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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
2009	Win Surachetpong	MAPK signaling regulation of mosquito innate immunity and the potential for malaria parasite transmission control
2010	Tara C. Thiemann	Evaluating trap bias in bloodmeal identification studies
2011	Sarah S. Wheeler	Host antibodies protect mosquito vectors from West Nile virus infection
2012	Brittany Nelms	Overwintering biology of *Culex* mosquitoes in the Sacramento Valley, California
2013	Kimberly Nelson	The effect of red imported fire ant (*Solenopsis invicta* Buren) control on neighborhoods in Orange County, California
2014	Thomas M. Gilbreath, III	Land Use Change and the Microbial Ecology of *Anopheles gambiae*
2015	Jessica M. Healy	Comparison of the efficiency and cost of West Nile virus surveillance methods in California
2016	Mary Beth Danforth	The impacts of cycling temperature on West Nile virus transmission in California's Central Valley
2017	Nicholas A. Ledesma	Entomological and Socio-behavioral Components of Dog Heartworm (*Dirofilaria immitis*) Prevalence in Two Florida Communities
2018	Kim Y. Hung	House Fly (*Musca domestica L.)* Attraction to Insect Honeydew
2019	Matteo Marcantonia	Revising alkali metals as a tool for mark-recapture studies to characterize patterns of mosquito (Diptera: Culicidae) dispersal and oviposition
2020	Adena Why	Semiochemicals associated with the Western mosquitofish, *Gambusia affinis*, and their effect on the oviposition of *Culex tarsalis*
2021	Vanessa Hill	Evaluation of residential property types for *Ades aegypti* habitats in Placer County, California
2022	Phurchhoki Sherpa	Asian longhorned tick, *Haemaphysalis longicornis* (Ixodida: Ixodidae), and optimal collection methods for the tick in the Northeast United States

Asian longhorned tick, *Haemaphysalis longicornis* (Ixodida: Ixodidae), and optimal collection methods for the tick in the Northeast United States[4]

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[4] Complete paper: **Sherpa, P., LC Harrington, NP Piedmonte, K Wunderlin, and RC Falco. 2021.** Optimal collection methods for Asian longhorned ticks (Ixodida: Ixodidae) in theNortheast United States. J. Med. Entomol. 58: 2255-2263.

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Abstract

Haemaphysalis longicornis, also known as Asian longhorned tick, New Zealand cattle tick, or bush tick, is a newly introduced, invasive tick species in the United States. An established population was first reported from New Jersey, in 2017. As of February 2022, *H. longicornis* has been recorded from sixteen additional eastern states. It is native to East Asia, mostly prevalent in China, Russia, Korea, and Japan. It is believed that *H. longicornis* spread to Australia and slowly invaded New Zealand and surrounding island nations in the 1800s. *Haemaphysalis longicornis* is a competent vector of pathogens that cause severe fever with thrombocytopenia syndrome (SFTS), Russian summer-spring encephalitis (RSSE), Q fever, babesiosis, rickettsiosis, and Theileriosis. The presence of *H. longicornis* poses potential risks of (i) new disease introduction and (ii) increased distribution of existing tick-borne diseases. Recent studies from Virginia reported that *H. longicornis* in the state could acquire and transmit *Theileria orientalis* Ikeda to cattle. Infection with *T. orientalis* can lead to reduced milk production, anemia, still birth, and even death in cattle, so further distribution of this pathogen has the potential to cause economic losses in cattle industry. The vector status of *H. longicornis* for endemic tick-borne pathogens is still not apparent. In a laboraqtory study, infected *H. longicornis* nymphs were unable to maintain the Lyme disease spirochete, *Borrelia burgdorferi*, when they molted into adults, but it is still unclear whether that remains true in the field. Study of *H. longicornis* tick biology, ecology, and disease risk is dependent on effective collection methods; however, studies comparing effectiveness of collection methods for this tick is limited globally and lacking in the US. Sherpa et al. (2021) assessed three commonly used tick collection methods (dragging, sweeping, and CO_2 traps) and three check distances (5 m, 10 m, and 20 m) for dragging and sweeping methods in southern New York state. The results indicated that shorter check distances (5 m) and the dragging method collected a greater number of *H. longicornis*. CO_2 traps were ineffective in *H. longicornis* collection; nymphs and adults were attracted to the trap, but 'lost interest' and crawled away. Although the density of ticks (that considers the fabric size difference between dragging and sweeping) was calculated and presented, results and conclusions were based on raw (average) data as not to compromise (i) the characteristic differences between the methods and (ii) the project goal of providing a directly translatable guide for field collections. When choosing a collection method there are also other factors to consider such as budget, project goal, and landscape. In our study, we were unable to compare landscape differences because of the low numbers of *H. longicornis* collected from the second location, but anecdotally we found that using the sweeping method was easier use under fallen trees or brushy/thorny shrubs. Similarly, we recommend using 5 m check distance if budget and personnel are available and the project goal is to estimate the public health risk. However, in case of budget constraints or a presence/absence study, using 10 m check distance is advised over opting for longer check distances.

Social Media Internship Program influences Gen Z in Placer County

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Abstract

Social media is one important element of the Placer Mosquito and Vector Control District (District) robust strategic public outreach program. The District has active profiles on Facebook, Twitter, Instagram, TikTok, Nextdoor and YouTube. During the COVID-19 pandemic, the District relied heavily on social media outreach because in-person outreach was no longer a possibility. To enhance the District's presence on these platforms during West Nile virus season, a social media intern was hired to help boost engagement on TikTok and Instagram, District platforms with the lowest engagement rates.

The internship program goal was to boost engagement and reach on TikTok and Instagram, reach Generation Z about important mosquito and vector information by hiring a Generation Z employee, provide a hands-on and resume building experience in the communications field and align all these activities with our District's strategic plan goal to spread awareness and educate the Placer County public about the risks from vectors and vector-borne diseases. Generation Z is known as the hardest to reach audience from a marketing perspective. These individuals were born from 1997-2012 and are currently ages 10-25. They are natural digital influencers, creative, unpredictable and trend-setters in the digital realm. The position was opened in hopes of hiring a local high school graduate or college student. Through the interview process, a local high school graduate was hired who produced their school's media program and was well-versed on social media content creation. The intern worked heavily on creative content development. The program's assignments ranged from internship and career goal setting, competitive analyses, social media account analyses, ideation and creation planning and storyboarding, strategic social media development, and presenting a closing evaluation. Each week featured a hybrid schedule and began with a creative meeting to guide content development. The intern worked independently with little oversight other than fact checking and proof-reading.

Due to the internship, the District's TikTok engagement increased with some videos reaching over 1,000 views. Three broadcast news style TikToks were posted along with six original videos and the District's TikTok follower count increased from zero to 37. The intern was able to develop four original reels, eight videos, highlights, and a digital quiz, guide and Linktree for Instagram. Overall, the District's TikTok and Instagram saw an increase in engagement. Due to the internship's success, the District plans to hire another social media intern for the 2022 West Nile virus season with the goal to continue to increase engagement across social media platforms.

Thank you to Olivia de Lamadrid, our 2021 social media intern, and the District's support to make this experience possible including district manager Joel Buettner and assistant manager Jake Hartle.

Elevating the conversation: Mosquito bites live on Instagram

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Introduction

Communications have changed dramatically because of the accessibility and variety of features on social media. Instagram continues to be a major platform in social media with as many as 1 billion active monthly users worldwide. Instagram Live has been a feature widely available since 2016, but use has increased markedly as a result of the COVID-19 pandemic. Many creators and brands gravitated to this feature as a way to continue creating personal engagement in the comfort of their homes. The San Gabriel Valley Mosquito and Vector Control District (District) leveraged this feature by creating a series that airs twice a month called Mosquito Bites Live. The District invites guests to talk about a topic on mosquito and vector control. The series also helps to amplify conversations in vector control, enhance current and new partnerships with other organizations, while having a live audience that tunes in during these discussions.

Methods

Mosquito Bites Live is hosted twice a month on Instagram with episodes lasting between 15 – 20 minutes on Wednesdays at 10 AM PT. A topic is selected based on the time of year, type of guest, or theme. Once the guest has been identified and confirmed 4-5 questions are usually created and confirmed by the host and guest. Guests are invited to add their own questions that can help drive the conversation. For adequate preparation time and message cohesion, the questions are sent ahead of time to the guest. The questions also are kept entertaining by asking each of the guests "if they were a mosquito what would their name be?" This helps to add constancy, 'fun', and creativity to the series. Topics covered on Mosquito Bites Live include *Aedes* mosquitoes, repellent use, mosquito prevention in Spanish, and the importance of public health.

For the production of the show, an iPhone on a tripod that has the District's Instagram account logged in is used. The background includes the text "Mosquito Bites Live", the District's logo, and a photo of Ada Eez the Mosquito. A demo is also offered to guests to build familiarity with the features of Instagram Live. This is usually done an hour before show time to greet the guest, confirm lighting, WIFI connection, and video.

Results and Discussion

Mosquito Bites Live is finishing season two with 15 -20 episodes, ranging with guests from other vector control districts to private organizations. With 15 episodes already aired, the series has generated an average of 94.5 views per episode with a total of 218 engagements on Instagram. Some of the most notable partnerships have been with the American Public Health Association and the NASA sponsored GLOBE Program. These new partnerships have helped to expand the district's reach and brand awareness. The majority of the other guests continue to be other vector control districts, continuing established partnerships.

Conclusion

Mosquitoes Bites Live has allowed the District to bypass the need to use publications to disperse educational information. By developing a talk show, like Mosquito Bites Live, the district is able to content that fits the platform and meet residents where they frequent. It also has given the District the opportunity to reach out to established and new organizations for partnership opportunities. The show continues to grow by developing brand awareness, creating a new platform for outreach and engagement, and a reason to develop new partnerships. Other vector control districts can benefit by being a guest on the show or developing their own Instagram Live series that can feature different aspects of vector control.

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What's the Buzz? A public outreach response to the spread of invasive mosquitoes

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Introduction

Invasive *Aedes aegypti* mosquitoes were detected initially within the Sacramento-Yolo Mosquito and Vector Control District (District) boundaries in 2019 in the city of Citrus Heights in Sacramento County. The following year, *Ae. aegypti* was discovered for the first time in Yolo County and there were additional detections in new areas within Sacramento County. At the beginning of 2021, it seemed extremely likely that invasive mosquitoes would continue to be found in all known sites and potentially in new areas. As expected, the first detection came in April, followed by a second finding in May in BG traps operated as part of our extensive year-round surveillance program. Fortunately both of these detections were from known sites; however it was extremely surprising to find *Ae. aegypti* this early in the season, because these mosquitoes typically are active in late summer and fall. By the end of 2021 invasive mosquitoes had been detected in completely new areas within Sacramento County and the infestations in known sites kept growing.

This presentation provides an overview of the various public outreach strategies and activities that have been used to communicate with residents and alert them of new invasive mosquito detections within their neighborhoods, especially in light of the COVID-19 pandemic that limited some of the District's traditional outreach efforts.

Methods

With the ongoing *Ae. aegypti* detections in our District, we immediately launched an extensive public outreach response to ensure that residents were well informed. After a new detection was made a press release discussing details of the new discovery and the District's response plan was issued. The response from the media was very favorable and throughout the season we secured good coverage regarding the areas where invasive mosquitoes were being detected. The best media opportunities and stories came from television news crews shadowing our staff as they visited homes looking for mosquito breeding sites, showing treatments to backyards and providing recommendations and tips for homeowners to reduce breeding sites.

Another key element of our outreach included contacting our local elected officials. After each new discovery of *Ae. aegypti*, an email was sent to inform them of our detection and our District response plan. We encouraged them to sign up for our treatment notifications and provided them information that they could use to help us disseminate content to their constituents. In addition our annual spring presentations with each city and county provided an update on West Nile virus activity, invasive mosquitoes and other relevant information going into the mosquito season ahead. A critical component of our outreach efforts included enhancing our social media efforts as a way to relay information to the public. Social media became even more important as invasive mosquitoes were detected in many new areas of Sacramento County to get information out quickly into affected communities. In addition we utilized Facebook ads that were 'geo-targeted' towards specific zip codes where invasive mosquitoes were detected. To ensure we reached out directly to all residents within each new detection area, we also mailed out postcards to each home. The postcard included facts about invasive mosquitoes, photos of breeding sites and a checklist for homeowners with tips and recommendations of what they could do to reduce larval habitat. As part of our public information campaign, we partnered with local media outlets to launch a digital and print advertising campaign along with utilizing other advertising elements such as radio/television spots, digital ads, and billboards to disseminate our messages. Due to ongoing COVID-19 concerns, participation in traditional outreach events was very limited; therefore our own District field technicians took on the enhanced role of public educators, becoming public outreach experts. Every time they conducted a home service inspection or had interaction with the public, they took extra time to educate and provide information. In addition, they also gave out District bags that contained repellent packets, brochures and other information that would normally be given out at community events or presentations. Because our office and members of the public were not able to pick up repellent wipes, our staff delivered boxes of wipes to senior centers, parks/recreation departments, and agencies that serve the homeless.

