity after sunset for each site (average of the data for the whole three-month season). Same color for the bars indicates that there is no statistical difference between those sites based on the overlapping of the error bars (95% CL).

peak of activity represented less than 10% of the total activity from sunset to sunrise. In the case of Locust Road, out of the 82 days when data were recorded, there were 36 days (which represents 42% of the time) when more activity was observed after midnight than before midnight. Operationally, those findings can make Districts reconsider when is the best time to conduct adulticide treatments (aerial or ground). New tools and new data available should help to maximize the impact of adulticide applications.

Table 1.—Results of linear regressions applied to each site (three-month season) to determine if the time (number of minutes) after sunset for the first peak of mosquito activity changed as a function of date over the summer within the same site.

	P-value / Intercept	Data
Locust	0.32	86
Philip	0.08	68
Pinto	0.01*	60
Amoruso	0.007*	72
Log Decks	0.30	74

* P-value < 0.05 indicated that the time (number of minutes) after sunset for the first peak of mosquito activity significantly changes over the course of the season.

Time (number of minutes) after sunset for the first peak of mosquito activity

Studies have suggested (Reisen et al.1997; Boisvert et al. 2018) that sunset and sunrise will trigger activity for a night active species such as *Cx. tarsalis*. Depending on different variables (temperature, relative humidity, wind, etc.), it is quite likely that the time (number of minutes) after sunset for the first peak of mosquito activity may vary on a daily basis. Although some daily variation was observed, an analysis was performed to determine if the time (number of minutes) after sunset varied significantly within a site during the course of the season. Results in Table 1 show that for three locations (Locust Road, Philip and Log decks) the time (number of minutes) after sunset for that first peak of mosquito activity did not change significantly during the course of the season (linear regression, date vs. time of first peak, P-value > 0.05). Pinto and Amoruso showed that the number of minutes after sunset for that first peak of mosquito activity can vary significantly during the course of the season (linear regression, date vs. time of first peak, P-value < 0.05).

The BG-Counter can count mosquitoes entering the trap, but cannot specifically differentiate among species. A

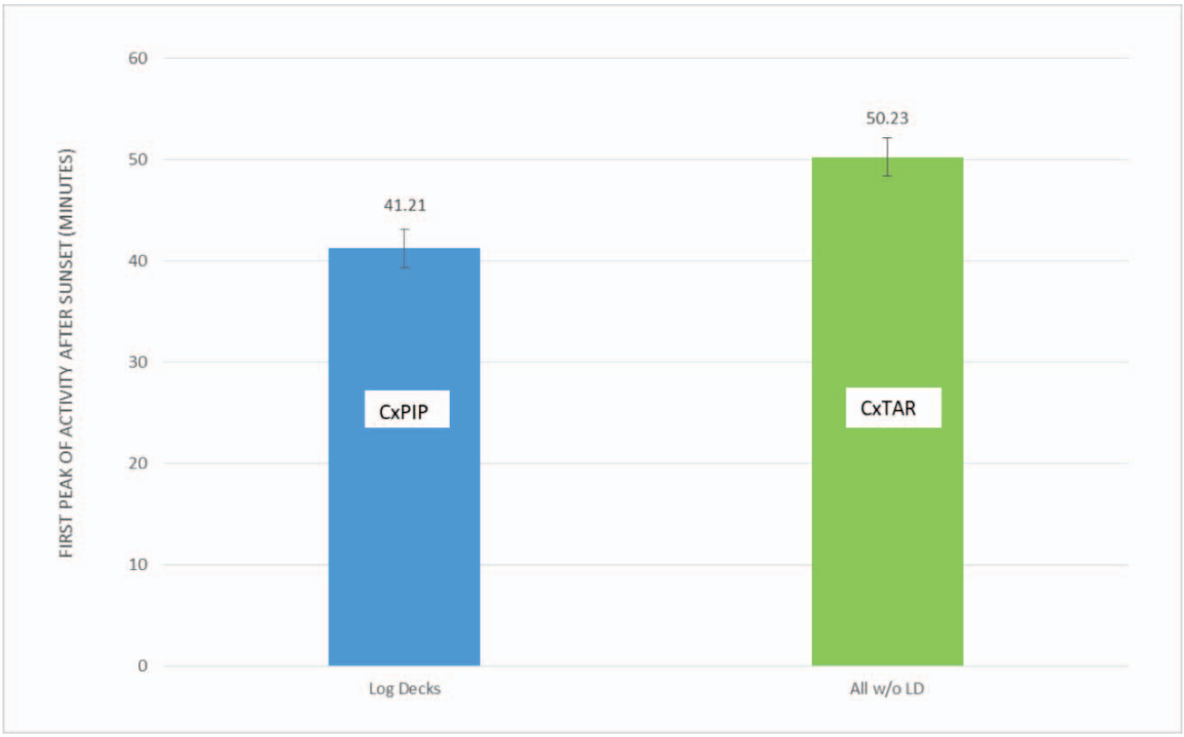

Figure 7.—Time (number of minutes) of the first peak of activity after sunset at the *Culex pipiens* site versus the combination of all the *Culex tarsalis* sites. There was a statistical difference between sites based on the non-overlapping of the error bars (95% CL).

thorough analysis of the Amoruso's data indicated that a very high peak of abundance of *Aedes melanimon* at the end of September (confirmed by our regular surveillance program) might have made that site "falsely" significant (i.e, appearing that timing of *Cx. tarsalis* activity changed over the season). Without those data (*Ae. melanimon*) the time (number of minutes) after sunset for Amoruso may have shown a non-significant result, indicating that the time of the first peak of activity after sunset did not change significantly during the 72 days of the experiment if we would have considered only *Cx. tarsalis* data.

We also were able to determine if the minutes after sunset for the first peak of mosquito activity was different among sites. Pinto and Log decks were both significantly different from each other and the three other sites (Figure 6). For Locust Road, Philip and Amoruso there were no significant differences in the number of minutes when the first peak appeared after sunset (47.30 to 49.56 minutes). Reisen et al. (1976) and Reisen and Aslamkhan (1978) also observed diel activity patterns in some culicidae in studies performed in Pakistan. Reisen et al. (1976) found that some culicine mosquitoes (*Aedes* and *Culex*) were most abundant during the evening crepuscular period and the first two hours of darkness.

As the Log decks site was very different than the rice or corn field sites and consisted mainly of a *Cx. pipiens*, the significantly different results were not necessarily "expected" but could be explained by those two facts.

Finally, if we compared all the *Cx. tarsalis* sites combined together to the *Cx. pipiens* site, we observed a significant difference ($P<0.05$) in the number of minutes after sunset for the first peak of activity (41.21 min for *Cx. tarsalis* versus 50.23 min for *Cx. pipiens*) (Figure 7).

Conclusion

In conclusion, the BG-Counter was a very useful new tool to collect data about the timing (first peak after sunset, peaks during the season) or patterns (curve patterns, activity throughout the evening and night) of mosquito activity throughout the night. Similar data (patterns, peaks) were observed over the 2-year trial, even though the second year the surrounding environment was very different. We documented that the abundance and peaks of activity of *Cx. tarsalis* varied greatly within and among sites.

Operationally, we did not observe enough variation between the time of the first peak of activity at the different sites to make a change in the time our aerial or ground sprayings are performed. Eventually, Districts might have to reconsider the time of the spraying based on the time period when the highest mosquito activity is found, meaning that the best time to treat might not be necessarily at the peak of activity after sunset and that use of trucks and drones might be better tools than planes to meet those narrow windows of operations.

Setting up the BG-Counters in the same locations for another season could help confirm results obtained in 2018. Analysis of other variables such as the age of the mosquitoes caught at different time of the day or the wind speed could also help better define and interpret the data.

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Utilizing new technology for analyzing host seeking mosquito activity: The BG Counter Trap

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Introduction

Determining peak mosquito flight activity is an important aspect for maximizing the efficacy of adulticide Ultra Low Volume (ULV) applications. The Sacramento-Yolo Mosquito and Vector Control District (District) performs over 500,000 acres of ULV mosquito adulticiding every year via aerial and ground based equipment. Although the majority of this is done in rural/agricultural areas, some treatments are done in urban/suburban habitats. In 2018 over 30,000 acres of ground based ULV treatments were done in this habitat due to high West Nile virus activity. Optimizing the efficacy of these applications is an important component of a good mosquito management program. During the 2018 season the District incorporated the Biogents Sentinel Counter trap (BG Counter) as part of our surveillance program. The BG Counter is a remote monitoring device which counts mosquito-sized insects entering the trap in real-time which can be viewed via a web application. Verifying the time of peak mosquito flight activity is an important factor in planning ULV activities, and can optimize the exposure of mosquitoes to a specific application. We evaluated flight activity in both rural/agricultural areas and urban/suburban areas.

Methods

Biogents counter traps record and size objects that pass through a light sensor. The trap distinguishes between three sizes: smaller than a mosquito, mosquito-sized and larger than a mosquito. Carbon dioxide (CO_2) is the mosquito attractant utilized in addition to the black and white color of the trap itself. The data are transmitted every 15 minutes to a web page that displays and records the data. Several aspects of the trap can be controlled remotely via the web interface including, the timing and volume of CO_2 emitted and operation of the fan and counter.

Counter traps were placed at ten different locations over the 2018 season. Seven of these locations were in primarily rural/agricultural habitat whereas three were in urban/suburban habitat. *Culex tarsalis* and *Anopheles freeborni* were the primary mosquito species present in the rural/agricultural habitat, whereas, *Culex pipiens* and *Culiseta incidens* were the primary species found in the urban/suburban habitat.

Results and Discussion

The rural/agricultural counter traps showed the expected activity, with peaks at dusk/early evening and dawn (Figure 1).

By comparison, the urban/suburban counter traps showed most flight activity after midnight in the early morning hours, far later than expected for the mosquito species found in this habitat (Figures 2 and 3).

The significance of detecting the early morning activity shown by the counter trap is that control applications in this habitat may be more effective in the late night and early morning hours. Normal adulticiding in this habitat is done at parks, greenbelts and similar areas during the dusk time frame with backpack, quad, and truck mounted equipment. Applications are often interrupted by the sudden presence of people in these areas. In agricultural areas particularly rice fields, an inversion layer needs to be present for the pesticide material to traverse the treatment area and stay close to the ground where mosquitoes are active. The need for an inversion layer to be present while performing ground applications in an urban/suburban habitat is not as critical as treatments usually are done underneath a tree canopy in dense vegetated areas. Data from the counter traps has indicated that early morning applications in urban areas, where inversions are not as critical, may be more effective than dusk applications, thus providing flexibility in the timing of mosquito control operations in urban/suburban habitats.

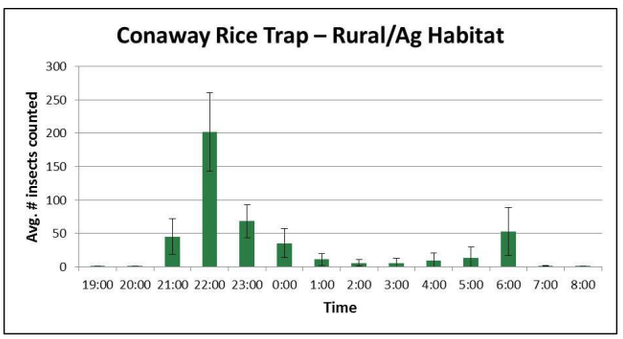

Figure 1.—The average hourly count of mosquito-sized insects collected in a counter placed in a rice field habitat from July 17-23, 2018. The standard error of the mean is shown.

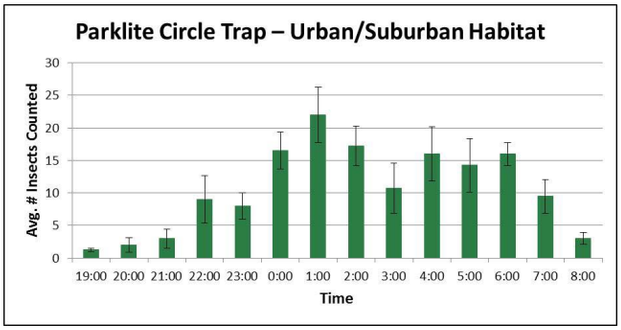

Figure 2.—The average hourly count of mosquito-sized insects collected July 19-22, 2018 from an urban/suburban habitat in Sacramento County. The standard error of the mean is shown.

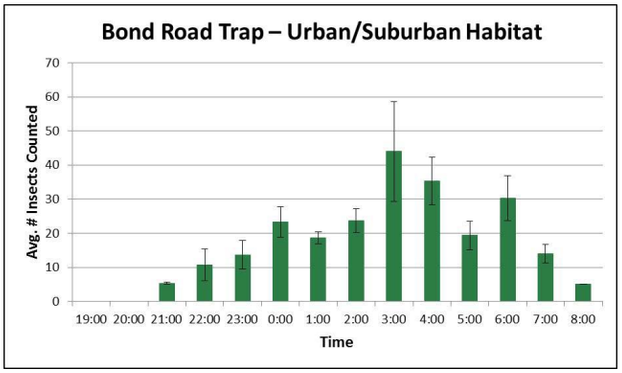

Figure 3.—The average hourly count of mosquito-sized insects collected August 10-12, 2018 from an urban/suburban habitat in Sacramento County. The standard error of the mean is shown.

Conclusion

The BG Counter trap provided valuable surveillance data for mosquito control operations, especially in determining when mosquitoes were active. Our data from a rice field habitat trap verified the dusk or post-sunset as the optimal time frame. Data for the urban/suburban area along with the weather and other environmental factors affecting adulticide ULV treatments in these habitats indicated a much wider range of potential treatment times. When virus activity necessitates urban/suburban adulticide ULV treatments, efficacious treatments may be possible in the early morning hours.

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Flea-borne typhus surveillance in Orange County: working together to protect the public

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Abstract

Since 2006, flea-borne typhus (= flea-borne rickettsiosis) has re-emerged as an important vector-borne disease in Orange County, California, with 191 reported human cases (no deaths) since its resurgence. The Orange County Mosquito and Vector Control District's (District) flea-borne rickettsiosis prevention and response program has conducted ecologic investigations (patient interviews, host animal and flea collections near putative exposure sites), qPCR testing for the presence of *Rickettsia* spp. bacteria in flea and host animal specimens, public education campaigns (door-to-door notifications, informational flyer distribution near disease cases), and coordinated with other stakeholders for rickettsial surveillance and disease prevention. The District has also provided guidance to other governmental agencies faced with recent outbreaks of flea-borne rickettsiosis in the cities of Long Beach, Los Angeles, and Pasadena. As part of this collaboration, the District tested fleas removed from Norway rats, cats, opossums, and stray dogs from the two typhus zones in Los Angeles County in 2018. This presentation summarized concerns from public health agencies regarding flea-borne rickettsiosis disease risk associated with feral cat Trap-Neuter-Return programs, and the future of uncontrolled Norway rat populations in impoverished metropolitan areas of southern California.

Detection of *Rickettsia* species in fleas collected from opossums, feral cats and Norway rats in Alameda County

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Introduction

Rickettsia species are gram-negative, obligate intracellular bacteria that cause rickettsial diseases worldwide and are transmitted to humans via an arthropod host, specifically fleas, lice, ticks and mites (Abad et al. 2011). *Rickettsia typhi*, the causative agent of murine typhus in humans, is transmitted by the Oriental rat flea *Xenopsylla cheopis,* and was the first species linked to human cases of flea-borne rickettsioses worldwide. *Rickettsia felis* is commonly associated with the cat flea, *Ctenocephalides felis* , and causes human flea-borne rickettsioses worldwide (Reif et al. 2009). Los Angeles and Orange Counties are known as endemic areas for flea-borne rickettsioses (Williams et al. 1992 and Maina et al. 2016). Previous studies conducted in Sacramento and Contra Costa Counties showed the presence of *R. felis* in fleas collected from cats (Billeter et al. 2016). However, to date no reports of flea-borne rickettsioses are known from Alameda County. In the present study, we assessed cat fleas (*C. felis*) and Oriental rat fleas (*X. cheopis*) recovered from animals in Alameda County in 2018 for the presence of *Rickettsia* species using PCR based assays.

Materials and Methods

Between January-August 2018, fleas were collected from rats, cats and opossums as a part of the Alameda County Vector Control Service District's surveillance program. Nuisance opossums were trapped in a small mammal trap (Tomahawk Live Trap, Tomahawk, WI) from 7 locations throughout the county. Feral cats were obtained from animal shelters and veterinary clinics within Alameda County. Norway rats were collected at several homeless encampments in the City of Oakland. Trapped animals were euthanized according to the American Veterinary Medical Association protocol. Fleas were combed from each animal using a fine-tooth comb. After identification they were sorted by species, sex and host animal and pooled (up to five per group). Fleas were washed first with 70% ethanol followed by molecular grade water. DNA was then extracted using MagMax DNA Multi-Sample Kit (Life technology) and KingFisher Duo purification system following the manufacturer's instructions. Quant Studio™ 5 Real-Time PCR System (Life Technologies) was used for molecular testing. Initially, all samples were screened for presence of *Rickettsia* using Pan-Rickettsia qPCR assay developed by the Centers for Disease Control (Karpathy et al. 2009). To identify the specific *Rickettsia* species, 1560 bp segment of ompB gene (Billeter et al. 2016) was amplified, sequenced and analyzed using data in the GeneBank (www.ncbi.nlm.nih.gov/genbank).

Results

The fleas in the study were identified as cat fleas (*C. felis*) and Oriental rat fleas (*X. cheopis*) using a standard taxonomic key (Lewis et al. 1988). In total, 439 cat fleas and 226 Oriental rat fleas were removed from 11 opossums, 12 feral cats and 51 Norway rats. Opossums had a higher *C. felis* infestation rate (24 per animal) compared to cats (10 per animal) and Norway rats (2 per animal). *X. cheopis* (6 per animal) were only found on Norway rats. All 665 fleas were tested in 177 pools for the presence of *Rickettsia* spp. using qPCR. Out of 177 DNA extracts, 36 were positive for *R. felis*. None were positive for *R. typhi*. The infection prevalence of *R felis* in cat fleas was higher in Norway rats (45%) than opossums (31.1%) and feral cats (18.8%). Only one pool of Oriental rat fleas was positive for *R. felis*. Our data indicate that the presence of *R. felis* is widespread in Alameda County. We found *R. felis* positive cat fleas from eight cities: Alameda, Oakland, Hayward, Union City, Fremont, Newark, San Leandro and Pleasanton. All three-animal species were hosts of *R. felis* infected cat fleas, suggesting an involvement in the *R. felis* transmission cycle. The classic rat-cycle of *R. typhi* was not detected in this study.

Conclusions

Rickettsia felis was found in cat fleas and oriental rat fleas collected countywide. These results demonstrate that opossums, feral cats and Norway rats in Alameda County are hosts to *Rickettsia* infected fleas which may pose a potential public health risk.

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Survey of ectoparasites collected from Norway rats, *Rattus norvegicus*, in homeless camps in the City of Oakland, California.

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Introduction

The City of Oakland is the largest city within Alameda County, encompassing 78 square miles, and serves as the economic center of the county including a major transportation and shipping hub. It is the 8th largest city in California, and the 45th largest city in the United States. Alameda County is located across the bay from San Francisco and is the 7th most populous county in the state of California. Over 1.5 million people live in Alameda County and it is comprised of 15 major cities. The Alameda County Vector Control Services District operates under the Department of Environmental Health and its mission includes preventing the spread of vector-borne disease, injury and discomfort to County residents associated with rodents and other vectors.

The number of homeless encampments within the City of Oakland has increased significantly in the past two years. A biennial count of homeless released in May 2017 found a 25% increase in the number of homeless people living within the city limits (Alameda County Health Care Services Agency, 2017). Currently, approximately 2,800 people are living in encampments throughout the city, mostly concentrated underneath freeway overpasses/transportation infrastructure and on adjoining lands. Upon inspection by District biologists, it was discovered that several of these encampments had active Norway rat populations as indicated by active burrows within, and adjacent to, the camps. These observations coincided with reports of rat sightings by residents of the encampments, surrounding businesses, and members of the public.

The Norway rat (*Rattus norvegicus*) was introduced to North America along the eastern seaboard around 1775 from sailing ships (Bourne 1998). Originally from China (Nowak and Walker 1991), they have become a worldwide pest and they are most strongly associated with urban ecosystems (Feng and Himsworth 2014). Norway rats are adapted to temperate climates and are well suited for cohabitation with people (Feng and Himsworth 2014). They can commonly be found living inside structures, feeding on refuse and discarded food. Firth et al. (2014) "suggest that urban Norway rats may be an important source of zoonotic pathogens". Norway rats are known reservoirs of several human pathogens, including *Bartonella* spp., SEOUL hantavirus, and *Leptospira interrogans* (Easterbrook et al. 2007a; Gundi et al. 2012; Himsworth et al. 2013; Feng and Himsworth 2014). Coupled with high levels of fecundity, fast growth rates and high population densities (Firth et al. 2014), ongoing monitoring of Norway rat populations is warranted.

Beginning in the fall of 2017, District biologists began live-trapping at several homeless encampments in the City of Oakland to ascertain ectoparasite diversity and abundance on corresponding Norway rat populations. Historically Norway rats have been found to harbor varying species of fleas, lice and mites in North America (Pratt and Good 1954). Because they are vectors of pathogens such as Murine typhus (*Rickettsia typhi),* flea-borne typhus (*Rickettsia felis*), and plague (*Yersinia pestis*) (Azad 1990; Krueger et al. 2016), District biologists focused on ascertaining flea abundance and species composition. Norway rats from four different encampments in the City of Oakland were trapped over a nine-month period and the abundance and species composition of fleas, mites and lice monitored.

Materials and Methods

Four homeless encampments with ongoing Norway rat activity, within the City of Oakland, were identified. From November 2017 through August 2018, staff members from Alameda County VCSD placed Tomahawk Live Traps (Tomahawk Live Traps, WI) at the sites. Traps were baited with a combination of canned mackerel and peanut butter. Traps were placed in the late afternoon, 14:00-16:30 h, and the following criteria were used to determine the best areas for trap placement: presence of Norway rat droppings; active Norway rat burrows (indicated by the burrow entrance being clean, smooth and free of cobwebs); rat "runs" evident through neighboring vegetation/debris; residents of the camps directing us to where they saw the heaviest activity.

After traps were set, they were covered with debris found nearby, i.e. discarded clothing, cardboard, blankets etc. This was done to mask any potential new odors present on the trap that would induce trap shyness by the rats. Traps were left out overnight and picked up the following morning between 0900 and 1100 h.

Table 1.—Changes in the flea index associated with Norway rats live-trapped from homeless encampments within the City of Oakland, California.

Homeless Encampment	Collection Date	Total Number of Rats Trapped	Total Number of Fleas Collected	Average Number of fleas/rat
Encampment 1 – High Street	5/1/18	13	0	0
Encampment 1 – High Street	11/7/18	25	24	0.9
Encampment 2 – Wood Street	1/12/18	6	7	1.17
Encampment 2 – Wood Street	2/6/18	13	0	0
Encampment 2 – Wood Street	8/28/18	13	12	0.92
Encampment 3 – E12th and 23rd St	8/15/18	32	179	5.6
Encampment 4 – Northgate	6/5/18	43	48	1.12

Live rats were brought back to the Alameda County VCSD laboratory and euthanized using CO_2 according to protocols outlined by the American Veterinary Medical Association. Once euthanized, the animals were placed into individual clear plastic bags to retain any ectoparasites associated with the host. Euthanized rats were sprayed with P.T. P.I. (BASF Corp., NC), a pyrethrin to kill any ectoparasites. Animals were left for at least one hour prior to combing for ectoparasites.

Rats were combed for ectoparasites using a fine bristle brush (Scotch Brite, 3M, St. Paul MN). Any ectoparasites collected were placed in 95% EtOH and set aside for identification using the following taxonomic keys: Fleas: Hubbard. Fleas of Western North America; Lice: USDA. Chewing and Sucking Lice as Parasites of Mammals and Birds; Mites: Pratt and Stojanocvich. Acarina: Illustrated key to Some Common Adult Female Mites and Adult Ticks.

Results and Discussion

Norway rats were trapped at four different homeless camps in the City of Oakland over approximately a nine-month period. The composition and abundance of ectoparasites changed over time at each encampment (Table 1). During the first collection event at Encampment 1 (High Street) (N=13), no fleas were recovered, but two of the rats had a louse load of over 200 lice per individual. The second collection (N=25) at the same encampment saw an increase in number of fleas, with a flea index = 0.9, but the number of lice dropped significantly to less than twenty per individual. At Encampment 2 (Wood Street), the average number of fleas did not change over time (average flea index = 1.05), but the number of mites increased over time; no lice were recovered from any of the rats over the three separate trapping events (N=32). At Encampment 3 (E12th and 23rd St.), the flea index = 5.6 (N=32 rats) and Encampment 4 (Northgate) the flea index = 1.12. Both cat fleas, *Ctenocephalides felis*, and Oriental rat fleas, *Xenopsylla cheopsis*, were collected. The number of lice and mites collected from these two encampments also varied. Because, each encampment was located several miles from each other, we believe these are distinct populations of Norway rats. Trap success rates ranged from 30-90% over the various trapping events. Data analysis is on-going. In order to establish a baseline of ectoparasite abundance and composition over time, we are continuing trapping at the same locations.

Conclusions

The species composition and abundance of ectoparasites found on Norway rats trapped at four different homeless encampments within the City of Oakland varied over a 9-month period. There was also a difference seen between encampments. It is unknown whether this variability is linked to normal changes that are seasonal in nature, or if other factors such as soil composition, ground vegetation, microclimate variations, or contact with dogs/wildlife influence these infestations. More research needs to be done to determine what constitutes an "average" ectoparasite load on Norway rats in the City of Oakland, California.

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Downslope movement of adult ticks, *Ixodes pacificus* and *Dermacentor occidentalis* (Acari: Ixodidae), under natural conditions in Alameda County, California, USA

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Introduction

The western black-legged tick, *Ixodes pacificus*, is the primary vector of the Lyme disease spirochete (*Borrelia burgdorferi* sensu stricto) in the western United States. The Pacific Coast tick, *Dermancentor occidentalis*, has recently been found to transmit *Rickettsia philipii*, the causative agent of Pacific Coast tick fever (Padgett et al. 2016). Both ticks are commonly found in the numerous public parks throughout Alameda County. Several investigators have noted that *I. pacificus* adults are more numerous on the uphill side of trails than on the downhill side (Kramer and Beesley 1993; Lane 1996). Li et al (2000) found that *I. pacificus* densities were higher on the uphill than the downhill sides of trails, but higher on the downhill side of most trails with level sides. They stated that the reasons of this pattern remained obscure. Herein, we described a mark and recapture study to determine if adult ticks move downhill from uphill areas to trail margins.

Materials and Methods

This study was conducted along a section of fire trail in Leona Heights Regional Park (37.789039, -122.174130), Oakland, Alameda County, California. This park, a popular dog walking area, was located on the west facing part of the Oakland Hills (California Coastal Range). The uphill study area was covered mostly with a thick stand of old-growth chaparral that was located on the edge of oak woodland. Coyote brush was the predominant plant in the uphill area.

The slope of the uphill section varied between 30° and 38°. Slope was determined using an Apple iPhone Compass app. Temperature, relative humidity and dew point readings were taken during each sampling event using a Extech Humidity Alert II, hygro-thermometer.

All ticks captured for mark and release *(I. pacificus* and *D. occidentalis*) were collected by flagging along the trail and environs prior to the study period. Adult ticks were marked on the dorsal side with a BIC® Wite-Out® Brand Correction Pen, Shake 'N Squeeze™ correction pen. This marker was found to be non-toxic and durable (Kramer et al. 1993; Walker and Wineriter 1981) and was easy to apply.

The release site was located 1 m below the upper part of the chaparral stand and 30 meters above the trail. Google Earth measuring tool was used to estimate the distance. Marked male ticks were released 1.5m laterally from the female release site.

Sampling for questing adult ticks was done by flagging with a 1.0 m^2 flannel cloth over low vegetation on the uphill margin along a 30m x 1m transect along the trail. The transect was divided into 5 m sub transects. The midpoint of the transect was directly below the release site. Unmarked and marked recaptured ticks were recorded per sub transect. This study was conducted from 2016 to 2018. Sampling along the transect was conducted between 0930 and 1300 hours from January to May on a weekly basis, weather dependent.

Results and Discussion

In 2016 of the 188 female and 114 male *I. pacificus* ticks marked and released, 11 female and one male *I. pacificus* ticks were recaptured at the trail transect line (Table 1). The first recaptures occurred on 4/6/2016 where a male and female *I. pacificus* were collected, demonstrating a movement of 1.6 - 1.9 m/day. Seven of the 11 (63%) *I. pacificus* were recaptured 43 to 62 days after the release.

In 2017 71 female and 52 male *I. pacificus* were collected, marked and released (Table 1). None of the ticks were recaptured during the 11 week period. Of the 81 female and 82 male *D. occidentalis* ticks marked and released, 15 females and 6 males were recaptured. The first recaptures of *D. occidentalis* were on 4/24/17 when four marked females and one marked male were recaptured. These ticks travelled between 0.7 to 3 meters per day.

In 2018 a total of 247 female and 287 male *I. pacificus* were collected, marked and released on 1/19/2018, of which 9 female and one male were recaptured (Table 1.). The first recapture was 32 days after release (1 female), travelling 0.9m per day. Seven of the 9 (77%) *I. pacificus* were recaptured between 49 and 73 days after release. The study was started earlier in 2018 when adult I. pacificus are more abundant (Salkeld 2014; Kramer and Beesley 1993) which allowed to collect, mark and release a larger number of ticks and to extend the recapture period up to 18 weeks after the release. Interestingly, 3 marked female and 2

Table 1.—Summary of number of *I. pacificus* (I.pac) and *D. occidentalis* (D.o) that were marked, released and recaptured at Leona Heights Regional Park, Oakland

	2016	2017	2018
Release date(s)	3/ 14 & 18	3/10-4/14	1/19/2018
I. pac ♀ released	188	71	247
I. pac ♂ released	114	52	287
D.o. ♀ released	NA	81	NA*
D.o. ♂ released	NA	82	NA*
Sampling dates	3/23-6/1	4/3 - 6/8	1/26 -5/22
Sampling events	11	11	21
1st recapture date	4/6/2016	4/24/2017	2/20/2018
1st recapture (days)	16-19	10-44	32
Movement (m/day)	1.6-.9	0.7-3	0.9
marked I. pac ♀	11(5.8%)	0	9(3.6%)
marked I. pac ♂	1(0.87%)	0	1(0.35%)
marked D.o ♀	NA	15(18.5%)	3*
marked D.o ♂	NA	6(7.3%)	2*
unmarked I. pac ♀	20	10	95
unmarked I. pac ♂	20	6	83
unmarked D.o ♀	NA	78	209
unmarked D.o ♂	NA	70	200

* no D.o marked in 2018

marked male *D. occidentalis* were recaptured even though no *D. occidentalis* were marked and released in 2018.

Our study was only concerned with the downhill movement of ticks and not the many factors which may influence tick movement. Recapture numbers only indicate the number of questing ticks at the time of sampling and do not reflect any "drop off" (Li and Dunley 1998), vegetation changes over the years along the transect, and differing collector techniques.

A number of marked *I. pacificus* females (3.6-5.5%) and males (0.4-0.9%) did travel down slope (30m) and were recaptured at the trail margin. A higher number of marked *D. occidentalis* were recaptured, 18.5% females and 7.3% males. However, the rate of movement for *I. pacificus* and *D. occidentalis* was not significantly different.

The *D. occidentalis* marked in 2017 and recaptured in 2018 indicated that male and female *D. occidentalis* can survive through to the next season in maritime habitats in Alameda County.

Conclusions

We demonstrated that downhill movement of *I. pacificus* and *D. occidentalis* adult ticks in a natural environment varies between tick species and that female ticks tend to move more compared to males. *I. pacificus* ticks moved downhill in chaparral habitat with the rate of 0.9 – 3.1 m/day. An uphill trail margin could play a role of a natural barrier which explains the higher adult tick abundance on the uphill sides of trails. Also, we found that *D. occidentalis* adults could survive one year in the maritime habitats of East Bay Regional parks.

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Geospatial risk of encountering *Ixodes pacificus* nymphs in Alameda County, California: Morning dryness determines bio-geographic boundaries in a semiarid climate.

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Abstract

The nymphal life stage of the western black-legged tick, *Ixodes pacificus,* is the primary vector of the Lyme disease spirochete *Borrelia burgdorferi* sensu stricto and closely related spirochetes in the far-western United States. Field surveillance was conducted to assess the potential geospatial risk of encountering *B. burgdorferi*-infected nymphs in three high-risk sites located on coastal and continental sides of the California Coast Range in Alameda County, California. During the study period between 2012 and 2016, California experienced a severe drought. The year 2014 set unprecedented precipitation records for the driest period statewide. Paradoxically, there was a countywide upsurge in host-seeking nymphs. Although nymphs are highly vulnerable to desiccation, drought related dryness was suspected as the cause for the nymphal increase. Climate patterns were examined with Dew Point Depression (DPD) and minimum Vapor Pressure Deficit (VPDmin) found to best predict the trends of increase in nymphs. Meteorological variables were estimated by Climatologically-Aided interpolation. This ground truthing field study helped to link geospatial and temporal variation in the abundance of host seeking nymphs to morning minimum dryness levels (or maximum ambient moisture).

Introduction

Among 2,890 *Ixodes pacificus* nymphs tested in 2009-2012 by Alameda County Vector Control Services District in California, 189 (6.5%) were positive for Lyme disease group spirochetes *Borrelia burgdorferi* sensu lato (Bbsl) (Fedorova et al. 2014). To better understand the potential geospatial risk for Lyme disease in Alameda County, sites having high rates of Bbsl infection (21.3% - 29.5%) in nymphs inhabiting coastal and continental aspects of the California Coast Range were monitored yearly (Fedorova et al. 2014).

California experienced a severe drought from 2011 to early 2017. During the driest year 2014, there was an unexpected countywide increase in *I. pacificus* nymphal collections. Although nymphs are highly vulnerable to desiccation, densities of nymphs in Alameda County and elsewhere in California are greater in warm, dry inland habitats (Eisen et al. 2003). Here, we feature minimum dryness derivatives, Dew Point Depression (DPD) and minimum Vapor Pressure Deficit VPDmin that are quantitative measures of moisture. Moisture is critical to the survival and the questing behavior of the nymphal life stage (Lees 1946, Gray 2009, Clover and Lane 1995). Because the distribution and abundance of the tick vector is basic to the risk of acquiring Bbsl disease agents, the intent herein was to link meteorological values to geographical variation in year-to-year nymphal abundance.