Results and Discussion

The implementation of ongoing public outreach strategies was useful in ensuring residents were informed

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about the spread of invasive mosquitoes. Some of the challenges that the District faced included continuous and ongoing detections starting in April and going thru October. This made for a very lengthy and intense season that the District had not anticipated. Another challenge was ensuring that the affected cities and appropriate city staff were kept up to date with the District's efforts. Due to COVID-19 restrictions, the District had limited opportunities to engage with residents in affected communities. Another challenge we faced was that while social media was a tremendous help to disseminate information, we realized that not everyone utilizes social media platforms, therefore our reach was limited. Lastly, even though District staff did a tremendous job with public outreach and it allowed for great reach into the community, it added another element to their daily workload.

Conclusion

Despite the ongoing spread of invasive mosquitoes, the District was able to successfully continue public outreach to keep residents informed about the spread of invasive mosquitoes. At the same time we were able to uphold our commitment to protect the health and welfare of residents in Sacramento and Yolo counties. Moving forward the District will continue implementing the strategies discussed and other innovative outreach efforts as the invasive mosquito *Ae. aegypti* will likely continue to spread into new communities.

Acknowledgements

The Sacramento-Yolo Mosquito and Vector Control District staff for their assistance in helping to ensure public outreach to inform residents of ongoing invasive mosquito detections.

Community outreach: Planning an effective program

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Abstract

Community Outreach is an integral component of the Best Practices for Integrated Mosquito Management. In addition to surveillance and inspections, vector control districts across the country provide community outreach and education for stakeholders to remove mosquito sources on their properties, and thereby reduce mosquito-borne disease in their communities. Most programs include methods to teach larval habitat reduction and behavior modification such as "Tip & Toss." Education programs also may include the use of personal protection measures, such as repellents and appropriate clothing. Although most general guidelines and objectives remain the same, community outreach should be customized to each agency based on the local situation and budget. This presentation examined three outreach programs with varying budget levels (low, mid-level, and high) from different mosquito control agencies, explored the platforms used to reach their stakeholders, examined the effectiveness of the programs, and discussed successes and challenges faced.

Benefits of establishing a good working relationship with homeowner associations in the Coachella Valley

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Introduction

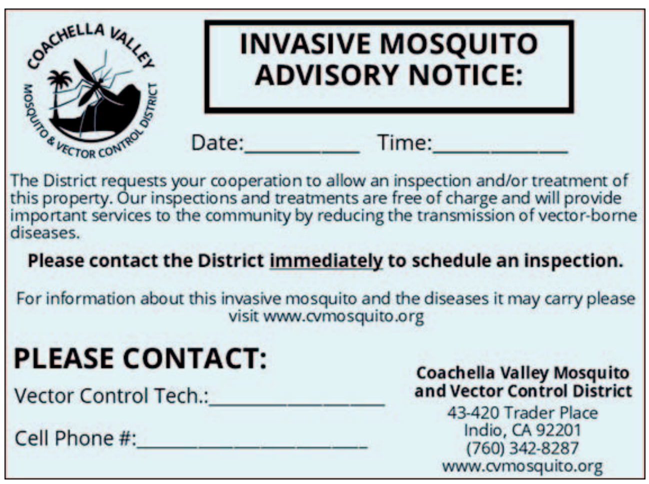
Coachella Valley's extreme summer weather poses two challenges for mosquito control inside home owner association (HOA) communities. Frequent extended irrigation leads to a high volume of drains with stagnant water and access to these potential larval sources becomes limited and time-consuming once the substantial seasonal population leaves. The 625 HOAs present a unique opportunity to collaborate with each management on developing sustainable mosquito control methods that benefits the whole of Coachella Valley. The current study aims to showcase the benefits of collaboration between the Coachella Valley Mosquito and Vector Control District (CVMVCD) and HOA managements.

Methods

The CVMVCD's *Aedes aegypti* and urban technicians conducted door to door inspection campaigns in two areas with high volumes of service requests. One campaign occurred every Wednesday for four weeks in the same residential neighborhood in Palm Desert. The second campaign was conducted for one day inside the Mirada Estates HOA in Rancho Mirage with the assistance of management notifying residents prior to the inspections. The CVMVCD's *Aedes* notice (Fig 1) was used in all "Notice Posted" entries to encourage residents to schedule a property inspection. Inspection data was collected utilizing the CVMVCD's Combined Vector Technician (CVT) tablet application (Petrovic 2010) to compare access between residential and HOA neighborhood access. Customized, educational newsletters created by CVMVCD's Public Information Officer were sent to HOA managements to circulate to all residents to notify them of mosquito issues in their area and how to address them. HOA managers and maintenance employees of Big Horn Country Club in Palm Desert, Mirada Estates in Rancho Mirage, and Andreas Hills Phase IV in Palm Springs were proactive participants in implementing mosquito management practices. CVT application was utilized to enter and collect data about the chemical treatments applied to drains within an HOA community before and after collaboration between technician and management to gage pesticide application reduction.

Results

After four weeks of attempting to inspect 75 properties in an open, residential neighborhood during summer season, only 43% of homes were inspected for mosquito sources. Overall 57% of properties received at least one *Aedes* notice, but did not contact the District to schedule an inspection (Fig 2). After one day of inspecting properties inside an HOA community where management granted permission to inspect all open (non-gated) homes and notified residents prior to technicians' arrival, 81% of 79 homes were inspected and 19% were locked with notices left at the gates (Fig 3). In the HOA community of Big Horn Country Club, our Public Information Officer created a newsletter with photos taken of common sources found throughout properties in that HOA (Fig 4). Engagement rate was low, and only 4 people opened the link sent by the HOA management. Government newsletters have low opening rates, but community-specific information has great potential for increasing HOA participation in mosquito control efforts. The Andreas Hills Phase IV HOA in Palm Springs had an issue with eleven French drains repeatedly positive for mosquito larvae throughout the community. Drain sites # 162473,


COACHELLA VALLEY MOSQUITO & VECTOR CONTROL DISTRICT

INVASIVE MOSQUITO ADVISORY NOTICE:

Date:__________ Time:___________

The District requests your cooperation to allow an inspection and/or treatment of this property. Our inspections and treatments are free of charge and will provide important services to the community by reducing the transmission of vector-borne diseases.

Please contact the District immediately to schedule an inspection.

For information about this invasive mosquito and the diseases it may carry please visit www.cvmosquito.org

PLEASE CONTACT:

Vector Control Tech.:______________

Cell Phone #:____________________

Coachella Valley Mosquito and Vector Control District
43-420 Trader Place
Indio, CA 92201
(760) 342-8287
www.cvmosquito.org

Figure 1.—*Aedes* Advisory notice left at homes where access is not possible for technicians during door-to-door inspection campaigns

Figure 2.—Open, residential neighborhood with high volume of mosquito Service Requests. Operations team conducted a door-to-door inspection campaign for 4 Wednesdays in one month, resulting in 43% of 75 homes being inspected for larval sources. 57% gave no response/access

162474, and 16275 were initially discovered in May 2020 and the additional eight drains were found in July 2020. For five to six months, each drain had been treated monthly with a 30-day larvicide. Out of 58 inspections, only 6 inspections did not warrant treatment (Table 1).Technician established a connection with management and advised on source reduction. The HOA maintenance crew added store-bought mosquito dunks to each French drain periodically. After 8 monthly follow-ups from December 2020 until December 2021, only 9 French drains were treated for larvae. These drains were positive for larvae because the Bti dunks were dissolved before maintenance was scheduled to replenish the product. The pesticide application by CVMVCD was reduced by 80% as a result of collaborating with the HOA employees (Table 2).

Table 1.—Frequency of monthly larvicide treatments after drains were discovered in May and July of 2020. Treatments applied prior to developing relationship with HOA manager.

Andreas Hills HOA, Palm Springs Pre-Collab Treatments							
SITE #	MAY '20	JULY '20	AUG '20	SEPT '20	OCT '20	NOV '20	TOTAL TREATMENTS
168500	N/A	1	0	1	1	1	4
168501	N/A	1	1	1	1	1	5
168499	N/A	1	1	1	1	1	5
168498	N/A	1	0	1	1	2	5
168497	N/A	1	1	1	1	1	5
168503	N/A	1	1	DRY	1	DRY	3
168504	N/A	1	1	1	1	1	5
168505	N/A	1	0	1	1	1	4
162473	1	1	1	1	1	1	6
162474	1	1	1	1	1	1	6
162475	1	1	1	0	1	1	5
							53

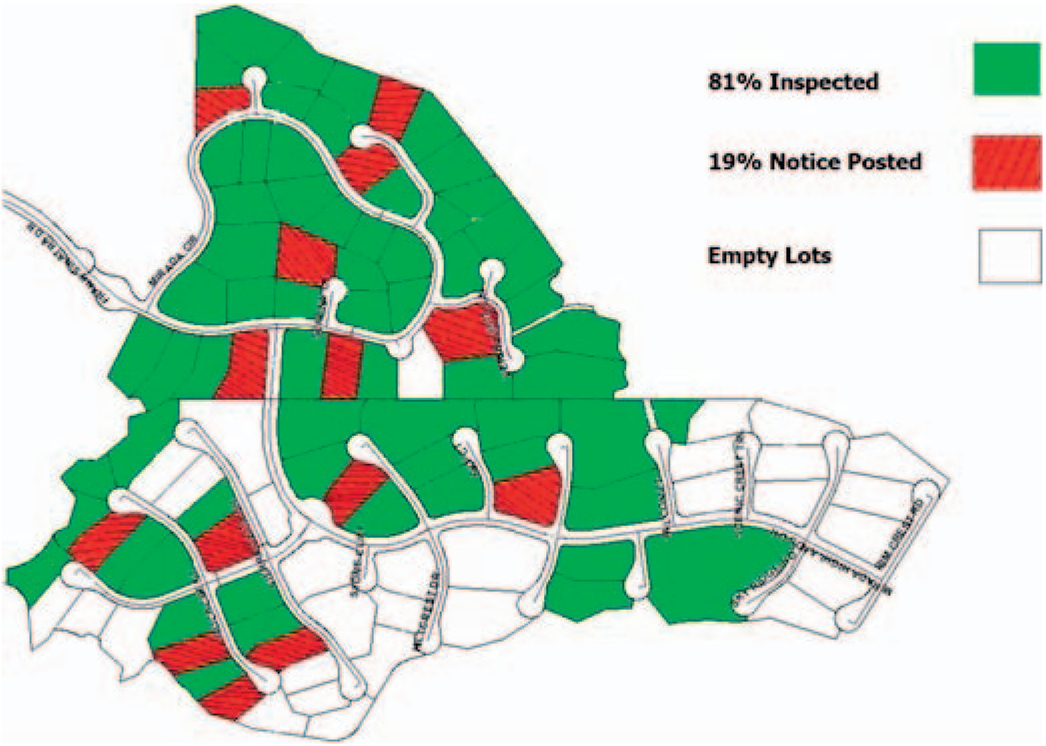


Figure 3.—Homeowner Association gated neighborhood with high volume of Service Requests. Operations team conducted a door-to-door inspection campaign in one day after management notified all residents, resulting in 81% of 79 homes being inspected for larval sources. 29% of properties were locked and not possible to access

Conclusions

Collaborative communication between HOA employees, technicians, and public outreach encompasses integrated vector control management practices through means of education, surveillance, and physical/chemical control of sources. Developing a relationship with HOA management reduces the seasonal barriers technicians face in the summer season. Mosquito control efforts made by HOA employees will continued to be monitored in 2022. Results will be used in developing a standard of procedures for future HOAs willing to work with CVMVCD in reducing mosquito infestations.

Acknowledgements

Tammy Gordon, Public Information Officer, Marco Medel and Miguel Vargas, Aedes Team Leads, Aedes

Table 2.—Infrequency of monthly larvicide treatments after drains were treated by HOA maintenance. 80% reduction in pesticide application after developing relationship with HOA employees.