Methods

Three ecoregions in Alameda County were monitored for long-term adult and nymphal tick surveillance (2009 - 2018): 1) cool evergreen California bay/Coast live oak woodland with limited Coast Redwood influence (Maritime); 2) dense deciduous hardwood forest along the east face of the Coast Range (East Face); and 3) savanna deciduous oak/woodland grass habitat which characterized much of the warm, dry Sunol Regional Wilderness (South East (SE) Wilderness). Tick collections were conducted along the margins of perennial creeks and park trails. These included Joaquin Miller, Anthony Chabot and Garin Regional parks in the Maritime climate zone; Augustin Bernal Park and Pleasanton Ridge Regional Park along the east face of the Coast Range; and the SE wilderness embraces Sunol and Del Valle Regional parks. Ticks were collected by dragging a light-colored 1.0×1.0 m^2 corduroy cloth that was weighted along its distal end by a chain hemmed in the cloth. The drag was inspected frequently for one hour long, monthly sampling occasions.

Climate

Maximum and minimum temperature (Tmax and Tmin), daily mean dew point (Tdmean), and maximum and minimum Vapor Pressure Deficit (VPDmax and VPDmin) were obtained online from the PRISM Climate Group (www.prism.oregonstate.edu). Geographic coordinates of sites with the highest densities of host-seeking nymphs and

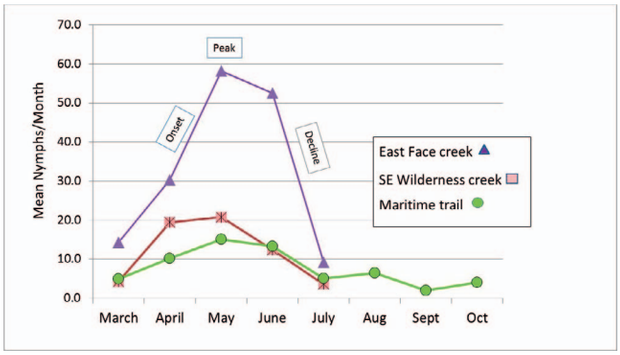

Figure 1.—The phenology for *Ixodes pacificus* nymphs for selected high risk sites in three ecoregions located on coastal and continental sides of the California Coast Range in Alameda County, California. Means of nymphs collected per month over the nymph season from 2011-2016.

Bbsl infection prevalence were selected from within each eco-region (Fedorova et al. 2014). Coordinates were entered into PRISM "Data Explorer" where the 4 kilometer range and interpolation were selected. Meteorological variables accessed at PRISM are estimated by Climatologically-Aided interpolation (Daly et al. 2015).

Dew Point Depression (DPD) and VPDmin are quantities that measure dryness in the morning, the time of day when ambient moisture typically is at maximum. DPD equals Tmin minus Tdmean. As the DPD increases, relative humidity (RH) decreases (an inverse relationship). DPD is more readily converted to relative humidity (RH) than is VPDmin. The same values accessed to derive DPD, Tmin and Tdmean were converted to RH online using a vapor pressure calculator (www.csgnetwork.com/vaporpressurecalc.html).

Results and Discussion

The mean catch of nymphs varied more among the three regions in Alameda County than did catch of adults. Notably the highest hourly adult collections per year exceeded 100 ticks/hour. Adult ticks are known to be key indicators of suitable habitats for *I. pacificus* (Eisen et al. 2018). More exactly, if good numbers of adults were detected, then the nymph life stage must also be present, even if it wasn't collected. The fact that adults were uniformly plentiful in the three regions indicated that variations in nymphal abundance could partly be explained by different climate factors known to be critical determinants in the nymphal questing response (Eisen et al. 2010, Eisen et al 2016).

Inland

Abundance of nymphs was strikingly different inland for the East Face of the Coast Range and the SE Wilderness areas (Table 1). Nymphs rarely were detected in the warmer and drier SE Wilderness (with the exception of one creek site). However, nymphs were commonly found along four miles of riparian habitat and along trails associated with the East Face of the Coast Range. The Riparian California Sycamore Alliance that had the greatest nymphal burden of the two inland regions was the habitat focus inland.

Coastal aspect

In the maritime climate zone, fewer nymphs were collected from creek bank sites compared to trail sites (Table 1). The geographic coordinates of a trail site in Anthony Chabot Regional Park were selected to analyze maritime exposure. The geospatial dichotomy of nymph abundance along creeks versus trails that was detected on coastal versus inland aspects of the Coast Range was subtly influenced by temperature and humidity (Eisen et al. 2016). The mean of the hourly collections of nymphs for each month of each nymph season over 6 years of sampling at high-risk sites reflected the timing and duration of population onset, peak, and decline (Figure 1). Determining temporal and geographical variation in the abundance of host-seeking ticks is critical to evaluating the risk for acquiring Lyme disease (Killilea et al. 2008, Eisen et al. 2016).

Relative humidity

Ixodes scapularis nymphs, closely related to *I. pacificus*, have been demonstrated to survive in low humidity

Table 1.—All *Ixodes pacificus* nymphs collected from the margins of creeks and trails in three eco-regions of Alameda County, California, 2011- 2016.

Eco-region	Habitat	Total no. of Nymphs Collected	No. of sampling occasions	Mean No. nymphs/hour
Maritime	Creeks	303	61	5.0
	Trails	849	82	10.4
East Face	Creeks	1022	40	25.6
	Trails	931	52	17.9
SE Wilderness	Creeks	409	31	13.2
	Trails	93	28	3.3

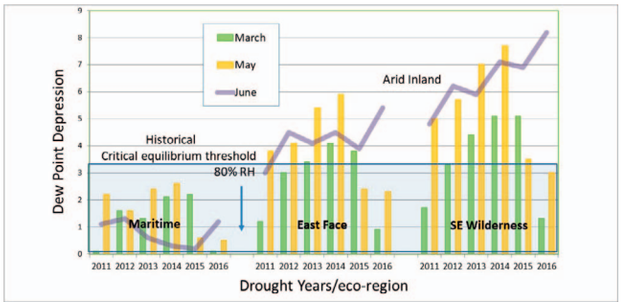

Figure 2.—Dew point depression measurements for three eco-regions located on coastal and continental sides of the California Coast Range in Alameda County during drought years 2011 to 2016. The Dew Point Depression value of 3.1 was converted to 80% relative humidity, which is known as the critical equilibrium for many *Ixodes* ticks.

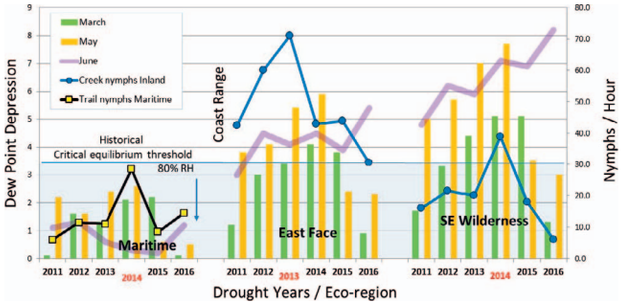

Figure 3.—The average of the two highest hourly *Ixodes pacificus* nymph collections per year in the selected high-risk sites. Increase in nymph populations are suggested to follow a pattern of increase in the local Dew Point Depression during the drought period 2011-2016.

conditions as long as they are returned daily to a critical RH equilibrium threshold above 82% (Rodgers et al. 2007, Berger et al. 2014). Herein, the DPD value below 3.2 indicated the mean monthly levels of RH above 80%. In Alameda County, the air mass on the maritime side of the Coast Range rarely fell below morning access to 80% RH. In contrast, 80% RH was not always attained daily along the warm dry inland side of the Coast Range (Figure 2). The DPD values for March and May inland demonstrated departure from the critical equilibrium during the early nymph season, suggesting that *I. pacificus* thrived in a lower RH domain during the drought years than would be expected for *I. scapularis*.

The average for the two highest yearly collections of *I. pacificus* nymphs is understood to embrace the bulk of the yearly population. These values presented a distribution of regional yearly abundance with the greatest values located along the east face of the Coast Range (Figure 3). Lower acarological activity in the Maritime zone and the savanna grassland habitat (the SE Wilderness) was consistent with prevous study from northwestern California (Eisen et al. 2010). Greater departure from the moisture equilibrium threshold (DPD values) likely inpacted the SE Wilderness population.

Early Drought Years

Nymphal abundance generally increased yearly between 2011 and 2014, the peak drought year (Table 2). A major contribution herein was documentation of the nymphal increase during the historical drought.

The increase and decrease of nymph abundance over the drought period coincided with the increase and decrease in the DPD for March, the month of onset, and May, the peak month of nymph season (Figure 3). These early months of the nymph season are critical to maintenance of the tick cycle (Padgett and Lane 2001). It is the time when most nymphs and larvae attach to their primary vertebrate hosts (e.g., lizards, birds, small mammals) (Lane and Loye 1989). It is proposed that warmer, drier conditions boosted tick/host interactions. Precipitation in the driest year (2014) was 400, 284, and 252mm for the Maritime, East Face, and the

Table 2.—The average of the two highest hourly *Ixodes pacificus* nymph collections per site per year (in April, May or June) in three eco-regions in Alameda County, California. All sites that were monitored are incorporated. Nymphal increase in 2014 was consistent with the exception of the creek site along the East Face that presented increase in 2013.

	Maritime				East Face				SE Wilderness			
Year	Creek nymphs	No. Sites	Trail nymphs	No. Sites	Creek nymphs	No. Sites	Trail nymphs	No. Sites	Creek nymphs	No. Sites	Trail nymphs	No. Sites
2011	4.5 (±2.5)	3	12.2 (±5.8)	3	42.5 (±14.8)	1	34.0 (±7.1)	1	14.0	1	4.3 (±0.9)	2
2012	5.2 (±4.6)	3	15.5 (±5.1)	3	60.0 (±1.4)	1	31.5 (±0.7)	2	21.5 (±0.7)	1	3.0 (±2.8)	2
2013	3.3 (±0.6)	2	10.5 (±1.0)	2	71.0 (±8.5)	1	20.3 (±7.6)	3	20.0 (±2.8)	1	2.0 (± 0.5)	2
2014	14.5 (±4.7)	3	28.2 (±16.2)	3	43.3 (±11.0)	2	46.5 (±16.3)	2	39.0 (±8.5)	1	7.0 (±2.8)	2
2015	10.2 (±6.0)	3	16.2 (±8.4)	3	33.0 (±12.9)	2	30.5 (±4.9)	2	18.0 (±7.1)	1	5.5 (±3.9)	2
2016	7.5 (±1.7)	2	14.0 (±4.2)	3	27.5 (±7.0)	2	22.0 (±9.4)	2	6.0 (±1.4)	1	3.5 (±0.7)	2

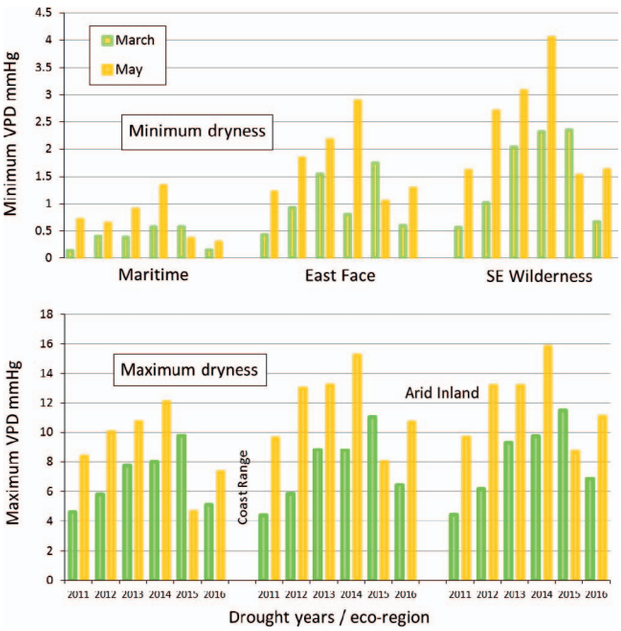

Figure 4.—Comparison of mean monthly minimum and maximum Vapor Pressure Deficit (VPD) for target sites on coastal and continental sides of the California Coast Range in Alameda County, California during the 2011-2016 drought.

SE Wilderness areas, respectively. Even with limited precipitation, the rainy season occurred in late winter and was in time to moisten the leaf litter (aspect of microclimate). Tmax during peak drought stayed within the "climate suitability envelope" (23 - 25°C in May) on both sides of the Coast Range (Eisen et al. 2018). This was due to the influence of the marine layer that moderates extremes in climate along the California coast. Because suitable conditions for ticks were maintained during the critical months of the nymph season, the early seasonal decline in June that might have been imposed by drought was not critical to the conservation of *I. pacificus* populations. In fact, the seasonal questing period is normally relatively short in California. The decline in host-seeking nymphs in June (Figure 1) differs from June as the peak month for nymphal activity on the East Coast USA (Gatewood et al. 2009).

The downturn in nymph abundance starting in 2015 may have been prompted by drought, but specifically by a sharp decrease in Tmax in May, from 23°C to 16.8°C along the Maritime zone and to 17°C inland. Suitable optimum temperatures have been shown to be 21- 25°C (Eisen et al. 2018). Lower nymphal densitie in maritime climate weresuggested to be due to temperatures generally below optimum. The 30-year norm for Tmax in this location was 20.9°C.

VPDmin confirmed the value of a minimum dryness measure to differentiate among geographical moisture levels in arid climates (Figure 4). Maximum VPD better evaluated abiotic changes along the cool moist Maritime, but did not distinguish climate differences for the arid inland areas.

The SE Wilderness area is an extremely arid region with generally very few host seeking nymphs captured by dragging leaf litter, even though the relatively large collections of adults flagged along the uphill margin of sympatric trails would indicate nymphal presence. The relative scarcity of nymphs might be explained by an alternative underground escape from this dry environment. It has been suggested that in dryer conditions *I. pacificus* ticks reside underground where temperatures are cool and humidity is higher, such as under rocks, in cracks of the ground or in rodent burrows (Padgett and Lane 2001). Nymphs are often located in crevices about the base of trees or under logs (Lane et al. 2007).

Conclusions

Ecologically diverse California is unique with respect to its heterogeneous landscapes and climate extremes. The historical drought that began in late 2011 offered a unique opportunity to examine the impact of extreme dryness upon populations of the western blacklegged tick in Alameda County and to compare local maximum daily ambient moisture measures verses nymphal tick abundance in 3 different ecoregions (a "biogeographic response to climate") (Rapacciuolo et al. 2014**)**.

Paradoxically, annual early spring increases in minimum dryness values during the early years of the drought best explained the trend for an increase in nymph populations. Because nymph abundance directly determines the risk of acquiring Lyme disease these data underscore the public health significance of the unexpected increase in nymphs. The minimum dryness values (DPD and VPDmin) quantified moisture in the morning when maximum daily ambient moisture is available to the nymphs in arid climates. We propose that quantifying ambient moisture conveys the geospatial risk of encountering nymphs of *I. pacificus* that potentially may be infected with Lyme disease spirochetes or other human pathogens including the agents of *Borrelia miyamotoi* disease and human anaplasmosis.

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Evaluations of bait-only programs for outdoor nuisance cockroaches in California

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Abstract

Cockroaches in the genus *Blatta* are commonly targeted as peridomestic pests during commercial pest control services in California. These cockroaches primarily live outdoors but readily enter structures in search of food, water, and shelter. Liquid insecticides are commonly applied to building perimeters to manage these pests, but this practice is under scrutiny due to surface water contamination issues, inconsistent control, and insecticide resistance concerns. Additionally, sensitive sites such as public schools and municipal properties may institute policies that restrict liquid insecticide sprays or certain active ingredients. We investigated the efficacy of insecticidal baits against *B. lateralis* and *B. orientalis* in the laboratory, instituting low humidity conditions and the associated gel bait desiccation that is likely within arid outdoor environments. Next, we evaluated baiting programs against *B. lateralis* in the field over the course of one year using self-contained, tamper-proof bait stations. Several commercially-available bait products, formulated as both gels and granules, were highly effective against these species in laboratory trials and in the field demonstrations. Laboratory data suggested that mortality may be due to contact toxicity as well as ingestion toxicity. Field data suggested that area-wide baiting programs have the ability to drastically reduce outdoor nuisance cockroach populations within one year. Such programs can be used to manage these pests without the need for liquid insecticides, mitigating potential negative impacts on communities and the environment.

Effects of short-term weather on the observed abundance of West Nile virus vectors

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Introduction

One of the major limitations in the current use of entomological surveillance to estimate human West Nile virus (WNV) disease risk is that estimates of vector abundance do not account for the short-term effects of weather, which can result in high variability. Instead, trap counts are taken at face value to represent the true abundance of vectors, then used to estimate risk for arbovirus transmission to humans. This could over- or under-estimate human WNV infection risk in a given area. Our study aims to use models to quantify and adjust for the relationship between nightly weather and trap counts to improve estimates of adult mosquito abundance.

Methods

We used daily gridded temperature and wind speed data to examine nightly weather conditions (minimum temperature as a proxy for the relative temperature of the trapping period and average wind speed during the trapping period) that were expected to affect relative abundance estimates for *Culex pipiens* adult females at traps in Sacramento and Yolo Counties. We applied a generalized additive model (GAM) to relate the number of mosquitoes collected per trap-night between 2006 and 2017 to the weather conditions experienced during the trapping event after controlling for the location of the trap (smooth two-dimensional function of geographic coordinates), seasonality (smooth function of day of year), and trap type (CO_2-baited trap, gravid trap, or mosquito magnet).

Results and Discussion

Cx. pipiens were readily collected by all three trap types included in the data, although the count per trap-night varied based on location (Figure 1). Gravid and mosquito magnet traps collected fewer mosquitoes per trap-night than CO_2-baited traps after adjusting for trap location, day of year, and weather (Table 1). Finally, after controlling for trap location, time of year, and trap type, increasing temperature was associated with a higher rate of collection of *Cx. pipiens* for any wind speed, whereas greater wind speed generally was associated with lower collection rates across temperatures (Figure 2). A number of other studies have examined the effect of weather on mosquito abundance through statistical models and have also demonstrated a positive association between temperature and *Culex* spp. abundance (Chuang et al. 2011, Deichmeister and Telang 2011, Wang et al. 2011, Karki et al.

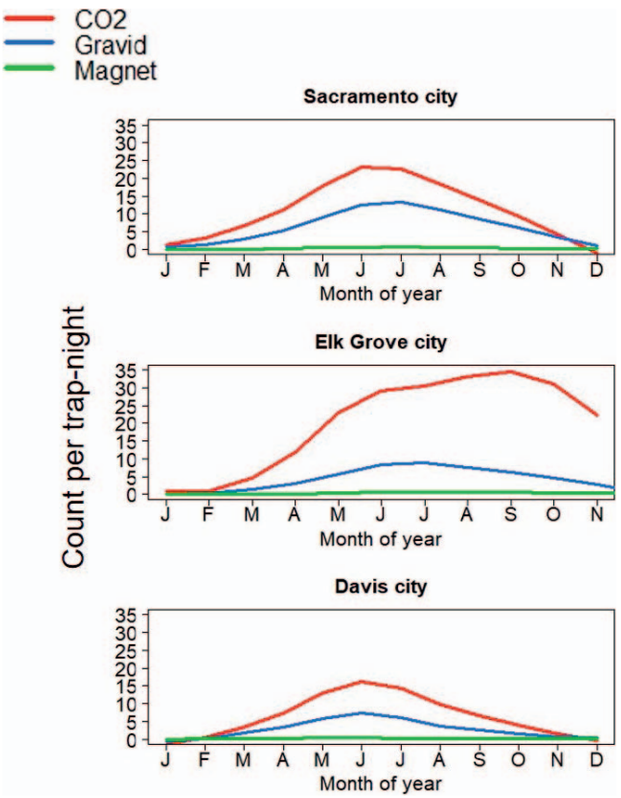

Figure 1.—Adult female *Cx. pipiens* per trap night during a typical season in three cities. Sacramento, Elk Grove, and Davis represent typical cities in urban, suburban, and rural environments, respectively, in Sacramento and Yolo Counties.

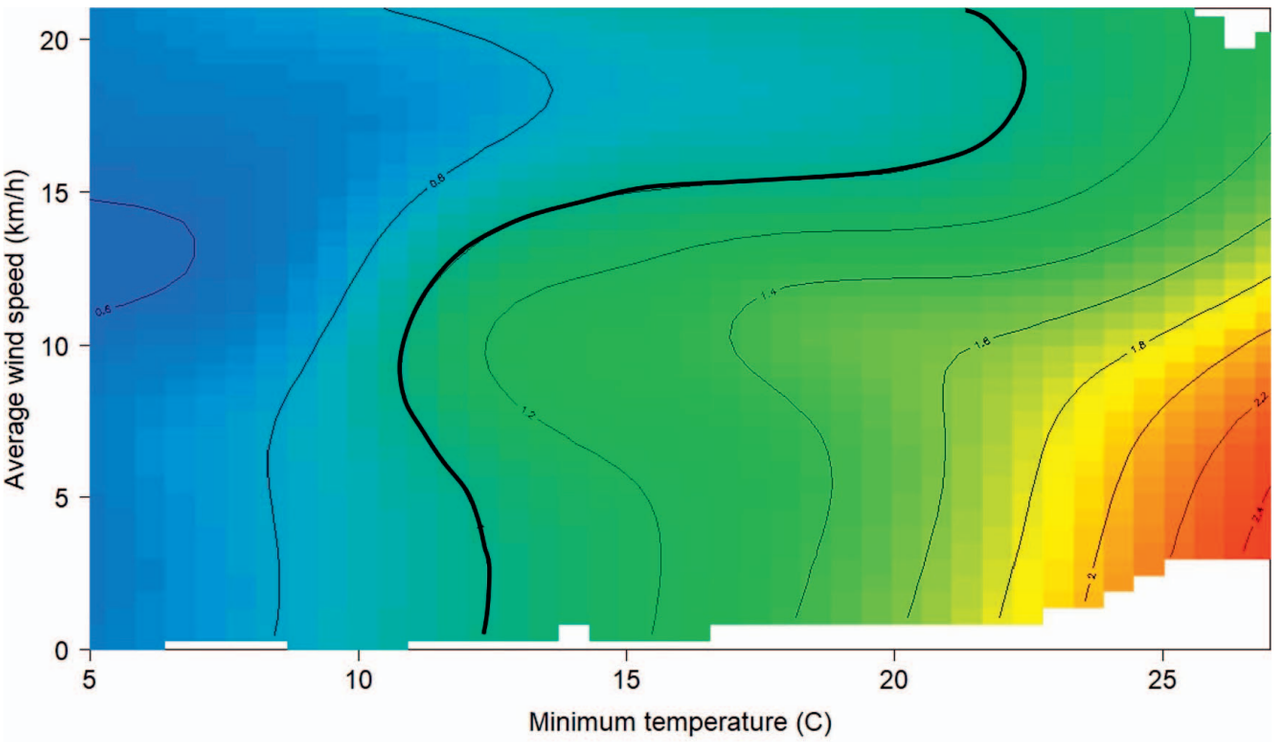

Figure 2.—Rate ratios for adult female *Cx. pipiens* per trap night in relation to different nightly temperatures and wind speeds at traps in Sacramento and Yolo Counties. Warmer and cooler colors indicate higher and lower collection rates, respectively, and numbers on contour lines indicate collection rate ratios versus the bolded reference line. This model adjusts for the effects of trap location, time of year, and trap type.

2016, Groen et al. 2017, Moise et al. 2018), but most investigate this effect at weekly time lags and few consider the effect of wind on mosquito flight activity and trap catch. These results indicate that weather conditions during the observation period, including wind speed, are important moderators of mosquito activity during the course of an observation period.

Conclusions

WNV risk estimates that do not account for the effect of weather on trap collections are missing an important source of variation resulting from the effects of short-term weather on mosquito activity that cannot be captured through weekly lags in weather conditions. These estimates also may be biased if location, seasonality, and trap type are not considered.

Table 1.—Differences in adult female *Cx. pipiens* per trap night by trap type versus CO_2-baited traps (reference). Models adjust for the effects of trap location, time of year, and weather conditions.

Year	CO_2	Gravid	Magnet
2011	ref	- 49%	- 93%
2012	ref	- 43%	- 90%
2013	ref	- 54%	- 91%
2014	ref	- 62%	- 93%
2015	ref	- 64%	- 95%
2016	ref	- 74%	- 96%

Acknowledgements

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Genomics of the western encephalitis mosquito, *Culex tarsalis*

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Introduction

In the western United States, *Culex tarsalis* is an important 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). *Cx. tarsalis* is ornithophilic and host selection among bird species is non-random with respect to host availability. For example, *Cx. tarsalis* fed on snowy egrets more than expected given availability at a specific site in Yolo County (Thiemann et al. 2011). There is also a well characterized increase in the frequency of mammalian blood meals starting in July and peaking in September, which is attributed at least in part to changes in host availability due to fledging chicks (Thiemann et al. 2011; Reeves et al. 1990; Tempelis et al. 1965). In this study, we hypothesized that opportunistic versus strictly ornithophilic subpopulations of *Cx. tarsalis* occur in varying relative frequencies in sympatry and that certain opportunistic genotypes would be over-represented among mammal-fed individuals.

Methods

Mosquitoes

For the assembly of the reference genome, high molecular weight DNA was extracted from a pool of two KNWR colony pupae (a single pupae was not sufficient). The KNWR colony was first established from females collected in 2002 at the Kern National Wildlife Refuge (35.7458 N, 119.6179 W) in Kern County. To elucidate the sex locus, we also sequenced the genomes (standard 'shotgun' sequencing) of a male and female adult mosquito from the KNWR colony. The population genomic analysis of host preference in *Cx. tarsalis* was performed on a sample population of bloodfed *Cx. tarsalis* individuals (N=38) that were field caught at a farmstead in Davis, CA in 2008 (Thiemann et al. 2011). The two pupae from the KNWR colony were used as input for the 10X genomics library preparation and draft genome assembly of *Cx. tarsalis*.

Draft genome assembly

High molecular-weight DNA was extracted by the UC Davis DNA Technologies core from a pool of two pupae from the KNWR *Cx. tarsalis* colony. The 10X Genomics Chromium sequencing library and Illumina sequencing was also performed at the UC Davis DNA Technologies Core. Raw linked reads were assembled using the Supernova 2.0.1 software (10X Genomics) on an Amazon Web Services (AWS) server with 480Gb on RAM and 64 logical cores. The assembly summary statistics were gathered from the supernova report summary file. To generate a fasta formatted reference sequence, we used supernova mkoutput with –style=pseudohap2. This option arbitrarily selects haplotypes across the genome resulting in two haploid reference genomes composed of a mosaic of paternal and maternal alleles. We selected the pseudohap with the most unique contigs for the reference genome (CtarK1). To assess genome completeness of CtarK1, we used BUSCO_V2 (Simão et al. 2015; Nishimura, Hara, and Kuraku 2017).

Annotations were performed using Maker (v.2.31.10) with the following inputs: *Culex tarsalis* transcriptome (Ribeiro et al. 2018), *Cx. quinquefasciatus* protein sequences (CpipJ2.4), and for repeat masking we provided the *Cx. quinquefasciatus* repeat libary (VectorBase) and the RepBase database (RepBaseRepeatMaskerEdition-20170127).

Genome resequencing and data processing

DNA from wild-caught bloodfed mosquitoes was extracted from head and thorax tissue using the QIAGEN Biosprint 96 system and QIAGEN blood and tissue kits (QIAGEN, Valencia, CA). DNA from the associated abdomens was analyzed previously for host identification (Thiemann et al. 2011). Genome resequencing libraries preparations were prepared with 25–50 ng genomic DNA input, using the Nextera DNA Sample Preparation Kit and TruSeq dual indexing barcodes (Illumina). Libraries were size-selected with Agencourt AMPure XP beads (Beckman

Coulter) and the final insert size distribution was assessed using the Bioanalyzer 2100 (Agilent). The final library concentration was measured with a Qubit 2.0 fluorometer (Life Technologies). Samples were sequenced on the Illumina HiSeq3000 or Nextseq platform with 75-100bp paired-end reads at the UC Davis DNA Technologies core. Raw reads were trimmed based on quality and known Illumina adapter sequences using Trimmomatic version 0.30 (Bolger, Lohse, and Usadel 2014), with the following parameters: LEADING:3, TRAILING:3, SLIDINGWINDOW:4:15, MINLEN:36. General read quality was inspected using FastQC version 0.10.1 (Babraham Bioinformatics, Cambridge, UK). Trimmed paired-end and orphan reads were mapped to the CtarK1 draft assembly using BWA-mem (Li 2013) and then the resulting bam files were merged using samtools (V1.8). Read group information was then added to the bam files using AddOrReplaceReadGroups and optical duplicates (identical left and right reads) were removed using MarkDuplicates (version 2.18.4, Picard Tools). Bam files were then sorted and indexed using samtools (v1.8). We used GATK's HaplotypeCaller (v4.0.8.1) to identify SNPs and indels on all bam files. SNPs were filtered with a minor allele frequency threshold of 0.1 and multiple sequencing depth thresholds were imposed (–max-missing-count 5 –min-meanDP 1 –max-meanDP 100 –maf 0.10 –max-maf 0.90).

We estimated the "SNP heritability" of host preference using filtered SNPs as described in (Wray et al. 2013). In short, SNP heritability is the correlation between the genome-wide genotypic variation and phenotypic variance (V(G) / V(p)). To estimate SNP heritability, the VCF file containing genome-wide SNP data for all samples was converted to PLINK with VCFtools (command "vcftools—plink") and then binary ped files (GCTA option: "—make-bed") for analysis with the Genome-Wide Complex Trait Analysis software (GCTA; (Yang et al. 2011)). To calculate "SNP heritability" with GCTA, we first generated a genetic relationship matrix. Then we calculated SNP heritability for host choice.

Bloodmeal analysis

To identify the bloodmeal source, we sequenced previously described DNA barcoding regions of the mitochondrial 16S and cytochrome B genes (Arulandhu et al. 2017). To make the PCR amplicons compatible with Illumina sequencing, we added adapter sequences to the 5′ end of each primer. To identify the host species for each mosquito from the Illumina sequencing reads we BLASTed (NCBI 2.7.1) each unique sequence to a modified mitochondrial database. In brief, we started with the latest NCBI mitochondrial database (mito.nt) and then removed species that are rare or absent from California and added human, dog, cow, chicken and goat. Low quality BLAST results were filtered out by an imposed threshold of e-60. In some cases, the result of a BLAST search with a unique hit (vertebrate with a single maximum Evalue) was used to select the most parsimonious host species among a set of equally likely BLAST hits for another sequence from the sample mosquito. Reads from forward and reverse reads from each locus were treated independently.

Results and Discussion

Draft genome assembly

We assembled the first *Culex tarsalis* reference genome (CtarK1) using linked-reads (10X genomics). The final assembly had a contig N50 of 24.3Kb and scaffold N50 of 119.42Kb. The estimated assembly size (scaffolds >= 10Kb) was 1.08Gb. Among the set of 1066 Arthropoda reference genes used to assess genome completeness, we identified 1029 (98.78%) in our assembly (see methods).

Population genomics

We resequenced thirty-eight bloodfed *Cx. tarsalis* mosquitoes that were collected in October, 2008 in Davis, CA. This sample included 13 mammal-fed, 23 bird-fed, and 2 lizard-fed. Each individual mosquito library was sequenced to an average of 26 million paired-end reads.

Reads were mapped to the CtarK1 assembly using BWA-mem with an average mapping percentage of 95%. For comparison, approximately 40% of reads mapped to the *Culex quinquefasciatus* assembly (CpipJ1). The median sequencing depth was 6.4x (Supplemental Table 1). Using GATK's HaplotypeCaller workflow, we identified 24 million SNPs and 262K were kept after filtering (see methods). Using these 262K SNPs, we estimated the "SNP heritability" of host preference among these thirty-eight *Cx. tarsalis* samples. The heritability estimate for host choice was $H^2 = 0.08$, SE=2.247, indicating that there is no evidence for a substantial genetic component underlying host preference among these samples. While a positive heritability estimate would be strong evidence for a genetic component underlying host choice in *Cx. tarsalis*, this negative result needs to be confirmed with a greater sample size from multiple locations to indicate a lack of a substantial genetic component for this phenotype.

AmpliconSeq for host ID and population genetics

Using paired-end reads from cytochrome B and 16S mitochondrial loci, we successfully identified the vertebrate host in 92% of bloodfed *Cx. tarsalis* (N=22/24). American crow was the most common host; present in 45% of samples (N=10/22). Two individuals (9%) were determined to have a mixed blood meal: cow with American crow and human with Brewer's blackbird.

The insecticide resistance allele *ace-1* in the acetylcholinesterase gene was genotyped and the susceptible allele was fixed in this sample population (N=24). The prophenoloxidase gene was another target locus and 5 nonsynonymous mutations were identified: Ala671Val, Asn667Asp, Pro666Ala, and Val664Ala. We observed an enrichment of cattle-fed mosquitoes among the pure "wild-type" individuals (dark blue, wt = genotype of KNWR reference genome; P=0.01, Fisher's Exact; Figure 1).