Andreas Hills HOA, Palm Springs Post-Collab Treatments									
SITE #	DEC ‘21	FEB ‘21	APRIL ‘21	MAY ‘21	JULY ‘21	SEPT ‘21	OCT ‘21	DEC ‘21	TOTAL TREATMENTS
168500	0	0	0	0	DRY	DRY	DRY	0	0
168501	0	0	0	0	DRY	DRY	DRY	0	0
168499	0	0	0	0	1	DRY	DRY	0	1
168498	0	0	0	1	1	1	0	0	3
168497	0	0	0	0	0	DRY	DRY	0	0
168503	0	0	1	DRY	DRY	DRY	DRY	0	1
168504	0	0	0	0	DRY	DRY	DRY	0	0
168505	0	0	0	0	DRY	DRY	DRY	0	0
162473	0	0	0	0	0	0	0	0	0
162474	0	0	0	0	1	DRY	1	0	2
162475	0	0	0	0	1	DRY	1	0	2
									9

Coachella Valley Mosquito
& Vector Control District
2021

Big Horn HOA do you hear the buzz? Mosquitoes in your neighborhood are finding a new home in catch basins.

The Coachella Valley Mosquito & Vector Control District has received an uptick in requests for service in your area. Vector Control Technicians have identified breeding sources and potential sources.

Please help reduce the risk of contracting these diseases for yourself and your community by educating yourself and taking action. Pictures from your neighborhood are included that show potential breeding sources and steps you can take to prevent further mosquito issues.

Overwatering grass and landscape is one reason these drains filling regularly. Without proper cleaning and maintenance, catch basins also fill with debris and create a perfect mosquito breeding habitat.

HOAs are responsible for cleaning and maintaining drains and catch basins. HOA management should take an active role by creating a maintenance schedule and educating residents about potential breeding sources and preventative measures.

Residents can also help reduce mosquitoes around their homes. Below is information for homeowners to protect themselves and their neighborhood from mosquitoes and the viruses they can carry.

Figure 4.—Homeowner Association newsletter created by Public Information Officer, Tammy Gordon. Photos of larval sources found inside the Big Horn CC HOA were used in the newsletter to educate residents on specific issues to be aware of to reduce breeding sources

"hot shot" crew, operations staff, Oldembour Avalos, Field Supervisor, Gregory Alvarado, Field Supervisor, Geneva Ginn, Lead Technician, Gonzalo Valadez, Lead Technician, Jennifer Henke, Laboratory Manager, Fernando Gutierrez, Community Liaison, Vincent Valenzuela, Lead Technician, Michael Martinez, Field Supervisor, Marko Petrovic, CVT Developer, Jeremy Wittie, General Manager

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Utilizing technology to facilitate student citizen science mosquito-borne disease surveillance

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Introduction

San Gabriel Valley Mosquito and Vector Control District's (District) EcoHealth Vector Education Program brings K-12 Next Generation Science Standards (N.G.S.S.)-aligned programs to over 550 schools within the District's boundaries. Operation Mosquito G.R.I.D. (Growth Reduction, Increased Detection) is a new California N.G.S.S.-aligned community science program available to all students within our District boundaries. Since 2011, our District has used citizen science to expand mosquito awareness. Operation Mosquito G.R.I.D. was created in 2020 and is an ongoing fall program that teaches citizen scientists source reduction strategies to reduce *Aedes* mosquitoes and provides tools to monitor *Aedes* activity in the San Gabriel Valley. In a time of extended social distancing requirements, the use of technology allows student citizen scientists in both remote and in-person learning to take part in citizen science without the need for face-to- face interaction with District staff.

Methods

Each fall in 2020 and 2021, 22 and 84 (respectively) student citizen scientists, grades 3 - 8, completed enrollment and received Operation Mosquito G.R.I.D. kits. Students inspect the area around their homes, collect stagnant water samples (starting in 2021), clear their properties of all potential sources, deploy an oviposition trap for two weeks, and report their results using digital platforms.

Online applications allow for program optimization during remote and in-person learning. Prior to the start of the program, logistics and project management applications were employed: Acuity Scheduling Platform for Teacher-Staff reservations, Wix for hosting the EcoHealth Vector Education website, HelloSign to capture parent permission signatures, and Monday.com for project management.

The 2021 program has an enrollment period of 1-month. Once enrolled, students complete assignments for 6 weeks. All communication between students and staff occurs via electronic communication. Communication applications include: MailChimp for eblast email instructions and reminders to students and parents, Google Classroom and Gmail for communication with students and teachers, SurveyMonkey for reporting and image submissions, Padlet for water analysis images and descriptions of results in the Virtual Lab, and YouTube for step-by-step, "how-to" video instructions. Pre-assessments are required to complete the enrollment process. Post-assessment surveys are collected at the 1, 3, and 6 month marks. Six-month survey data is collected in the spring (the fall 2021 will be collected in April 2022). Program assessments utilize SurveyMonkey and Google Forms for data collection.

Results and Discussion

Other organizations that have utilized oviposition cups in citizen science programs include: Beach Mosquito Control's "Trapping Young Minds'' program in Panama City Beach, Florida; the USDA-ARS' "The Invasive Mosquito Project"; and the Smithsonian Science Education Center's Science for Global Goals project, "Mosquito!" Our program is unique in its use of technology for communication with participants, collection of water samples during source reduction activities, and the reduction of face-to-face teacher or staff involvement.

The use of digital technology increases accessibility for distance learners to participate and facilitates collaboration with other citizen scientists across the San Gabriel Valley. G.R.I.D., employs technology already available and familiar to most students, and empowers students to engage in environmentally safe mosquito control by turning their backyard into a living laboratory. The use of the District's EcoHealth Virtual Lab provides participants with a digital platform to publish their findings and see the results of students at other schools, while honing their N.G.S.S. science and engineering practices. The Virtual Lab gives participants access to a wider student network beyond their own school and promotes a sense of community teamwork and accomplishment. Through the process, students discover they can solve an insect pest problem without the use of broad-spectrum pesticides. In addition to providing education for students and their families, the project also provides *Aedes* eggs for the District's surveillance department.

Student responses to pre- and post-assessment, collected by Google Forms in the 2020 pilot program, revealed increases in positive results in knowledge gain and environmental modification. Students demonstrated knowledge gain in the role of stagnant water and containers in mosquito proliferation. The number of students stating that 'baby' mosquitoes grow in stagnant water increased by 22.2%; from 81.8% to 100%. The number of students correctly identifying *Aedes* mosquitoes' preference to lay eggs in small containers increased by 69.8%; from 54.5% to 100%. Students gained efficacy in preventing mosquito production on their property. The percentage of students stating they "Strongly Agree" or "Agree" that they know how to stop mosquitoes from growing in their yard increased by 69.2%; from 59.1% to 100%. Data for 2021 is pending completion of the program.

Setting up the program did have its challenges. Each school district's information technology department operates independently and relies on their learning management system of choice. Technological challenges such as blocked emails and school district-established user limitations created communication barriers between vector control staff and students. As a result, web pages dedicated to each step of the process were developed. Teachers provided students the link to each step and parents received e-blasts to their personal emails.

Conclusion

Utilizing technology successfully facilitated communication and engagement in Operation Mosquito G.R.I.D.. Whether learning remotely or in-person, digitizing the program increases access to science opportunities and promotes source reduction strategies and *Aedes* surveillance within the District. Although school districts' cyber security restrictions and technical support staff created barriers to the initial setup of the program, we used both website-based communication strategies such as Monday.com and Google Classroom applications to adjust to differences in school district student communication policies. Moving forward, the education program plans to streamline communication channels for all schools. Future education programs with multiple touch points could benefit from using similar technology for communication channels and project management. Agencies should provide ample time to work with school IT departments on communication barriers prior to the initiation of a digitized citizen science program.

Acknowledgements

SGVMVCD would like to thank Gimena Ruedas and Melissa Doyle from the Surveillance department for establishing the *Aedes* Super Cup Program in 2019. Their knowledge and experience greatly supported the expansion of the program that became Operation Mosquito G.R.I.D..

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Delta MVCD strikes back! Funding to fight the *Aedes aegypti* in Tulare County

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Abstract

New invasive mosquito species, such as *Aedes aegypti,* have placed an additional burden on the budgets of local mosquito control agencies, including the Delta Mosquito and Vector Control District in rural and politically conservative Tulare County. The Delta MVCD decided to fight back. District staff successfully led the implementation of a new assessment, passed with 57% support, to fully fund *Aedes aegypti* services - but it was not without it challenges. Tulare County is known for political skepticism. This presentation included a step-by-step case study of this successful assessment balloting, including polling and feasibility analysis, ballot measure development, balloting, tabulation, and community outreach.

Ethics and ethical practice in mosquito control – an introduction

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Abstract

Local government is not immune from requests for equitable services. As agencies responsible to our residents and with trustees and directors who are elected or appointed by governing bodies, mosquito control districts answer to public officials whose constituents are asking for greater accountability and transparency of our work. Some government agencies have been tasked with the responsibility of examining how and where employees are spending their time and who is receiving their services. For instance, some mosquito control districts are experiencing community changes that have required an update in the languages used for communication. For others, although not a legal requirement, districts are considering the need to update their materials.

The introduction to the current symposium included some definitions of terms and resources used by the speakers to conduct their work. Here, the term 'equality' was defined as the fairness of equal access and opportunity, whereas 'equity' was defined as realizing that some individuals may need more resources than others to achieve the fairness of access (Kranich 2005). The Coachella Valley Mosquito and Vector Control District has a vision to progress towards a future free of vector-borne disease using scientific, technical and educational strategies which are financially and environmentally sound. Using the example of libraries, equality would examine whether constituents garner equal returns on their investment into the District, whereas equity would acknowledge that some communities or members will have different needs to be addressed to achieve equal results.

The state of California has several resources which are available for mosquito control districts who are examining their services. CalEnviroScreen 4.0 (October 2021) is a mapping tool that tracks pollution sources. It includes environmental, health, and socioeconomic information which is used to calculate a score for the pollution burden of a community, allowing government agencies to compare different communities. Examples of indicator maps that may be of interest include pesticide use, poverty, education and linguistic isolation.

The California Department of Water Resources has a Disadvantaged Communities Mapping Tool. The intent is to assist local agencies such as water districts to achieve their responsibilities related to the California Water Plan, Integrated Regional Water Management and Sustainable Groundwater Management Act. Disadvantaged communities, and severely disadvantaged communities are defined using the statewide median household income and recent census information. Knowing the locations of these communities may improve the ability of mosquito control agencies to meet the needs for mosquito control services. Further, communities with additional needs are eligible for funding through partnerships with local Integrated Water Management Regions. Financial opportunities are also available through the California Governor's Office of Emergency Services.

CalEnviroscreen 4.0. https://experience.arcgis.com/experience/11d2f52282a54ceebcac7428e6184203/page/Draft-CalEnviroScreen-4.0/

DAC Mapping Tool. https://gis.water.ca.gov/app/dacs/

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The importance of public opinion surveys for mosquito and vector control districts

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Why Conduct a Jurisdiction-wide Survey?

According to Merriam-Webster, a public-opinion poll is "taken by sampling a cross section of the public in an effort to predict election results or to estimate public attitudes on issues." Mosquito and Vector Control Districts conduct surveys to gain insight into public attitudes on issues that impact their work as public health agencies. Districts want to know if their constituents understand that mosquitoes can transmit the causative agents of disease, that skunks are a reservoir of rabies, what a vector is, and that their local mosquito and vector control district is a public health agency. The number of questions can vary, but the goal of the survey is to find out who a district's constituents are and what they think about a district and the issues the district faces. This information should be used to make improvements to agency services, response times, outreach, and protecting public health.

Who Conducts the Survey?

When hiring a survey company, it's useful to ask other district managers and staff who they have used to conduct a large survey, because then you know the company has previous experience asking questions about vector-related issues. We received recommendations for three companies: Smith Johnson Opinion Research out of Sacramento, Goodman Simon Strategic Research out of Oakland (a company our District used many years ago), and J. Wallin Opinion Research out of Irvine. These companies provided proposals for conducting the survey using mailed letters, online posts, and telephone landlines, cell phones and texting. The cost estimates ranged from $19,000 – $45,850.

How is the Jurisdiction-wide Survey Conducted?

The goal was to survey a cross section of citizens. J. Wallin Opinion Research was selected. The company's standard procedure is to use county voting records for a sample size of 400 citizens. Voting records not only provided contact information, they also allowed for analysis based on gender, political party, place of birth and age. The survey was conducted using telephone land lines, cell phones and texting, with questions asked in English or Spanish depending upon the language spoken by the citizen. Justin Wallin was available to provide unlimited reports and speak to our Board of Trustees for no additional cost beyond the $20,000 cost for asking 25 questions.

Who Receives the Jurisdiction-wide Survey?

For the Contra Costa Mosquito & Vector Control District (CCMVCD, District,) survey conducted in 2021, 47% of the respondents were men and 52% were women. There was no information on gender identification. 77% of the respondents were born in the United States, 17% were born outside of the US and 5% chose not to answer. 25% of the respondents were 18-34 years of age, 31% were 35-54, 16% were 55-64, and 26% were > 65.

Results of the CCMVCD 2021 Survey

Only 11% of respondents knew that a vector could transmit a pathogen that may cause disease. Alternatively 8.8% answered a vector is related to Area/Location/Section, 8.5% Directional/Angles/Lines, 7.2% Pest Control/Removal, 6.0% Insect/Mosquito/Bugs, and 2.0% Company Name/Service. 6.8% provided "other" answers, and 49.8% were unsure or refused to answer.

When residents did not know the subject they were asked about, the survey included information so that in the future they could provide a more informed answer. Ultimately, the survey provided CCMVCD with important information, while providing an opportunity to educate District constituents.

The next question included the definition of a vector. Once the citizens knew what a vector is:

66% said they were concerned about vectors. Specifically, 25.8% of the respondents were Very Concerned, 40.3% Somewhat Concerned, 19.5% Not Very Concerned, and 14.0% Not at All Concerned. 0.5% were unsure or refused to answer.

Respondents then were asked what people can do to prevent mosquito issues. 46.5% said they use Mosquito Repellents, 45.5% Remove Standing Water, 41.2% Install or Repair Window or Door Screens, 14.8% Report Standing Water, 11.0% Use Mosquitofish, 9.0% Call a Government Agency for Assistance, and 8.2% Report Neglected Swimming pools. 14.8% said none of these options would prevent a problem with mosquitoes, whereas 3.8% were unsure or refused to answer.

District-Specific Questions

When asked if they had heard of CCMVCD: 53% responded No, 44.8% said Yes, and 1.7% were unsure or refused to answer. After the survey explained that the CCMVCD is a public health agency dedicated to the health and welfare of Contra Costa County's more than 1 million residents and that the District works to protect residents from harmful and potentially disease-carrying insects and other animals by providing services for mosquitoes, ticks, ground-nesting yellowjackets, rats, mice, and skunks, the survey asked if the resident approved of the job CCMVCD is doing. With this new information, surveyed citizens responded that 44.5% Strongly Approve, 35.8% Somewhat Approve, 2.8% Strongly Disapprove, and 2.0% Somewhat Disapprove. 14.9% were unsure or refused to answer.

On the subject of invasive mosquitoes and Innovative Technologies related to these mosquitoes, the survey asked if residents were aware that there are invasive species in California. 18% responded Yes, 80.3% answered No, and 1.8% were unsure or refused to answer. As we have not yet found invasive mosquitoes in Contra Costa County and have had limited communication about them, we were not surprised with the response. Following a description of how invasive species can impact the lives of Californians, the survey asked residents if they, "approve of introducing new technology that can be used to control non-native mosquitoes, including genetically modified mosquitoes?" 48.2% Strongly Approve, 28.5% Somewhat Approve, 8.0% Strongly Disapprove, 5.0% Somewhat Disapprove, and 10:3% were unsure or refused to answer.

The next question introduced the idea of Integrated Vector Management (IVM). 53.0% of residents responded that IVM is Very Important, 37.2% Somewhat Important, 1.8% Not at All Important, and 3.2% Not Very Important. 4.8% were unsure or refused to answer.

Remember to Ask About Equity

The last question asked if the respondents feel CCMVCD serves all parts of the jurisdiction equally. 40.% agreed with the statement, "The District serves all portions of Contra Costa County equally." 20.5% agreed with the statement, "The District serves the eastern portion of Contra Costa County better than the western portion." 39.0% were unsure or refused to answer. From an operations standpoint, the eastern part of the County tends to be warmer and produces more West Nile virus activity, but the District cannot let the residents on the western side of the County feel they are not provided equal service.

Mailed Survey Cards

A jurisdiction-wide survey is a large and important project that is best to budget every three to five years. Another way to find out how constituents feel about an agency's services is to mail survey cards. CCMVCD mails postage-paid tri-fold style survey cards that are perforated so that residents can tear off the survey portion to complete and return it to the District. CCMVCD mails up to 30 cards per week at a current cost of $31.80. When possible, cards are mailed equitably to residents of each city in the County.

The survey card asks residents about their experience with the District employee who provided the District service, whether the vector issue was resolved, and their level of satisfaction. Residents also have the opportunity to write a comment. The survey card includes information about the District's services, history, and the fact that CCMVCD is a special district and not a part of county government. In addition, there's information on how residents can view the District's online content and sign up for the District's online communication options including the District's website, District social media channels, e-newsletters, news releases, adult mosquito control notifications, and Board of Trustees agendas.

The response rate for mailed survey cards remains at 15-20% per week. There is little research to suggest an appropriate response rate for mailed survey cards; however, online survey response rate data indicates that 5-10% is a good response rate.

Any adverse feedback from residents regarding service is sent to the General Manager, the Public Affairs department and Operations Supervisors for further review, discussion, and additional communication with the resident, if needed.

Conclusions:

- Mosquito and Vector Control Districts conduct surveys to gain insight into public attitudes on the issues that impact our work as public health agencies
- Choose a vendor who has worked with Mosquito & Vector Control Districts in the past
- Budget for a jurisdiction-wide survey every 3-5 years
- Conduct Surveys equitably
- Traditionally mailed, self-addressed and postage-paid survey cards provide insight into what constituents are thinking about the services District employees provide in real time.
- Use survey information to improve your agency's services, response times, outreach, and how your agency protects public health

Equitable, effective practices for mosquito abatement in Alameda County: Challenges and solutions

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Abstract

The Alameda County Mosquito Abatement District (ACMAD) protects the health and comfort of Alameda County residents from mosquito-borne disease and mosquito nuisance. It employs a system of integrated pest management that includes outreach and public education, surveillance, and physical control. It is one of over 70 such agencies in California and is funded through property and benefit assessment taxes.

Native mosquito species in California can transmit West Nile, St. Louis encephalitis, and Western equine encephalitis viruses. Although not yet detected locally, invasive *Aedes* species introduced elsewhere in California can transmit Zika and dengue viruses. Mosquitoes also impact health in ways beyond disease, particularly by preventing people from enjoying outdoor space near their homes.

Our analysis has two parts: First, through interviews and a literature review, it identified three populations that are particularly vulnerable to these negative health effects in Alameda County: low-income individuals, recent immigrants, and unhoused individuals. Second, this analysis developed and applied a "service score" model that aggregates data across ACMAD's three branch services (outreach, surveillance, and control) to highlight areas of high or low service. It found that although ACMAD is doing well in delivering services to different areas and groups across the county, there is still room for improvement. Geographic clusters of lower service for vulnerable populations suggest that ACMAD could address more services specifically to these communities and areas.

To address service gaps, meet the needs of marginalized populations, and prepare for these future threats, ACMAD and other MADs in similar situations should: Hire an additional full-time staffer that focuses entirely on community connections; partner with local homelessness outreach organizations to provide unhoused individuals with mosquito repellent and information about how to contact ACMAD about a mosquito problem; hold more events and/or shift surveillance traps to service gaps or marginalized population areas; make both printed and online educational materials available in additional languages; and use aerial reconnaissance data to identify areas particularly at risk for invasive *Aedes* invasion.

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Analyzing vector control program services through a health equity lens

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Abstract

Health Equity is the principle that everyone should have the same opportunities to live as healthily as possible. It differs from health equality in that resources may need to be distributed in an unequal manner to achieve equity in communities that are disadvantaged or underserved. The County of San Diego Vector Control Program (VCP) assessed the number and distribution of vector complaints and requests for service throughout the county and cross-referenced them with the California Healthy Places Index (HPI) and CalEnviroScreen 2.0 databases. Recognizing that many factors influence complaints and requests for service, including local vector conditions as well as awareness of and ability to request vector services, the VCP identified areas and demographics with lower numbers of requests for services that potentially need additional resources to achieve equity. The VCP used these results to develop and direct resources to potentially underserved areas to promote equitable access to vector services. Actions included making materials available in multiple core languages used in the county, hypertargeting media campaign content directly to specific communities, and proactively connecting with community groups.

A One Health perspective on the resurgence of flea-borne typhus in Texas in an era of climate change and human population growth

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Introduction

Flea-borne typhus (FBT) is an infection caused by the bacteria *Rickettsia typhi* and *R. felis*. It is typically an acute undifferentiated febrile illness, but about one-quarter of patients suffer respiratory, neurologic, renal, hepatic, cardiac, ocular or other complications (Tsioutis et al. 2017, Anstead 2021a). The infection is transmitted to humans by a flea bite or by the inoculation of a bite site, a skin abrasion, or mucous membranes with feces from fleas infected with these rickettsiae (Azad 1990, Brown et al. 2016). In the last decade, the incidence of FBT has increased in both Texas and California (TDHS 2021, Murray et al. 2017, CDPH 2020).

Methods

In the current paper, county-level epidemiological data for the number of cases of FBT occurring in Texas for two decades, 1990-1999 (TDH 2001) and 2010-2019 (TDHS 2021), were compared with respect to county of residence, urbanization, and climatic region. County-level data was obtained from the Texas Department of Health Services.

Results and Discussion

The One Health concept focuses on issues at the intersection of human and animal health and the environment (Sleeman et al. 1981). The current characteristics of the vectors, pathogens, and reservoirs of FBT, the environment, and human population trends wereintergrated to derive a One Health model to explain the increase in the number of FBT cases in Texas that has occurred in the new millennium. In this schema, it is proposed that the principal driver of the increased number of FBT cases in Texas is ecosystem disruption, due to human population growth/urbanization and climate change. The current epidemiologic trends of FBT in Texas are dependent on two versatile opportunists, the cat flea *Ctenocephalides felis* and the Virginia opossum *Didelphis virginiana*. Furthermore, increasing populations of cats (*Felis catus*) and dogs (*Canis familiaris*), including stray and feral animals, also may be affecting epidemiologic trends. The increasing populations of these mammals are being driven by human population growth. Greater mammalian host abundance may increase cat flea populations, which also may be increased by climate change. By the 1980s, FBT in Texas underwent an ecological transition from rats as the predominant reservoirs to opossums, dogs, and cats (Older 1970, Schriefer et al. 1994).

The human population of Texas is increasing at a rapid rate. In 1990, the population of the Lone Star State was 17 million; in 2019, the population stood at 29 million, a 70.7% increase. The urban population of Texas grew 83%, even more during this time period; therefore, the proportion of Texans living in a metropolitan area increased from 1990 to 2019, from 83.4 to 89.4%.

From 1990-1999, there was an average of 30.7 cases of FBT/year from 26 Texas counties, whereas during 2010-2019 there was an average of 375 cases/year occurring in 91 Texas counties (Fig. 1). From the recent data, 90.5% of cases occurred in counties classified as urban. The six counties that had the most FBT cases during 2010 to 2019 were Hidalgo (major city McAllen) > Nueces (Corpus Christi) > Cameron (Brownsville) > Bexar (San Antonio) > Harris (Houston) > Travis (Austin); these six counties had 2,824 cases, comprising 75% of the total Texas FBT cases. Compared to 1990-1999, cases have increased in Hidalgo, Nueces, Cameron, Harris, and Bexar Counties in 2010-2019 by 8.5-, 5.0-, 17.5-, 252-, and 211-fold, respectively. (Travis County had zero cases during 1990-1999). The population of Bexar, Cameron, Harris, Hidalgo, Nueces, and Travis Counties increased 67.1, 62.7, 66.3, 126, 24.2, and 121%, respectively, from 1990 to 2019.

Texas is divided into 10 climate divisions (Texas Water Development Board 2012) (Fig. 2). In the period 2010-2019, the northern "cooler" set of four climate divisions had only 6.1% of the total cases. Texas FBT cases were focused in the warmer South Central (includes Bexar, Travis, and Nueces Cos.) and Lower Valley (includes Hidalgo and Cameron) climate divisions (39.1 and 36.4% of the cases, respectively), accounting for 75.5% of the total cases. In 2010-2019, cases increased in 8/10 climatic divisions, especially in the Upper Coast (includes Harris County), as compared to 1990-1999.