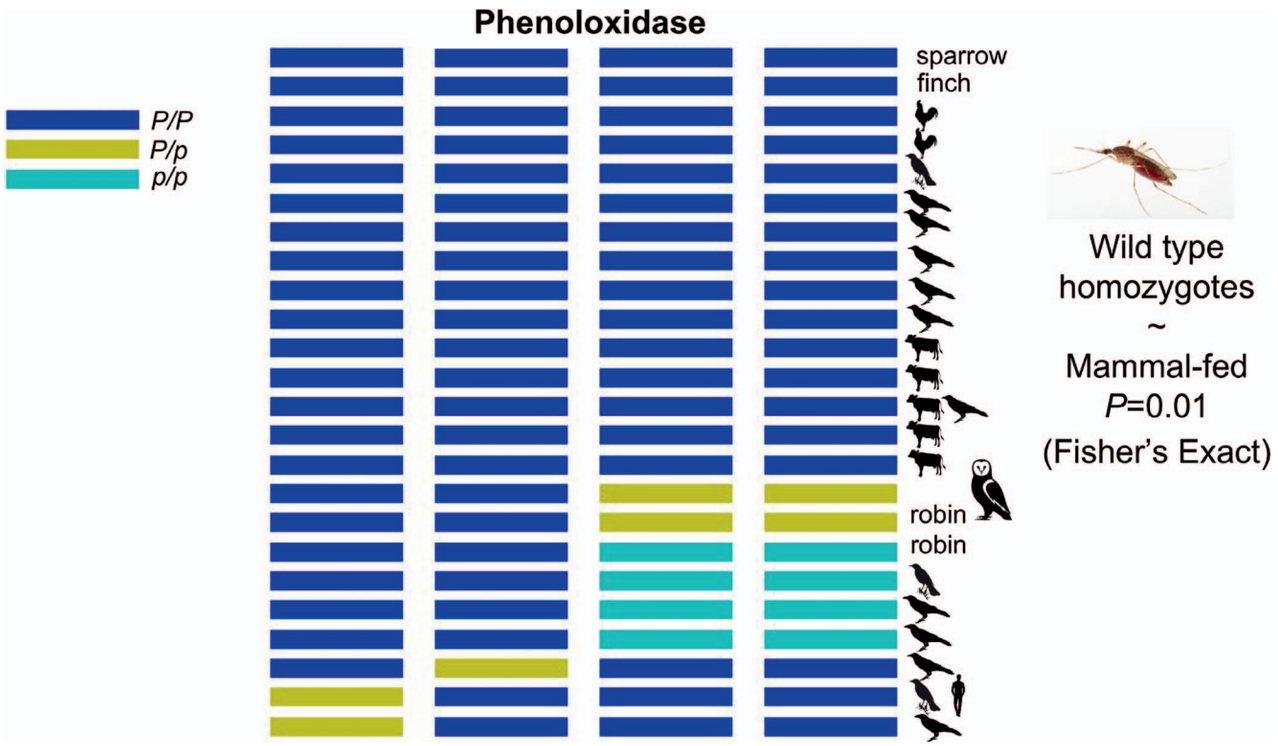

Figure 1.—Host species and mosquito genotype at the prophenoloxidase gene. The blue, aqua and gold colored boxes represent "wild-type" (*P/P*), heterozygote(*P/p*), and homozygous alternate (*p/p*) genotype for a given mosquito at four non-synonymous mutations: Ala671Val, Asn667Asp, Pro666Ala, and Val664Ala (columns from left to right, respectively). The *wt* allele at a given locus was arbitrarily determined based on the genotype of the *Cx. tarsalis* reference genome (KNWR). Host species pictured from top to bottom include: chicken, Brewer's black bird, American crow, cow, barn owl, and human.

Conclusions

We did not find evidence for a genetic component underlying host preference among our sample of 36 *Cx. tarsalis* genomes. However, this sample size is small and was only collected from one location at one time point. Furthermore, our population genetic analysis using AmpliconSeq revealed a relationship between specific genotypes at the *prophenoloxidase* gene and cattle-feeding. Future studies will involve genotyping more loci from larger mosquito populations across CA. In addition, we will sequence *Cx. tarsalis* populations early and late in the season to assess whether distinct genotypes contribute to the increase in mammal-feeding behavior.

The fixed susceptible allele at *ace-1* indicates that we did not detect any evidence for resistance to organophosphates in this population. Further genotyping (including *kdr*) and bottle bioassays from populations across California will elucidate the state of insecticide resistance in *Cx. tarsalis*.

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Table 1.—Identified blood meal hosts for 22 of the 38 *Cx. tarsalis* females field-collected from Davis, CA in October 2008.

Bloodmeal source species	#
Corvus brachyrhynchos	9
Bos taurus	4
Euphagus cyanocephalus	2
Gallus gallus	2
Bos taurus + Corvus brachyrhynchos	1
Euphagus cyanocephalus + Homo sapiens	1
Haemorhous mexicanus	1
Passer domesticus	1
Turdus migratorius	1

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West Nile virus dead bird testing: an evaluation of sample preservation systems

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Abstract

Dead bird surveillance plays an important role in the early detection of West Nile virus (WNV) activity. In an effort to streamline this program, we have assessed the suitability of two sample preservation systems as compared to the system currently in place at the Sacramento-Yolo Mosquito and Vector Control District. These sample preservation systems have the advantage of virus inactivation and room temperature storage, allowing samples to be shipped for testing without dry ice. These preservation systems were assessed across multiple dead bird sample types including: oral swab, brain tissue, cranial swabs, and maggots. Additionally, they were assessed across a wide range of bird species. Overall, both sampling methods preserved the viral nucleic acid and may serve as useful tools for dead bird surveillance programs.

Introduction

At the Sacramento-Yolo Mosquito and Vector Control District (SYMVCD), the dead bird surveillance program plays an important role in the early detection of West Nile virus (WNV) activity. Therefore, continuous effort is made to improve the program. This has included expanding the types of samples collected due to condition and species of the bird. The latest effort focused on evaluating whether two different sample preservation systems, RNASound cards and PrimeStore MTM tubes, can be adapted to the wide range of samples routinely collected. These sample preservation systems have the advantage of virus inactivation and room temperature storage, allowing samples to be shipped for testing without dry ice. RNASound cards are currently utilized by the California Dead Bird Surveillance Program for oral swabs, but herein we expand the types of samples evaluated.

Methods

Using known WNV-positive dead birds collected in 2018, PrimeStore MTM tubes and RNASound cards were compared to the current methods of sample storage including MagMAX lysis buffer and viral transport media (VTM). Four sample types were collected: oral swab, brain tissue, cranial swabs collected from desiccated birds, and maggots.

Oral swabs

Oral swabs were collected by opening a bird's mouth, inserting a sterile polyester applicator tip swab, and twisting the swab back and forth to collect buccal secretions. Oral swabs were preserved in VTM (the current sampling method used for oral swabs at SYMVCD), PrimeStore MTM tubes, and RNASound cards. When preserving in VTM and PrimeStore MTM tubes the swabs were inserted into the collection tube, and the applicator stick was snapped off. All swab samples were mixed using a mini bench top vortex mixer prior to the extraction process, then supernatant was collected for testing. RNASound card samples were collected by applying a swab to the card focusing sample deposition on the perforated circles, after application the cards were allowed to fully dry prior to storage. To test the RNASound cards, the perforated circles were punched free from the card using a clean pipette tip. Perforated circles were deposited into 1.0 ml of MagMAX lysis buffer and then mixed by bench top vortex prior to RNA extraction.

Brain tissue

Brain tissue aspirates were collected primarily from non-crow species using a 3mL syringe with an 18 gauge needle. The needle was inserted into the side of the cranium, and brain aspirates were collected by pulling back the plunger and creating a vacuum inside the cranium. Collection of aspirates was enhanced by carefully moving the tip of the needle up and down. Brain tissue samples were preserved in both MagMAX lysis buffer and PrimeStore MTM tubes, samples were homogenized prior to RNA extraction using two 5mm glass ball bearings and a mixer mill (Spex CertiPrep, Metuchen, NJ). Brain tissue was not applied to RNASound cards, but oral swabs collected on RNASound cards were compared to brain tissues collected from the same bird. Although brain aspirates provide a sensitive sample, collection requires extra training and safety precaution that may limit general use. Therefore, it was important to know how brain aspirates compared to oral swabs, especially with non-crow species that tended to have lower viral loads.

Cranial swabs

Occasionally, dead birds were collected in a desiccated state. These birds were sampled by swabbing the inner cranium, a sample type only used for desiccated birds. Collection was done by first preparing the swab by dipping it into the selected preservation agent, for RNASound cards the swabs were dipped in water. The back of the swab was used to puncture a hole through the ocular orbit into the cranium, then the applicator tip was inserted into the cranium, and a sample was collected from inside the cranium. This sample was preserved in MagMAX lysis buffer, PrimeStore MTM tube, and RNASound card. Samples were introduced into each preservation method as described for oral swabs.

Maggots

Often dead bird carcasses are exposed to the environment for multiple days prior to carcass collection, and are often colonized by fly maggots. Maggots can be utilized for WNV surveillance. This was done by selecting 5-6 maggots with noticeable material within the gut that often appeared as a spot of blood. Maggots were preserved in MagMAX lysis buffer, PrimeStore MTM tube, or RNASound Card. Maggot samples in MagMAX lysis buffer and PrimeStore MTM tubes were processed as described for brain tissues. However, maggot samples were preserved on RNASound card by using the applicator tip of a swab to rupture the integument and gut of one maggot onto each of the perforated circles. Excess tissue was removed and the card was allowed to dry prior to storage. Maggot samples on RNASound cards were processed as described for oral swabs.

Sample storage

Samples preserved by VTM, MagMAX lysis buffer, and RNASound cards were stored at 4°C and tested within two weeks of collection. Samples preserved in PrimeStore MTM tubes were stored on the laboratory bench at ambient temperature and initially tested within two weeks of collection. To test the long term stability of samples stored in PrimeStore MTM tubes, brain samples preserved by this method were stored on the laboratory bench at ambient temperature and were retested monthly for up to three months post-collection.

WNV detection

RNA was extracted using MagMax 96 viral RNA kits and the MagMax Express 96 Deep Well Magnetic Particle Processor (Applied Biosystems, Foster City, CA). Extracted RNA were tested for nucleic acid specific for WNV by RT-qPCR using previously published primers (Lanciotti et al. 2000) and a 7500 Real Time PCR System (Applied Biosystems, Foster City, CA). All samples were run for a total of 40 PCR cycles; samples that crossed the threshold within 35 cycles were considered positive for WNV.

Statistical analysis

To compare preservation systems RT-qPCR Ct values were evaluated by One-Way ANOVA (analysis of variance) across multiple dead bird sample types. When the compared preservation systems displayed significant differences ($P<.05$), pairwise multiple comparisons using the Holm-Sidak method were utilized. Statistical analyses were conducted using the statistical software SigmaPlot (Systat Software, San Jose, CA).

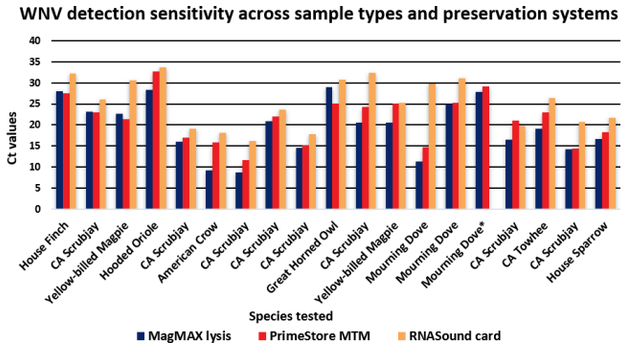

Figure 1.—Ct values of brain tissue collected from individual birds preserved in MagMAX lysis and PrimeStore MTM tube, and oral swabs from the same birds preserved on an RNASound card.

Results and Discussion

Initially VTM, PrimeStore MTM tubes, and RNASound cards were compared using oral swabs from five known WNV positive American crows. West Nile virus was detected with all three sample preservation systems. The RT-qPCR Ct values were most similar between VTM (mean=19.22, SD=1.93) and PrimeStore MTM (mean=18.76, SD=0.67) samples, with the Ct values from RNASound cards 1 - 3 Ct higher (mean=21.72, SD=2.40) that the other two preservation systems. Although the RNASound card Ct values were elevated, there was not a statistically significant difference ($P=.052$) in Ct value across preservation types when evaluating oral swabs from American crows. Subsequent testing evaluated the performance of additional bird species and sample types, focusing primarily on brain tissue, cranial swabs and maggots.

Brain tissues collected either in MagMAX lysis buffer or PrimeStore MTM tubes were compared to oral swabs preserved on RNASound cards. RNASound cards were not used for brain tissue due to possible incomplete inactivation of WNV in thick tissue samples. Further work is needed to document inactivation of WNV using this method. Because some agencies used RNASound cards to preserve oral swabs from non-crow species, the sensitivity of this collection method was compared to brain aspirate samples in MagMAX lysis buffer or PrimeStore MTM tubes. West Nile virus was detected in all three preservation systems across samples collected from nine bird species (Figure 1), except for one oral swab (5.3%) collected from a mourning dove and preserved by RNASound card. Overall, the brain tissue stored in MagMAX lysis buffer (n=19, mean=19.59, SD=6.40) produced lower Ct values than the PrimeStore MTM tubes (n=19, mean=21.40,

SD=5.62) and RNASound cards (n=18, mean=25.28, SD=5.83). The only statically significant difference in Ct value was brain aspirates preserved in MagMAX lysis buffer compared to oral swabs preserved on RNASound cards (P=.016). Despite the false negative and elevated Ct values, RNASound cards were well suited to preserving oral swab samples from non-crow species, and are recommended for both crow and non-crow species.

Among cranial samples collected from desiccated birds, WNV was detected with all three sample preservation systems, but two of the eight samples (25.0 %) preserved by RNASound cards resulted in false negatives. The Ct values were very similar between samples preserved in MagMAX lysis buffer (n=8, mean=26.33, SD=4.61) and PrimeStore MTM tubes (n=8, mean=27.76, SD=4.85), but Ct values for sample preserved by RNASound cards were slightly higher (n=6, mean=30.83, SD=4.68). Although there were no statically significant differences between the Ct values (P=.23) of cranial samples preserved across the three sample types, RNASound cards are not recommended for this sample type due to an elevated proportion of false negatives.

When comparing the Ct values of maggots collected from the same bird but preserved in three different preservation methods, Ct values for MagMAX lysis buffer and PrimeStore MTM tubes were similar, and RNASound cards were slightly higher (MagMAX n=14, mean=23.52, SD=5.59); PrimeStore MTM tubes n=12, mean=23.29, SD=4.81; RNASound card n=14, mean=26.77, SD=4.64). West Nile virus RNA was detected in all maggot samples stored in MagMAX lysis buffer and on RNASound cards, but storage in PrimeStore MTM tubes resulted in 2 out of 14 false negatives (14.3 %). Although the RNASound card Ct values were slightly higher, WNV was consistently detected. There was no statistically significant difference (P=.22) in Ct values in maggots preserved across the three preservation systems.

The final assessment evaluated the stability of samples stored in PrimeStore MTM tubes, held at ambient temperature, and tested monthly over a three-month period. The Ct values of consecutive samples tested over time were compared to the original sample Ct value. There was no statistically significant difference in Ct value overtime (P= 0.996) as compared to the original sample results (n=19, mean=21.41, SD=5.62); month-1 results (n=17, mean=21.14, SD=6.15); month-2 results (n=19, mean= 21.06, SD=6.02); month-3 (n=19, mean=21.47, SD=5.97). Our results demonstrated that brain samples stored in PrimeStore MTM tubes were highly stable overtime (Figure 2). The PrimeStore MTM tubes not only protected the samples from degradation, but it also eliminated the need for any special shipping requirements such as dry ice.

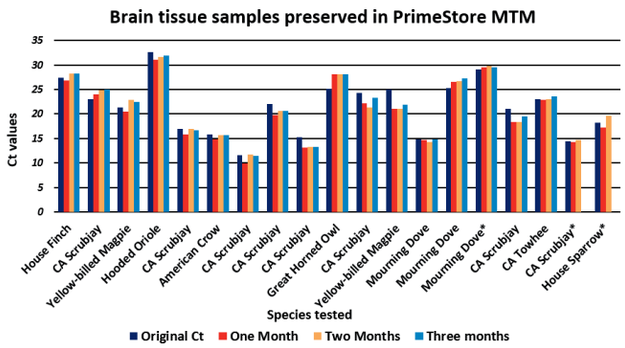

Figure 2.—Samples stored in PrimeStore MTM tubes were stored on a laboratory bench at ambient temperature and were tested monthly to determine sample stability overtime. *These birds were collected late in the season and were only available for resampling on two occasions.

Conclusions

New sample preservation systems could enhance WNV dead bird surveillance. Although MagMAX lysis buffer samples consistently produced slightly lower Ct values when compared to the PrimeStore MTM tubes and RNASound cards, there were advantages to using the RNASound cards and the PrimeStore MTM tubes. The RNASound cards are extremely easy to use and the cards detected 94% of all the positive bird samples regardless of sample type, including oral swabs collected from non-crow species. The added benefits of the PrimeStore MTM tube included storage at room temperature and sample stability over time; additionally, the Ct values for samples stored in PrimeStore MTM tubes were most comparable to the preservation systems currently used by SYMVCD.

Acknowledgements

We want to thank Chris Helm, from Longhorn Vaccines & Diagnostics, for providing some of the PrimeStore MTM tubes and Leslie Foss from, CA Dept. of Public Health, for providing some of the RNASound Cards used the evaluation. Also, thank you to the SYMVCD Laboratory Technicians for all their hard work in the collection and the organizing of all the dead birds brought into the District for testing.

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Evaluation of a residual insecticide in the field: Should we use residual adulticides to reduce mosquito abundance and West Nile virus activity?

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Abstract

Foliar applications of Suspend Polyzone, a residual insecticide containing the active ingredient deltamethrin, were conducted in four urban parks. Applications were assessed to 1) determine whether applications lead to a reduction in mosquito abundance and/or WNV infection rate, 2) assess whether residual efficacy was impacted by leaf type/plant species, and 3) quantify the impact of pyrethroid resistance on product efficacy. Study sites were divided into control and treatment areas, and Polyzone was applied at a mid-label rate to treatment areas. Mosquito abundance and West Nile virus prevalence was compared between treatment and control areas. Inspection of regression coefficients of a generalized linear model that predicted mosquito abundance based on species, weeks post-treatment, and study site indicated that that there was a 25% reduction in *Culex pipiens* and *Culiseta incidens* mosquito abundance in treatment areas as compared to untreated controls. There was no difference in West Nile virus activity between treatment and control areas. Residual efficacy was not affected by leaf type, but was greatly reduced in a pyrethroid-resistant population.

Introduction

Residual insecticides can be used strategically to target mosquitoes where they rest, providing flexible application parameters and extended periods of residual activity. Our study evaluated the efficacy of foliar applications of Suspend Polyzone (Bayer Environmental Science, Research Triangle Park, NC) at reducing mosquito abundance and West Nile virus infection rates. Suspend Polyzone (hereafter Polyzone) contains 4.75% deltamethrin, and the label states that this product may provide residual control for up to 90 days. Previous work demonstrated that Suspend SC and Suspend Polyzone applied at a mid-label rate maintained residual action for up to 29 weeks, as determined by a leaf bioassay using pyrethroid-susceptible *Culex pipiens quinquefasciatus* Say (CQ1) colony mosquitoes, indicating that these residual insecticides may provide great utility in a mosquito control program (Wheeler et al. 2018). The current work evaluated the performance of Polyzone in the field and addressed three critical questions pertaining to residual insecticide application: 1) do residual applications lead to a reduction in mosquito abundance and/or WNV infection rate, 2) does the residual efficacy of Polyzone vary by leaf type or plant species, and 3) how are residual applications affected by insecticide resistance?

Methods

Four urban parks were selected for application trials. The parks were divided into treatment and control areas. All foliage that was not actively flowering was sprayed with Polyzone at a rate of 0.75oz/1000sqft. Applications were made using a 50 gallon power spray system set to 300 psi, powered by a 5.5 horsepower gas engine (Honda 3X160), pump (Hypro D30), gear reduction box (Hypro 9910KIT1640), and spray gun (Hudson Green Garde JD9-C). The spray system was mounted on a 6X6 John Deere Gator equipped with turf tires.

Residual efficacy was assessed at each study site weekly by collecting leaves from three designated locations in both the treatment and control areas. Leaf bioassays were performed using a susceptible *Cx. p. quinquefasciatus* colony (CQ1) as previously described (Wheeler et al. 2018). Impact of application on local mosquito abundance was measured by trapping from May - July 2018, for eight to ten weeks post-application. Traps were set in the vicinity of each leaf collection site so that there were three traps in each treatment and control area. At each trapping site one CO_2-baited trap and one gravid trap was run overnight on a weekly basis. Trapped mosquitoes were sorted to species, enumerated, and every-other week female *Culex tarsalis* Coquillet and *Cx. pipiens* complex mosquitoes were pooled separately in batches up to 50 mosquitoes. Pools were tested for West Nile virus, Saint Louis encephalitis virus, and western equine encephalitis virus using previously published methods (Brault et al. 2015). To assess reduction in mosquito abundance, *Cx. pipiens* complex and *Culiseta incidens* Thomson were used as indicator species, as these were the most common mosquitoes trapped at our study sites.

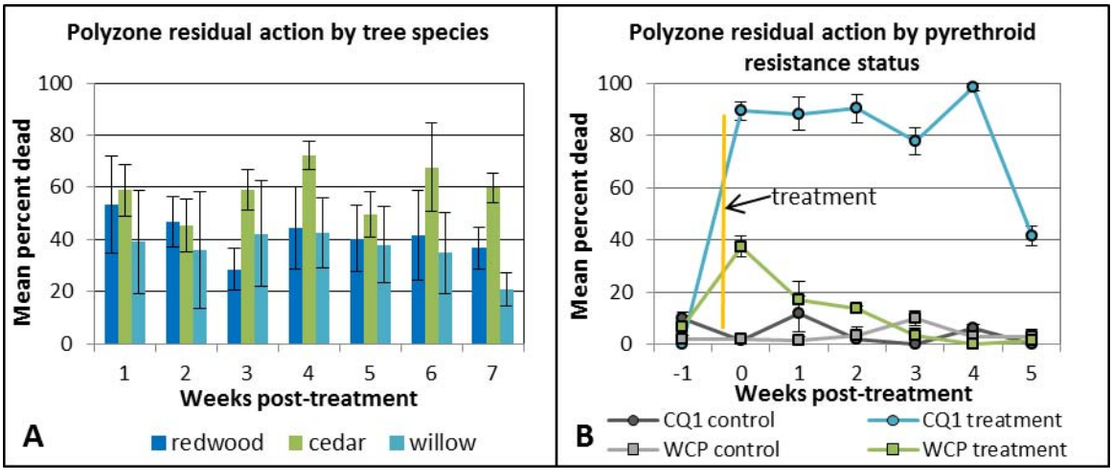

Figure 1.—A. Mean percent mortality was calculated by tree species [weeping willow (*Salix babylonica*), coastal redwood (*Sequoia sempervirens*), and Deodar cedar (*Cedrus deodara*)] over time, error bars represent the standard error of the mean. **B**. Mean percent mortality for pyrethroid susceptible (CQ1) and pyrethroid resistant (WCP) mosquitoes exposed to Polyzone treated leaves at increasing intervals post-treatment, error bars represent the standard error of the mean.

Although all the routine leaf bioassays were performed with pyrethrin/pyrethroid susceptible mosquitoes (CQ1 colony), insecticide resistance is a growing problem (Scott et al. 2015). Therefore to better understand how residual treatments performed against resistant populations, a small trial was conducted. Foliage was treated with Polyzone as described above. Leaf bioassays were conducted using both pyrethrin/pyrethroid susceptible (CQ1 colony) and resistant (WCP colony) *Cx. pipiens* complex. Leaf bioassays were conducted just prior to application, immediately after application (once product had dried), then weekly thereafter for five consecutive weeks.

While conducting previous work on residual insecticides (Wheeler et al. 2018), an anecdotal observation was made indicating that plants with needle-type leaves had reduced residual efficacy. To better understand the impact of leaf type on residual efficacy trees treated as part of our larger park treatments were opportunistically selected for enhanced sampling. The tree species selected were weeping willow (*Salix babylonica*), coastal redwood (*Sequoia sempervirens*), and Deodar cedar (*Cedrus deodara*). The weeping willow has deciduous lanceolate leaves, the coastal redwood had flattened nettle-type leaves, and the Deodar cedar has fascicles of needle-type leaves. Three individuals of each species were selected, treated with Polyzone as described above, and leaves were collected and tested for residual efficacy on a weekly basis.

To analyze the effect of Polyzone application on mosquito abundance a Poisson generalized linear model was fit to relate mosquito abundance to Polyzone application status (treatment vs. control), with mosquito species, weeks post-treatment, and study site as covariates. To determine whether leaf type had an impact of Polyzone residual efficacy a linear regression model was performed where the mean proportion of dead mosquitoes in the leaf bioassay was predicted by the tree species, individual tree, and weeks post-treatment. Statistical analyses were conducted in R (Core Team and Others 2013).

Results and Discussion

To increase statistical power, all four sampling sites were combined and *Cx. pipiens* and *Cs. incidens* abundance and the number of *Cx. tarsalis* and *Cx. pipiens* positive WNV positive pools were compared between treatment and control areas. The number of WNV positive pools was almost identical between control (23 of 639 WNV-positive) and treatment areas (22 of 666 WNV-positive). Treatment and control areas were immediately adjacent to each other allowing for unrestricted bird movement that may have confounded any effect our application had on WNV activity. Model coefficients indicated that the overall abundance of *Cx. pipiens* and *Cs.incidens* was reduced in treatment areas by 25.2% as compared to untreated controls ($P<.001$), indicating that foliar application of Polyzone lead to reductions in mosquito abundance, but not West Nile virus activity. However, a 25% reduction in mosquito abundance fell short of operational targets, especially given the potential for building insecticide resistance due to sub-leathal exposure.

Polyzone residual efficacy was compared across three tree species with differing leaf morphology. Although the mean mortality was generally highest in mosquitoes exposed to Deodar cedar leaves (Fig. 1A), there was no significant effect of leaf type on mortality ($P>0.1$), indicating that the residual efficacy of Polyzone application was not altered by the leaf morphology assessed here.

To better understand the impact of insecticide resistance on Polyzone efficacy, pyrethroid-susceptible (CQ1) and pyrethroid-resistant (WCP) *Cx. pipiens* were exposed side-by-side to polyzone-treated leaves weekly for up to five weeks post-treatment (Fig. 1B). The mean percent

mortality in CQ1 was greater than 75% for the first four weeks then dropped to 41.5% at five weeks post-treatment. In contrast, WCP had a mean percent mortality of 37.3% immediately after treatment, then dropped to less than 20% and remained indistinguishable from the untreated control after one week post-treatment. These results indicated that the levels of pyrethroid resistance present in some field populations may be sufficient to overcome Polyzone exposure via leaf surfaces and greatly reduced product efficacy.

Summary

Residual adulticide applications can provide operational flexibility in terms of application time. We measured a 25% reduction in mosquito abundance over eight weeks following a Polyzone application. Although mosquito abundance was marginally reduced, WNV activity was not impeded. This study indicated that spraying the foliage in parks with the products tested herein did not sufficiently reduce mosquito abundance and warrant use in this manner, especially given the lack of efficacy against pyrethroid-resistant mosquitoes. Pyrethroid resistance is a growing problem in *Culex* mosquitoes and in the interest of good pesticide stewardship product usage should always be considered with regard to limiting insecticide resistance. Therefore usage of this and other residual pyrethroid-based products should be combined with bioassays of the target populations to ensure the best application outcomes.

Acknowledgements

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Monitoring for naled resistance in Sacramento and Yolo counties.

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Introduction

Naled, an organophophate insecticide primarily used in the formulation of adult mosquito control products, has been registered in the U.S. since 1959. The Environmental Protection Agency estimates that naled has been applied by air to about 16 million acres per year within the mainland U.S. as part of routine efforts to control mosquitoes and interrupt the cycle of vector borne diseases. The Sacramento-Yolo Mosquito and Vector Control District (District) contains 46,707 acres of rice and utilizes ultra-low volume (ULV) aerial applications to control adult mosquito populations in these and other areas. Because naled has been used routinely for decades resistance is a concern. However, one of the primary characteristics of naled is its short half-life in the environment, a characteristic that reduces the potential for resistance development. Nonetheless, resistance monitoring is part of all routine operations at the District. During an urban adulticide application conducted in Sacramento County in June 2018, it was observed that the adult *Culex tarsalis* Coquillett sentinels collected from a rice field exhibited less than 100% mortality. The current study was undertaken to explore the possible reasons for that outcome.

Methods

Four populations of *Cx. tarsalis* were collected from varying habitats. Two were from rice fields (Natomas [Sacramento County] and Conaway [Yolo County]), a third was from a wetland on the Vic Fazio Refuge in Yolo County, and a fourth was from Brannan Island in the Delta region of South Sacramento County. Field collected mosquitoes were sorted by sex and assessed for naled sensitivity in the field by deployment of sentinel cages and in the laboratory by CDC bottle bioassay.

Field assessments were conducted over three nights in North Yolo County rice fields. Trumpet EC containing 78% naled was applied at a rate of 0.75 fluid ounces/acre by ULV aerial application. Sentinel cage monitoring stations were deployed at six locations within the spray block. At each monitoring location a tripod was stationed with a sentinel cage array affixed with a wind vane and ball bearings so that cages always pointed into the wind. Each cage array held both field populations and a *Cx. tarsalis* reference colony that is susceptible to Naled (Kern National Wildlife Refuge; KNWR). Not all populations were available on each spray event. Sentinel cages contained 25 mixed aged female *Cx. tarsalis*. The cage array design ensured that all populations at each monitoring station received similar pesticide exposures and was based on similar wind vane style sentinel cage holder designed by Clarke (St Charles, Il). The susceptible population (KNWR) was used as biological indicator of naled exposure in the field. Cages from each monitoring location were included in the analysis only when 100% mortality was observed in KNWR sentinel cages.

Bottle bioassays were conducted on a minimum of 60 mixed-age females from each population. Bottles were prepared with 25 micrograms of technical grade naled (Chem Service, West Chester, PA; item# N-12640) per bottle and were prepared according to the CDC protocol (Brogdon and Chan Accessed 6 June 2019). Field populations were run in conjunction with the KNWR reference colony.

Sentinel cage data was analyzed to determine whether the proportion of dead mosquitoes varied by population. Because this dataset failed the Shapiro-Wilk normality test, data were analyzed using the non-parametric Kruskal-Wallis Analysis of Variance on Ranks followed by the Dunn Multiple Pairwise Comparisons Procedure. Bottle bioassays were interpreted using CDC guidelines, where a diagnostic time was generated for KNWR tested concurrently. The CDC guidelines for assessing bottle bioassay results (McAllister and Scott 2019) state that when there is 97% - 100% mortality in a tested population by the diagnostic time (time at which 100% of susceptible population is dead) a population can be considered susceptible. Mortality between 90% - 96% indicates developing resistance and mortality $<$90% suggests resistance.

Results and Discussion

Sentinel cages deployed in the field are often exposed to confounding factors, such as weather, that impact the efficacy of pesticide application. To isolate these factors we only assessed mortality of field populations when co-deployed cages containing KNWR had 100% mortality. The Brannan Island *Cx. tarsalis* were collected from an

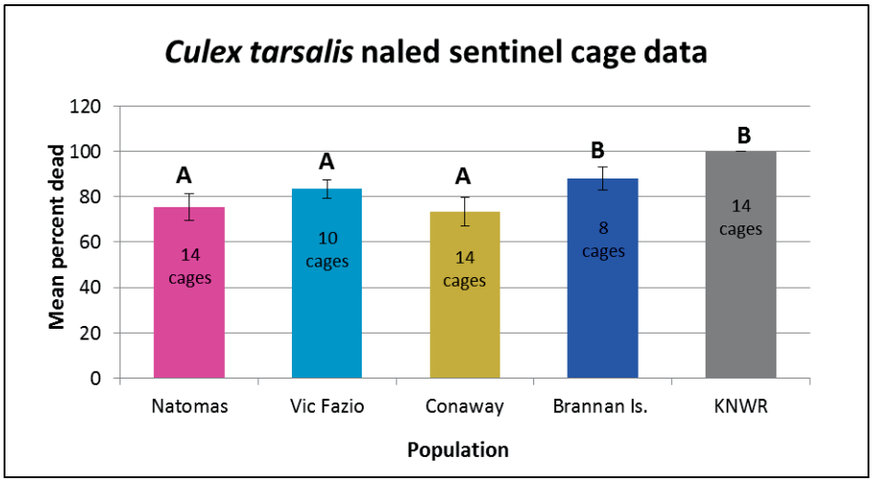

Figure 1.—*Culex tarsalis* were exposed to naled by aerial ULV application (0.75floz/acre). Only cages from locations where 100% mortality was observed in susceptible cages (KNWR) were included. The error bars represent the standard error of the mean. Letters denote populations that are statistically significantly different ($P<0.05$).

area where naled rarely has been applied, and sentinel cage mortality (Figure 1) was not statistically significantly different than KNWR (mean 88.1%, P=0.2). The three field populations collected from areas where naled has been routinely applied had sentinel cage mortality (Figure 1) that was statistically significantly different than KNWR (Natomas mean 75.5%, $P<0.001$; Vic Fazio mean 83.4%, P=0.004; Conaway mean 73.5, $P<0.001$).

According to these criteria, by end of the diagnostic time (105 minutes) the Brannan Island (99.4% +/- 0.43 [percent mortality +/- standard error of the mean]) and Vic Fazio (98.0% +/-1.42) populations were susceptible, whereas the Conaway (96.2% +/- 1.42) and Natomas (90.9 +/- 4.33) populations had developing resistance. These results agree in part with the sentinel cage data, where resistance was indicated in Natomas, Conaway, and Vic Fazio. The standard error of the mean for the Vic Fazio and Conaway populations indicated that Vic Fazio may actually have growing resistance and that Conaway may actually be susceptible, highlighting the importance of sample size and repeated sampling.

Both sentinel cages and bottle bioassay are artificial systems for assessing product efficacy. Bottle bioassay allows for evaluation of active ingredients under controlled exposures, so that populations can be directly assessed for pesticide tolerance. Sentinel cage assessments most closely replicate operational control efforts and are most likely to indicate product performance in the field. Given these considerations both the bottle bioassay and sentinel cage data indicated that there may be low levels of resistance forming in the areas where naled has been routinely applied. Future work will continue to monitor naled

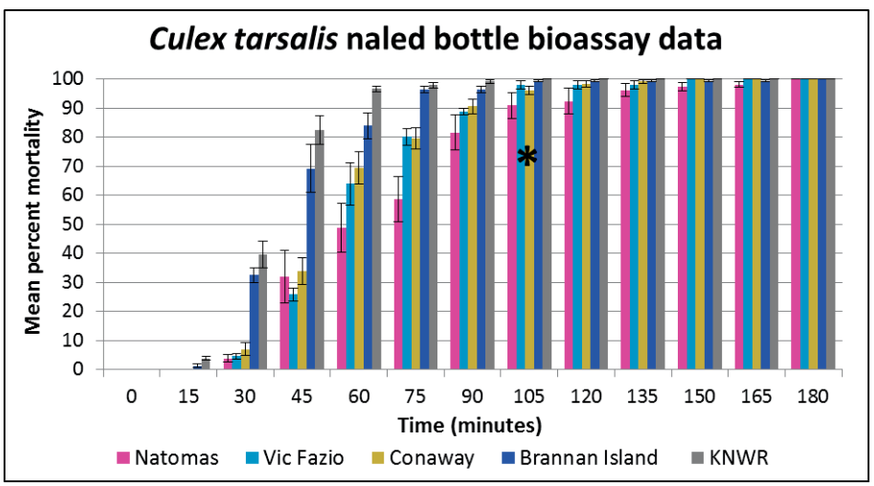

Figure 2.—Percent mortality plotted as a function of exposure time. The asterisk denotes the diagnostic time where 100% mortality was observed in the susceptible *Culex tarsalis* colony (KNWR); Error bars represent the standard error of the mean.

resistance levels in the field to better understand and impact of emerging resistance on mosquito control operations.

Conclusion

Naled has been used for decades with limited evidence of resistance. This product is an important rotational tool in our control efforts, especially given the mounting resistance to pyrethroids observed in *Culex* species. The data herein show that low levels of resistance may be emerging in some field populations. We will continue to work to quantify and confirm this finding additionally further work is planned to better understand the operational differences between bottle bioassay and sentinel cage findings.

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Determining pyrethroid resistance and resistance mechanisms in Northern California populations of *Culex tarsalis*

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Introduction

Culex tarsalis is a known vector of West Nile Virus (WNV) and other encephalitis viruses in the Western United States. Although pyrethroid resistance has been documented in another WNV vector, the *Culex pipiens* complex, very little has been published on *Cx. tarsalis*. This ongoing study seeks to identify the levels of pyrethroid resistance and resistance mechanisms in several Northern California populations of *Culex tarsalis*.

Methods

Female *Cx. tarsalis* were field-collected from 16 selected populations within five Northern California counties: San Joaquin, Sacramento, Yolo, Placer, and Lake. To determine resistance status, bottle bioassays were conducted by confining mosquitoes in glass bottles coated with a diagnostic dose of permethrin or permethrin synergized with piperonyl butoxide (PBO) and recording mortality every fifteen minutes for two hours. To investigate methods which may be involved in resistance, colorimetric enzyme assays are being conducted to measure levels of the enzymes alpha-esterase, beta-esterase, oxidase, glutathione-S-transferase, and acetylcholinesterase. A qPCR assay will also be used to identify any *kdr* mutations.

Results and Discussion

There was a wide range of permethrin susceptibility observed among the 16 *Cx. tarsalis* populations tested, with two-hour mortality ranging from 8.6% to 96.2% in bottle bioassays. The use of the oxidase inhibitor, PBO, in addition to permethrin increased efficacy, resulting in >90.0% mortality in all but one population fromWinters, which reached 88.7% mortality. In preliminary enzyme assays of mosquitoes from three sites, oxidase levels were found to be elevated in two populations, Locust from Placer County and Escalon from San Joaquin County and acetylcholinesterase levels in all three sites were significantly higher than the Bakersfield Field Station (BFS) susceptible laboratory strain. Enzyme assays and qPCR *kdr* assays are currently being conducted for the remaining populations.

Conclusions

The 16 investigated Northern California populations of *Cx. tarsalis* have varying levels of resistance to permethrin. The use of an oxidase enzyme inhibitor, PBO, increased the efficacy of permethrin in all populations, suggesting that oxidases are contributing to pyrethroid resistance. Preliminary enzyme assays conducted thus far have also shown that oxidases were significantly elevated in two of three populations and acetylcholinesterase levels were higher in all populations when compared to the BFS laboratory colony. Moving forward, enzyme and qPCR *kdr* assays will be completed to characterize all 16 populations.

Acknowledgements

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Placer County Mosquito and Vector Control District

Lake County Vector Control District

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New CalSurv Gateway tools for management and analysis of bottle bioassays

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Introduction

Bottle bioassays are an important tool for monitoring insecticide resistance, and the California Vectorborne Disease Surveillance System (CalSurv) Gateway has introduced new tools to aid in the data management and analysis for these assays. Rather than recording the data on one-off spreadsheets or paper worksheets, the new tools will provide a unified format for data entry with error checking to improve data accuracy.

Methods

This project sought to leverage the existing CalSurv Gateway data management platform (CalSurv Development Team 2019) to collect and standardize bottle bioassay data. The Gateway provides a data entry web interface powered by an Apache / PHP / PostgreSQL web stack. Once in the system, the data can be easily retrieved and graphed using the D3 javascript library (Bostock 2019).

Results and Discussion

The bottle bioassay data entry tool was intended to aid in recording bottle bioassay results by providing an easy to use interface that helps correct errors and allows for comparisons of data across space and time. The newly updated bioassay module adds value to the data by providing the ability to easily graph and compare bioassay results from different field-derived populations or lab colonies (Fig. 1). The system also calculates median and 95^{th} percentile for knock-down times (KD_{50} and KD_{95}) with statistical confidence intervals for each assay.

Conclusions

The new bottle bioassay module makes it easier to store bioassay results in the CalSurv Gateway. As more results are collected, it will provide new opportunities for analysis and mapping of insecticide resistance throughout California. As one example, we are developing new mapping capabilities that will users to relate results from insecticide resistance assays to pesticide usage by mosquito control agencies and other sources.

Acknowledgements

We want to acknowledge key input on the bioassay data modules provided by Dr. Paula Macedo of Contra Costa Mosquito & Vector Control District and Dr. Mary Sorensen of Placer Mosquito & Vector Control District that has helped to guide our development. Funding for this project has been provided through the Pesticide Applications and Resistance Testing (PART) project from the Mosquito and Vector Control Association of California, Epidemiology and Laboratory Capacity for Infectious Diseases Cooperative Agreement no. 6 NU50CK000410 from the U.S. Centers for Disease Control and Prevention, and CalSurv Gateway funding provided by the state of California for fiscal year 2018-2019.

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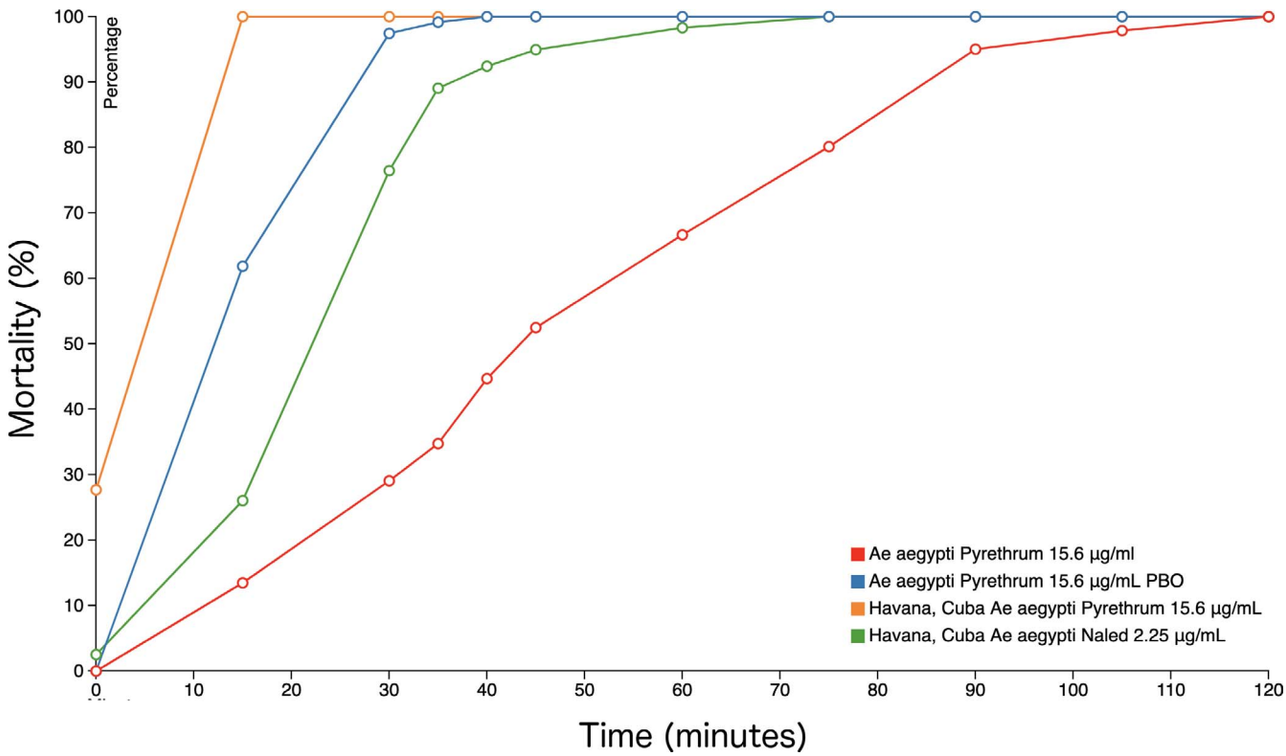

Figure 1.—Example graph from the CalSurv Gateway bottle bioassay calculator comparing results from several assays for *Ae. aegypti*.

Mosquito and Vector Control as Special Districts: Opportunities and challenges

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Abstract

According to the 2017 National Association of County and City Health Officials (NACCHO) Report: *Mosquito Control Capabilities in the U.S.,* dedicated mosquito and vector control districts provide more competent mosquito control than local health departments and other city or local governmental agencies. Dedicated mosquito and vector control districts mean "special districts" with a board and staff focused exclusively on this issue. In California, most mosquito and vector control agencies are organized as independent special districts. My presentation will address several important questions: What makes this form of government so special? What threats do special districts face in our current political environment? And, how can special districts and other leaders promote their story and ensure the continued delivery of effective mosquito abatement and vector control?

Opportunities with the advancement of tidal marsh restoration in the San Francisco Bay Area

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Abstract

The San Francisco Bay Area is a dynamic region where environmental stewardship is championed. It is not only home to the largest tidal wetland restoration project on the West Coast, but also has an unprecedented nine-county parcel tax which was approved with a 70% voter majority. Measure AA, the San Francisco Bay Clean Water, Pollution Prevention and Habitat Restoration Measure, will raise approximately $25 million annually, or $500 million over twenty years, for the restoration, enhancement, protection, and enjoyment of wetlands and wildlife habitat in the San Francisco Bay and along its shoreline, including associated flood management and public access infrastructure. As the Bay transitions from dumping grounds to a revitalized resource, there are many opportunities to reaffirm the importance of water management that reduces mosquito breeding potential. New restoration design techniques are focused on creating an adaptive shoreline which is both resistant to rising sea levels and provides habitat by expanding the horizontal footprint of a levee creating a gradual vegetated slope to break the waves. With a new funding resource, available wetland restoration will continue to progress in the Bay Area and the Alameda County Mosquito Abatement District is fostering partnerships to keep the public health perspective included.

Gone Phishing - Basic lessons in information security

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Abstract

The next attack on your network is already sitting in your inbox, waiting for a click. Experts predict a new business is attacked every 14 seconds, and costs to respond will reach $11 billion by the end of 2019. The majority of ransomware and password stealing "phishing" attacks happen over email. This presentation covered email security best practices, including how staff can be trained to recognize attacks and repel them by using testing methods and creating a culture of security. In addition, simple steps to better information security and available resources for performing a security self-audit we recovered.

Coordinating with various land managers to control mosquitoes in Benton County, Washington.

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Abstract

Benton County Mosquito Control District covers 350 square miles of southeastern Washington at the confluence of the Yakima and Columbia Rivers. Historically famous for the decommissioned Hanford nuclear production complex, Benton County is also known for outdoor recreation and wine production. This presentation will focus on the collaboration between the Benton County Mosquito Control District and local, state, and federal land managers as we work together to find a balance between nature and the human environment.

Succession Planning: Cultivating our future leaders

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Introduction

With 10,000 baby boomers retiring from the workforce each day (Pew Research center 2018), the vector control industry must avoid the knowledge vacuum that occurs when senior staff leave the agency before others are trained to take over their roles. Succession planning is key to ensuring smooth management transitions without negative impacts to operations and productivity.

Methods

The Greater Los Angeles County Vector Control District implemented a supervisory training and development program to provide on-going mentorship to supervising field staff and to cultivate fundamental leadership skills. The program was open to internal staff with state certifications in all four continuing education categories and a minimum of four years of experience as a full-time operational field control employee with a vector control district. The recruitment process was competitive and some selected individuals served only two-year limited term assignments as a Vector Control Specialist IV with a modest temporary salary increase. Duties included supervising and evaluating seasonal staff, working on special projects, participating in management meetings and training, preparing reports, and participating on seasonal staff hiring panels.

Results and Discussion

To date, eight employees have participated in the program and two have been promoted to full-time management positions. Overall evaluations of the program have been extremely positive, with many participants indicating they learned life skills and gained a better understanding of how management decisions are made.

Conclusions

The District will continue the program as an employee development and succession planning strategy so long as internal staff are interested in the opportunity. Considerations will be made to expand the program to additional District departments.

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Developing novel RT-PCR markers for detecting dog heartworm (*Dirofilaria immitis*) and other filarial worms

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INTRODUCTION

The incidence of dog heartworm (*Dirofilaria immitis*) infection in northern California is increasing (Weinman and Garcia 1974, 1980; Sacks 1998) and determining the distribution and abundance of mosquitoes infected with filarial worms is necessary for monitoring filariasis in a given region (Tahir et al. 2017). Dog heartworm is primarily of veterinary concern for domestic and wild canids, and to a lesser extent domestic and wild cats. Infection in humans can occur, causing small lesions on the lungs or other tissues. Lesions are not known to cause disease in humans, but can show up on medical scans potentially leading to false diagnoses and unnecessary medical intervention (Simon et al. 2012).

Molecular genetic methods, principally quantitative polymerase chain reaction (qPCR), are commonly used to detect and identify filarial worm species infecting mosquito vectors and there are a number of qPCR markers designed to detect *D. immitis* and other filarial worm species (Mar et al. 2002; Huang et al. 2009; Latrofa et al. 2012; Albonico et al., 2014; Tahir et al. 2017). However, some markers are potentially unable to distinguish between *D. immitis* and closely-related taxa. For example, the ribosomal 5s RNA gene (5s rRNA) is a commonly used genetic marker for detecting various filarial worm species (Xie et al. 1994), but the 5s RNA gene is also highly conserved (Xie et al. 1994) making it unsuitable for species-specific determinations. In addition, the relative specificity of genetic markers can be affected by variation at the genomic level, potentially leading to false positive or false negative results (Yao et al. 2006).

To better understand the presence and animal health consequences associated with dog heartworm, we developed molecular probes for identifying filarial worms, including dog heartworm, using quantitative qPCR. Our goals were to develop novel probes to determine the relative abundance of tree-hole mosquitoes (*Aedes sierrensis*) infected with *D. immitis* and also to determine if there were other filarial worms present in *Ae. sierrensis* inmosquito populations collected in Placer County, California. We developed two novel qPCR markers targeting the highly-conserved 5s RNA gene which detected a wide range of filarial worm genera as well as the rapidly-evolving mitochondrial CO1 locus that should be specific for *D. immitis*. Using this approach, we detected *D. immitis* and non-*D. immitis* taxa because any sample testing positive for the 5s RNA, but not the CO1 marker, indicates the presence of a filarial worm species other than *D. immitis*.

MATERIALS AND METHODS

Beginning in 2012, Placer Mosquito and Vector Control District (PMVCD) began collecting *Ae. sierrensis* for dog-heartworm testing and as of September 2017, PMVCD collected 3,123 *A. sierrensis* distributed into 378 pools of up to 50 individuals. Genomic DNA was extracted from each pool using a guanidine thiocyanate/paramagnetic bead protocol modified from Lijtmaer et al. (2012). Mosquito samples were then amplified using the qPCR protocol and *D. immitis*-specific CO1 marker developed by Tahir et al. (2017). However, initial qPCR reactions, using negative controls (water) and positive controls (DNA extracted from *D. immitis* larvae), failed to amplify all known positive controls using the Tahir et al. (2017) marker. Failure to detect all known positive samples could be due to the PCR reactions not being optimal or perhaps there is a DNA sequence mismatch between the Tahir et al. (2017) marker nucleotide sequence and the CO1 locus of *D. immitis* in northern California (e.g. Wilcox et al. 2013). Because we were unable to detect *D. immitis* consistently, we developed a novel species-specific CO1 marker for *D. immitis*.

Quantitative PCR markers for filarial worms in general and *D. immitis* in particular were generated using the following approach.First, we used BLAST searches (Zhang et al. 2000) to align filarial 5s RNA nucleotide sequence data from GenBank, determined a consensus sequence, and then used the Primer3 software (Koressar and Remm 2007; Untergasser et al. 2012) to design custom TaqMan primers and probe based on the *D. immitis* 5s RNA gene consensus sequence. This highly-conserved 5s RNA marker will detect a wide range of filarial worm genera and species. Similarly, we used *D. immitis* CO1 nucleotide sequence data from GenBank to generate a consensus sequence and then used Primer 3 to design custom TaqMan primers and probe that targets the CO1 locus. The mitochondrial CO1 gene is rapidly-evolving, thus the CO1 marker should be specific for *D. immitis* only. (Table 1).

Table 1.—RT-PCR primers and probes including genetic locus, primer and probe name, sequence, and reporter and quencher dyes.

Locus	Primer name	Sequence (all 5′ to 3′)
CO1	Dim_CO1_PQS_F	TTACTTTTGTTGCGTTGTTG
CO1	Dim_CO1_PQS_R	TGACCCTCTACACTCAAAGG
5s RNA	Fil5s_PMVCD_F	TACCACGTTGAAAGCACGAC
5s RNA	Fil5s_PMVCD_R	CCAAGTACTAACCAGGCCCA
Locus	Probe	Reporter dye-sequence-quencher
CO1	Dim_CO1_PQS_Pr	CAL Fluor Orange 560- GGGGGTCCTGGGAGTAGTTGA-BHQ
5s RNA	Fil5s_PMVCD_Pr	Quasar 670-CGTCCGATCTGTCAAGTTAAGCAACGT-BHQ

RESULTS AND DISCUSSION

We tested both the 5s RNA and CO1 markers on a test panel of known positive and known negative samples. All know positives were detected with either marker, whereas neither marker was positive for negative samples (not shown). Of the 378 *Ae. sierrensis* pools tested here, 68 (∼18%) were positive for the 5s RNA marker but only three pools (∼0.8%) were positive for the CO1 marker, indicating the presence of non-*D. immitis* filarial worms in these mosquitoes In addition, the 68 pools that were positive for the 5s RNA were subjected to secondary testing using the CO1 marker, but only the same three pools were positive for *D. immitis* .

Our initial results indicate that our combined approach of using both a highly conserved marker in conjunction with a rapidly-evolving marker enabled us to detect *D. immitis* and non-*D. immitis* filarial worms infecting *Ae. sierrensis* mosquitoes. Reactions using the COI and 5s RNA markers developed here successfully identified all known *D. immitis* positive pools and also indicated the presence of filarial worms in samples that tested negative for *D. immitis*. However, initial experiments to combine the CO1 and 5s RNA markers into a duplex reaction were only marginally successful, additional experiments are necessary to optimize for a *D. immitis* CO1 plus 5s rRNA duplex reaction.

CONCLUSIONS

Although our initial survey of *Ae. sierrensis* mosquitoes revealed a relatively low level of dog heartworm infection, testing *Ae. sierrensis* mosquitoes for the presence of *D. immitis* and other filarial worms may be useful for determining the potential threat to humans and their canine companions. In addition, an unanticipated result was the much higher prevalence of non-*D. immitis* filarial worms infecting *Ae. sierrensis* mosquitoes in Placer County, California. Future research should be focus on determining the species composition of the filarial worms present in mosquito vectors inhabiting populated areas in order to determine the potential health impacts on humans, their animal companions and livestock.

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Comparison of *in vitro* and *in vivo* blood-feeding in *Culex quinquefsciatus* (Diptera: Culicidae)

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Abstract

Hematophagous arthropods such as mosquitoes, ticks, fleas and others depend on blood-meals to reproduce. Under laboratory conditions, options of preferred blood-feeding hosts, namely *in vivo* feeding, are not always available. Therefore, various techniques and devices have been developed to provide *in vitro* feeding options, mostly on stored blood with anticoagulant *In vitro* feeding techniques have been used in studies on vector-pathogen interactions or recently in screening of mosquito repellent. The primary obstacles associated with *in vitro* feeding are simulation of host skin through which the blood-sucking arthropods feed, and maintenance of constant temperature in blood meals throughout the feeding period. In the current studies, we compared a Hemotek Membrane Feeding System (Hemotek Ltd., Blackburn, UK) with live chicks to feed the southern house mosquito, *Culex quinquefaciatus*. The following parameters were used to compared *in vitro* and *in vivo* feeding: Blood-feeding rate, quantity of blood meal ingested per female, size of egg raft, number of eggs per milligram blood meal, oviposition rate and egg hatchability. The *in vitro* feeding system substituted with fresh chicken skin as feeding membrane allowed the mosquitoes to feed more readily, imbibe larger blood meals, and ovposit at a higher rate, but produced a lower number of eggs per milligram of blood meal as compared with *in vivo* feeding. There were no considerable differences in hatchability of eggs from *in vitro* and *in vivo* feedings. The membrane feeding system as developed by Hemotek Ltd. with substitution of fresh chicken skin is recommended for *in vitro* feeding, especially when considering the dependability of blood-feeding hosts, need of animal use protocol and cost of live animal husbandry.

Unexpected observations in catch basins treated with extended release briquettes

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Introduction

Many extended release briquettes are labeled to provide 5–6 months of larval control, which is ideal, considering the thousands of catch basins (CB) that must be treated in a season. However, post-treatment inspections frequently found larvae in treated CB. A study by the North Shore Mosquito Abatement District, IL showed similar findings (Harbison et al. 2017). The aim of our study was to gain insight as to why extended release briquettes were not working for as long as expected and why a high number of CB were found with unacceptable quantities of mosquito larvae.

Methods

The larvicide products that were evaluated, Altosid XR-Briquets, FourStar Briquets, FourStar Bti Briquets, and Natular XRT Tablets, were reported by the manufacturers to control mosquito larvae in CB for 150, 180, 150 and 180 days post treatment, respectively. The primary active ingredient in each larvicide was methoprene, *Bacillus sphaericus*, *Bacillus thuringiensis* subspecies *israelensis*, or spinosad, respectively. Of the four active ingredients in the larvicide products that were evaluated, only methoprene does not kill larvae, and instead prevents larval molting or adult mosquito emergence from pupae. Consequently, to evaluate the efficacy of Altosid XR-Briquets for controlling mosquitoes in CB, mosquito larvae were collected from CB and adult mosquito emergence was recorded. Each larvicide was evaluated separately in a CB that contained mosquito larvae during the year prior to the present study (n = 20 CB per larvicide tested). The treated CB were inspected for the presence of mosquito larvae once every two weeks. The average water depth inside basins was 50 cm. Because all the CB in the study contained mosquito larvae during the prior year and preventing the emergence of adult mosquitoes is the primary goal of our District, untreated control CB were not included in the present study. CB that were found to never contain larvae during the study were removed from calculations. The reported error values are standard error of the mean.

Results and Discussion

On average, 55 ± 14% of the CB contained mosquito larvae two weeks after the product was placed into the CB (Table 1; range of 10 – 85%). At four weeks post-treatment, 61 ± 12% of the treated CB contained mosquito larvae (Table 1; range of 25 – 90%). At both time points, CB that were treated with a Natular XRT Tablet were least likely to contain mosquito larvae. As expected, the proportion of CB that contained mosquito larvae was highest for those that were treated with an Altosid XR-Briquet. Although FourStar Briquets and FourStar Bti Briquets contain active ingredients that quickly kill mosquito larvae, over half of the CB that were treated with those products contained mosquito larvae. When mosquito larvae that were collected from the CB two weeks post treatment were reared in the laboratory, we found that adult mosquitoes would have successfully emerged from 41 ± 14% of the treated CB (Table 2; range of 0 – 78%). For mosquito larvae that were collected from CB four weeks post treatment, 56 ± 9% of

Table 1.—Proportion of CB that contained larvae two and four weeks after treatment with larvicide. The row that is labeled "# of CB lacking larvae" includes the inspection immediately prior to treatment and two- and four-weeks post treatment.

	Altosid XR	FourStar	FourStar Bti	Natular XRT
2 weeks post treatment				
% CB with larvae	85	60	65	10
# CB with larvae	17/20	12/20	13/20	2/20
4 weeks post treatment				
% CB with larvae	90	55	75	25
# CB with larvae	18/20	11/20	15/20	5/20
# CB lacking larvae	2	3	5	9

Table 2.—Adult mosquito emergence from water samples that were collected from treated CB that contained mosquito larvae.

	Altosid XR	Four Star	FourStar Bti	Natular XRT
2 weeks post treatment				
% CB with emerged adults	35	50	78	0
# CB with emerged adults	6/17	6/12	10/13	0/2
4 weeks post treatment				
% CB with emerged adults	28	64	53	80
# CB with emerged adults	5/18	7/11	8/15	4/5

the treated CB would have allowed adult mosquitoes to emerge (Table 2; range of 28 – 80%). Adult mosquitoes emerged from more than a quarter of the mosquito larva samples that were collected from CB treated with an Altosid XR-Briquet and more than half of those that were treated with a FourStar Briquet or FourStar Bti Briquet. Although relatively few CB that were treated with a Natular XRT Tablet contained mosquito larvae, 80% of those that did would have allowed adult mosquitoes to emerge.

Conclusion

Products that claim to control mosquitoes in CB for several months sounds perfect, but our current results and others have shown that this claim is too good to be true. Our study emphasized the importance of post-treatment inspections and proper product selection.

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Mapping the KDR mutation in *Culex pipiens* throughout Alameda County

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INTRODUCTION

The development of resistance to insecticides that target adult mosquitoes can hinder a District's ability to successfully kill mosquitoes that are infected with arboviruses, making insecticide resistance a public health threat. *Culex pipiens* mosquitoes represent a heightened risk of arboviruses transmission to humans, because their preferred larval habitats are often located within urban centers and near to people. Their proximity to people and the propensity of *Cx. pipiens* to take blood meals from people makes it crucial to eliminate arbovirus-carrying *Cx. pipiens* when they are detected. Knowing the status of genetic resistance of these mosquitoes to the pyrethroid class of insecticides would help vector control workers select the most effective pesticide for adulticide operations. Genetic resistance to pyrethroids can be evaluated in *Cx. pipiens* using an assay that detects the mutant knockdown resistance (*kdr*) single nucleotide polymorphism (SNP) in the voltage-gated sodium channel gene that confers resistance to pyrethroid insecticides.

METHODS AND MATERIALS

The mosquitoes were collected throughout Alameda County,California during 2018 for the purpose of determining mosquito abundance and arbovirus prevalence. Up to five females from each collection were saved individually and later tested by the *kdr* SNP test using standard quantitative polymerase chain reaction (qPCR) methods. Nucleic acids were extracted using the MagMAX – 96 Viral RNA Isolation Kit (ThermoFisher Scientific) and the *kdr* SNP evaluated using qPCR. All genotyping calls were determined automatically using the QuantStudio Design & Analysis Software (ThermoFisher Scientific). In some cases, the *kdr* genotype was evaluated at the same site on multiple occasions during 2018.

RESULTS DISCUSSION

A total of 390 *Cx. pipiens* were analyzed for the *kdr* SNP. When combined into a single group, 33.3% were homozygous susceptible, 38.5% were heterozygous susceptible/resistant, and 28.2% were homozygous resistant. To test whether the location of mosquito collection affected the proportion of mosquitoes with the mutant *kdr* SNP, the collections sites were combined into three regions of Alameda County based upon natural and anthropogenic barriers: north (north of Interstate 238 and west of the East Bay Hills), south (south of Interstate 238 and west of the East Bay Hills), and east (east of the East Bay Hills). The northern region of the county lacks extensive agriculture and pyrethroids have not been applied by vector control agencies. In agreement, the northern region had the lowest proportion of homozygous resistant mosquitoes (Table 1). In contrast, the eastern region of Alameda County, home to several vineyards, farms and pastures, haD the highest proportion of *Cx. pipiens* with the homozygous resistant *kdr* SNP (Table 1). *Culex pipiens* that were collected from the southern region of the county displayed an intermediate proportion of specimens that contained the mutant kdr SNP (Table 1), which may result from the dispersal of resistant mosquitoes from the eastern region of the county via highway corridors that follow natural valleys in the East Bay Hills to the southern region of the county (e.g. State Route 84 and Interstate 680). These data are useful for determining the most suitable pesticide to use, by region, for controlling adult mosquitoes.

Table 1.—Geographic Distribution of the *kdr* SNP in Alameda County, CA

County Region	Homozygous Susceptible	Heterozygous Susceptible/Resistant	Homozygous Resistant	Total Number of Mosquitoes
North	50.8%	38.5%	10.7%	123
South	38.7%	40.9%	20.4%	137
East	11.5%	35.9%	52.7%	130

Bloodfeeding patterns of *Culex quinquefaciatus* from various habitat types in San Bernardino County

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Abstract

Culex quinquefasciatus is a primary vector of West Nile virus (WNV) in Southern California. Because they are opportunistic feeders, primarily biting on birds, humans, and other mammals, describing their feeding patterns is critical to understanding the transmission of the virus. The purpose of this project is to determine the feeding patterns of *Cx. quinquefasciatus* in a variety of habitat types in San Bernardino County, and examine the relationship between feeding patterns and WNV transmission. This study utilized over 800 blood-fed samples collected from 14 different sites during 2011 in inland southern California. The collection sites were categorized as urban, rural, rural-dairy, and suburban agriculture. DNA was extracted from the bloodmeals of individual mosquitoes and was amplified to target the cytochrome c oxidase I (COI) mitochondria gene. This gene contains a "barcode" region that was used to identify host species. These data were then used to assess bloodfeeding patterns in the various habitat types and was compared to WNV infection data collected as part of the West Valley MVCD arbovirus surveillance program.

Wolbachia infections in mosquitoes of Merced County California

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Abstract

Wolbachia are bacteria present in mosquitoes as well as in many other arthropods. These bacteria can influence reproduction and vector competence of mosquitoes, and are currently employed in mosquito release programs for *Aedes aegypti* control. Several subgroups and strains exist for *Wolbachia*, and they have yet to be characterized for some mosquito species in the Central Valley of California. In this study, the presence of *Wolbachia* was examined in mosquitoes found in Merced County. To accomplish this, mosquitoes were trapped in different habitats in Merced County and then identified to species. Ten mosquito species were abundant in the region, and these were the focus of this study. DNA was extracted from mosquitoes. Traditional and quantitative PCR were used to investigate the presence or absence of *Wolbachia*. *Wolbachia* strains will be sequenced and compared to others previously characterized.

Making *Aedes aegypti* eggs count

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Introduction

The Coachella Valley Mosquito and Vector Control District has had *Aedes aegypti* in its region since 2016. As part of the *Aedes* surveillance program, we place ovicups throughout the Coachella Valley to monitor mosquito activity and to detect *Aedes* in areas where we have not detected them before.

Materials and Methods

Ovicups were set along with CO_2 and gravid traps and at additional locations within neighborhoods. Ovicups were lined with germination paper (Anchor Paper Company, St Paul, MN) custom cut to fit in black stadium cups: 24 oz. (708 mL), 5.625" × 3.375" (14.3 × 8.6 cm). Bait for the cups was made by adding 2 lbs (0.99 kg) hay to 32 Gal (121.1 L) water and letting this incubate outdoors for 1 week. Ovicups were held in place by a holder which was a 3" (7.6 cm) ABS coupling cut in half and attached to a 2.3" (5.9 cm) counter sunk bolt. Ovicups were located at the base of a CO_2 stand or they are set separately in a secure location. Every week, germination papers were replaced in the 68 ovicups and new bait water added. Eggs were counted, and data entered into CalSurv Gateway. Eggs on germination papers were stored with moisture inside a plastic, zipped bag in the refrigerator (4°C) for up to 6 months. Germination papers were discarded after soaking in 10% bleach for 3 hours or saved for studies by external researchers. Eggs will be used to start a colony in the near future.

Results and Discussion

In 2017, we collected eggs from weeks 13-50 (March – December) (Fig. 1). Total number of eggs from the ovicups from the entire district peaked in early–October. We collected an average of 87 eggs per week, with a maximum of 180 eggs per cup. In 2018, we collected eggs from weeks 8-50 (February – December) (Fig. 1). Total eggs collected from ovicups from the entire district peaked from September to October. We collected an average of 249 eggs per week, and a maximum of 450 eggs per cup. There were more eggs collected in 2018 in total than in 2017.

The disadvantages of ovicups were that they were potential breeding sources and the ovicups can easily be tampered or stolen. *Aedes aegypti* eggs have a similar appearance to *Ae. albopictus* eggs, and if we had an introduction of *Ae. albopictus* into our area, these ovicups would not be able to detect them. In the summertime, the cups dried quickly, because summer temperatures in the Coachella Valley can reach up to 120°F (49°C). On the other hand, ovicups were inexpensive and easy to deploy, low maintenance (ovicup papers are switched out once a week), and easy to set up, giving them an advantage over other traps used for *Aedes* surveillance. We use them throughout our district, with ovicups at 68 different locations throughout the residential areas of Coachella Valley, and plan to continue using them for the foreseeable future.

Acknowledgements

Thanks to Arturo Gutierrez for designing the ovicup holder and to the surveillance team for their ovicup collections and help with developing this presentation.

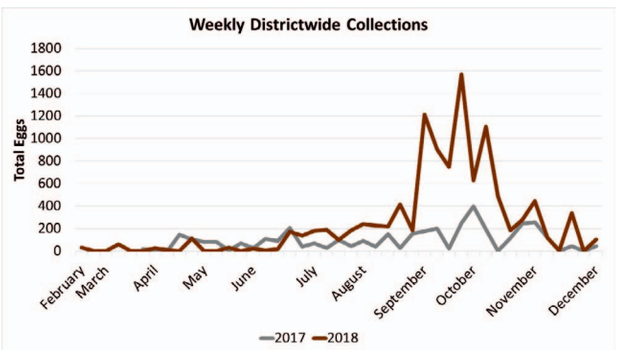

Figure 1.—Weekly total egg collections from ovicups during 2017 and 2018.