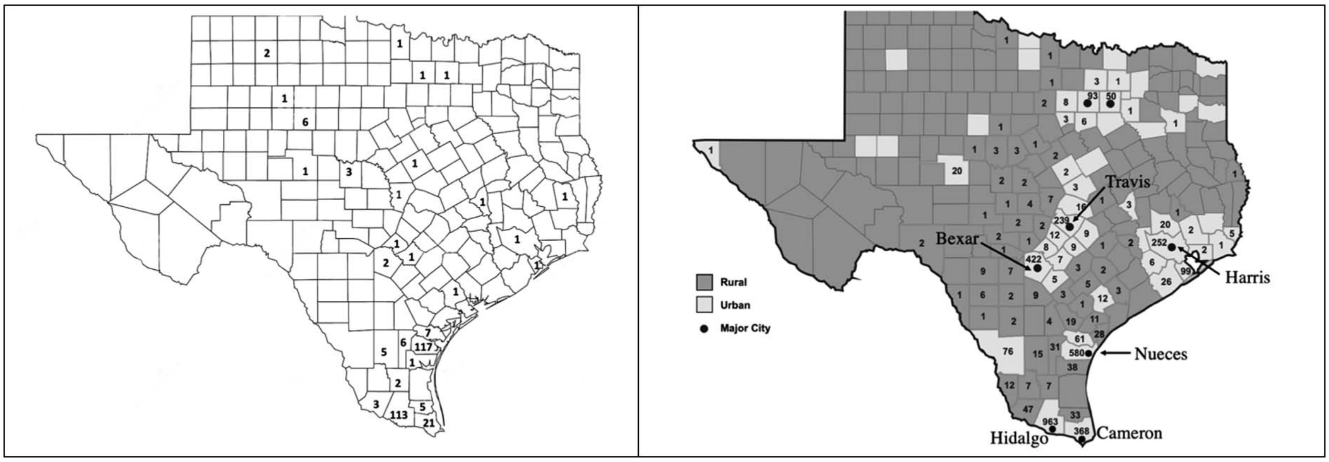

Figure 1.—Left panel. County of residence of flea-borne typhus cases in Texas, 1990-1999. Right panel: County of residence of flea-borne typhus cases in Texas, 2010-2019, and urban-rural distribution. The six counties with the highest number of cases are indicated. On both maps, the Texas Panhandle has been omitted because there were no cases in this Northern region.

Temperatures in Texas have increased almost 1.5 °F since the beginning of the 20th century. Although there is no overall trend in the number of hot days, the number of warm nights was particularly high during the 2010s. The urban heat island effect has amplified this problem in Texas cities (NOAA 2022, Xie et al. 2010).

A One Health model is presented to explain the increase of cases of FBT in Texas in the last decade. Increasing human population growth in Texas has been accompanied by an increase in pet dogs and cats and strays. For every person in Texas, there are approximately 0.27 pet dogs and 0.21 pet cats. Therefore, there were about 7.8 million pet dogs and 6 million pet cats in Texas in 2019. The number of stray and feral dogs is difficult to determine (Bergman et al. 2009). The number of stray and feral cats is estimated to be one-third to two-thirds of the owned cat population (Slater 2005); if an estimate of 40% is made, there are also 2.4 million stray and feral cats living in Texas in 2019. The urban/suburban environment also has promoted opossum population growth compared to rural areas (194 opossums/km^2 in urban Nueces Co., TX versus 6.8/km^2 in adjacent rural San Patricio Co.)(Boostrom et al. 2002, Gerht et al. 1997), likely due to greater food availability in the urban area (Yao et al. 2011). The significance of opossums in the epidemiology of FBT is amplified by the huge number of fleas that they can host (Cummings et al. 2014). Increasing temperatures in Texas may affect various aspects of the cat flea lifecycle. Data from various locales indicate that cat flea infestation of cats, dogs, and opossums is more common during warmer months (Xhaxhiu et al. 2009, Durden et al. 2005, Mohr and Morlan 1959); therefore, warmer weather due to climate change may increase the period of high flea infestation.

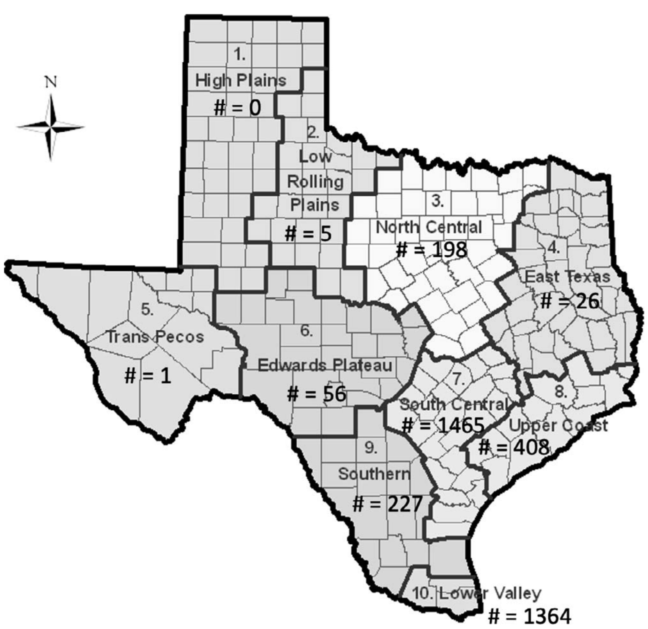

Figure 2.—The ten climatic regions of Texas, with the number (#) of cases of flea-typhus indicated for each climate region for the decade 2010-2019.

In general, fleas increase the frequency of blood feeding with increasing air temperature (Krasnov 2008). Increased adult flea feeding will increase quantities of flea feces in the environment that will supply additional food for larval fleas. Warmer temperatures also stimulate the mating frequency of cat fleas (Hsu and Wu 2001). Higher temperatures also accelerate the cat flea lifecycle from egg to adult (Kern et al. 1999). Furthermore, rickettsial replication within the cat flea also is increased by higher temperatures (Farhang-Azad et al. 1984). Warmer temperatures also mayincrease the number of potential opossum litters from two per year to three (Krause and Krause 2006).

CONCLUSIONS

Human population growth in Texas promotes FBT by increased urbanization and the abundance of pet dogs and cats, stray/feral dogs and cats, and opossums. Increasing temperatures in Texas in the new millennium increase the flea-borne transmission of typhus by promoting host infestation and flea feeding and defecation, accelerating the flea life cycle, and increasing rickettsial replication within the flea. Increased opossums and stray cats and dogs in the urban/suburban landscape increase the risk of flea transfer to humans and their pets. The incidence of FBT can be decreased by the use of flea control products on pets and the control of stray/feral cats and dogs and urban opossums.

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GIS exploration allows for a new look at an old disease: Flea-borne typhus in California, 2011-2019

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Abstract

This study assessed environmental variables (e.g., weather, land use) associated with flea-borne typhus (FBT) cases in the Los Angeles Basin of Southern California. FBT case surveillance data were retrieved from the California Reportable Disease Exchange (CalREDIE) for individuals with a reported age greater than 18 years, residence in Los Angeles or Orange County, and illness during January 1, 2011 to December 31, 2019. Land use by type was extracted from National Land Cover Database layer files (NLCD; 2011-2019) using circular buffers around reported case residential addresses. Daily historical weather records of temperature (minimum, maximum, average), precipitation, average wind speed, and fog coverage were retrieved up to 90 days prior to case onset dates or report dates via the Global Historical Climatology Network (GHCN). The associations of FBT cases with land coverage and weather records were estimated for each buffer using multivariable logistic regression models and compared based on their R^2 values to determine the best scale for observing this relationship.

Increased availability of geographical data has made it possible to approach studies of FBT ecology with a new perspective, specifically a One Health approach that takes into account environmental variables at a large scale. This approach may inform local public health jurisdictions and vector control districts to prioritize prevention in areas at greater FBT risk. Future studies should consider evaluating the role of socioeconomic status in reported FBT cases. Additionally, the methods presented here should be replicated using animal host and vector geographical data wherever available to ensure these findings were not spurious because based on human surveillance data.

An introduction to Siphonaptera: A brief look at the anatomy, biology, and tools associated with fleas

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Abstract

Insects in the order Siphonaptera are unique and highly specialized ectoparasites, with none of their anatomy being easily mistaken for that of any other arthropod. Fleas are highly adapted to live in the pelage, feathers and dens/nests of their mammalian and avian hosts, and have small size, laterally compressed bodies, and subtle differences among the distinguishing morphological characteristics of individual species. In combination these complexities create challenges associated with obtaining specimens, preparation for examination, and identification of species. Two digital documents have been created that utilize photography as a visual aid to provide users with easy and effective tools to identify and preserve flea specimens. The first, a guide entitled *How to Mount Your Flea: a guide to the preservation, preparation, clearing, and mounting of Siphonaptera*, discusses previously developed, but obscure techniques, covering the preservation as well as the clearing and mounting of flea specimens onto microscope slides. The second, a photographic key of common and medically important flea species collected from urban wildlife and pets in Orange County, California entitled *Pictorial Key to Some Common Fleas of Southern California*, utilizes images taken through a microscope combined with short descriptions to guide the user through the identification process. The key and mounting guide, which are freely available online at Orange County Mosquito and Vector Control District's website, provides users with a guide to the processes of preserving, clearing, mounting, and identifying flea specimens. Flea genera included are *Cediopsylla*, *Ctenocephalides, Oropsylla* (originally *Diamanus), Echidinophaga, Hoplopsyllus, Leptopsylla, Nosopsyllus, Orchopoeas, Pulex*, and *Xenopsylla*. Examined hosts include cats, coyotes, dogs, mice, opossums, rabbits, raccoons, rats (including roof rats, Norway rats, and woodrats), skunks, and squirrels.

Detection of *Rickettsia* species in cat fleas collected from feral cats in Alameda County

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Abstract

Feral cats, commonly referred to as "community cats", is a term used to describe any *Felis catus* that is unowned and lives outdoors. Feral cats are ubiquitous in Alameda County, resulting in numerous nuisance complaints to our Vector Control Services District for a range of issues, including flea infestations and concerns for disease. As a result, we began an investigation into quantifying the disease risk feral cats and their associated fleas pose to the public in our service district. Flea-borne typhus, also known as murine typhus, is an infection transmitted to humans when a bite site is inoculated with feces from fleas infected with *Rickettsia* species. *Rickettsia typhi* presently causes most human flea-borne rickettsioses worldwide; however, another rickettsia found in cat fleas, *Rickettsia felis*, has been implicated as a potential human pathogen. The objective of this study was to investigate the presence and infection rate of *Rickettsia* species in cat fleas (*Ctenocephalides felis*) collected from feral domestic cats in the non-typhus endemic region of Alameda County, California. From 2019-2021, 160 pools of fleas were collected from 66 feral cats throughout Alameda County. Polymerase chain reaction indicated that 127 of these pools were positive for *Rickettsia* infection. DNA sequencing has not yet been performed to identify *Rickettsia* species.

County-wide surveillance of *Rickettsia* species in Alameda County associated with wildlife and commensal rodents in urban settings

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Abstract

Rickettsia typhi, the causal agent of murine typhus, historically has been found throughout northern and southern California, but the pathogen has not been detected to date in Alameda County. In 2018, Alameda County Vector Control Services District began a county-wide surveillance program to ascertain what *Rickettsia* species were present in the county. Our study focused primarily on fleas from commensal rodents associated with homeless encampments and from wildlife from urban areas. To date we have confirmed the presence of *Rickettsia felis* in Alameda County and plan to continue our surveillance for the foreseeable future.

Introduction

Flea-borne typhus (FBT), *Rickettsia typhi*, the causative agent of murine typhus and *Rickettsia felis*, the causative agent of flea-borne spotted fever, have been linked to cases of human pathogenicity worldwide, and together they are referred to as flea-borne typhus. (Anstead 2021). There has been a resurgence of FBT cases in the United States and California over the last few decades. *Rickettsia typhi* was considered an "emerging disease" affecting the southern United States and California from 1910-1945 (Anstead 2021). This was due to unfavorable conditions and substandard housing throughout the southern U.S., which led to a proliferation of rodents and fleas. Flea-borne typhus is transmitted to humans via two different ways: 1. through the bite of an infected flea, and 2. from the feces of the infected fleas being inoculated into mucous membranes and skin abrasions, or the site of the flea bite itself (Anstead 2021). The majority of human cases manifest as fever, headaches, body aches, rash and other milder symptoms, but 26 - 28% of cases can manifest as acute, affecting multiple organ systems (Anstead 2021). Flea-borne spotted fever, caused by *R. felis,* has been found worldwide and is considered a common cause of fever in humans in Africa (Brown et al. 2016).

Historically, the primary flea vector in the southern U.S. was the Oriental rat flea, (*Xenopsylla cheopsis* Rothschild 1903) and the vertebrate hosts were the Norway or sewer rat (*Rattus norvegicus* Berkenhout 1769) and the Roof rat (*Rattus rattus* Linnaeus 1758) (Anstead 2021). It is estimated that 42,000 cases of FBT were reported between 1931-1946, but the actual number of cases may have been three-fold higher (Anstead 2021). The increase in cases was so alarming that a control program was started by the US Public Health Service from 1945-1952 and included the use of insecticides, rodenticides, and environmental remediation and sanitation (Anstead 2021). Federally funded rodent-control programs continued in the southern U.S. until the mid-1980s (Anstead 2021).