Temporal patterns of host-seeking mosquitoes in the Coachella Valley

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Introduction

The Coachella Valley is located 120 miles east of Los Angeles in southern California. It is a low desert environment surrounded by mountains to the north and southwest. There is also a thriving community in the eastern section of the valley. Therural habitat is influenced by large agricultural operations and marshes near the Salton Sea. These areas can be sources for huge numbers of nuisance and virus-carrying mosquitoes. St. Louis encephalitis virus and West Nile virus are regularly detected in this region (Reisen et al. 2002, 2008); therefore, this is where most adulticide applications are performed. To better understand night-time mosquito activity patterns here,a collection bottle rotator trap was operated during 2018. .

Methods

A collection bottle rotator (CBR) trap (John W. Hock Company, Gainesville, FL) was operated at a rural site near the north rim of the Salton Sea where we regularly detect virus activity. Beginning in July 2018, the trap was set weekly from an hour before sunset until just after sunrise to have a record of year-round temporal patterns of the mosquitoes in this rural area. A weather station (Kestrel 5700, Boothwyn, PA) was deployed at the CBR trap site to record temperature, wind, and relative humidity during the collection periods.

The CBR trap runs on three 6-volt batteries; two batteries run the CBR motor and one battery powers the fan. The trap holds 8 collection cages, and wasset to rotate one position every hour. To accommodate a total collection period longer than eight hours, two CBRs were used or the collection cages were swapped out mid-way through the evening. . Dry ice attractant was suspended over the CBR in a 1-gallon water jug three inches away from the fan opening.

Trap collections were converted into percent females captured per hour on that trap-night. The percent captures were then averaged across trap-nights for the season.

Results

At total of 17,847 mosquitoes in seven species, of which; the most abundant were *Culex tarsalis* and *Psorophora columbiae*. Here we report the results for the top 4 species collected from the CBR trap from July 2018 thru January 2019. *Ps. columbiae* activity was greatest in August and relatively low in late summer and early fall. *Cx. tarsalis* activity was low during the mid-summer and peaked in late September. In July and August, mosquito activity peaked about one hour after sunset or around midnight. In the fall and winter months, the peak mosquito activity occurred between 0-2 hours after sunset. The mosquitoes collected were primarily unfed females.

For the summer collections from July 19 – September 21 (Figure 1a), the nightly temperatures averaged 87.3°F, ranging from 99.7°F to 74.8°F, average wind speed was relatively constant at 3.1 mph, and average relative humidity RH was 41.3%, ranging from 33.7% to 50.6%. Peak activity period for all 4 species appeared between 0-2 hours after sunset, with a slight peak 4-6 hours after sunset.

For the fall collections from September 26 – December 13 (Figure 1b), nightly temps averaged 60.7°F, ranging from 77.3°F to 47.1°F, average wind speed was relatively constant at 1.9 mph, and average RH was 60.7%, ranging from 48.3% to 71.6%. Peak activity period for *Cx. tarsalis*, *Cx. erythrothorax*, and *Ps. columbiae* were 0-2 hours after sunset. Peak activity period for *Ae. vexans* was from 1 hour before to 2 hours after sunset.

For the winter collections from December 20 – Jan 30 (Figure 1c), nightly temps averaged 49.8°F, ranging from 63.2°F to 44.5°F, average wind speed was relatively constant at 0.9 mph, and average RH was 77.1%, ranging from 57.3% to 83.6%. Peak activity period for *Cx. tarsalis* was 0-2 hrs after sunset; *Cx. erythrothorax* was between 0-1 hours after sunset, and *Ae. vexans* was 1 hour before sunset. No *Ps. columbiae* were collected during this period

Discussion

Peak mosquito activity for these 4 species occured within 0-2 hrs after sunset, but there appeared to be variations. These variations may depend on the changing temperature, wind, and relative humidity throughout the night, but further analysis is needed to confirm this. Reisen et al. (1997) observed *Cx. tarsalis* activity was highest immediately after sunset in the spring and fall and was consistently active throughout the night in the late summer. Their spring and fall observations were similar to we observed, but

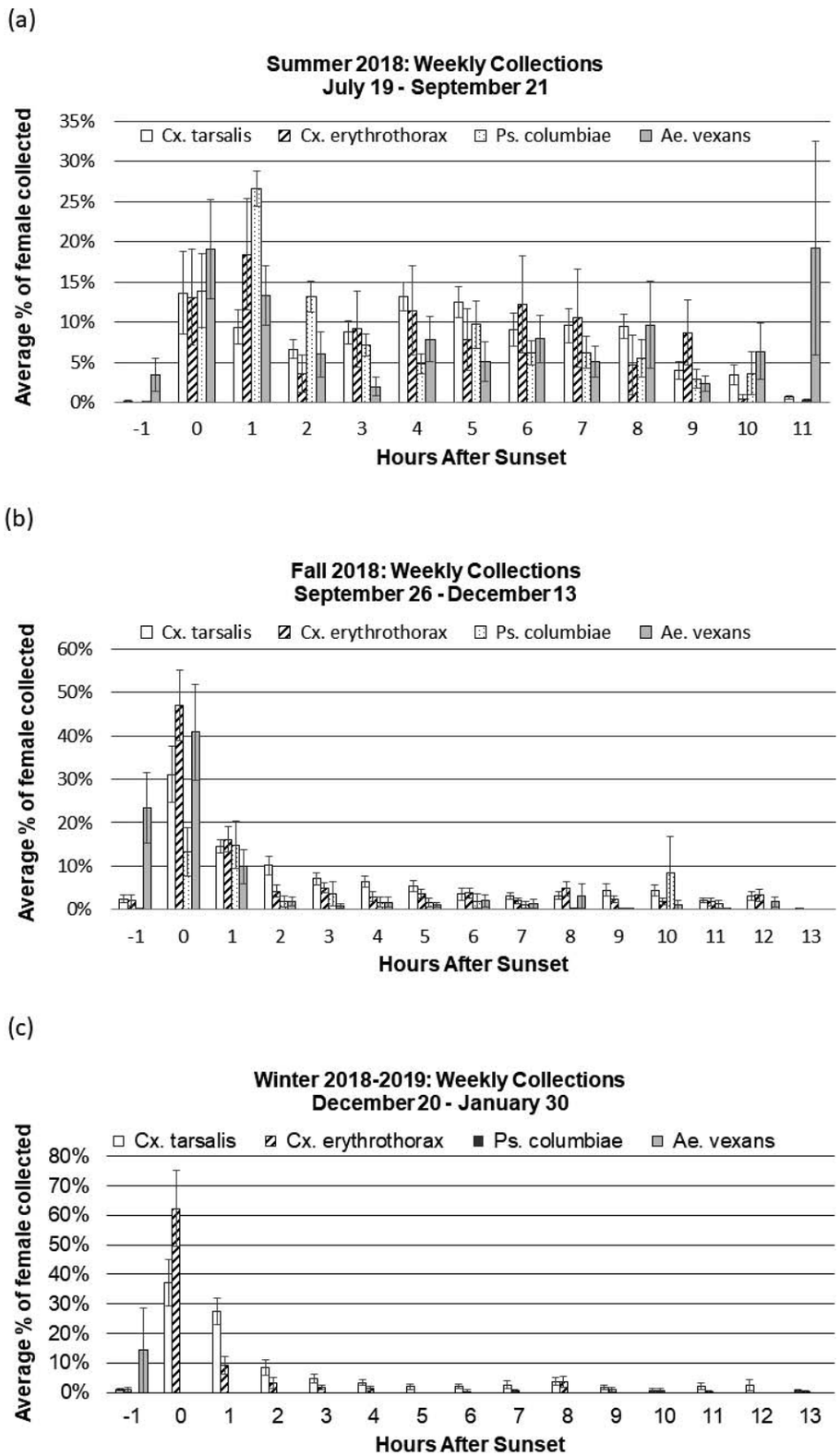

Figure 1.—Average percentage of total mosquitoes collected per hour each weekduring (a) summer, (b) fall, and (c) winter of 2018-2019.

during the late summer, it appeared that there were two peaks of activity, one immediately after sunset, and another small peak 4-6 hrs after sunset. We plan to continue collections to get a full year of weekly trap data. The results from this study will help us better understand nighttime mosquito activity throughout the year and help us better determine the optimal time for adulticide applications.

Acknowledgements

Thanks to assistance from Melissa Snelling on the poster design and the laboratory staff at CVMVCD for their valuable feedback.

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Adult longevity of colony reared *Culex tarsalis* and *Culex quinquefasciatus* mosquitoes

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Introduction

Mosquito control districts utilize pesticide-susceptible mosquitoes as controls for testing pesticide resistance and efficacy. Those tests, usually in the form of bottle bioassays and/or field trials, require a relatively large number of mosquitoes. Placer Mosquito and Vector Control maintains two mosquito colonies, *Culex quinquefasciatus* (CQ1) and *Cx. tarsalis* (BFS), using overlapping generations, so therefore the longevity of individual mosquitoes is not known. Using metrics, such as the measurement of the adult tibia size and longevity of our colony, will allow the generation of a baseline measurements that can be used to optimize our larval and adult rearing by testing the effect of different variables such as larval density, temperature, humidity and light-dark cycles.

Methods

Four replicates were reared, maintained throughout adulthood, and measurements recorded for each species. Each replicate consisted of 100 pupae that were added to an adult rearing cage (100 pupae/per cage (x) 4 replicates, for each species). Pupae that did not eclose within 48 hours were discarded and the emerged adults were maintained in our insectary until death. The adults were provided a weekly blood-feeding for 8 hours using chicken blood in a Hemotech blood warmer. They also had access to water for oviposition 3 to 4 days after blood -feeding for *Cx. tarsalis* and *Cx. quinquefasciatus,* respectively, as well as 10% sugar-water that was available continuously as a food source. Insectary environmental conditions were kept near 26° C with a 15.5:8.5h L:D cycle without a crepuscular period; humidityand larval densities were not recorded in this experiment. Dead adults were removed daily from the cages and the age, sex and tibia lengths were recorded. Tibia length measurements were used instead of wing lengths because previous district studies had used this metric and continuing to use this measurement allowed us to compare these results to previous studies.

Results and Discussion

Female *Cx. quinquefasciatus* lived significantly longer than female *Cx. tarsals,* with an average of 57.5 days versus 28 days, respectively (Fig. 1). Male *Cx. quinquefasciatus* lived an average of 47.7 days, which was significantly longer than the male *Cx. tarsalis* who averaged 27.2 days. In contrast there was no significant difference in life span between males and females within either species (Fig. 1). It is still unknown if these

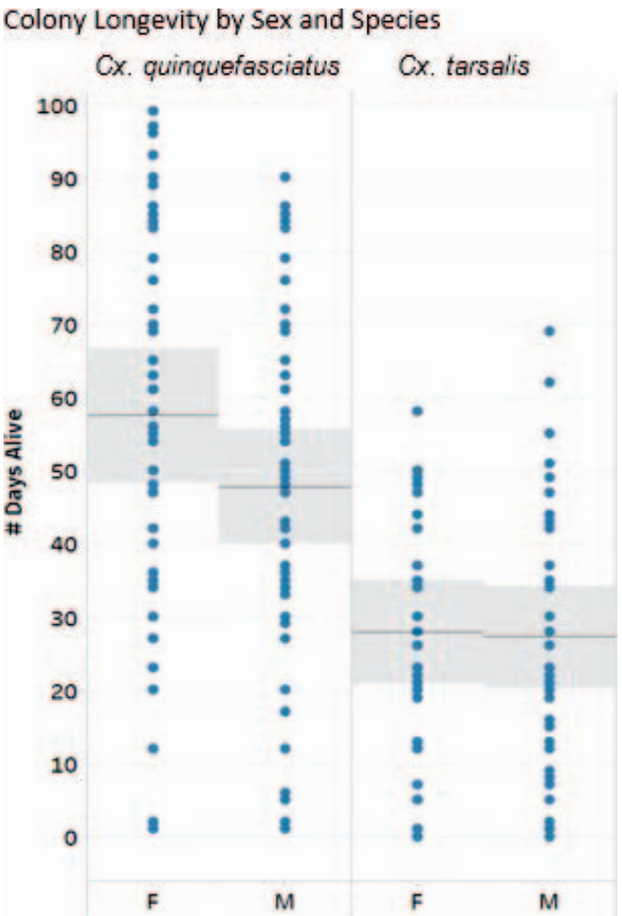

Figure 1.—Individual days to death are plotted for each sex and species with grey bars showing the mean life span and shaded regions representing 95% confidence intervals. A significant difference in longevity was found between these species but not necessarily between males and females within either species. Differences between species may be due to species variation or differences in rearing conditions.

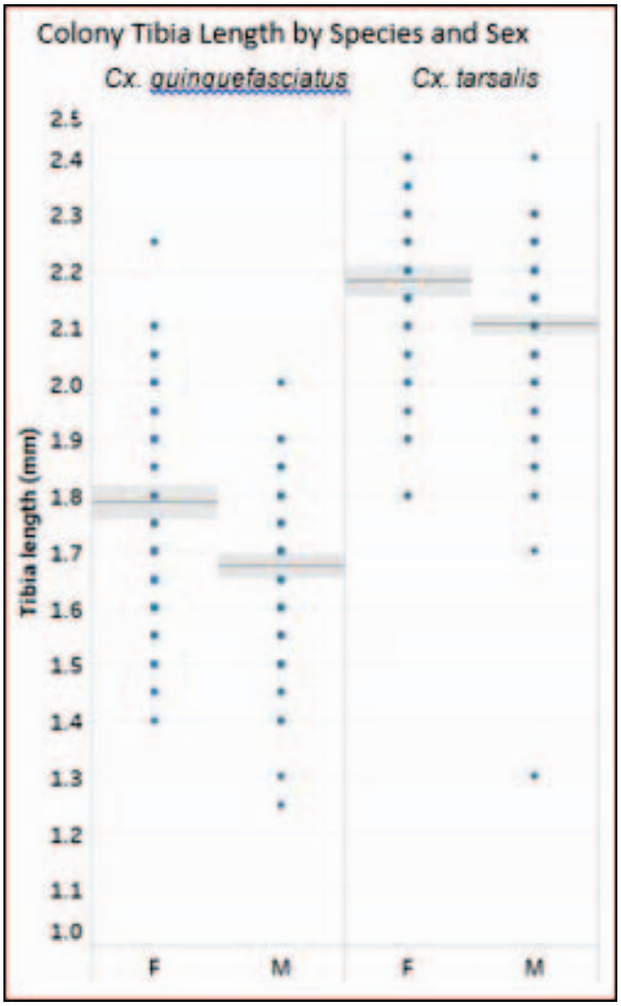

Figure 2.—Measured tibia lengths by species and sex are shown with grey bars representing mean values and shaded areas showing 95% confidence intervals. Tibia lengths for male and female *Culex tarsalis* were significantly larger than their *Cx. quinquefasciatus* counterparts, while females were significantly larger than males within both species. The size difference between species could be due to variation in rearing conditions as well as species specific variation.

differences in longevity are simply due to variations of the different species natural life spans or if the differences can be attributed to different rearing conditions experienced by the two species, such as temperature, humidity or larval density. Bock et al. (1983) found the life expectancy for male *Cx. tarsalis* was 29.9 days, which was very similar to the average longevity observed in our experiment of 27.2 days (Fig. 1).

For each species, females were significantly larger than males, while for both males and females *Cx. tarsalis* was significantly larger in size than *Cx. quinquefasciatus* (Fig. 2). Along with natural variation between species, differences in size may be due to our current larval rearing protocol for *Cx. quinquefasciatus*. Actual larval densities were not measured and even though *Cx. quinquefasciatus* were maintained at an obviously higher density than *Cx. tarsalis,* they pupated at a similar time frame and produced more pupae than the *Cx. tarsalis* colony. Higher larval densities can produce smaller sized adults (Reisen et al. 1984, Dodson et al. 2011), thus the difference in larval densities used here could have contributed to the smaller *Cx. quinquefasciatus* adults. In addition, adult size can also be affected by temperature, with higher rearing temperatures producing smaller adults (Andreadis et al. 2014). The temperature here for the rearing rooms of both species was controlled by a shared thermostat so the differences should have been minimal and unlikely to create such a large difference in size between the species as recorded here. However, the true value of this information will be seen after future experiments are completed, where one rearing variable is changed for one species and the resulting size and longevity can be compared to that species measurements, allowing that specific variables effect on the mosquito development to be determined.

Conclusions

Our experiments provided some baseline information on adult longevity and adult size in relation to rearing conditions. Optimizing mosquito production depends on determining rearing conditions that are most favorable for mosquito growth and longevity, thus future experiments should focus on determining the conditions having the greatest effect on adult size and longevity. In addition, optimizing mosquito production will help improve efficacy testing by providing a large number of healthy adults for testing purposes.

Acknowledgements

Thanks to Tom Moore for the hard work in helping to complete this experiment.

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Evaluation of Natular T30, a tablet formulation of spinosad, against *Culex* mosquito larvae in simulated catch basins

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Abstract

Mosquitoes are found abundantly in catch basins in urban areas, posing a public health threat for humans in the vicinity and necessitating control efforts. Catch basins vary in construction and can have depths of up to ten feet or more and hold a variable amount of water. Several formulations based on the reduced-risk pesticide spinosad, consisting of spinosyn A and D, are available under the trade name Natular® and may be used as mosquito larvicides. Focusing on the Natular T30 tablet containing 8.33% spinosad, previous studies have shown that when the tablet was placed directly in catch basins to control mosquitoes, the product sank and failed to reach the larvae dwelling by the water surface. However, high efficacy was achieved when the tablet was suspended at the water surface. To elucidate the differing mechanisms of efficacy between the applications described above, nine simulated catch basins were set up, filled with water and enriched with organic matter. One tablet was placed in each of six basins, three were floated by attaching them to a wine cork with fishing line, while three were applied directly and sank immediately after application. The remaining three basins were used as untreated controls. Water samples were taken from the top 1" from each basin and placed in 8-OZ Styrofoam cups, along with 50 3rd instar *Culex quinquefasciatus* larvae. Water sampling and bioassay were conducted at multiple time intervals for 21 days. Larval mortality was recorded after 24 or 72-hour exposure. Basins with "floating" tablets showed a high mortality from day one post-treatment, whereas basins with "sinking" tablets did not exhibit a difference in larval mortality when compared to untreated controls shortly after treatment. Although larval mortality in basins with "sinking" tablets increased gradually over time and reached 85.3% on day 16, the risk of resistance development to spinosad should be considered during the period when sublethal conditions prevailed shortly after application.

INTRODUCTION

Mosquitoes are known vectors of many vector-borne diseases including Zika, West Nile virus infection, dengue, and others. Urban catch basins present ideal larval habitats for *Culex* mosquitoes (Su et al. 2004, Harbison et al. 2018). Because the catch basins can have a depth >10 feet, storm water can accumulate and create optimal mosquito development conditions. Catch basins are numerous in urban areas and the mosquitoes produced provide a nuisance and arbovirus health risk. For example, in Orange County, it was estimated that if only 10% of the catch basins supported mosquito development, these would be the most significant mosquito development habitat in the urban areas (Su et al. 2004). However, manholes and catch basins cannot be eliminated because they are an integral component of the urban drainage system, and therefore a method to control larval mosquitoes must be developed.

Mosquito larvicides based on spinosad have become available recently. Spinosad is derived from the soil-dwelling actinomycetes bacteria *Saccharopolyspora spinosa* Mertz and Yao, which produces spynosyns during fermentation (Bond et al. 2004). Commonly developed spinosyns are spinosad and spinetoram, the former consists of spynosyn A and D and the latter contains spynosyn J and L. Spinosad acts as neurotoxins activating primarily the acetylcholine receptor and secondarily the gamma-amino-butyric acid (GABA) receptor in mosquito larvae, which causes over excitation of the larval nervous system, ultimately leading to paralysis and death. Spinosad has been proven to be successful against mosquito larvae and has many benefits, because it is relatively target specific (Nasci et al. 2017). In fact, spinosad was classified by the United States Environmental Protection Agency (US EPA) as a reduced risk pesticide (Thompson et al. 2000). The end-use products based on spinosad are viable options to control mosquito larvae breeding in urban drainage systems.

Some spinosad products have drawbacks mainly associated with physical format, dispersal and release behavior. Natular products labeled to control mosquito larvae have different formulations such as emulsifiable concentrate, granules, tablets and briquets with various residual efficacy. Natular T30, a 30-day slow release tablet, has not been shown to be effective in field catch basins per label application method (Su et al. 2014). Due to weight and density, the tablets sink immediately when placed in water. This is not an issue when placed in shallow containers,

however, catch basins can have a water depth ≥10 ft., which makes it less likely for spinosad to impact the larvae dwelling mostly around the water surface. To improve the utility of Natular T30 tablets, research on product performance in catch basin environments is warranted.

Previous research has been done on catch basins with Natular T30 tablets and has demonstrated success against mosquito larvae when suspended (Su et al. 2014). However, data on ingredient dispersal under "floating" and "sinking" application conditions is needed to explain the differences in field efficacy (Su et al. 2014). In the current study, Natular T30 tablets were applied by "floating" and "sinking" them in simulated catch basins, where the surface water layer was analyzed by bioassay.

MATERIALS AND METHODS

Habitat

The catch basin habitat was simulated by placing nine plastic basins (Rubbermaid 27" Height x 19.5" Diameter, 35-gallon, Home Depot, Ontario, CA) outdoors in a suburban location in Ontario, California. Each of the basin lids was cut with seven circular openings with 3" in diameter to simulate a catch basin cover. Window screen was glued to each hole to prevent gravid females from entering the container and ovipositing. Basins were filled with tap water to the full volume of 35 gallons with a depth of 27 inches. Preliminary observation indicated non-treatment related larval mortality in some basins, suspiciously related to unknown materials that leached after flooding these new plastic basins. Prior to the beginning of the formal experiment, the basins were soaked and rinsed for 4 times in 3 days to remove any leachates that could affect the results of the later bioassays. Upon flooding, 30 grams of rabbit food pellets were added to each catch basin as organic enrichment. Three of the basins were designated as untreated control (UTC), which contained only water and the organic matter. Three basins were labeled as "sinking" and contained water, organic matter, and a Natular T30 tablet which settled to the bottom. The remaining three basins were labeled as "floating", and contained water, organic matter, and a Natular T30 tablet floating on the surface.

Pesticide

The pesticide used was Natular T30 (Clarke, St. Charles, IL), a pharmaceutical grade tablet containing 8.33% spinosad. Each of the six tablets had a 1-mm diameter hole through the center, to make the tablets used for "floating" and "sinking" treatments identical. To float the tablet, a wine cork was attached to the tablet by clear nylon fishing line (Fig. 1).

Mosquitoes

Mosquito larvae used in the bioassays are mostly *Culex quinquefasciatus* Say collected in an urban area of Southern California. These mosquitoes are known to be susceptible to spinosad (Su and Cheng 2014). Egg rafts were collected

Figure 1.—A Natular T30 tablet that is tied to a wine cork by a piece of Nylon fishing line.

by organic infusion (1: 1 ratio of tap water and infusion concentrate, which was made by adding 100 g rabbit pellets to one gallon of tap water and fermented outdoors for 2 weeks). Hatched larvae were reared to late 3rd – early 4th instars for bioassay.

Water sampling

Water sampling was conducted by taking 4 of 50 mL samples from each basin by a turkey baster to avoid disturbing the water and tablet inside the basins. Each 50 mL sample was collected by sweeping half of the water edge along the basin rim and aspirating from the top 1 inch of the water, which was repeated 4 times to achieve 200 mL in total sample volume. One turkey baster was used for each basin to avoid cross contamination of treatments. Water was sampled on days 1, 2, 3, 7, 8, 9, 10, 14, 15, 16, 17 and 21 post-treatment for each 24-hour bioassay, whereas samples on day 4, 11, and 18 post-treatment were used for 72-hour bioassays.

Water temperatures

Minimum and maximum water temperatures were monitored by a Taylor Min-Max Duel Reading Thermometer (Oak Brook, IL), which was placed to the midpoint of the water depth in one of the nine basins.

Laboratory Bioassay

Bioassays were conducted to test the toxicity of the water samples against mosquito larvae. The water samples taken from each basin were placed inside an 8-ounce Styrofoam cup containing 50 *Cx. quinquefasciatus* larvae. The larvae remained in the cups with treated water at a temperature of 85° ± 2°F. The surviving larvae were counted at the end of each 24- or 72-hour period to calculate mortality. Data was composited from the three replicates per treatment. Larval mortality and standard errors were calculated and charted.

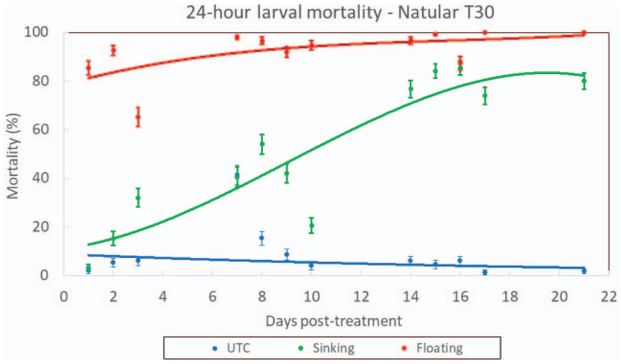

Figure 2.—The mean (±SE) 24-hour *Culex quinquefasciatus* larval mortality in bioassays of water samples from the surface of simulated catch basins treated with "floating" and "sinking" tablets (Ontario, CA; July 18 – August 7, 2018). utc = untreated control. Trends in larval mortality were presented by fitting by polynomial curve at degree 3.

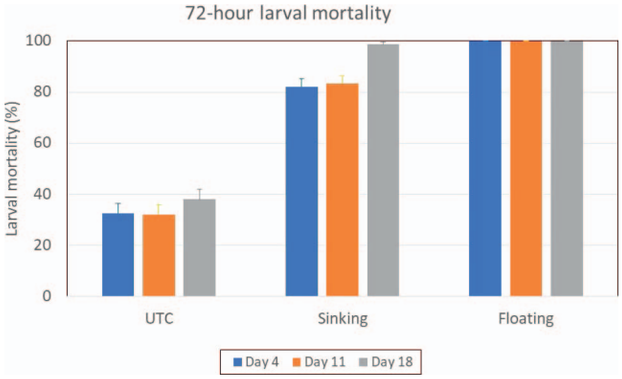

Figure 3.—Mean (±SE) 72-hour *Culex quinquefasciatus* larval mortality in bioassay water samples from the surface of simulated catch basins treated with "floating" and "sinking" tablets (Ontario, CA; July 18 – August 7, 2018). utc = untreated control

Residual weight

The original weight of each tablet was recorded before placing into the water in each basin. Upon completion of study, all tablets were retrieved carefully, air-dried for 48 hours and residual weight determined.

RESULTS

24-hour bioassay

Water samples from the basins with "floating" Natular T30 tablets resulted in a high mortality from day one post-treatment (85.3%) (Fig. 2). When affected by the tablet, larvae displayed a period of 'over-excited' activity, followed by paralysis and death. Larval mortality in water from basins with "floating" tablets slightly increased throughout the study period until 100% was reached. Conversely, the water inside the basins with "sinking" Natular T30 tablets caused a significantly lower mortality in comparison with the basins with "floating" tablets, although mortality in these basins with "sinking" tablets gradually increased over time (Fig. 2). Mortality in treated basins were considerably higher than that in UTC basins, with exception to day 7, where mortality at UTC was close to that in water from basins with "sinking" tablet (Fig. 2).

72-hour bioassay

When the exposure time was extended to 72 hours, water samples from the basins with "floating" tablets demonstrated 100% in mortality for all three samples from 4, 11 and 18 days post-treatment, whereas those in the "sinking" basins displayed a mortality above 80% at the same time periods (Fig. 3). Larval mortality in the UTC also increased as compared with 24-hour assays (Fig. 3).

Water temperatures

Water temperature inside the basins recorded was relatively stable. There was a slight increase during days 8-10 post-treatment, but temperature stayed within the range of 76° to 89°F (Fig. 4).

Residual weight

The average residual weight of each tablet was calculated upon completion of study. On day 21 post-treatment when the test was concluded, 1.97 ± 0.06 g (36.7% of original) remained for the "floating" tablets, whereas 3.52 ± 0.09 g (65.5% of original) remained for the "sinking" tablets (Fig. 5).

DISCUSSION

With spinosad being a relatively target-specific pesticide with many benefits, it is important to know its true performance under field conditions. Best efforts were made to quantify differences in larval mortality when Natular T30 was applied differently to simulated catch basins. Basins initially were cleaned and rinsed to avoid any other substances affecting larval mortality. Turkey basters were

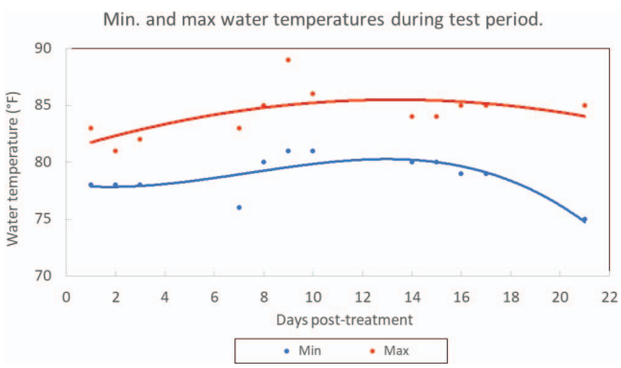

Figure 4.—Minimum and maximum water temperatures during study period (Ontario, CA; July 18 – August 7, 2018). Trends in temperature were presented by fitting by polynomial curve at degree 3.

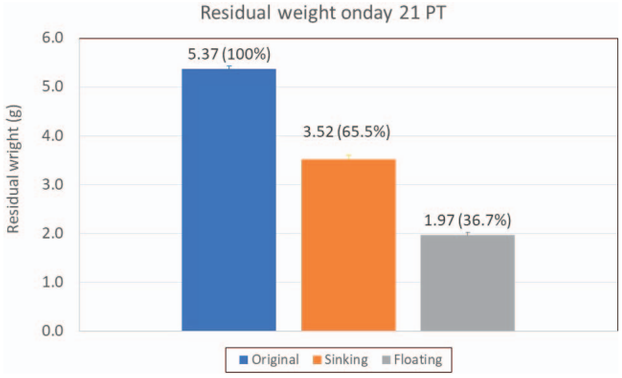

Figure 5.—Mean (±SE) weight of tablets in grams pre and post study with percentage lost for the average "floating" and "sinking" tablets tested.

used to collect water samples from the surface of the basins to minimize the disturbance of water layers and movement of tablets by sampling, which could affect the release and mixing of the pesticide. Water samples were taken only from the top 1" of the basins, because that is where *Culex* larvae mostly dwell and feed.

If an overall efficacy target of >85% mortality was desired in mosquito control operations, then the tablet applied directly and allowed to sink, showed poor results. When the initial bioassays were conducted 24 hours post treatment, the "sinking" tablets produced a mortality of 3.3%, considerably lower than the "floating" tablets which showed a mortality of 85.3% at the same sampling time post treatment. The larval mortality associated with "sinking" tablet increased as a function of time post treatment and reached 85.3% by day 16. This process led to the sublethal exposure of mosquito larvae to spinosad, and conditions that have been shown to develop resistance to spinosad in *Culex* (Su and Chang 2014).

On day 7 post-treatment, the untreated control had an unusually high mortality, almost equivalent to that of the "sinking" tablet water sample, which was attributed to scum accumulated at the water surface of the basin during peak eutrophication time post-flooding. Microbial flora have been shown to peak in density in a similar microcosm system (Nguyen et al. 1999). In this case, day seven appeared the peak day for bacterial growth which affected the larval survivorship, i.e. probably killed by physical occlusion from air by the surface scum, even though the water did not contain any pesticide.

Water temperature is the most significant abiotic factor in the environment to affect larvicide dispersal and degradation through physical and chemical processes, as well as microbial activities. Temperatures measured during the evaluation ranged from 76°-89°F, which is believed to be conducive to product dispersal.

Exposure duration in the bioassay affected larval mortality. At 72-hour exposure, differences in mortality between assays of water taken from "floating" and "sinking" treatments were reduced compared with results read at 24 hours. Nevertheless, 72-hour assays still showed efficacy differences between the two application methods on days 4 and 11 post-treatment.

The "floating" tablets dissolved more rapidly than "sinking" tablets based on tablet weights upon the completion of the study. This is in agreement with greater mortality in bioassays of water from basins with "floating" tables throughout the study. The difference in weight loss also could have been due to water temperature differences between the surface and the bottom of the basins. Water temperature fluctuations and movement were more profound at the surface than at the bottom of the basin.

In summary, Natular T30 tablets can be used as an efficient larvicide when applied under the correct conditions. Application in urban catch basins as intended by its manufacturer will unlikely provide an optimized solution for larval control. Under "floating" conditions, the product can be utilized safely and efficiently as a mosquito larvicide against *Culex* mosquitoes, providing a high mortality soon after application. However, under "sinking" conditions, Natular T30 tablets not only performed poorly, but may potentially create sublethal conditions allowing mosquito larvae to develop tolerance or resistance. Further research is necessary to improve the tablet's buoyancy to deliver the active ingredients mostly to the feeding zone of mosquito larvae for better product performance.

ACKNOWLEDGMENTS

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Impact of storage and handling temperatures on the activities of mosquito larvicides

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Abstract

Larvicides based on *Bacillus thuringiensis israelensis* de Bajac, *Lysinibacilus shaericus* Meyer and Neide (formerly *Bacillus sphaericus* Neide), *Saccharopolyspora spinosa* Mertz and Yao and insect growth regulators (IGRs) such as methoprene are the most commonly used products in the United States and elsewhere in mosquito control operations, because of their relative specificity and high effectiveness on target organisms, safety on non-target species and compatibility with the environment. Using a standard laboratory bioassay, we evaluated the loss of larvicidal activities of various formulations after being exposed to different storage and handling temperatures. Transportation of products under outdoor conditions for an extended period of time caused more activity loss compared with other conditions. Results are discussed based on their active ingredients as well as physical and chemical properties of the formulations. Recommendations are made for proper inventory control, storage and handling of larvicides to minimize the loss of larvicidal activity.

Evaluation of EverGreen ULV 5-25 Ground applied at below label rate

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Introduction

EverGreen ULV 5-25 Ground (pyrethrins 5% and piperonyl butoxide 25%) is an adulticide product labeled for use at a rate of 0.0018 to 0.0025 lbs of pyrethrins per acre. However, the manufacturer suggested that using the product at below-label rate may be effective. Consequently, we examined whether applying at a rate of 0.0015 lbs/acre would provide effective mosquito control at our district. Our objective was to compare EverGreen ULV 5-25 Ground applied at 0.0015, 0.0020, and 0.0025 lbs/acre.