Over the last few years, cases of FBT have been increasing in California and Texas. In 2010-2018 there were a total of 868 cases of FBT in California, averaging 96.4 cases per year, a 574% increase compared to the prior decade (Anstead 2021). The urban transmission cycle of the disease occurs between the Oriental rat flea (*X. cheopsis*) and the Norway rat (*R. norvegicus*) and Roof rat (*R. Rattus*) (Anstead 2021).

Billeter and Metzger (2017) collected cat fleas, (*Ctenocephalides felis* Bouché 1835*)*, from Los Angeles, Sacramento and Contra Costa counties in 2016. *R. felis* was confirmed in the fleas via PCR, but *R. typhi* was not detected. In October 2018, Los Angeles Co. of Public Health issued a FBT alert for Los Angeles County as nine people living and working in downtown Los Angeles were confirmed as having FBT. Six of the nine people reported experiencing homelessness or living in interim housing during this period (Anstead 2021, Los Angeles Co. Dept. Public Health 2019).

Rickettsia felis was first described as a human pathogen from the United States in 1991 (Anstead 2021). Since then, *R. felis* has been found to be widely distributed and this has been attributed to the cosmopolitan nature of its main vector, the cat flea, *Ctenocephalides felis* (Anstead 2021). To date, *R. felis* has been found in more than 40 species of fleas, ticks, mosquitoes and mites, but the cat flea is the only proven vector of *R. felis* (Thepparit et al. 2013, Anstead 2021). In suburban areas, the opossum (*Didelphis virginiana* Linnaeus 1758) and feral cats have replaced rats as the primary reservoirs of FBT, with the cat flea (*C. felis*) as the main flea vector (Anstead 2021). In Alameda County we have the main flea vectors of murine typhus and FBT, the Oriental rat flea (*X. cheopsis*) and the cat flea (*C. felis*) and all of the animal reservoirs: Norway rats (*R. norvegicus*), Roof rats (*Ra. rattus*), opossums (*D. virginiana*) and feral cats.

Materials and Methods

Norway rat and Wildlife Trapping

In 2019, Norway rats were trapped at 14 different locations in and around the City of Oakland and 1 location in the City of Berkeley. In 2020, trapping changed to 4 homeless encampments in the City of Berkeley and 12 locations in the City of Oakland. This trapping protocol changed in 2021 due to the COVID-19 pandemic, with most trapping events during two nights, with 20-30 traps deployed each night. The number of traps deployed was based on the size of the encampment and the amount of observed rodent activity. The goal was to trap N=30 Norway rats from each encampment.

Pro Rat Tomahawk Live Traps (Tomahawk Live Traps, WI) were baited with a combination of canned mackerel and peanut butter. Traps were placed in the late afternoon, 14:00-16:30h. The following criteria were used to determine the best areas for trap placement: presence of fresh Norway rat droppings; active burrows (indicated by the burrow entrance being clean, smooth and free of cobwebs/debris); rat "runs" evident through neighboring vegetation/debris; and residents of the camps directing us to where they saw the most activity. After traps were set, they were covered with debris found nearby, i.e. discarded clothing, cardboard, blankets etc., 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 09:00-11:00h. Live rats were transported to the Alameda County VCSD laboratory and euthanized using CO_2 according to protocols outlined by the American Veterinary Medical Association (AVMA Guidelines 2020).

Alameda County Vector Control Services District is legally authorized to remove sick, injured and nuisance wildlife from private and commercial residences countywide, including striped skunks (*Mephitis mephitis* Schreber 1776), opossums and raccoons (*Procyon lotor* Linnaeus 1758). We also have a U.S.D.A. Wildlife Services Specialist on staff, who is federally permitted to trap and handle wildlife. Wildlife that is trapped in accordance with these policies are brought back to Alameda County VCSD and euthanized using CO_2 according to protocols outlined by the American Veterinary Medical Association (AVMA Guidelines 2020).

Table 1.—Flea Species that were tested for *Rickettsia* in 2019 from Norway rats.

Flea Species	Total Number of Fleas	Total Number of Flea Pools (Up to 5 fleas/pool)	Number of Positive Flea Pools	Minimum Infection Prevalence (%)
Xenopsylla cheopsis	327	134	10	3.0
Ctenocephalides felis	88	48	7	8.0
Nosopsyllus fasciatus	226	112	4	1.7
Pulex simulans	7	5	0	0
Leptosylla segnis	5	4	0	0

Euthanized rats and wildlife were bagged individually and sprayed with P.T. P.I. (BASF Corp., NC). Animals were left for at least one day prior to combing to ensure that all ectoparasites were dead. Animals were combed for ectoparasites using a fine bristle brush (Scotch Brite, 3M, St. Paul MN) and any ectoparasites collected were placed in 95% EtOH and set aside for identification. Fleas were identified using the taxonomic keys in Hubbard (1947).

Molecular Analysis

Fleas were sorted by collection locality, species and sex. Identified fleas were washed with 95% EtOH, followed by DI H2O, and pooled into groups of up to 5 fleas. Ceramic beads and FastPrep-24 homogenizer (MP Biomedicals) were used to disrupt tissues. DNA was purified using MagMax DNA Multi-Sample Kit Ultra 2.0 (Applied Biosystems) and KingFisher Duo purification system (ThermoFisher) according to manufacturer's recommendations. All samples were screened using the Pan-Rickettsia qPCR assay (Kato et al. 2013), and positive pools were confirmed with a species-specific qPCR (Leulmi et al. 2014).

Results

Fleas collected from Norway rats

A total of N=653 fleas were collected from Norway rats (*R. norvegicus*) in 2019 comprising five different species, including the Oriental rat flea (*X. cheopsis*) and the cat flea (*Ctenocephalides felis*) (Table 1). The majority of fleas collected in 2019 were the Oriental rat flea (*X. cheopsis*) (N=327), and the second most common flea was the Northern rat flea (*Nosopyllus fasciatus*) (N=226). We only collected N=88 cat fleas (*C. felis*), but from those 7 flea pools tested positive for the presence of *Rickettsia* and had a Minimum Infection Prevalence (MIP) of 8%. Of the *X. cheopsis* pools that were tested, 10 pools were positive with an MIP = 3.0%. Only N=4 pools of *N. fasciatus* came back as positive for a MIP of 1.7% (Table 1). None of the false human fleas, *Pulex simulans* (Baker 1895), or European mouse flea, *Leptosylla segnis* (Schönherr 1811), were positive for *Rickettsia* (Table 1).

In 2020, we collected 610 fleas comprising four species from Norway rats (Table 2). The majority of fleas collected were *X. cheopsis* (N=291) and *N. fasciatus* (N=223). We collected 85 cat fleas (*C. felis*) and tested N=39 pools for a

Table 2.—Flea Species that were tested for *Rickettsia* in 2020 from Norway rats.

Flea Species	Total Number of Fleas	Total Number of Flea Pools (Up to 5 fleas/pool)	Number of Positive Flea Pools	Minimum Infection Prevalence (%)
Xenopsylla cheopsis	291	101	1	0.3
Ctenocephalides felis	85	39	16	18.9
Nosopsyllus fasciatus	223	128	5	2.2
Leptosylla segnis	11	10	0	0

MIP of 18.9%. This was a much higher MIP than recorded for the Oriental rat flea (*X. cheopsis*), MIP = 0.3%, and the Northern rat flea (*N. fasciatus*) MIP = 2.2%, even though more *X. cheopsis* and *N. fasciatus* fleas were tested when compared to the number of cat fleas tested (Table 2). None of the *L. segnis* fleas tested positive for *Rickettsia*. These results were similar to the results in 2019.

Fleas Collected from Wildlife

In 2019 we tested a total of 440 fleas collected from skunks, opossums and raccoons in Alameda County (Table 3). From opossums, 293 cat fleas were tested in 66 pools; we also collected 13 *Pulex* and tested these in 3 pools. Overall, 45 flea pools from opossums tested positive for *Rickettsia* with an MIP of 15.4%. We also tested 25 flea pools from raccoons with 9 positive; 6 pools of *C. felis* for a MIP = 12.2% and 3 pools of *P. simulans* for a MIP of 8.1%. We tested 48 *Pulex* fleas from skunks and 1 pool was positive for *Rickettsia*, with a MIP = 2.0%. (Table 3).

In 2020, we tested 284 fleas from wildlife, with the majority (N=253) being cat fleas (N = 247) collected from opossums (Table 4). Of the fleas tested from opossums, all of the *C. felis* pools (N=37) were positive with a MIP of 15%; but none of the *Pulex* (N=6) tested positive. The fleas collected from one raccoon (N=5 *C. felis* with MIP=20%) were positive for *Rickettsia,* but the cat flea (N=1) from the skunk was not. No *Pulex* fleas were collected from raccoons in 2020, and of the N=2 pools of *Pulex* tested from skunks, both were positive for *Rickettsia* (MIP=8%) (Table 4).

Table 3.—Fleas combed from wildlife and tested for Rickettsia in 2019

	Total Number of Fleas	Total Number of Flea Pools (Up to 5 fleas/pool)	Number of Positive Flea Pools	Minimum Infection Prevalence (%)
Opossums				
C. felis	293	66	45	15.4
P. simulans	13	3	0	0
Raccoons				
C. felis	49	14	6	12.2
P. simulans	37	11	3	8.1
Skunk				
P. simulans	48	11	1	2.0

Table 4.—Fleas combed from wildlife and tested for *Rickettsia* in 2020.

	Total Number of Fleas	Total Number of Flea Pools (Up to 5 fleas/pool)	Number of Positive Flea Pools	Minimum Infection Prevalence (%)
Opossums				
C. felis	247	54	37	15.0
P. simulans	6	4	0	0
Raccoons				
C. felis	5	1	1	20.0
Skunk				
P. simulans	25	2	2	8.0
C. felis	1	1	0	0

Discussion

Alameda County Vector Control Services District surveyed flea diversity on Norway rats and wildlife (opossums, skunks and raccoons) in 2019 and 2020, and collected a total of 1,986 fleas. Most (N=1,263) fleas were collected from Norway rats; 723 fleas were collected from skunks, opossums and raccoons. Fleas from both Norway rats and wildlife tested positive (N=138 pools) for the presence of *Rickettsia*. Of the five flea species tested, the most positive pools were from the cat flea (N=112). Fleas were sent for molecular sequencing to determine what species of *Rickettsia* were present, and *Rickettsia felis* was confirmed for fleas that were associated with Norway rats, opossums, raccoons and skunks. No pools were positive for *R. typhi*.

Between 2019 and 2020, 300 *X. cheopsis* and 200 *N. fasciatus* were collected from Norway rats. Even though *C. felis* were not the most abundant flea species collected from Norway rats, they had a higher MIP than *X. cheopsis* or the *N. fasciatus*. This pattern aligns with the shift in the transmission cycle of flea-borne typhus, that has been documented previously in southern California (Anstead 2021). The urban cycle of flea-borne typhus historically has been between Norway rats and fleas, specifically *X. cheopsis*. The suburban cycle involves opossums, feral cats, and *C. felis* and the transmission of flea-borne spotted fever (*R. felis)* (Anstead 2021, Nelson et al. 2015) and was indicated to be occurring in the area of reported human cases of flea-borne Rickettsiosis in 2020 in Pasadena (CDPH 2020). Our research confirms the presence of *R. felis* in cat fleas in Alameda County. Opossums have been shown to carry a high flea load (Anstead 2021, Nelson et al. 2015) and a single opossum can harbor several hundred fleas. This can cause an issue in terms of potential disease transmission to humans when the animals start harboring in and around man-made dwellings.

Alameda County VCSD plans to continue to collect fleas from Norway rats, skunks, opossums and raccoons in Alameda County and test them for the presence of *R. felis* and *R. typhi* as part of our on-going County-wide disease surveillance program. We do not anticipate that the number of unhoused and housing-insecure individuals to decrease in the County in the foreseeable future and our District's mission is to provide disease surveillance and control to all of the residents of Alameda County.

Acknowledgments

AMW would like to thank the staff at Orange Country Mosquito and Vector Control District for their assistance with this project. Alameda Co. Vector Control would specifically like to thank Bob Cummings and Laura Kreuger at Orange County VC for their technical assistance and guidance. AMW would also like to thank the staff at Alameda Co. VC for their continued hard work and dedication to this project, including Dr. Lucia Hui, our former Chief and our current Chief Robert Gay; this project took shape under their tenure.

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Response to fleas and flea-borne typhus by the Orange County Mosquito and Vector Control District

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Abstract

Flea-borne typhus (FBT) is increasing in prevalence in southern California. An effective strategy to prevent flea-borne typhus is to avoid exposure to fleas and flea feces. Although it is unclear exactly how most FBT victims acquire infection, many are exposed routinely to fleas around the home from their pets. Pets acquire fleas from backyard wildlife and free-ranging animals in the community. In 2021, the Orange County Mosquito and Vector Control District defined four FBT scenarios that staff would respond to in Orange County including: 1) infestation of fleas in a public space such as a school, park, or worksite; 2) human case of typhus; 3) cluster(s) of human cases of typhus; and 4) detections of flea-borne typhus bacteria in fleas. The response to these scenarios included an inspection of the site for evidence of flea or animal activity, control of fleas, determination of the intervention area, and notification of impacted residents. The notification process included placement of FBT notification signage at major intersections within 0.48 km (0.3 mi) of an intervention area, and distribution of FBT postcards to parcels within the intervention area advising residents of typhus activity and to keep pets on flea control. Additional responses to FBT include flea control at impacted property(s); distribution of topical flea control medication to residents; inspection of public places for flea activity; and property inspections of parcels adjacent to human case detections.

INTRODUCTION

From 2006-2020, the Orange County Mosquito and Vector Control District (District) responded to 221 human cases of flea-borne typhus with reported exposure sites in Orange County, California (CDPH, 2022). Lessons learned from the investigations of human case exposure sites and experience gained from notifying impacted communities led the District to define specific responses to flea infestations in public spaces and detections of flea-borne typhus in people or fleas in Orange County. The Flea-borne Typhus (FBT) Response Plan documents a set of predetermined procedures conducted by each of the four District Departments (Operations, Scientific and Technical Services, Communication and Administration) in response to the following: 1) infestation of fleas in a public space such as a school, park, or worksite; 2) human case of typhus; 3) cluster(s) of human cases of typhus; 4) detections of flea-borne typhus bacteria (*Rickettsia typhi* and *R. felis*) in fleas. Our paper summarizes the District's overall strategy for FBT prevention and control, and details of the response to fleas and flea-borne typhus in Orange County during 2021.

District Strategy for FBT Prevention and Control

The strategy to control a pest can be defined as the integrated use of various control tactics against an entire pest population within a geographical area to achieve economic control, defined as the density at which losses exceed cost of control (Barclay et al. 2011). The concept of economic control is not used in public health response to vector-borne disease in the same way as crop management. Public health agencies responding to vector-borne diseases define economic control as a cost-efficiency analysis of the resources necessary to lower the number of vectors, the number of infected vectors, or the number of human cases (Stafford 1993). The CDPH defines economic control for plague, a flea-vectored disease, as the use of available methods to reduce the number of fleas on host animals to less than one per animal (CDPH 2021). To control FBT, the tactics used must accomplish the following: reduce flea density, host density, and/or the prevalence of FBT bacteria (*R. typhi* or *R. felis*) in fleas; eliminate the pathogen from humans, hosts, and fleas (through vaccination or microbial treatment), and modify human behavior to reduce exposure to fleas. However, the ecology of fleas and host animals in urban environments, and the lack of area-wide interventions targeting backyard wildlife limits the effectiveness of many of the potential tactics for FBT control, both at the area-wide and single residential property level.

The primary goal of the District's FBT Response is to educate Orange County residents that fleas found on cats, dogs, opossums, rabbits, raccoons, skunks, squirrels, and coyotes can carry the bacteria that cause FBT in humans. The best way to prevent FBT is to keep pets on flea-control medication year-round and restrict backyard wildlife from nesting or feeding on residential and commercial property.

Table 1.—The "toolbox" for integrated flea management and control of flea-borne typhus.

Personal protection measures	Avoid contact with fleas or flea feces Protective clothing Synthetic chemical repellants Natural product-based repellents Permethrin-treated clothing
Treatment of human disease	Antibiotic use
Landscape/vegetation management	Xeriscaping/hardscaping to limit flea development sites Removal of animal harborage and nests Trimming of fruit trees and removal of fallen fruit
Flea control Landscape Pets Rats/Mice Wildlife[1] Cat Colony[1]	Synthetic pesticides (IGR, pyrethroids) Botanically-based pesticides Pesticide dusts applied to animal burrows Biological agents and biopesticides (entomopathogenic fungi, nematodes) Regular application of topical or oral pesticides to control fleas on pets Oral systemic flea control pesticide (IGR) in cat food[1]
Host animal management Habitat modification Pets Rats/Mice Wildlife	Xeriscaping/hardscaping to limit attractive harborage and food for wildlife, rats/mice, feral cats Fencing or other exclusion to limit backyard wildlife and feral cats Rodenticide bait boxes to control rats/mice Feed pets indoors Spay/neuter pets

[1] Strategy not available but needed for effective FBT control.
Modified from Stafford (2017).

Epidemiologic analyses of human case investigations have found that greater than 80% of cases have an exposure to fleas in their own homes and yards, and therefore keeping pets on flea control year-round is recommended for all residents of Orange County (OCMVCD 2022).

The FBT education materials produced by the District recommend the following to reduce exposure to fleas: ensure pets (cats, dogs, rabbits, rats) are free from fleas and consult your veterinarian about flea control options, keep pet cats indoors, remove outside food sources, cover garbage containers, trim vegetation around buildings to discourage wildlife, report dead opossums, cats, and other animals in public spaces to local animal care agencies for removal, or use personal protection when removing dead animals from property. The District maintains a robust notification program that provides FBT educational materials to impacted city jurisdictions, animal control agencies, and properties adjacent to known exposure and detection sites. Additionally, signage indicating the presence of FBT in the community is hung within a defined intervention target area. For clusters of human cases, mailers delivering FBT public health messages are sent to parcels within the intervention area (OCMVCD 2022).

The main barrier to effective flea-bite prevention for people and pets is the constant vigilance necessary to prevent bites and infestations, because all life stages of fleas are active year-round in Southern California. The current available "toolbox" for control of fleas and FBT in the United States is listed in Table 1. The District continues to advocate and support research and development of new tools to reduce the prevalence of FBT in flea populations and reduce the population of fleas on host animals (pets, rats, wildlife, cats in colonies). To reduce flea density at FBT exposure sites or adjacent properties, the District's strategy includes providing topical flea control medication free of charge and conducting flea control when fleas are present with permission from the owner/resident.

Human cases of FBT are reported to the Orange County Health Care Agency (OCHCA) and shared with the District through a process outlined in a Memorandum of Understanding adopted in 2012. As per the MOU, all District staff are trained annually in HIPAA compliance, and the District has discretion to share information with local animal care agencies for assistance, when needed, as determined during investigations of suspected exposure sites.

The foundation of the District's FBT Response is the inspection and investigation of exposure sites for conditions conducive to FBT. The investigation relies on permission from the case, family member, or property owner, to inspect the exposure site for fleas, feeding of wildlife or pets outside, presence of vegetation or harborage for backyard wildlife. If fleas are present, they can be collected and submitted for testing at the District's Laboratory (Rangel et al. 2019). Properties with an active flea infestation can be treated with a residual insecticide to control eggs, larvae, and adult fleas. If fleas or host animals are present, the District works collaboratively with property owners to reduce pest populations using the process outlined in the District's Vector Reduction Manual (OCMVCD 2010).

The District does not remove, or cull, vertebrate animals present at exposure sites, due to concerns associated with flea population emergence following animal removal. Results from the site investigation, flea test results (when available), and presence of additional human cases within 0.5 mi (0.8 km) in the last 6 months, determine the target area and scope of the intervention. Following target area determination, signage and notifications are distributed to properties in the area, advising residents that FBT has been detected in the neighborhood and to ensure pets are up to date with flea medications. Notification of activities within the target area are provided to the city, community, and District Trustee. In response to public requests generated from signage and notification campaigns, the District educates residents to keep their pets on flea- control medication year-round, and if residents indicate a need, and live within the target intervention area, topical flea control medication can be provided. The topical flea control medication provided by the District is available over the counter (OTC), and residents sign a liability waiver stating they are aware the product is available OTC and to consult a veterinarian prior to application, if they have concerns. If conditions such as cat colonies, feral animals, or animal hoarding are present or suspected, at time of inspection, the

District coordinates transfer of information to animal care agencies and shelters.

Response to a Human Case or Detection of Fleas Positive for FBT

Human FBT cases received from OCHCA or positive flea detections by the District will be forwarded to the District's Operations Supervisor who will coordinate the response with input from a District Vector Ecologist. Inspection of the exposure site (index property) is arranged with permission of the case, family, or other resident or property owner. The Operations Supervisor or Vector Ecologist contacts the case and sets up a time for the exposure site inspection. During inspection, the case is queried regarding flea exposure and the presence of wildlife and feral cats in the neighborhood, place of work, school or other potential exposure site visited in the 14 days prior to illness onset. The case interview includes questions, such as: Do you own pets? Are your pets current with their flea medication? Are there fleas present on the property? Do any neighbors feed unowned cats? Did you recently adopt a pet? Do you see backyard wildlife? Do you see or hear signs indicating animals are living in the walls or underneath the home?

If conditions conducive to FBT are present at the exposure site, the Operations Supervisor or Vector Ecologist provides recommendations to abate the infestation and provides topical flea control medication for pets on site, if requested. If a flea infestation is present outside the home, staff will coordinate pesticide application and schedule a follow up treatment in two weeks, if warranted. The property owner will be referred to private pest control if they have fleas inside the residence in the absence of a pet, or if more than two exterior flea-control treatments are needed. Outreach materials notifying residents that FBT has been detected in the neighborhood and instructing them on the best way to protect their property by making sure their pets are on flea control. If requested, the adjacent properties are offered topical flea control medication for pets. Findings from the exposure site and surrounding area are assessed to determined if conditions conducive to fleas and backyard wildlife are present (hoarded homes, presence of animal food on street or sidewalk, presence of cats on front lawns or street, obvious sanitation issues, such as open dumpsters).

If these conditions are present in the neighborhood, an escalated response may be necessary, and includes increasing the intervention target area, and referrals to agencies responsible for enforcing local health and safety codes (city code enforcement, local animal control jurisdictions, and the OCHCA). The District works collaboratively with property owners to abate significant vector sources through the process outlined in the District's Vector Reduction Manual.

The notification process for a single case includes notifying the city, District Trustee, and local animal care agency of the FBT detection and target area for intervention. City and animal care agencies are leveraged to coordinate inter-agency messaging. Signs indicating FBT in the community are placed within 0.48 km (0.3 mi) of the exposure site. District Communications staff are informed of the FBT intervention area to ensure resident's questions are answered, and the website is updated with a map detailing year-to-date FBT activity (OCMVCD, 2022).

Response to a Cluster of Human FBT Cases

The District defines a human cluster of FBT as two or more human cases occurring within 0.8 km (0.5 mi) over six months. Clusters of more than two human cases within 0.8 km (0.5 mi) have been identified historically in Orange County. The size of the intervention area increases during the response to a cluster of human cases (0.5 mi), as compared to the area for a single case response (0.48 km, or 0.3 mil). For clusters of two cases, signs are placed within a 0.8 km (0.5 mi) diameter intervention area. For three or more cases, signs are placed within the entire 0.8 km (0.5 mi) diameter intervention area that encompasses all cases. For clusters with three or more cases, a direct mail campaign distributed to parcels in the intervention area is considered. A press release indicating FBT is active in the affected area may be distributed.

Response to Fleas in Public Spaces

Reports from residents of fleas in public spaces are received at the District as a Service Request. The Service Request is forwarded to an Area Supervisor who will arrange an initial inspection with a Vector Ecologist whose primary role is to advise on safety issues and potential personal protective equipment for District staff. The Area Supervisor or Vector Ecologist will inspect adjacent properties and provide written findings and flea abatement recommendations to the property owner or management. If conditions conducive to FBT are present in the neighborhood, an escalated response may be necessary and includes increasing the intervention target area, and referrals to agencies responsible for enforcing local health and safety codes (city code enforcement, local animal control jurisdictions, OCHCA). The District works collaboratively with property owners to abate significant vector sources through the processes outlined in the District's Vector Reduction Manual.

Preventing Flea and Flea-borne Typhus Exposure to District Staff and Personal Protective Equipment Requirements

Inspections for FBT target the exterior or outside areas of a property. District staff are advised not to inspect the interior of homes, enter confined spaces, sub-areas, or other interior spaces, such as garages or sheds. If an interior inspection is necessary, an N100 or P100 respirator and eye protection should be worn.

Appropriate Personal Protective Equipment (PPE) to be worn on an exterior inspection include: long-sleeved uniform shirt, pants and closed-toed shoes. An EPA Registered insect repellant should be applied to the uniform prior to site inspection. To prevent flea exposure, pant legs should be tucked inside socks. If fleas are present, cease the inspection until flea control occurs at the site, or until assessment of the property by a Vector Ecologist. If fleas are removed from the uniform, they can be placed in a vial of 70% isopropyl alcohol and submitted to the District Laboratory for testing with a designated specimen identification number and typhus case code. Flea bites that occur during work time should be reported to a Supervisor.