Methods

Cages were deployed in three replicate transects 100 ft from each other and 100, 200, or 300 ft from the spray route (Figure 1). A pair of cages was positioned at each location and contained either 25 females from the *Culex quinquefasciatus* (CQ1) susceptible colony or wild F1 females for a total of 9 cage pairs per spray pass. The wild mosquitoes were *Cx. quinquefasciatus* collected from Indio and La Quinta, CA, sites with prior evidence of resistance to pyrethroids. We mounted droplet impingers with Teflon slides next to 7 of the 9 sentinel cages to characterize the density and size of droplets arriving at the cages: 2 impingers at 100 ft, 3 impingers at 200 ft, and 2 impingers at 300 ft from the spray route. Three pairs of control cages and 1 impinger were set 150 feet upwind from the spray area. We sprayed each application rate in triplicate on 3 separate days between 0630-1030h during April-May 2018 with a truck-mounted Guardian sprayer. Cages were exposed to the treatment for 1 hr before collection. New cages were used for each spray pass. We counted the mortality at 15 min, and then at 6, 24, and 48 hrs after trap collection. Temperature, wind speed, and percent relative humidity were recorded at 1-minute intervals using a weather station (Kestrel model 5700, Boothwyn, PA).

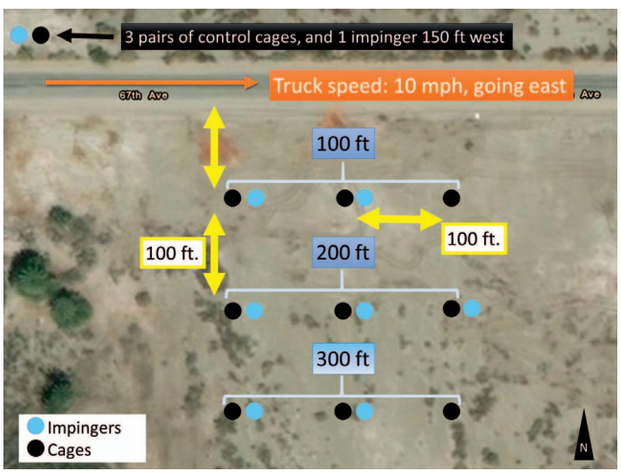

Figure 1.—Map of cage and impinger placement and the truck route.

Results and Discussion

At 48 hrs post treatment, greatest mortality was observed at the below-label rate (0.0015 lbs/acre) in both the susceptible colony and wild *Cx. quinquefasciatus* mosquitoes (Table 1). Unexpectedly, mortality was lower at the higher application rates, likely due to differing wind and relative humidity between each day of application. The weather conditions for treatments at 0.0015 lbs/acre were ideal with winds below 5 mph and percent relative humidity was $\leq$40% (Table 2). For the remaining two treatment rates, temperatures were warmer and humidity was higher. The storm that passed when treating at 0.002 lbs/acre delayed the start time of the 2nd and 3rd spray; thus, the temperatures were warmer later in the day. For all treatments, the mortality of the wild mosquitoes was lower than the colony mosquitoes at their respective application rates and distances, demonstrating resistance (Fig. 2). Mosquito mortality at the control cages ranged from 0.41-1.8% for the colony mosquitoes and 0.26-9.26% for the wild mosquitoes (Table 3). Droplet size did not show variation across the treatment transect for the different rates, but droplet density was lower at 300 ft from the spray

Table 1.—Mean percent mortality (±standard error of the mean) of colony and wild *Cx. quinquefasciatus* 48 hrs post-exposure.

Application rate (lbs/acre)	Colony ± SE	Wild ± SE
0.0015	94.5 ± 4.3	88.7 ± 4.4
0.002	52.1 ± 5.4	25.1 ± 4.5
0.0025	69.6 ± 6.7	20.5 ± 5.9

Table 2.—Weather data for the sentinel cages' 1 hr exposure period. Values are the average of recordings.

Rate; Date		Time start	Wind Speed (mph)	Temp (°F)	% Relative Humidity
0.0015 lbs/acre; April 18, 2018	**Spray 1**	6:20 AM	2.5	49.0	40.4
	Spray 2	7:30 AM	4.1	63.7	25.8
	Spray 3	8:30 AM	4.2	73.1	17.5
	Mean		**3.6**	**62.0**	**27.9**
0.002 lbs/acre; May 2, 2018	**Spray 1**	6:35 AM	1.2	56.2	58.3
	Spray 2	9:05 AM	7.7	66.4	42.5
	Spray 3	10:15 AM	4.9	70.0	37.4
	Mean		**4.6**	**64.2**	**46.1**
0.0025 lbs/acre; May 3, 2018	**Spray 1**	6:23 AM	3.4	58.8	63.0
	Spray 2	7:45 AM	4.4	67.4	49.6
	Spray 3	8:58 AM	3.0	75.5	38.5
	Mean		**3.6**	**67.2**	**50.4**

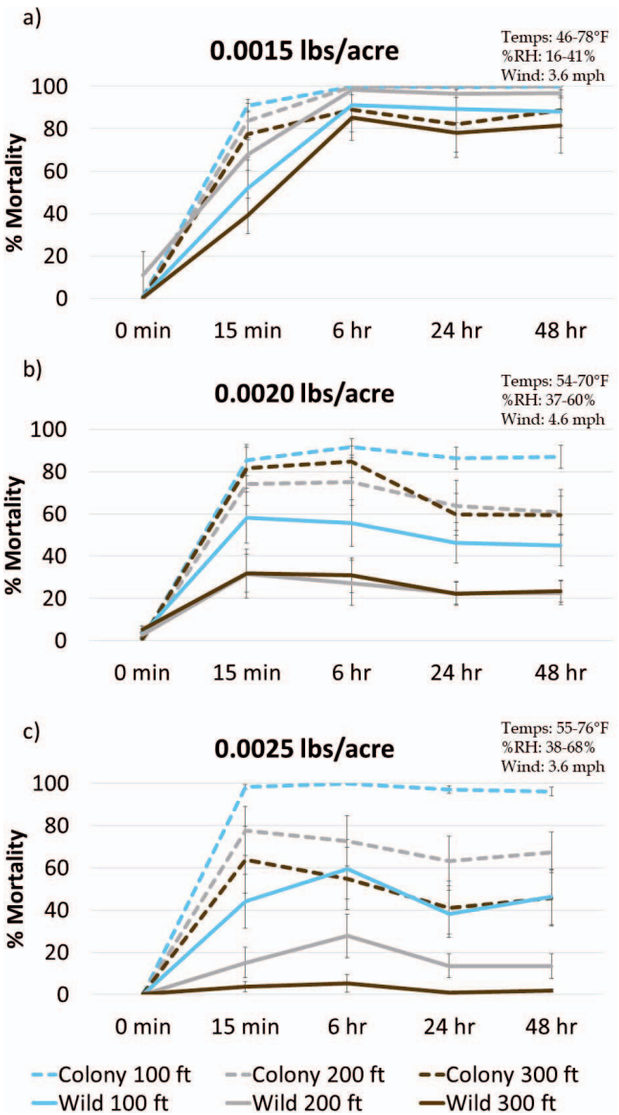

Figure 2.—Mean percent mortality of colony and wild *Cx. quinquefasciatus* mosquitoes post-exposure at (a) 0.0015, (b) 0.002, and (c) 0.0025 lbs/acre. A total of 9 cages were sprayed at 100 ft, 200 ft, and 300 ft from the truck route for each application rate, which is depicted as the blue, gray and brown lines, respectively.

Table 3.—Mean percent mortality of the colony and wild *Cx. quinquefasciatus* at the control sites 48 hrs post-exposure.

Rate	Strain	Mean % mortality
0.0015	Colony	0.41
	Wild	4.48
0.002	Colony	1.48
	Wild	9.26
0.0025	Colony	0.83
	Wild	0.26

Table 4.—Mean droplet size and density captured by the Teflon impingers for each spray rate at 100 ft, 200 ft, and 300 ft away from the spray route.

Rate (lb/acre)	Distance	Mean droplet size (μm)	Mean density (drops/mm^2)
0.0015	100 ft	15.84	2.17
	200 ft	16.29	2.01
	300 ft	14.80	0.60
0.002	100 ft	15.54	4.24
	200 ft	15.45	2.44
	300 ft	14.82	3.14
0.0025	100 ft	15.00	4.19
	200 ft	13.83	2.86
	300 ft	14.05	0.44

route at 0.0015 and 0.0025 lbs/acre (Table 4). Correspondingly, cages that were closer to the spray origin had higher mortality than those placed further away (Figure 2).

Conclusions

Because the mosquitoes did show greatest mortality at the lowest rate, we plan to use the lower approved label rate in our mosquito control applications. Future plans entail repeating this study for reproducibility as well as performing bottle bioassays to investigate mosquito resistance to the product.

Acknowledgements

Jennifer Williams and Brian Meagher from MGK® provided the pesticide product and project discussions. Jacob Tarango assisted in the field. Enrique Giron assisted with cage assembly.

Using a Remotely-Operated Vehicle ("Rover") for mosquito surveillance and control in underground storm drains

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INTRODUCTION

Underground storm drain systems (USDS) in southern California are composed of a complex matrix of catch basins and a variety of subsurface stormwater conveyance infrastructures. These systems collect stormwater and irrigation runoff from residential and commercial properties and direct flows to larger open flood control channels. During the warm summer - fall months, most of the water entering the USDS in urban southern California comes via small inflows from the surrounding landscapes. Without forceful flushing, the USDS in southern California can produce thousands of adult *Culex quinquefasciatus* (Figures 1, 2) in portions of the system with structural damage or minimal gradients, and where subsurface water drainage is slow and irregular (Kluh et al. 2001, Su et al. 2003).

Culex quinquefasciatus is the primary urban vector of West Nile virus (WNV) in southern California (Kwan et al. 2010), and control of this mosquito in problematic USDS is of major importance for mosquito control agencies. In 2014, during Orange County's largest WNV outbreak on record [264 cases with 9 deaths (OCHCA 2014); 8.8 WNV cases/100, 000 people], 17.4% (88 of 506) of the County's WNV-infected mosquito pools were from *Cx. quinquefasciatus* collected within underground drains. Overall, WNV infection rates in mosquitoes and people occur at levels several times higher in landscapes with problematic USDS than in other areas of Orange County (Cummings et al. 2016).

Because of the difficulty in controlling mosquitoes in the USDS (Mulligan and Schaefer 1981, Kluh et al. 2006), mosquito control agencies need to look for collaboration and innovation from the stormwater community to help address this serious public health threat. The potential benefits of this collaboration could lead to new technologies customized for better mosquito surveillance and control in the USDS. One option proposed in this paper is to modify remotely-operated vehicles (i.e., "rovers") equipped with closed-circuit TV used by wastewater management companies and governmental agencies to monitor sewer systems (Figure 3) for mosquito surveillance and control in areas of the USDS not reached by insecticides with current application methods. These modified rovers could enhance mosquito surveillance and if supplied with customized spray equipment, improve mosquito control in the USDS.

METHODS

The Orange County Mosquito and Vector Control District's (District) mosquito suppression program in the USDS includes year-round applications on 10-day cycles of EPA-approved refined oils or microbial pesticides through holes in the drop inlet covers of a drain (Figure 4). However, even under routine treatment, per-trap night collections of mosquitoes can still number in the hundreds in some USDS with low water flow, minimal grade, and

Figure 1.—Mosquitoes emerging from a USDS drop inlet after removal of the cover.

Figure 2.—Mosquitoes on the walls of a USDS tunnel.

Figure 3.—Remotely-powered rover with closed-circuit TV used for inspection of sewer systems.

impounded water due to debris accumulation. In mid-2018, Plumbers Depot, Inc. (Hawthorne, Calif.) conducted a free demonstration of a remotely-operated rover (CUES Camera Transporter model SPRII, CUES, Inc., Orlando, FL) (Figure 5) placed in several problematic USDS in Orange County. Figure 5 shows the rover as it was lowered into a drain; Figure 6 shows closed-circuit TV monitoring and remote control of the rover from a support vehicle.

RESULTS AND DISCUSSION

Inspection of several USDS with the rover revealed a refuse-choked, 1900s-era corrugated steel pipe with stagnant water (Figure 7) and extensive mosquito production and adult mosquitoes flying throughout this drain and another problematic USDS (Figure 8), even after many treatment cycles of pesticides through the drop inlets. In

Figure 4.—Inspector from the Orange County Mosquito and Vector Control District applying anEPA-approved, larvicidal spray through a drop inlet hole to the USDS.

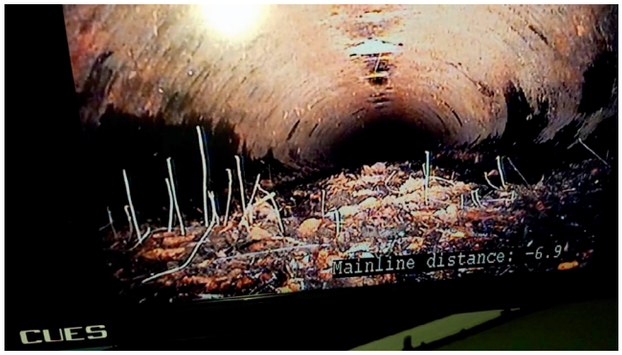

Figure 5.—Underground rover being lowered into a USDS.

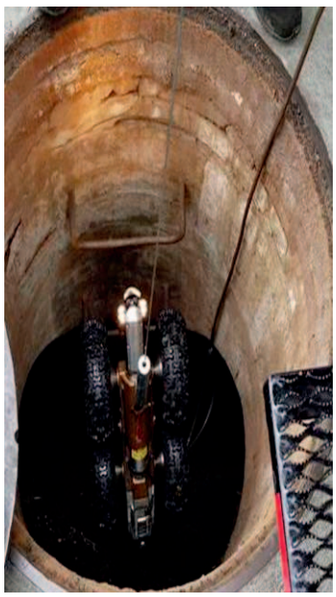

Figure 6.—Closed-circuit TV monitoring and control of a rover traveling through the USDS.

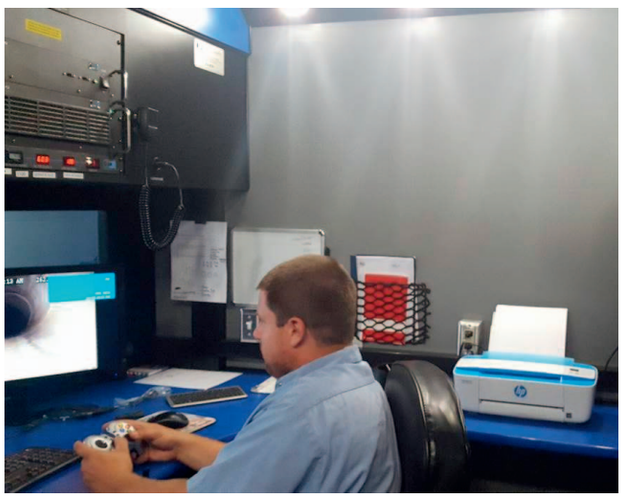

Figure 7.—Picture taken by underground rover of a difficult-to-control USDS in Santa Ana, California.

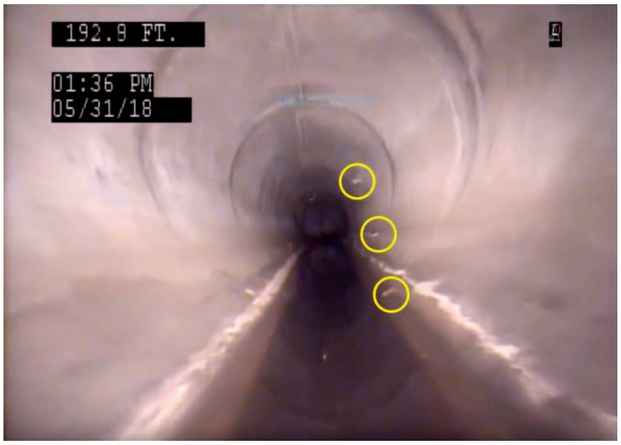

Figure 8.—Adult mosquitoes flying in a routinely-treated USDS. Many mosquito larvae were also observed in the slow-moving drainage water in locations beyond reach of the larvicidal treatment at the drop inlet.

many USDS, the District's current treatment approach is inadequate.

Unmanned aerial systems (UAS, drones) are under development to survey, map, inspect and apply pesticides to wetlands, large riparian habitats, and potentially, mosquito breeding sources in populated areas (AMCA 2019, Carrasco-Escobar et al. 2019). As this drone technology gets integrated into the vector control tool box, underground rovers should also be receiving similar consideration as UAS technology. In urban areas with high WNV activity linked to problematic USDS, underground rovers outfitted with spray equipment could provide an innovative solution for targeted pesticide applications to control mosquitoes in inaccessible portions of the USDS.

CONCLUSIONS

Underground rovers with ranges ca. 610 m (2,000 ft.) for inspection of USDS are currently available; however, the technology for rovers to apply mosquito control products has not yet been developed. The District plans on partnering with Plumbers Depot to design a pesticide application system fitted to a rover for precision mosquito control in the USDS and ultimately, improved protection of public health.

ACKNOWLEDGEMENTS

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A Case Study: Documenting the presence of the Turkestan Cockroach, *Blatta lateralis*, in Santa Clara County

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INTRODUCTION

Nonnative species periodically arrive in new regions, but their presence can go undetected. The purpose of this study is to provide a framework for the detection, identification and documentation of the arrival of new species. On July 30, 2018, the Santa Clara County Vector Control District received a Service Request from a resident who lives in Glenoaks Park Villas, a townhouse complex in Cupertino, CA. The resident reported that recently there had been an unusually large number of cockroaches in the landscaping in her complex and she was concerned because she was now finding cockroaches in her garage and occasionally in her kitchen.

The resident provided a photo and information about the cockroaches. The characteristics of the cockroaches did not match those of cockroach species known to occur in Santa Clara County, including the American Cockroach, the Oriental cockroach, the German cockroach and the Three-lined cockroach. We decided that a field investigation would be needed to determine which species was present.

MATERIALS AND METHODS

Our first goal was to collect specimens for identification. We contacted our regional Urban Integrated Pest Management Adviser with the University of California Cooperative Extension, Dr. Andrew Sutherland, an expert on cockroaches. He suspected that we were dealing with Turkestan cockroaches, an invasive species, and provided instructions on how to make and place traps designed to capture them. We made traps by wrapping masking tape around the outside and over the lip at the top of ½ pint glass mason jars (Figure 1). This would allow Turkestan cockroaches to climb up the rough masking tape to get into the jar, but once inside, they would be unable to climb up the slippery glass to get back out. A small piece of bread was placed in each jar as bait. A business card was taped to each trap for identification.

Six traps were deployed. The traps were placed next to walls, fences and utility boxes because cockroaches follow the edges of structures while foraging. The traps were set out after 1800 h and collected before 0700 h the following day. Specimens were collected (Figure 2) and a photo was submitted to Dr. Sutherland for primary identification.

RESULTS AND DISCUSSION

In August 2018, the presence of a new species of cockroach was documented in Santa Clara County following a routine nuisance complaint by a resident. During the first trapping with negative results, we found that it was important to set the traps out late in the day and collect them early the following day, because when traps are left out for too long, other animals, e.g. crows, would eat the bread bait and possibly any cockroaches that had been trapped in the jars. The second trapping was successful and four specimens were collected. Photos of the specimens were sent to Dr. Sutherland who identified them as Turkestan cockroaches: an adult male, 2 adult females and a nymph.

The specimens were then sent to the California Department of Food and Agriculture (CDFA) for secondary confirmation. The CDFA responded with a Pest Detection Report which confirmed the identification and recorded the detection. We then contacted the resident and provided information for the effective control of the Turkestan cockroaches.

The Turkestan cockroach was first reported in California in 1978 at Sharpe Army Depot in Lathrop, San Joaquin County (Kim and Rust 2013). Since then it has become widely distributed from Sacramento County southward (K. Beucke, pers. com.). The Turkestan cockroach is "C" rated by CDFA's Action Oriented Rating System, which means that there is no state enforced action required to control it and therefore no surveillance trapping. This makes it difficult to estimate when the Turkestan cockroach first arrived in Santa Clara County. In the San Francisco Bay Area, prior to documenting the presence of the Turkestan cockroach in Santa Clara County, its presence had been documented in four of the nine Bay Area counties: Alameda (A. Why, pers. com.), Napa (A. Donohue, pers. com.), San Mateo (K. Keyser, pers. com.) and Solano (Rios and Honda 2013). Our team was the first to go through the formal process to document its presence in Santa Clara County.

After providing control information to the resident of the complex, the resident deployed cockroach pesticide bait stations near her unit. Another trapping was attempted in order to collect more specimens, but it was unsuccessful, probably due to the use of the pesticide bait. Traps were

Figure 1.—Turkestan cockroach trap

also deployed at a neighboring apartment complex but no specimens were collected.

CONCLUSIONS

To prevent the establishment of harmful invasive species, it is important to perform field investigations to identify the potential presence of new species. Early detection allows rapid assessment and rapid response, for possible containment or eradication, if needed (National Invasive Species Council 2008).

The following guidelines are suggested to other programs:

- Be aware of the possibility that new animal or plant species may arrive in your district.
- Know the appearance and habitats of species that occur in your district, so that you are prepared to recognize an anomaly.

Figure 2.—Turkestan cockroaches: an adult male, 2 adult females and a nymph

- Learn about species that might arrive from neighboring areas or counties, but also be aware that species not previously seen may also appear.
- Explore available resources for information on collecting and identifying specimens.
- Obtain confirmation through an authoritative process that will formally confirm and record the find.

This study demonstrated that it is important to perform field investigations because a routine Service Request resulted in the detection of a new species of cockroach in Santa Clara County.

ACKNOWLEDGEMENTS

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Determining the effectiveness of a sticky Light trap for collecting and evaluating mosquitoes for insecticide resistance

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Introduction

EVS CO_2 traps are widely used by vector control workers to evaluate mosquito abundance, however, these traps are bulky and require dry ice to attract mosquitoes. *Culex pipiens* breed in street-side catch basins (CB) that contain water, but it is difficult to place large EVS CO_2 traps in CB to monitor mosquito abundance. Mosquitoes are attracted to LED lights (Bentley et al. 2009; Gonzalez et al. 2016) and *Cx. pipiens* can be highly resistant to pyrethroid insecticides (Scott et al. 2015). We evaluated the efficacy of a lighted adhesive trap that could be placed inside CB to capture mosquitoes that could be tested for pyrethroid resistance with the *knock down resistance* (*kdr*) single nucleotide polymorphism (SNP) quantitative polymerase chain reaction (QPCR) assay.

Methods

To construct a sticky light trap (SLT), a Catchmaster universal fly glue board (AP&G Co., Inc., Bayonnne NJ) was first trimmed to produce 10 cm^2 glue boards and four 18 mm circular magnets were attached to each corner on the non-sticky side of the glue board using extra strength hot glue (Figure 1A). LED lights with holder were inserted with the lit side facing the sticky side of the glue board and powered using a CR2032 coin battery (Figure 1B). Electrical tape was used to attach the coin battery to LED lights and the electronics were subsequently secured using duct tape. The magnets affixed to the SLT allowed the trap to be attached to the underside of a CB grate or any ferromagnetic surface (Figure 1C). SLT were lit continuously by the LED lights for at least 3 weeks by a single coin battery (data not shown). To evaluate the efficacy of SLT for capturing mosquitoes in the field, SLT were placed in CB throughout Alameda County (CA, USA; $n = 139$). The data on the abundance of the collected mosquitoes was mapped using ArcGIS Desktop software (version 10.6). To evaluate the amount of time that mosquitoes adhered to a SLT could be analyzed for the *kdr* SNP, wild-type *Cx. pipiens* from the pyrethroid-susceptible laboratory colony SM-S1 were placed on a SLT, collected daily for 16 days and subsequently tested for the *kdr* SNP using standard QPCR methods. The *kdr* SNP genotype was determined using ΔRn values (homozygous resistant if $\Delta Rn > 1.80$, heterozygous if $0.92 < \Delta Rn < 1.5$, homozygous susceptible if $\Delta Rn < 0.5$). The impact of time on trap to gene amplification efficiency was evaluated using Prism software (version 8.0.2; GraphPad, San Diego, CA).

Results and Discussion

A significant reduction in the efficiency of *kdr* allele amplification was observed as the time that the mosquitoes were on the SLT increased ($n = 32$, $Y = -0.1348X + 0.4396$, R square $= 0.4005$, $F = 20.05$, $Dfn = 1$, $DFd = 30$, $P = 0.0001$, Figure 1D). However, the *kdr* allele could be amplified and detected in mosquitoes that had been on the SLT for at least 16 days (Figure 1D). Consequently, for subsequent studies, SLT were placed in the field for no longer than 15 days.

More than half (56%) of the *Cx. pipiens* that were collected on SLT from 14 different locations throughout Alameda County were homozygous susceptible for the *kdr* SNP ($n = 84$, Figure 2A). Although from 2014 – 2018 vector control agencies in Alameda County applied pyrethroids only 6 times in areas less than 0.2 km^2, 17% of the *Cx. pipiens* that were collected on SLT were homozygous resistant for the *kdr* SNP and 27% were heterozygous (Figure 2A). When the same trap sites were sampled in June and again in August, the proportion of mosquitoes with the heterozygous *kdr* allele increased from 0% to 14 %, while the proportion of homozygous resistant mosquitoes did not change substantially (33% and 29%; Figure 2B). Mosquitoes that were homozygous for the susceptible and resistant allele or heterozygotes may have mated to increase the heterozygous population.

Conclusion

Both laboratory colony *Cx. pipiens* and field-caught *Cx. pipiens* could be successfully tested for the *kdr* allele after being caught on a SLT. The successful trapping of mosquitoes on the SLT, as well as the detection of the *kdr*

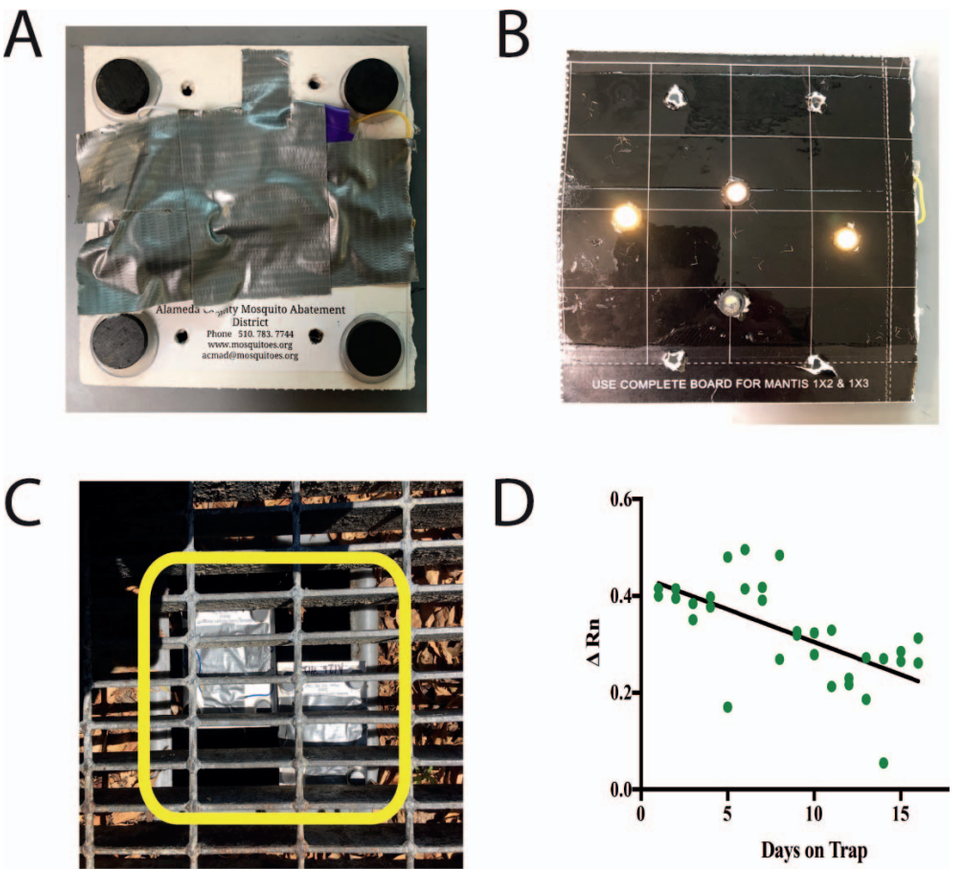

Figure 1.—Preparing and evaluating the SLT. (A) Rear side of SLT that attaches to ferromagnetic surfaces. (B) Front sticky side of SLT with lit LED lights. (C) Two SLT shown in yellow box attached to the metal grate that covers a catch basin. (D) Relationship of *kdr* allele amplification efficiency as measured by ΔRn value to the number of days wild-type *Cx. pipiens* from the pyrethroid-susceptible laboratory colony SM-S1 resided on the SLT.

allele in *Cx. pipiens* indicated that SLT may be a useful alternative to EVS CO_2 traps to monitor insecticide resistance using molecular genetic methods. More testing is needed to determine whether the mosquitoes collected on SLT can be successfully tested for arbovirus infection.

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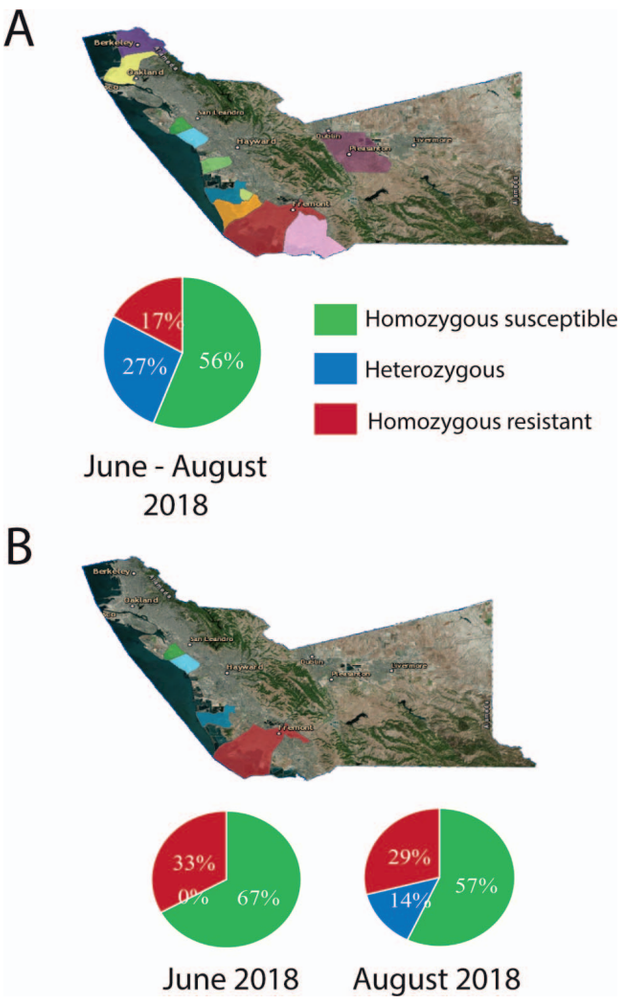

Figure 2.—Geographic distribution of the *kdr* allele *for Cx. pipiens* collected on SLT in Alameda County. Polygons overlaying satellite imagery show the location of SLT placements. Circle graphs show the proportion of *Cx. pipiens* collected from the SLT with the homozygous susceptible, homozygous resistant or heterozygous *kdr* alleles. (A) Aggregate SLT placed throughout Alameda County from June – August of 2018. (B) Repeated sampling of sites with SLT in June and August of 2018.

Use of a float device to improve efficacy of tablet and briquet mosquito control applications

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Introduction

The ubiquitous mosquito larval sites in urban areas present challenges to the Operations Departments at Coachella Valley Mosquito and Vector Control District (District). Innovative approaches to solving some of these challenges include delivering control products in a sustainable way to mosquito larval sources, including catch basins, dry wells, pools and water fountains. Most of the long term products are in tablet and briquette formulations that tend to settle at the bottom and can easily be buried under dirt, organic debris and trash. Such eventuality will hinder product dissolution and dispersal into the water column, making it difficult, if not impossible, to determine the amount of the product present on follow up inspections. Therefore, the objective of this report is to show how using a chemical flotation device system could improve the efficacy of mosquito larvicides and better determine the longevity of extended mosquito larvicide product formulations in tablets and briquets.

'Floating' long term chemical products was accomplished by dangling the product by a string instead of dropping the product into the source. When the water levels rise and fall, installing a floating device allowed the product to remain suspended in the best position in the water column close to the surface where the larvae are located. Keeping the product suspended may be effective in reaching the manufacturers recommended residual period and meeting District goals of maintaining cost low while keeping mosquitoes under control.

Materials and Methods

The study sites were selected that included catch basins in the City of Indio, California from December 2017 through October 2018. Five sites were treated with Altosid XR Extended Residual Briquets and five sites were treated with Natular ® XRT Mosquito Larvicide/Extended Release Tablet. The materials used for product flotation included wine corks for buoyancy, a protective netting made of polyethylene (Uline, USA), long zip ties used to secure netting to corks, and string to anchor the device to limit drift and make it easy to retrieve. Cutting tools used were scissors and wire cutters, 17 gauge galvanized electric fence wire used as the anchor to tie string between the float device which sealed the end of the netting and anchored it to the surface (Figure 1).

Applications of larvicides were made to mosquito sources where trash and organic debris was sparse. After treatment, the sites were inspected with each site dipped 3 times with a standard dipper to estimate the average number of larvae present. With Natular XRT the product was considered to be working if the inspection showed only 1st instar larvae. Pupae samples were not taken for emergence tests from the Altosid XR sites, because the sites had less than 10 pupae present on any given inspection. Upon inspection the date was noted and a visual estimation of how much product remained was determined. None of the sites were re-treated during this study.

Results and Discussions

The sites treated with Natular XRT in floatation devices had physical product present longer compared to the sites with Altosid XR (Fig. 2, and 3, respectively). An example of a favorable outcome was a 1.5 ft. depth catch basin treated with Altosid XR on January 27, 2017. A pupal sample taken on 3 April, 2017, 125 days post treatment,

Figure 1.—Materials used to construct flotation device for long term larvicide products.

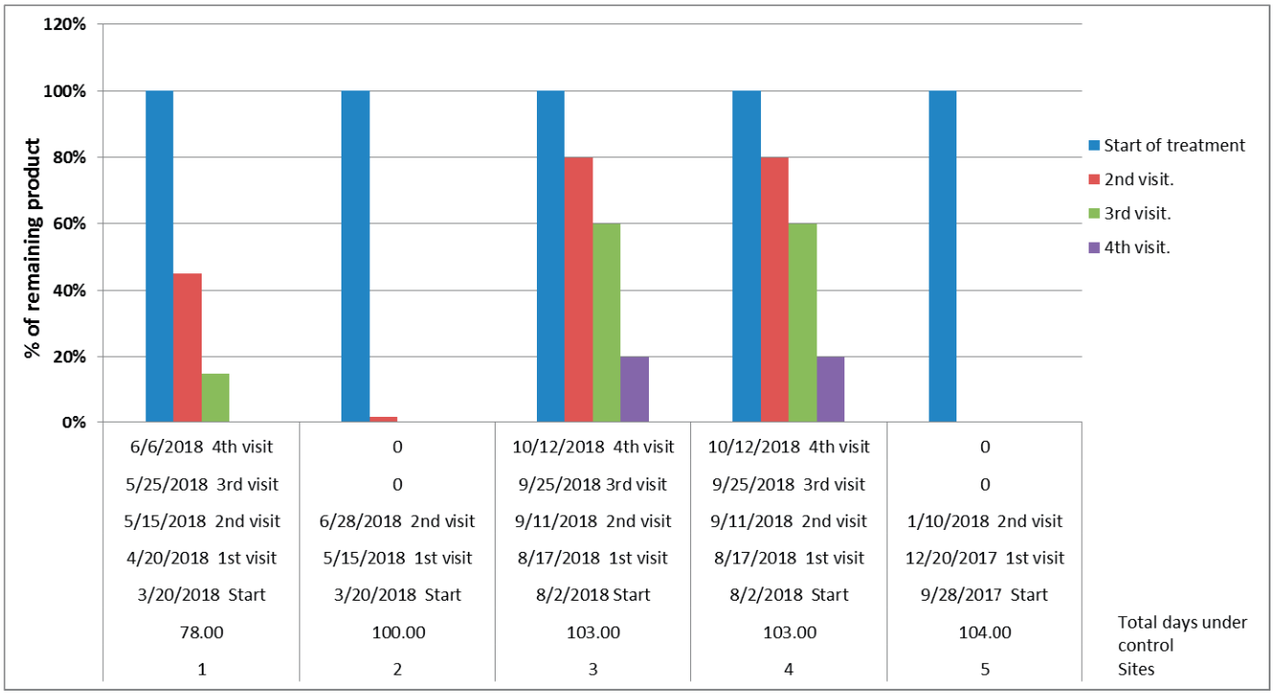

Figure 2.—Visual estimate of the amount of Altosid XR briquet present at each sampling occasion (N = 5 catch basins).

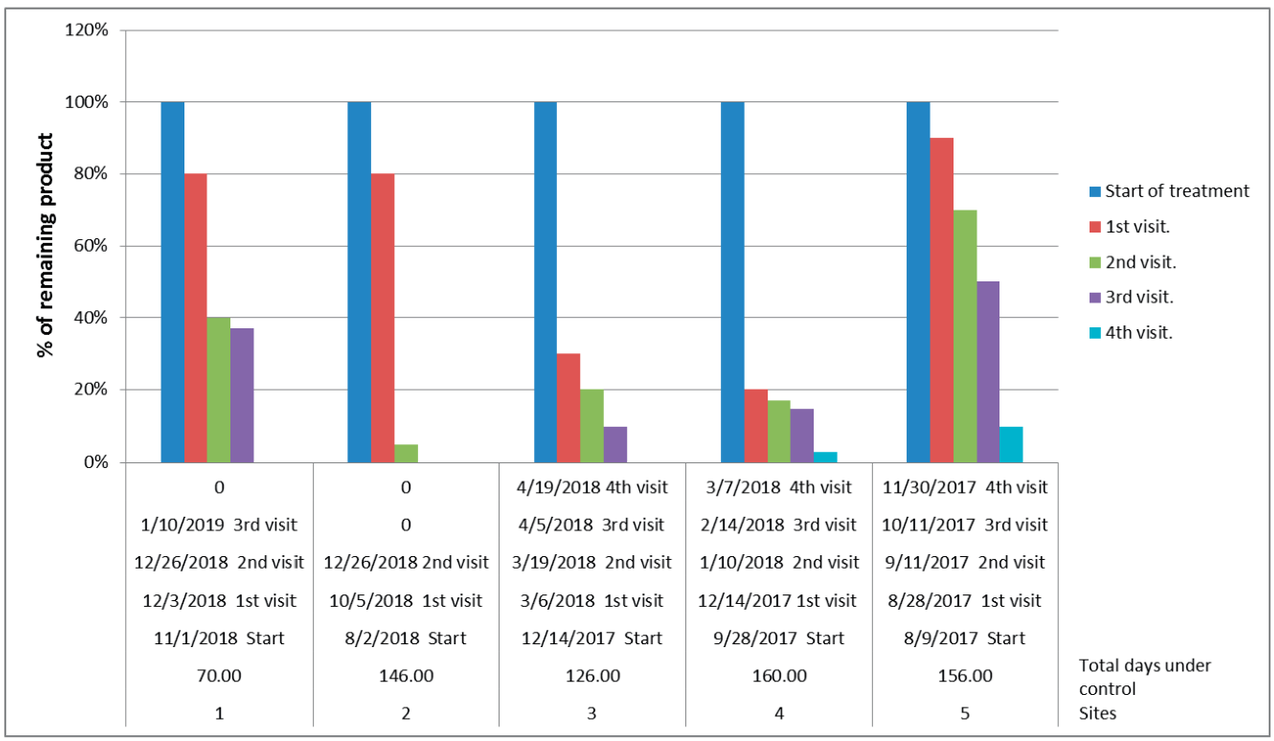

Figure 3.—Visual estimate of the amount Natular XRT tablet present at each sampling occasion (N = 5 catch basins).

had 95% mortality and the briquet was about 10% of its original size. Applications of Natular XRT in flotation devices showed that product was present effectively controlling mosquito larvae at five different sites for 70, 124, 126, 160, and 156 days after application (Figure 3). In comparison, five sites that received Altosid XR in flotation devices showed presence for 78 days, 100, 103, 104, and 103 days after applications (Figure 2). Residual activity

was determined by observation of product present during technician re-inspection. Presence of product at a site was an indication of potential residual activity, especially for sites treated with Natular XRT that mostly had early instar larvae present implying that the product is still active at the site. Flotation devices may be effective in helping to extend the effectiveness of the long term products in habitats where product may otherwise be buried in silt or debris.

Conclusion

Application of long term larvicide in tablet or briquet formulations in floating netting, anchored to the side of the mosquito source that is easily retrievable by a technician helps achieve optimal mosquito control by placing the product adjacent to the target species. The observation of residual product at the end of the floating device assures the field technicians of product persistence during re-inspections reducing costs and labor related to retreatment. Additional studies are planned for the 2019 mosquito season.

Acknowledgment

The authors thank the rest of the Operation staff for their support, and thank Geneva Ginn, Lead Technician, and Rod Chamberlain, former Lead Supervisor for their guidance. In addition, we thank the Operations Manager, J. Wakoli Wekesa, and General Manager, Jeremy Wittie, for their support in conducting this study and in putting together this extended abstract.

Field management application software for operations work flow in the Coachella Valley

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Introduction

The Coachella Valley Mosquito and Vector Control District (District) developed a web-based application to enable field technicians to collect and manage their daily work flow for *Culex* and invasive *Aedes* mosquito control. The application aggregates data for neglected pool management, mosquito brood management, surveillance data for West Nile and St. Louis Encephalitis Virus activity (referred to as Trap Heat), the invasive *Aedes* program, and pesticide inventory. This application has been instrumental in providing critical information to field technicians and in helping organize an efficient vector management system overseen by the Operations, Laboratory, and Information Technology Departments. This has made best management practices for control of mosquitoes a reality for our District.

Results and Discussion

The field management application software, generally referred to as "the Ops Application," consists of a main menu where fifteen different sections can be accessed, including the Coachella Valley Technician (CVT), Laboratory, Modification Forms, Service Requests, Pesticide Inventory, Red Imported Fire Ant Scheduler, Meetings, Gas, Employees, Orders, Time Off/Meeting Scheduler, Travel, Operations/Supervisors, Technicians, and Settings (Figure 1). The CVT icon from the Main Menu opens up a sub menu and the Pesticide Inventory. In the CVT menu important functions that are focused on technician activities include Swimming Pool Management, Invasive *Aedes* Management, Brood Management, and Virus Trap Heat Map (Figure 2).

The Swimming Pool Management tool enables the user to keep track of swimming pools and informs the technicians when pools are due for inspection. Clicking on a pool enables the user to see all the history associated with that pool site. When pool surveillance data captured by aerial imaging is completed, data uploaded, and all the sites are mapped into the system these pools sites are also accessed through this system. The Invasive *Aedes* Management section enables the user to see *Aedes* positive locations, either through trapping or positive larval identification. Once a location becomes positive for invasive *Aedes*, a point of interest (POI) is placed on the location, and a 450 feet buffer can be drawn around the site to include all properties that may need inspection. Brood Management section enables the user to query sites that need to be inspected, depending on the chemical product used and the residual ability of the product formulation. The query can be done incorporating the quick kill products, short term products, long term products, or all products at once.

Virus Trap Heat enables the user to a create buffer zone around a mosquito virus positive trap up to a mile in radius and shows every known mosquito breeding site that needs to be inspected within a specified time frame. In addition, the Virus Trap Heat section provides information on the most recent trapping data, including the number of mosquito species collected, their gender, and reproductive status. Finally, the Pesticide Inventory section enables the pesticide user to check out products currently in their "pesticide locker" based on a set minimum amount. At the end of the working day, the user can record the amount of used and unused product into the pesticide inventory. The Pesticide Inventory section of the Ops Program also helps in reconciling monthly inventory for all pesticide products used for the month district-wide with the total product in stock.

Conclusion

The Operations Application has been instrumental in helping streamline the District operations, enhancing our ability to locate the history of our work and providing a central reference for work conducted by District staff in different areas of our program. The efficiency of tracking the pesticide inventory has been greatly improved.

Acknowledgements

I would also like to thank my colleague Field Technicians, popularly knows as the "Operation Crew" that include Geneva Ginn, Gonzalo Valadez, Rene Delgado, Jess Lucia, Fernando Gutierrez, Vincent Valenzuela, Marina Espejo, Ramon Gonzalez, Trinidad Haro, Victor Scrima, Marco Medel, Arnold Khakali, Salvador Becerra, Carlos Hernandez, Jonathan Leung, Jeff Rushing, Jonathan Zamaniego, Rosendo Ruiz, Linda Petersen, Paul Bustamante, Fernando Fregoso, Jonathan Herrera, Erica Frost.

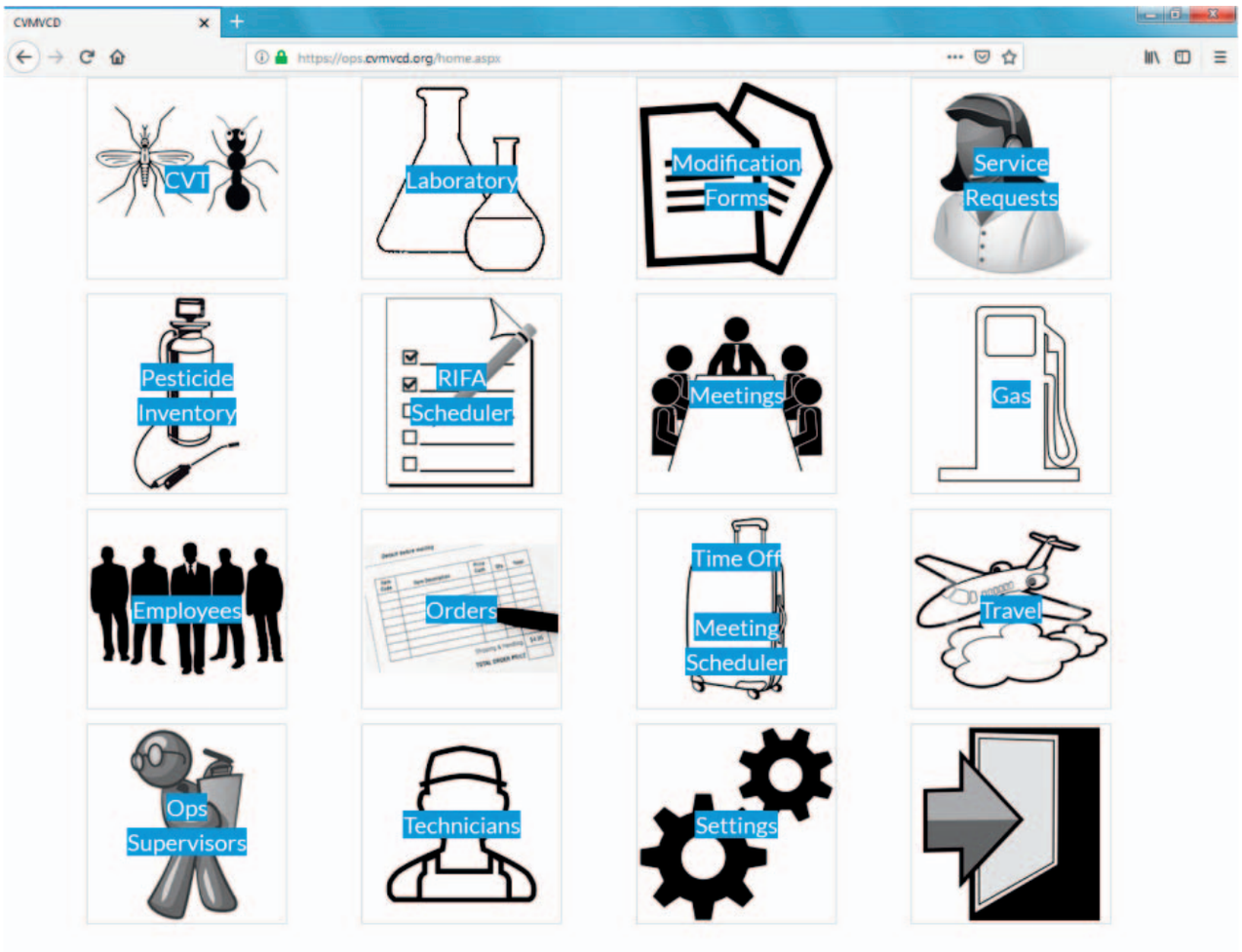

Figure 1.—Main menu of the Operation Application Software

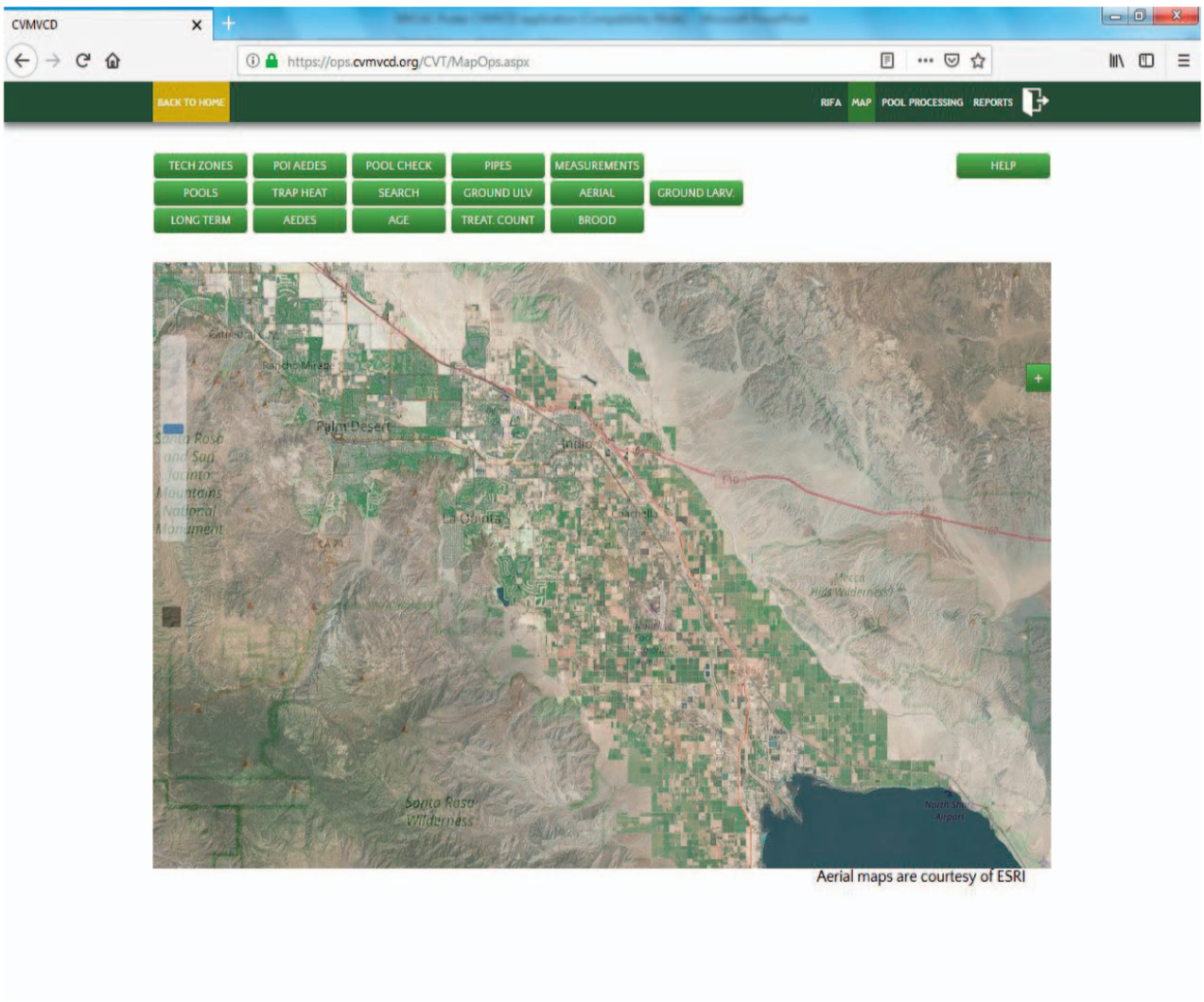

Figure 2.—Coachella Vector Technician menu

Spatial analysis of aggregation behavior in larval *Aedes sierrensis* in response to environmental stimuli

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Introduction

Larval clustering, or aggregation, is a commonly observed though uncommonly studied behavior in many mosquito species (Hocking 1953; Nielsen and Nielsen 1953; Renshaw et al. 1995, Shorrocks et. al. 1984). In salt marshes, *Aedes taeneorhynchus* cluster in balls of hundreds to thousands of individuals (Nielsen and Nielsen 1953) and in laboratory colonies *Aedes atropalpus, Ae. sierrensis,* and *Culex* spp. larvae have been observed clustering in rearing pans (personal observation, pers. comm. D. Woodward). Our study investigated this clustering phenomenon in *Aedes sierrensis* (Western treehole mosquito), with the goals of determining whether clustering occurs, the effect of specific environmental stimuli on this behavior, and where clusters are most likely to occur within the container.

Methods and Materials

For each 40-minute observation, 100 larvae, obtained from the Marin-Sonoma Mosquito and Vector Control District in Cotati, CA, were placed into a 19.05×30.48 cm. transparent glass tank filled with 1 in. of distilled water and labelled on the bottom with a numbered 55 square grid. Observations were recorded using a GoPro HERO4 Black camera (GoPro Inc., San Mateo, CA, USA) mounted above the tank and set to capture an image every 60 seconds. Treatments included 10 pupae added to the 100 larvae (pupae), artificial flower petals as a non-edible material (material), a shadow cast over half the tank (shadow), and a control with no added treatment. Larvae were hand-counted in each image and location of the head was used to assign each larva to a cell or, when a single cell held the majority of larvae and prevented accurate counting of individuals, the remainder larvae were counted and the result subtracted from the total to determine the number contained in that cell. Data were analyzed in R (R software, Vienna, Austria), using a multivariate regression with Poisson distribution.

Results and Discussion

Larvae clustered in the corners of the tank with 32% ($p<0.01$) more larvae found in corners than in the middle and 2% more larvae found on edges than in the middle. Larval distribution was significantly associated with all treatments with 24% ($p<0.01$) more larvae found in cells containing material, 53% ($p<0.01$) more larvae in cells containing pupae, and 22% ($p<0.01$) more larvae in cells covered by shadow compared to cells without treatment (Fig. 1). Therefore, although clustering of *Ae. sierrensis* did occur, it was unclear whether it occurred because larvae seek to congregate together or individual larvae independently seek the same location. Understanding the cause of clustering behavior could allow us to exploit this behavior to improve larval control.

Acknowledgements

We would like to thank the Lake County Board of Trustees for their support with this and other research projects that improve our districts ability to protect the health of Lake County residents and visitors, Dave Woodward for providing information on *Aedes sierresnsis* field aggregations, Kristin Holt of the Marin-Sonoma Mosquito and Vector Control District for providing the *Aedes sierrensis* larvae used in this project, and the Lake County Vector Control staff for assistance throughout this project.

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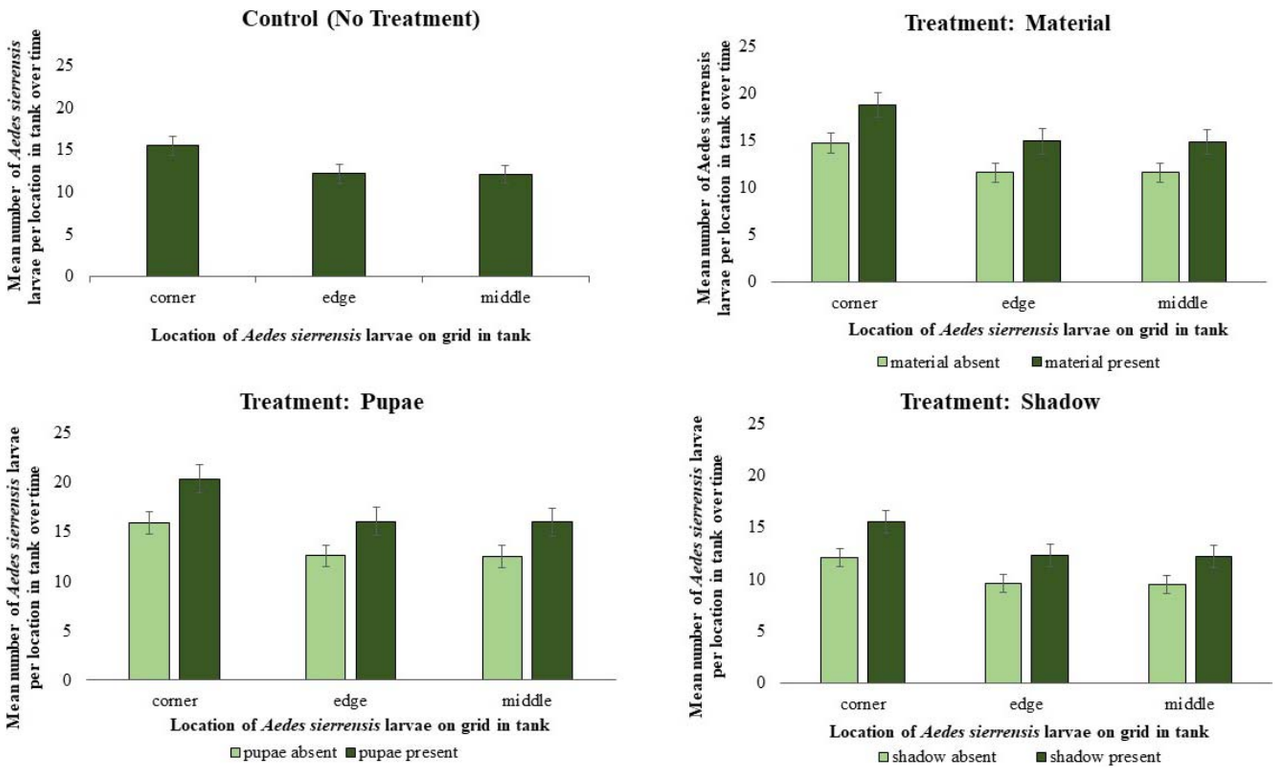

Figure 1.—Larvae of *Aedes sierrensis* were placed into a glass tank over a 55-square grid and exposed to three treatments plus a no-treatment control and their location measured within the tank in association with each treatment.

Jumping into the future: An analysis of 50 years of flea data from mammalian wildlife collected during three Flea-borne Rickettsioses surveys in Orange County, 1967 – 2017

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Abstract

Fleas infesting backyard mammalian wildlife have been epidemiologically linked to the transmission of flea-borne rickettsial pathogens in urban and suburban areas of Orange County. To understand the risk of flea-borne rickettsioses in an area, surveys of wildlife were conducted to determine flea species distribution, flea population size, and prevalence of rickettsial pathogens. This study highlights the medically-important species of fleas found on backyard mammalian wildlife (domestic cats and dogs, skunks, opossums, squirrels, raccoons, and commensal rodents) collected in residential neighborhoods of Orange County, California, during three study periods from 1967 to 2017 in response to locally-acquired human cases of flea-borne rickettsioses. This information has been used to guide decisions regarding risk management and potential intervention strategies to reduce and prevent the transmission of flea-borne pathogens. Results from this study show that the average number of fleas per opossum and striped skunk has increased considerably from 1967 to 2017 in Orange County.

INTRODUCTION

Orange County is a coastal county in Southern California, neighboring Los Angeles, San Bernardino, Riverside, and San Diego counties. As of 2017, approximately 3.2 million people lived in Orange County (US Census 2017). The county has a Mediterranean climate with rainfall occurring primarily in the winter and hot temperatures in the summer. Historically, Orange County was primarily agricultural, most noted for orange orchards. Over time the agricultural industry declined and the county became a largely urbanized region. Rapid urbanization has favored the increase of opossum (*Didelphis virginiana*) populations (Wright et al. 2012) and has led to increased contact between opossums, commensal rodents, other mammalian wildlife, pets, and humans (Krueger et al. 2016). Backyard mammalian wildlife in southern California are often heavily parasitized by fleas, which can infest companion animals and serve as hosts for fleas capable of transmitting rickettsial pathogens (Krueger et al. 2016). Worldwide, the majority of human flea-borne rickettsioses cases report at least one pet cat or dog in the patient's home (Clark et al. 2018). Collectively, the abundance of urban mammalian wildlife and domestic pets, coupled with a regionally mild climate, provides a favorable environment for year-round flea development, especially for the cat flea, *Ctenocephalides felis* (Rust et al. 1997), the primary vector of the rickettsial pathogens responsible for flea-borne rickettsioses (Azad et al. 1997).

The Orange County Mosquito and Vector Control District (District) provides service for mosquitoes, rats, fire ants, and flies, and responds to human cases of rickettsioses and other zoonotic pathogens as outlined in a Memorandum of Understanding with the Orange County Health Care Agency (OCHCA 2012). In response to a total of 180 reported human cases of locally-transmitted flea-borne rickettsioses since 1967 (Cummings et al. 2014, CDPH 2017), the District collected ectoparasites from backyard mammalian wildlife and domestic pets around putative exposure sites. Animals examined for ectoparasites have included the California ground squirrel (*Otospermophilus beecheyi*), fox squirrel (*Sciurus niger*), roof rat (*Rattus rattus*), house mouse (*Mus musculus*), opossum, striped skunk (*Mephitis mephitis*), raccoon (*Procyon lotor*), domestic cat (*Felis catus*), and domestic dog (*Canis familiaris*). This paper analyzes collections of fleas from urban-adapted mammals from three study periods (1967-1968, 1986-1987, 2016-2017) and summarizes the rickettsial species detected in fleas during 2016-2017.

METHODS

In all three study periods, Tomahawk® animal cages (Tomahawk, WI) were used to live-trap animals in residential neighborhoods of Orange County. Cats were not trapped intentionally and were released when found. Instead, pet cats and dogs were combed for fleas, and their

sleeping areas inspected, with owner permission at the home. Trapped wild animals were humanely euthanized in a chamber filled with carbon dioxide gas, sealed in plastic bags, and stored in a refrigerator at 1.6° C (34.9° F). Carcasses were held until flea death (> 48 hrs.) and examined in a Level 2 biological safety hood. Fleas were removed with combs and forceps, stored in flame-sealed 2 ml glass ampules at -70° C (-94° F) (1967–1968) or ethanol (1986-1987 and 2016–2017), identified by sex and species, and enumerated. Fleas collected in 1967-1968 were processed according to methods outlined by Adams et al. (1970). From 1986-1987, only opossums were collected. During 2016–2017, the District removed fleas from opossums, striped skunks, squirrels, roof rats, house mice, domestic cats and dogs, and raccoons following investigations of 28 human cases of flea-borne rickettsioses, and also obtained fleas from incidental mammal collections from other urban areas of Orange County. Cat fleas from human hosts were collected off the protective clothing of District staff during case investigations. After processing, if sufficient numbers of fleas were recovered and identified, fleas were pooled (1-10) by species from each animal for testing by qPCR according to protocols for detecting rickettsial DNA in fleas (Maina et al. 2016).

Historical data from animal and flea surveillance were entered into Microsoft Excel® for analyses. The mean number of fleas per animal, referred to as the flea index, was used to estimate flea-borne disease risk (Frye et al. 2005). The species-specific flea index, for flea species of medical and veterinary importance found on domestic pets and backyard wildlife, was calculated to further estimate flea-borne disease risk (Krasnov et al. 2004). All fleas in the study were identified using standard taxonomic keys (Pratt 1947, Campbell 2017).

RESULTS

Flea Species Composition and Flea Indices on Mammalian Wildlife in Orange County

Flea data from mammals collected during the surveys are summarized in Table 1. For the nine mammal species where comparisons can be made (1967-1968 and 2016-2017), the flea indices were higher for ground squirrels (7.8 vs. 12.2), striped skunks (8.1 vs. 22.4), opossums (13.9 vs. 69.8), similar for cats (6.5 vs. 6.25), and lower for roof rats (2.14 vs. 0.18), and dogs (27.7 vs. 5.0) in 2016-2017 than in 1967-1968 (Table 1). From 1987-1988, the flea index for opossums was 27.4 fleas/animal (range 0-236); no other mammals were recorded in this study. Fox squirrels were not sampled in 1967-1968 or 1987-1988 because they were not found in Orange County prior to 1998 (King 2004). During 2016-2017, OCMVCD collected 27 fox squirrels and recorded a flea index of 1.6 fleas/squirrel (range 0-15).

The cat flea was found on seven of the nine sampled mammal species during 2016-2017. Of the fleas removed from opossums, the cat flea represented 98.4% (984/1,000) and 92.7% (3,172/3,421) of all fleas collected from 1967-1968 and 2016–2017, respectively (Table 1). Cat fleas comprised 23.9% (107/447) of all fleas removed from 20 striped skunks during 2016-2017, while none (0/65) were found on eight striped skunks in the 1967-1968 study. The sticktight flea, *Echidnophaga gallinacea*, was found on ground squirrels, opossums, roof rats, and striped skunks during 2016-2017 and was the most abundant flea on ground squirrels (78%; 298/378). During 1967-1968, only four sticktight fleas (0.4%; 4/1,000) were collected off 72 opossums, whereas 212 sticktight fleas (6.7%; 212/3,172) were obtained from 49 opossums during 2016-2017 (Table 1). The human flea, *Pulex irritans*[1], was found on three mammal species (opossum, striped skunk, and raccoon) and represented 100% (65/65) and 53.7% (240/447) of all fleas collected off striped skunks in 1967-1968 and 2016-2017, respectively. In contrast, *P. irritans* was found on opossums in comparatively small proportions, 1.2% (12/1,000) and 1.2% (37/3,172) of all fleas during 1967-1968 and 2016-2017, respectively. Five rodent fleas, *Hoplopsyllus anomalus,* were collected off striped skunks during 2016-2017 (Table 1). Two species of fleas, the northern rat flea, *Nosopsyllus fasciatus,* and the squirrel flea, *Orchopeas howardii*, were found on fox squirrels in Orange County. This is the first detection of *O. howardii* on wildlife in Orange County. The European mouse flea, *Leptopsylla segnis*, was collected from roof rats and domestic cats during 1967-1968, but was not found on the mammalian wildlife examined during 2016-2017.

Flea Test Results for Rickettsial Pathogens

No fleas tested positive for rickettsial pathogens using inoculated weanling mice and fluorescent antibody techniques during the 1967–1968 study (Adams et al. 1970). Fleas were not tested during 1987–1988. Of 826 flea pools tested by qPCR for *Rickettsia felis* and *Rickettsia typhi* during 2016-2017, 106 (12.8%) were positive for *R. felis* and 4 (0.5%) were positive for *R. typhi* (Tables 2–3). *Rickettsia felis* was detected in two flea species, *C. felis* (18.6%; 105/566 pools) and *H. anomalous* (3.4%; 1/29 pools). Three *C. felis* pools (0.53%; 3/566) and one *P. irritans* pool (1.1%; 1/93) tested positive for *R. typhi*. One pool of *C. felis* (0.2%; 1/566) tested positive for both *R. felis* and *R. typhi*.

Twenty (20/49) opossums, five (5/11) striped skunks, two cats (2/4), one California ground squirrel (1/12), and one human (1/5) were hosts for *R. felis* positive fleas; two opossums (2/49), one striped skunk (1/11), and one domestic cat (1/4) were hosts for *R. typhi* positive fleas (Tables 2–3). The *C. felis* pool that was positive for both *R. felis* and *R. typhi* was removed from an opossum (Table 2).

DISCUSSION

Flea indices, in conjunction with host abundance data, provide indicators or clues to the geographical localization of the vectors, their hosts, and risk of pathogen transmission for humans (Dennis et al. 1999, Krasnov et al. 2004, Frye et al. 2015). Unlike bubonic plague, where the California Department of Public Health (CDPH) provides a

Table 1.—Flea numbers and indices by species on mammals collected in Orange County during 1967-1968, 1986-1987, and 2016-2017.

Host Animal (Flea Species)	1967-1968			1987-1988			2016-2017		
	Host No.	Total Fleas	Flea Index (Range)	Host No.	Total Fleas	Flea Index (Range)	Host No.	Total Fleas	Flea Index (Range)
Domestic cat	58	377	6.5 (0-34)				4	25	6.25 (5-12)
(*C. felis*)		374	6.5 (0-34)					25	6.25 (5-12)
(*P. irritans*)		1	0.02						
(*C. inequalis*)		1	0.02						
(*L. segnis*)		1	0.02						
Domestic dog	4	111	27.7 (6-52)				2	10	5 (0-10)
(*C. felis*)		111	27.7 (6-52)					10	5 (0-10)
Fox squirrel							27	42	1.6 (0-15)
(*N. fasciatus*)								31	1.1 (0-15)
(*O. howardii*)								11	0.41 (0-7)
Ground squirrel	101	786	7.8 (0-48)				31	378	12.2 (0-134)
(*C. felis*)								1	0.032
(*E. gallinacea*)		202	2 (0-45)					298	9.6 (0-134)
(*O. montanus*)		497	4.9 (0-48)					32	1.0 (0-12)
(*H. anomalous*)		26	0.26 (0-9)					47	1.5 (0-23)
(*H. glacialis*)		61	0.6 (0-30)						
House mouse	8	2	0.25 (0-1)				5	0	
(*N. fasciatus*)		1	0.125						
(*C. felis*)		1	0.125						
Opossum	72	1,000	13.9 (0-90)	96	2,634	27.4 (0-236)	49	3,421	69.8 (0-1,166)
(*C. felis*)		984	13.7 (0-90)					3,172	64.7 (0-1,166)
(*E. gallinacea*)		4	0.06 (0-1)					212	4.3 (0-94)
(*P. irritans*)		12	0.17 (0-3)					37	0.8 (0-9)
Raccoon							2	2	1
(*C.felis*)								1	0.5
(*P. irritans*)								1	0.5
Roof rat	78	167	2.14 (0-38)				73	27	0.37 (0-13)
(*C. felis*)		1	0.01					13	0.18 (0-13)
(*E. gallinacea*)		7	0.09 (0-2)					1	0.014
(*O. montanus*)		1	0.01					13	0.18 (0-13)
(*L. segnis*)		109	1.4 (0-38)						
(*N. fasciatus*)		21	0.27 (0-5)						
(*H. glacialis*)		2	0.03 (0-2)						
(Unknown)		26	-						
Striped skunk	8	65	8.1 (0-31)				20	447	22.4 (0-151)
(*C. felis*)								107	5.4 (0-24)
(*E. gallinacea*)								95	4.8 (0-40)
(*P. irritans*)		65	8.1 (0-31)					240	12 (0-112)
(*H. anomalus*)								5	0.25 (0-5)

flea index target (<1 flea/animal) for control operations aimed to reduce plague disease risk (CDPH 2016), there is no flea index target for control operations in areas with transmission of flea-borne *Rickettsia*. Nonetheless, cases of flea-borne rickettsioses are prevented by limiting human exposure to fleas and flea feces (Clark et al. 2018).

The total flea indices on opossums and striped skunks appear to have increased in Orange County since 1967.

Table 2.—Prevalence of *R. felis* and *R. typhi* in pooled fleas removed from domestic cats, dogs, humans, opossums, raccoons, roof rats, and striped skunks collected in Orange County, 2016-2017.

Host Animal	*R. felis*-positive				*R. typhi*-positive			
	% Host with Pos. Flea Pools (No.)	% Pos. Pools *C. felis* (No.)	% Pos. Pools *E. gallinacea* (No.)	% Pos. Pools *P. irritans* (No.)	% Host with Pos. Flea Pools (No.)	% Pos. Pools *C. felis* (No.)	% Pos. Pools *E. gallinacea* (No.)	% Pos. Pools *P. irritans* (No.)
Domestic cat	50% (2/4)	8.0% (2/25)			25% (1/4)	4.0% (1/25)		
Domestic Dog	0 (0/2)	0 (0/10)			0 (0/2)	0 (0/10)		
Human	20.0% (1/5)	16.1% (5/31)			0 (0/5)	0 (0/31)		
Opossum	40.8% (20/49)	19.4% (88/452)	0 (0/57)	0 (0/20)	4.1% (2/49)	0.44% (2/452)	0 (0/57)	0 (0/20)
Raccoon	0 (0/2)	0 (0/1)		0 (0/1)	0 (0/2)	0 (0/1)		0 (0/1)
Roof rat	0 (0/1)		0 (0/1)		0 (0/1)		0 (0/1)	
Striped skunk	45.5% (5/11)	21.3% (10/47)	0 (0/39)	0 (0/72)	9.1% (1/11)	0 (0/47)	0 (0/39)	1.4% (1/72)
Total	37.8% (28/74)	18.6% (105/566)	0 (0/97)	0 (0/93)	5.4% (4/74)	0.53% (3/566)	0 (0/97)	1.1% (1/93)

Table 3.—Prevalence of *R. felis* and *R. typhi* in pooled fleas removed from ground squirrels and fox squirrels, 2016-2017.

	R. felis-positive					*R. typhi*-positive				
Host Animal	% Hosts with Pos. Fleas (No.)	% Pos. Pools *H. anomolous* (No.)	% Pos. Pools *D. montanus* (No.)	% Pos. Pools *E. gallinacea* (No.)	% Pos. Pools *O. howardii* (No.)	% Hosts with Pos. Fleas (No.)	% Pos. Pools *H. anomolous* (No.)	% Pos. Pools *D. montanus* (No.)	% Pos. Pools *E. gallinacea* (No.)	% Pos. Pools *O. howardii* (No.)
Ground squirrel	8.3% (1/12)	3.4% (1/29)	0 (0/18)	0 (0/22)		0 (0/12)	0 (0/29)	0 (0/18)	0 (0/22)	
Fox squirrel	0 (0/1)				0 (0/1)					0 (0/1)

Flea indices for all flea species/host increased from 8.1 fleas/striped skunk and 13.9 fleas/opossum in 1967-1968 to 22.4 fleas/striped skunk and 69.8 fleas/opossum, respectively, during 2016-2017; species-specific indices also increased, with *C. felis* and *P. irritans* being the dominant fleas on opossums and striped skunks, respectively (Table 1). The implications of the increase in flea index and changes in flea species composition on opossums and striped skunks from 1967-1968 to 2016-2017 is unknown. However, the prevalence of cases of human flea-borne rickettsioses has shown a marked increase since 2005 in Orange County [20 reported cases from 1967–2005 (Cummings et al. 2014); 165 reported cases from 2006–2017 (CDPH 2017)].

The percent of *R. felis* positive pools (18.6%) in *C. felis* flea samples from 2016-2017 is similar to the 13-32% *R. felis* positive rate reported in previous studies from southern California (Abramowicz et al. 2012, Eremeeva et. al. 2012, Billeter et al. 2016). This study reports the first detection of *R. felis* positive *H. anomalus* fleas in Orange County and warrants the need for further analysis of *H. anomalus* flea populations collected from backyard mammalian wildlife. Only three *C. felis* pools (0.53%) tested *R. typhi* positive in 2016-2017, indicating that the prevalence of *R. typhi* in *C. felis* samples is low. The *R. typhi* positive rate ($< 2\%$) in flea samples from cats and opossums found in this study is consistent with results from past investigations of this infectious agent in Southern California (Eremeeva et. al. 2012, Billeter et al 2016).

Recent studies show that domestic dogs are hosts to fleas infected with *R. felis* and *R. typhi* (Nogueras et al. 2013, Teoh et al. 2018) and merit further investigation. Logistically, flea collections from dogs in Orange County have been more difficult to obtain than from cats and other mammals. The District has found that most dogs are on some form of flea treatment and offer few to no fleas to collect, whereas the natural hunting behavior of cats, if allowed to roam, increases their exposure to infectious, parasitic, and zoonotic disease, including flea infestations (Ogan and Jurek 1997, AVMA 2018).

Cat fleas, opossums, striped skunks, and cats pose the greatest risk for transmission of flea-borne rickettsioses to humans in Orange County. *Ctenocephalides felis* was the most ubiquitous flea species collected and represented 98.2% (108/110 pools) of all *Rickettsia*-positive flea pools. Opossums, cats, and striped skunks had the highest *C. felis* flea indices (64.7, 6.25, and 5.4, respectively) and the highest number of individual animals with *Rickettsia*-positive fleas (42.9%, 75%, and 54.5%, respectively). Other backyard mammalian wildlife and commensal rodents in Orange County have low flea counts or are parasitized by flea species with low vectorial capacity for rickettsial pathogens.

CONCLUSION

To reduce the flea-borne rickettsioses disease risk, residents of Orange County should be educated about flea-borne disease prevention and control of fleas and host populations. Community and local animal control agencies and city jurisdictions need to be made aware of the infectious agents associated with cat fleas from mammalian wildlife, which also infest pet cats and dogs. An outreach program targeting areas of Orange County with the highest abundance of urban mammalian wildlife and human disease cases would educate residents on preventative measures designed to reduce contact with infected fleas, as well as best practices to reduce and exclude populations of flea animal hosts from residential properties. Because no flea control products are available for application to backyard mammalian hosts, such as opossums and striped skunks, the best means of flea-borne rickettsioses disease prevention is to educate residents to apply flea control products to their pets year-round.

[1]*Pulex irritans/P. simulans* are morphologically similar and were not identified as separate species during the studies.

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Mosquitofish holding/culturing system at the West Valley Mosquito and Vector Control District

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INTRODUCTION

Mosquitofish, *Gambusia affinis* (Baird & Girard), the most effective predacious biological control agent, has been used to control immature mosquitoes since the early 1900s in the USA and elsewhere. The advantages of ease in culture, hardiness during transportation and handling, as well as tolerance to broad range of water quality associated with mosquito habitats have been well recognized. Even though concerns over safety to non-target organisms restrict application of mosquitofish, this biocontrol agent will continue to play an important role in integrated mosquito control in the foreseeable future. At West Valley Mosquito and Vector Control District, we innovated our mosquitofish program in 2017. The system consists of one Quarantine Tank, two Grow-Out Tanks, and one Brood-Stock and Fry Tank. This system functions well to maintain the water quality, hold and culture the mosquitofish for District's needs on a year-round basis.

WORK FLOW

Starting with Quarantine Tank, field-collected mosquitofish are held for 10-14 days to ascertain of any health issues before release into the holding/culturing system. Gravid females are transferred to Brood Stock and Fry Tank for reproduction. Juveniles from the Quarantine Tank are transferred to the Grow-Out Tanks for further growth and development. Gravid females from the Grow-Out Tanks are transferred to Brood Stock and Fry Tank to augment the reproduction populations. Fry are transferred from Brood Stock and Fry Tank to the Grow-Out Tanks to grow and develop to mature adults. In the Brood-Stock and Fry Tank, automatic separation of gravid females and fry occurs, which allows us to easily breed the needed quantity with limited man hours. It also eliminates the need of fry trays.

QUARANTINE TANK

A 200-gallon galvanized water trough facilitated with 1/3 horsepower cast-iron submersible sump pump (Gamma Vacuum, Shakopee, MN) has a carrying capacity of up to 500 mosquitofish. Aeration is provided by a LT19 Whitewater Lear Air Pump (Pentair, Apopka, FL). Field-collected fish are quarantined in this tank to sort diseased and unhealthy fish before further processing.

GROW-OUT TANKS

Each of two circular Grow-Out tanks holds 450 gallons and can house 2000 mosquitofish. Water that is pulled out from the bottoms of the tanks goes through a physical filter to remove the solid material, while water that is pulled out from the middle point goes through a biofilter to breakdown the ammonia, nitrites, nitrates and other toxic wastes. The filtration is an open system and water flow is driven by a MEDO LA-120 air pump (Nitto Kohki, Roselle, IL) and gravity. A UV sterilizer (Aqua Ultraviolet, Temecula, CA) is installed in the common flow of the filtration system to minimize infestations of algae, parasites, bacteria and other microorganisms.

BROOD STOCK AND FRY TANK

The Brood Stock and Fry Tank is a 325-gallon rectangular tank configured to create an environment which provides shelter for gravid females to give birth to their fry and minimize the cannibalism of fry by adults. The gravid females remain in the "Brood Stock" portion of the tank, while fry swim upward and penetrate through the 3/16" cloth mesh, and then are skimmed to the "Fry" portion of the tank by surface flow. Aeration is provided by a LT19 Whitewater Lear Air Pump. This system negates the need of fry trays commonly used in mosquitofish production system, which saves materials and labor.

FISH FOOD

In 2017, a study was conducted to compare koi granules (1/4" in. diameter, 32% crude proteins, Arthur Aquatics, Evansville, IN) with tropical flakes (41% crude proteins, Aqueon, Franklin, WI) as a function of fish growth. Tropical flakes supported fish growth more efficiently and were chosen as food for mosquitofish – a surface feeding minnow.

SUMMARY

Since the innovation of the holding/culturing system in June 2017, fish populations have been stabilized and met the needs in our District. Key water quality parameters such as water temperatures (semi-outdoor, no heating), dissolved oxygen, electrical conductivity and pH have been maintained within healthy ranges for mosquitofish.

The Development and Use of a Duplex Real-Time PCR for the Detection of *Rickettsia typhi* and *Rickettsia felis* in Fleas Collected in Orange and Los Angeles Counties, California

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Abstract

Our goal was to develop a duplex real-time PCR assay to test fleas for *Rickettsia typhi* and *Rickettsia felis*, the pathogenic bacteria responsible for human cases of flea-borne typhus. After the reemergence in 2006 of flea-borne typhus in Orange County, CA, the Orange County Mosquito and Vector Control District (OCMVCD) has been performing singleplex real-time PCR on specimens to test for the presence of *R. typhi* and *R. felis* separately. However, with the continuation of flea-borne typhus cases in Orange and Los Angeles Counties and the concomitant need to evaluate more fleas, we were prompted to develop a duplex real time-PCR to create a more efficient protocol to test for *R. typhi* and *R. felis* simultaneously. Fleas were collected off euthanized peridomestic host animals trapped by OCMVCD or pest control personnel, and opportunistically in the field from pet animals or human hosts during flea-borne typhus case investigations. Fleas were processed using ZR BashingBeads and PrepMan Ultra for DNA extraction and tested for rickettsial pathogens with an Applied Biosystems 7500 Fast Real-Time PCR System. Fleas with cycle thresholds (Ct) $\leq$ 36.0 were considered positive for either rickettsial species. All fleas were tested first using the singleplex real-time PCR and retested with the duplex real-time PCR, which yielded similar results. A DNA sequence template synthesized to target the sequences of the primers and probes of both singleplex qPCR reactions, along with previously extracted *R. typhi* and *R. felis* genomic DNA from infected fleas, were used as positive controls to test for the efficiency of the duplex PCR. To determine the sensitivity of the assay, serial dilutions of our positive controls were used to evaluate the primers and probes in the duplex reactions. Of five species of fleas examined in this study, the cat flea, *Ctenocephalides felis*, was the predominant flea that tested positive for *R. felis* (13.6%), whereas the predominant flea testing positive for *R. typhi* was the Oriental rat flea, *Xenopsylla cheopis* (8.9%). Our duplex real-time PCR assay was effective and specific for detecting *R. felis* or *R. typhi* in several different species of fleas.

INTRODUCTION

Murine typhus, caused by the Gram-negative bacterium *Rickettsia typhi* [antigenically, a typhus group *Rickettsia*], continues to be an endemic disease in southern California since the first reported case of typhus was documented in California in 1915 (Meleney and French 1945). A similar, but clinically indistinguishable disease, flea-borne spotted fever, caused by the Gram-negative bacterium *Rickettsia felis* [antigenically, a spotted fever group *Rickettsia*] has been recognized recently as an emerging vector-borne disease in Africa and other parts of the world (Parola 2011, Ahmed et al. 2016, Brown and Macaluso 2016). However, flea-borne spotted fever is considered a rare disease in the U.S, and no flea-borne rickettsial disease cases attributed to infection with *R. felis* have been diagnosed in California (Billeter et al. 2016, Billeter and Metzger 2016). Because antibodies from humans infected with *R. felis* cross-react with *R. typhi* antigen in diagnostic immunofluorescence and western blot assays currently used in the U.S., the actual etiologic agent(s) for human cases of these typhus and typhus-like illnesses has not been definitively identified (Green et al. 2011, Eremeeva et al. 2012). The California Department of Public Health (CDPH) now classifies these non-Rocky Mountain Spotted fever diseases as flea-borne typhus to address this uncertainty (CDPH 2019a).

The cat flea, *Ctenocephalides felis*, is the most common species of flea on cats, dogs, opossums, and other backyard mammalian wildlife in southern California; it feeds frequently and indiscriminately, changes hosts often, and readily bites humans (Rust and Dryden 1997). The cat flea is considered the putative vector of flea-borne typhus in southern California (Adams et al. 1970, Williams et al. 1992, Eremeeva et al. 2012). Both *R. typhi* and *R. felis* are found in individual *C. felis* fleas (Noden et al. 1998), but co-infections are rare (Maina et al. 2016). The detection of *R. felis* and *R. typhi* in *C. felis* fleas in southern California highlights the potential risk for human infection with either

Table 1.—Primers and probes used in duplex real-time PCR to detect *R. typhi* and *R. felis*.

R. felis	
Forward primer:	5′ d GCAACCATCGGTGAAATTGA 3′
Reverse primer:	5′ d GCCACTGTGCTTCACAAACA 3′
Probe:	5′ d VIC-CCGCTTCGTTATCCGTGGGACC-BHQ-1 3′
R. typhi	
Forward primer:	5′ d- TGGTATTACTGCTCAACAAGCT 3′
Reverse primer:	5′ d- CAGTAAAGTCTATTGATCCTACACC 3′
Probe:	5′-6-FAM- CGCGATCGTTAATAGCAGCACCAGCATTATCGCG-BHQ-1 3′

of these pathogens (Maina et al. 2016). Thus, in efforts to properly survey for the presence of rickettsial pathogens associated with human disease in the region, the Orange County Mosquito and Vector Control District (OCMVCD) tests for both *R. typhi* and *R. felis* in fleas (all species) collected from domestic and peridomestic animals.

Until recently, OCMVCD tested fleas using real-time PCR (qPCR) in a singleplex system, where fleas were assessed separately for *R. typhi* and *R. felis* using protocols established by Henry et al. (2007) and Leulmi et al. (2014), respectively. However, with the significant increase in the demand for flea testing for both Orange and Los Angeles Counties due to the region's relatively high number of human flea-borne typhus cases (ca. 1,000 since 2001, CDPH 2019b), we were prompted to make changes to our protocol by developing a duplex real-time PCR assay that could test individual fleas for both *R. typhi* and *R. felis* simultaneously, thus shortening the time required to evaluate specimens. Fleas from five species [*C. felis, Echidnophaga gallinacea*, *Pulex* spp. (*Pulex irritans* and *P. simulans* are morphologically indistinguishable and were not identified as separate species in this study), *Orchopeas sexdentatus*, and *Xenopsylla cheopis*] collected during 2018 off ten species of domestic and peridomestic host animals, including humans, were tested using both singleplex qPCR and retested using our duplex qPCR.

METHODS

Flea processing and DNA extraction

During 2018, animals collected for OCMVCD's flea-borne typhus surveillance program were processed according to Penicks et al. (2019, *in press*). Briefly, peridomestic mammals endemic to southern California were collected in the field and humanely euthanized in a chamber filled with CO_2 gas, placed in plastic bags, and stored at 4°C. Carcasses were held until flea death ($>$ 48 hrs.) and examined in a Level 2 biological safety hood, where fleas were removed with combs and forceps. During flea-borne typhus case investigations, fleas were removed from a case patient's pet cat or dog (if present) after owner permission, or collected by inspecting pet sleeping areas for fleas. Fleas from human hosts were removed off protective clothing of OCMVCD staff during flea-borne typhus case investigations. Fleas were placed in ethanol and held at 4° C until they were identified to sex and species (Campbell et al. 2018), enumerated, and tested individually.

Disinfection of fleas was accomplished by removing ethanol and washing three times with distilled water. Single fleas were placed in 1.5 microcentrifuge snap cap tubes along with eight small ZR BashingBeads (Zymo Research part # SKU-S6003-50, Irvine, CA) and 100 ul of PrepMan Ultra (Applied Biosystems, part # 4318930, Thermo Fisher Scientific Corp., Foster City, CA). Tubes then were placed in an 8000D Mixer/Mill (SPEX Sample Prep, Metuchen, NJ) for 2 min to macerate the flea. Tubes then were spun in a small tabletop centrifuge to bring contents to the bottom of the tube, followed by boiling at 100° C for 10 min. Samples were removed from heat and let cool at room temperature for 2 min. Samples were then centrifuged at 12,000 rpm (Allegra™ X-22R Beckman Coulture centrifuge, Brea, CA) for 2 minutes. After centrifugation, the supernatant containing genomic DNA was transferred into a processing plate and preserved at -20° C until testing by qPCR.

Detection of *R. felis* and *R. typhi*

The primers and probe used to detect the presence of *R. felis* were described previously by Leulmi et al. (2014) and are specific for the protein phosphatase gene that produce an amplicon size of 118 bp. The primers and probe used to detect the presence of *R. typhi* were previously described by Henry et al. (2007) and are specific for the outer membrane protein B (ompB) gene that produces an amplicon size of 122 bp. The same primers and probes for the singleplex reactions were used in combination for the duplex qPCR and are listed in Table 1.

Conditions for duplex Real-Time qPCR

Probes for *R. typhi* and *R. felis* were labeled at the 5′ end with the fluorescent reporter dyes FAM and VIC, respectively. Both probes were labeled with the BHQ quencher at the 3′ end. Ambion® Path-ID™ qPCR Master Mix (Thermo Fisher Scientific, part # 388644) was used in the PCR reactions and performed in 25 µl volumes. The following were included per tube: 12.5 µl of Ambion® Path-ID™ qPCR Master Mix, 4 µl with Nuclease-free Water (Applied Biosystems, part # 4388514), 3.5 µl of primer and probe working stock and 5 µl of extracted DNA. The primer and probe working stock consisted of 0.3 µM of each *R. typhi* primer and 0.2 µM of *R. typhi* probe, along with 0.5 µM of each *R. felis* primer and 0.4 µM of *R. felis* probe. Cycling conditions using the Applied Biosystems™ 7500 Fast Real-Time PCR System were as follows: one cycle at 50° C for 2 min, one cycle at 95° C for 10 min, 45

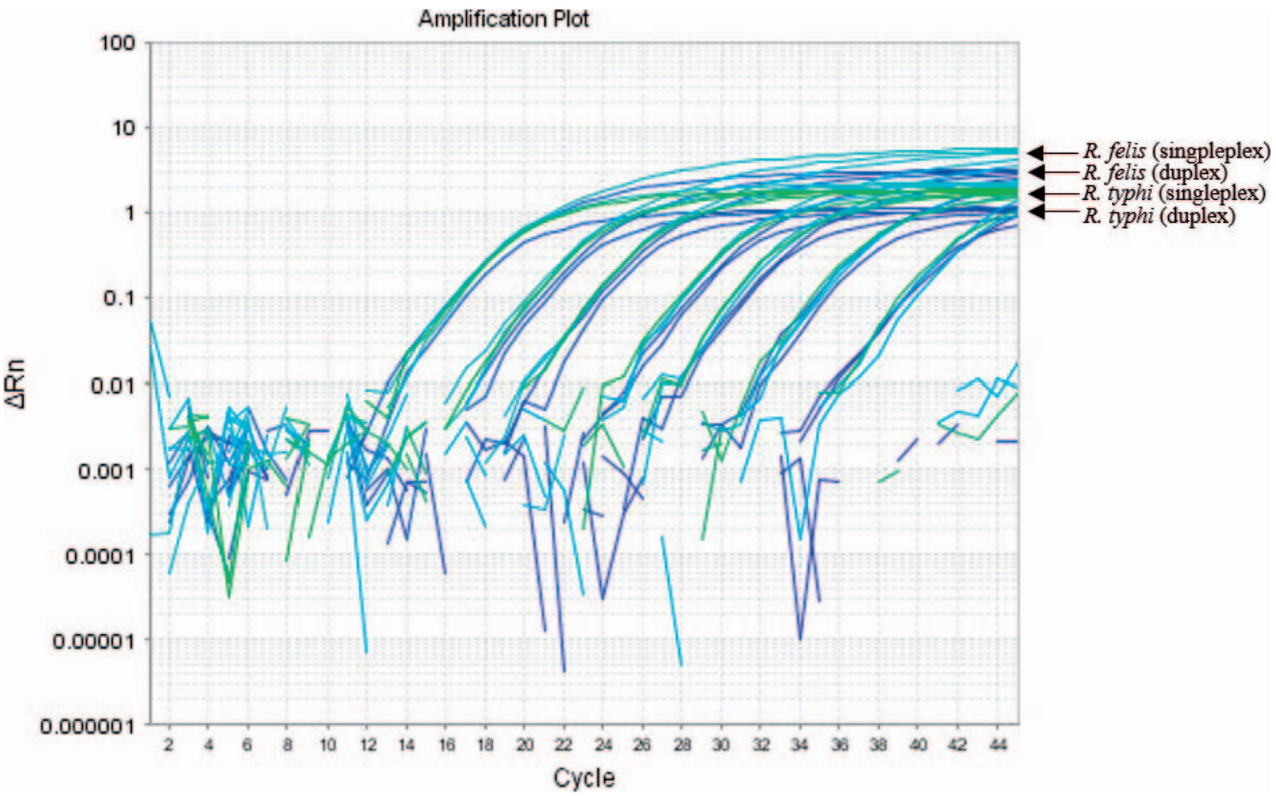

Figure 1.—Amplification curves of singleplex and duplex real-time PCR to detect *R. typhi* and *R. felis*. Light blue indicates amplification of DNA positive control using the *R. felis* singleplex PCR; green indicates amplification of DNA positive control using the *R. typhi* singleplex PCR, and dark blue amplification of DNA positive control using the *R. felis* and *R. typhi* duplex real-time PCR, as depicted by arrows. DNA positive controls diluted from 10^7 to 10^1 produced amplification curves as shown.

cycles at 95° C for 15 sec. and a final extension step at 60° C for 1 min. qPCR baselines to determine cycle thresholds (Ct) were set manually according to the recommendations of the manufacture (Thermo Fisher Scientific) such that threshold line passed through the geometric phase of the curve and above background signals.

The Rickettsial Diseases Division, U.S. Naval Medical Research Center (Silver Spring, MD), provided a DNA template synthesized to target the sequences of the primers and probes of both singleplex qPCR reactions (DNA positive control) that was used as a positive control to determine optimization of primer and probe concentrations for the duplex qPCR. In addition, previously extracted *R. typhi* and *R. felis* DNA from infected fleas also were used as positive controls to determine optimization of primer and probe concentrations for the duplex qPCR. Previously extracted DNA from negative fleas was used as a negative control. Master mix alone and nuclease-free water alone were used as no-template controls. Test pools with Ct $\leq$ 36.0 were considered positive.

Table 2.—2018 flea and host composition of *R. typhi* and *R. felis* positives confirmed by real-time PCR.

	R. felis-positive					
Host Animal	% Host with Pos. Flea Pools (No.)	% Pos. Pools *C. felis* (No.)	% Pos. Pools *E. gallinacea* (No.)	% Pos. Pools *Pulex spp*. (No.)	% Pos. Pools *O. sexdentatus* (No.)	% Pos. Pools *X. cheopis* (No.)
Big free-tail bat	0% (0/4)				0% (0/14)	
Domestic cat	42.9% (3/7)	22.9% (11/48)	0% (0/1)			
Domestic dog	100% (3/3)	40% (14/35)				
Rabbit	50% (1/2)	100% (1/1)	0% (0/8)			
Woodrat	0% (0/6)		0% (0/10)		0% (0/16)	
Human	60% (3/5)	9.3% (7/75)				
Opossum	64.9% (24/37)	13.3% (51/384)		0% (0/5)		
Coyote	12.9% (4/31)	9.4% (6/64)		0% (0/148)		
[1]Norway rat	0% (0/15)		0% (0/108)			0% (0/79)
Striped skunk	15% (3/20)	1.7% (1/60)	3.4% (2/58)	0% (0/86)		
Total	31.5% (41/130)	13.6% (91/667)	1.1% (2/185)	0% (0/239)	0% (0/30)	0% (0/79)

Duplex real-time qPCR

All fleas were tested initially using the singleplex qPCR and retested with the duplex real-time qPCR. Various concentrations of primers and probes (Table 1) were tested to optimize the duplex qPCR protocol. Serial dilutions of our DNA positive control ranging from 10^7 to 10^1 were performed to demonstrate accurate amplification of the target (Figure 1).

RESULTS

Comparison between singleplex and duplex real-time qPCR assays

After evaluating various concentrations of primers and probes between 0.2 – 0.9 μM, we determined that 0.3 μM of each *R. typhi* primer and 0.2 μM of the *R. typhi* probe, along with 0.5 μM of each *R. felis* primer and 0.4 μM of the *R. felis* probe, were optimal for the duplex qPCR. In the duplex assay for *R. typhi*, primer concentrations were increased from 0.2 to 0.3 μM and probe levels were reduced from 0.3 to 0.2 μM relative to the singleplex *R. typhi* assay. In the duplex assay for *R. felis*, primer concentrations were increased from 0.4 to 0.5 μM and probe levels were reduced to 0.4 μM relative to the singleplex *R. felis* assay.

The DNA positive control was useful in determining that the sensitivities of the duplex qPCR and the respective singleplex qPCR assays were comparable (Figure 1). DNA samples extracted from fleas infected with *R. typhi* and *R. felis* also confirmed that both singleplex and duplex qPCR assays results were similar, indicating that these two primer and probe sets can be used in the same reaction, without nonspecific amplification, regardless of flea species.

Flea species and host composition

The cat flea was found on the domestic cat (*Felis catus*), domestic dog (*Canis familiaris*), European rabbit (*Oryctolagus cuniculus*), Virginia opossum (*Didelphis virginiana*), coyote (*Canis latrans*), striped skunk (*Mephitis mephitis*), and humans (Table 2). The sticktight flea, *E. gallinacea*, was found on Bryant's woodrat (*Neotoma bryanti*), Norway rat (*Rattus norvegicus*), domestic cat, and rabbit. *Pulex* spp. fleas were found on opossums, coyotes, and striped skunks. *Orchopeas sexdentatus* fleas were found on the big free-tail bat (*Nyctinomops macrotis*) and the woodrat. We detected *X. cheopis* only on *R. norvegicus*. We also detected co-infections of both *R. typhi* and *R. felis* in a single *C. felis* flea collected from an opossum and in one *X. cheopis* flea collected from a Norway rat.

Real-time qPCR results of *R. felis* and *R. typhi*

Of 1,200 fleas tested (first by singleplex and then by duplex qPCR), 7.75% (93/1,200) were positive for *R. felis* and 1.08% (13/1,200) were positive for *R. typhi* (Table 2). *Rickettsia felis* was detected in two flea species, namely *C. felis* (13.6%; 91/667) and *E. gallinacea* (1.1%; 2/185); *R. typhi* was also detected in *C. felis* (1.3%; 5/384) and *E. gallinacea* (0.54%; 1/185), as well as in *X. cheopis* (8.9%; 7/79). Three cats (3/7), three dogs (3/3), one rabbit (1/2), three humans (3/5), twenty-four opossums (24/37), four coyotes (4/31), and three striped skunks (3/20) were hosts for *R. felis* positive fleas. Three opossums (3/37) and four Norway rats (4/15) were hosts for *R. typhi* positive fleas (Table 2).

DISCUSSION

The primer and probe sequences used in this duplex qPCR assay target genes that have been shown to be species-specific for *R. felis* and *R. typhi* and do not produce false positives (Henry et al. 2007, Leulmi et al. 2014). Various primer and probe concentrations were evaluated until the optimal concentrations were established to be used in the duplex qPCR. We then tested serial dilutions of our positive controls, and the resulting amplification curves demonstrated accurate amplification of the targets and comparable sensitivity between the singleplex and the duplex assays for both rickettsial pathogens in multiple flea species. All positives were also confirmed by retesting. Threshold baselines to determine Ct values were set manually, such that the threshold line passed through the geometric phase of the curve.

The cat flea, *C. felis*, was the most abundant flea collected, and had the highest positive rate for *R. felis*

Table 2.—Extended.

			R. typhi-positive		
% Host with Pos. Flea Pools (No.)	% Pos. Pools *C. felis* (No.)	% Pos. Pools *E. gallinacea* (No.)	% Pos. Pools *Pulex spp*. (No.)	% Pos. Pools *O. sexdentatus* (No.)	% Pos. Pools *X. cheopis* (No.)
0% (0/4)				0% (0/14)	
0% (0/7)	0% (0/48)	0% (0/1)			
0% (0/3)	0% (0/35)				
0% (0/2)	0% (0/1)	0% (0/8)			
0% (0/6)		0% (0/10)		0% (0/16)	
0% (0/5)	0% (0/75)				
8.1% (3/37)	1.3% (5/384)		0% (0/5)		
0% (0/31)	0% (0/64)		0% (0/148)		
26.7% (4/15)		0.9% (1/108)			8.9% (7/79)
0% (0/20)	0% (0/60)	0% (0/58)	0% (0/86)		
6.2% (8/130)	0.75% (5/667)	0.54% (1/185)	0% (0/239)	0% (0/30)	8.9% (7/79)

infection. Most of the *R. felis* positive cat fleas (13.3%) were collected off opossums, similar to what has been reported in earlier studies (Abramowicz et al. 2012, Eremeeva et. al. 2012). Although fewer in flea numbers, the high *R. felis* positive rate reported in this study for cat fleas removed from pet cats and dogs (22.9% and 40%, respectively, Table 2) illustrated the flea-borne typhus disease risk posed by not having companion animals on flea control. Although some *C. felis* fleas also tested positive for *R. typhi*, the highest percentage of *R. typhi* positive fleas were observed in the Oriental rat flea, *X. cheopis*. Interestingly, we also detected two fleas that were positive for both *R. typhi* and *R. felis*: one *C. felis* flea collected off an opossum and one *X. cheopis* flea from a Norway rat. The observation of a flea being co-infected with these two rickettsial species has been reported previously (Noden et al. 1998, Eremeeva et al. 2012).

Overall, this duplex real-time PCR assay was effective at detecting *R. felis* and *R. typhi* in fleas and has now become OCMVCD's method of choice when testing specimens for rickettsial pathogens.

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