Summary of OCMVCD FBT Response, 2021

The Flea-borne Typhus Response Plan documents the pre-determined set of procedures and processes utilized by the District to respond to FBT in Orange County. The plan aims to rapidly mobilize services that strive to slow or stop the transmission of FBT in impacted communities, informing residents that FBT is detected in the area and that pets should be treated with flea control products. In 2021, the District investigated 24 human cases of FBT in nine cities. The case investigations resulted in notification of 177 properties adjacent to exposure sites, distribution of 45 pet topical flea control doses, and treatment of fleas at five residential properties. An additional 579 notices were placed on properties within 0.48 km (0.3 mi) of cases. Teams hung 438 FBT notification signs in impacted communities; the signs were removed at the end of the year (December). In response to a cluster of three or more cases within a 0.8 km (0.5 mi) dia. area, the District mailed 5,413 notices to parcels in the intervention area. The signage generated 21 public calls to the District requesting more information. Twenty-one service requests for fleas in public spaces were reported in four cities. The District tested 1,069 fleas, of which 51 (4.7%) were positive for *R. typhi*, 61 (5.7%) were positive for *R. felis*, and 10 (0.01%) were found co-infected with both *R. felis* and *R. typhi*. The FBT Response Plan will be reviewed and updated annually.

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Challenges of responding to flea-borne typhus cases: lessons learned after 15 years of investigations in Orange County

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Abstract

Since 2001, flea-borne typhus (FBT) has reemerged as an important vector-borne disease in Los Angeles and Orange counties. The reasons for this resurgence are unclear, but this rise has been accompanied by changes in how public/private animal control groups manage "nuisance" animal populations, especially opossums and feral cats, the primary hosts for the cat flea, *Ctenocephalides felis*, a key vector of FBT in southern California. These changes include no longer recognizing the removal of opossums and feral cats as a flea control strategy and implementation of "return to field" programs that place, in lieu of euthanization, impounded cats in neighborhoods without on-going flea control treatments. Since the first reported FBT case in 2006 in Orange County (the first since 1993), the Orange County Mosquito and Vector Control District (OC Vector) has investigated 237 of the 249 reported FBT cases in the county. Investigations have included patient interviews, ecologic assessments of putative exposure sites, testing of flea and animal specimens collected from these sites for rickettsial bacteria, and public outreach on FBT prevention in affected areas. These investigations have shown that peridomestic animals, such as opossums and feral cats, support populations of fleas infected with *Rickettsia typhi* and *R. felis*, the bacteria responsible for human FBT. OC Vector and other local health care agencies have recognized a link between feral cat feeding sites and heightened disease risk in FBT-affected neighborhoods. Despite this observation, local human health care agencies, animal care groups, and city code enforcement agencies have been unwilling, or unable, to implement policies to stop the proliferation of feral cat colonies in areas with high FBT disease risk. The continued rise of FBT has proven difficult to mitigate because of conflicting perspectives among governmental agencies and animal rights advocates, who perceive the zoonotic disease risk associated with cat rescue programs at significantly different thresholds of concern.

INTRODUCTION

Since 2001, flea-borne typhus (FBT) has reemerged as an important vector-borne disease in California, with 1,506 human cases (0 deaths) reported through 2021 (CDPH 2012, CDPH 2020, CDPH 2022a). Flea-borne typhus is the second most common vector-borne disease in the state after West Nile virus disease (7,471 cases, 359 deaths, CDPH 2022b), with most FBT typhus cases (95.7%) occurring in Los Angeles and Orange counties [1,192 (79.1%) and 249 (16.5%) cases, respectively, Figure 1] (CDPH 2012, CDPH 2020, CDPH 2022a). Over the last nine years (2013-2021), more locally-acquired FBT cases have been reported in Ventura (3), San Bernardino (13), Riverside (11), and San Diego (8) counties compared to previous decades (CDPH 2012, CDPH 2020, CDPH 2022a). In affected areas, FBT cases appear year-round, with most occurring from May to October (CDPH 2021). Human FBT cases are reportable to the California Dept. of Public Health (CDPH) under Title 17 of the California Code of Regulations, Section 2500 (CDPH 2021). The Orange County Health Care Agency (OC Health Care) reports FBT cases to the Orange County Mosquito and Vector Control District (OC Vector) following guidelines outlined in a 2012 Memorandum of Understanding (MOU) between the two agencies for the confidential exchange of health care information (HIPAA 1996, OC Health Care 2012).

Flea-borne typhus is caused by infection with either of two rickettsial bacteria, *Rickettsia typhi* and *R. felis*, which are transmitted to humans by infected fleas, principally the oriental rat flea (*Xenopsylla cheopis*) and the cat flea (*Ctenocephalides felis*), respectively (Azad et al. 1997, Azad and Beard 1998, Reif and Macaluso 2009). Most infections are reported to be mild or asymptomatic, but for clinical cases, symptoms typically appear 7-14 days after exposure and include high fever, headache, chills, rash, myalgia, and other non-specific symptoms; in more severe cases, respiratory distress, renal failure, encephalitis, and endocarditis can occur (CDPH 2019). Fatalities are rare ($< 1\%$) when treated with appropriate antibiotics (typically, doxycycline), depending on the age and co-morbidities of victims (CDPH 2019). In Orange County, approximately 85 - 91% of FBT victims have been hospitalized, with a median length of stay of 5 days (range 1 - 368 days) (Cummings et al. 2014, OC Health Care 2019).

In southern California, most human FBT cases have been attributed to the suburban rickettsial transmission cycle involving the cat flea and its primary mammalian hosts,

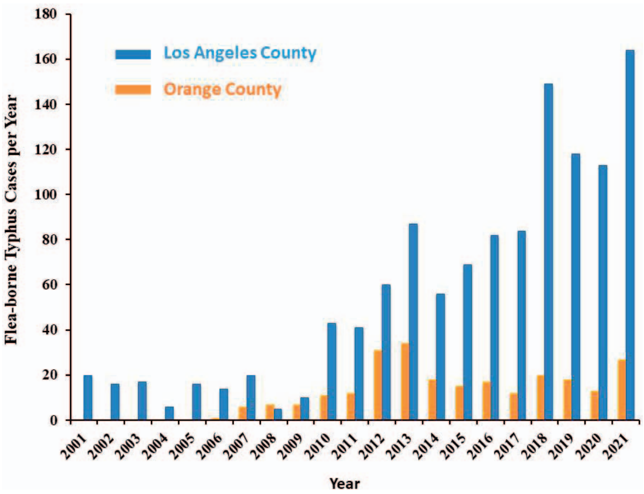

Figure 1.—Flea-borne typhus cases per year in Los Angeles and Orange Counties, 2001-2021.

opossums and cats (Adams et al. 1972, Sorvillo et al. 1992, Eremeeva et al. 2012, Krueger et al. 2016, Maina et al. 2016, Jia et al. 2021). With the exception of a few case clusters in Los Angeles County, the urban (murine) rickettsial transmission cycle, involving the oriental rat flea, commensal rodents (e.g., Norway, roof rats) and *R. typhi* (Dyar 1944), now plays a much smaller role in outbreaks in the region (Adams et al. 1972, Schwan et al. 1985, Sorvillo et al. 1992, Civen and Ngo 2008, Karpathy et al. 2009, Abramowicz et al. 2011, Eremeeva et al. 2012).

In contrast to the urban FBT cycle, *R. felis* may be the primary pathogen in the suburban transmission cycle. *Rickettsia felis* is found substantially more often than *R. typhi* in cat fleas and tissues of domestic and peridomestic animals in southern California (Williams et al. 1992, Eremeeva et al. 2012, Krueger et al. 2016, Maina et al. 2016, Mullins et al. 2018, Penicks et al. 2019, Penicks et al. 2020). However, Wiggers et al. (2005) (Texas), Billeter and Metzger (2017), and Blanton and Walker (2017) (Texas) attribute most FBT cases in Texas and California to infections with *R. typhi*. The serologic cross-reactivity of *R. felis* with the *R. typhi* antigen in standard diagnostic tests (IgM/IgG IFAs) used in the U.S. has precluded differential diagnoses in humans (Schriefer et al. 1994, Wiggers et al. 2005, Civen and Ngo 2008, Eremeeva et al. 2012). In contrast, discriminatory serologic studies in Spain (Nogeras et al. 2006), Mexico (Pérez-Osorio et al. 2008), Australia (Teoh et al. 2016), and Taiwan (Yang et al. 2021) have implicated *R. felis* as an important pathogen for FBT. Although molecular methods offer specificity for diagnosis of acute rickettsial disease, samples of whole blood are often not taken or tested before administration of antibiotics, making direct molecular determination for either *R. typhi/R. felis* infection unlikely; furthermore, rickettsemias are short-lived in the blood of humans (Caravedo-Martinez et al. 2021). To date, no flea-borne rickettsial disease cases attributed to infection with *R. felis* have been diagnosed in California (Billeter and Metzger 2017).

The causes for the increase in FBT cases, the geographical spread, and the specific etiologic agent(s) responsible in affected southern California counties are not well-defined. However, this rise has been accompanied by changes in how public and privately-sponsored animal control groups manage "nuisance" animal populations, especially opossums and feral cats, the most important hosts for *Rickettsia*-infected cat fleas (Eremeeva et al. 2012, Krueger et al. 2016, Penicks et al. 2020, Li et al. 2021). The 1998 passage of the Hayden Law (SB 1785, CA Chapter 752, Statutes of 1998) has required animal shelters to adopt strategies to reduce shelter euthanization rates and pet overpopulation. These strategies have included cessation of euthanasia of uninjured peridomestic wildlife (e.g., opossums, raccoons) and an emphasis on wildlife rehabilitation, and pet adoptions and sterilization.

To address the issue of having to reduce the euthanization of healthy, unadoptable cats surrendered to shelters, public and private animal care agencies have often instituted "Trap-Neuter-Return" (TNR) and "Return-to-Field" (RTF) programs in partnership with cat advocacy and rescue groups. TNR is a program in which feral cats are captured in the field, sterilized, treated for feline diseases and fleas, and returned to the community in which they were found. RTF refers to the practice of sterilizing and releasing non-feral cats not adopted within a specified time period to the field instead of euthanasia. Once cats are relinquished to a designated animal rescue group, TNR/RTF cats are to be cared for in 'cat colonies' by volunteer caretakers (Slater 2004). (By definition, a feral cat is free-roaming and, unlike stray and pet cats, not socialized to people, CA FAC Sec. 31752, 1998). The Orange County Animal Care Agency (OC Animal Care) initiated a pilot TNR program in 2009 in partnership with Feral Alley Cats and Friends of SPCA as an alternative to euthanasia and in an attempt to reduce cat overpopulation; the pilot program became fully operational as "Feral FREE" in 2013 (Cummings et al. 2016).

In southern California, several local public health agencies (health departments, vector control agencies) have expressed concern that these publically/privately-supported TNR programs may be contributing to the rise of FBT cases within their jurisdictions (LAC DPH 2009, OC Vector 2014, OC Grand Jury 2015a, Wekesa et al. 2016, Nelson et al. 2018, LAC DPH 2019). The current paper will outline some of the challenges OC Vector has faced in addressing the rise of FBT in the county and will discuss the development of its comprehensive FBT surveillance and mitigation programs.

Challenge: Defining FBT High Risk Areas

Mapping the spread of FBT in Orange County

From the 1960 to 2000, cases of FBT averaged < 20 cases and < 2 cases per year in Los Angeles and Orange counties, respectively (Wekesa et al. 2016a, Cummings et

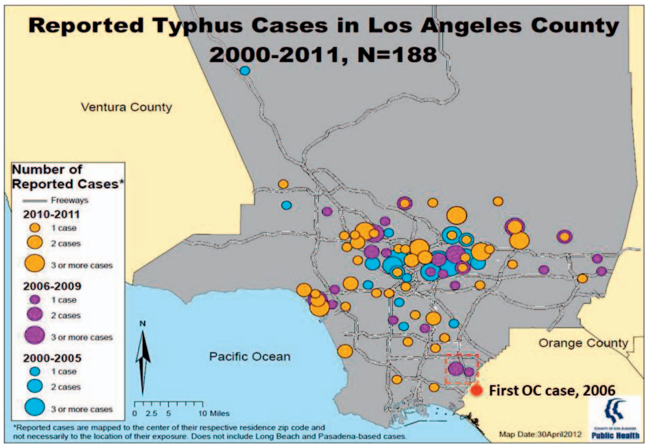

Figure 2.—Location of flea-borne typhus cases in Los Angeles County (2000 – 2011) and the first case identified in Orange County, 2006 (red circle). The OC County case was located near the Los Angeles/Orange County border and was randomly offset by 0.5 km (0.3 mi) for display per HIPAA and OC Health Care/ OC Vector MOU.

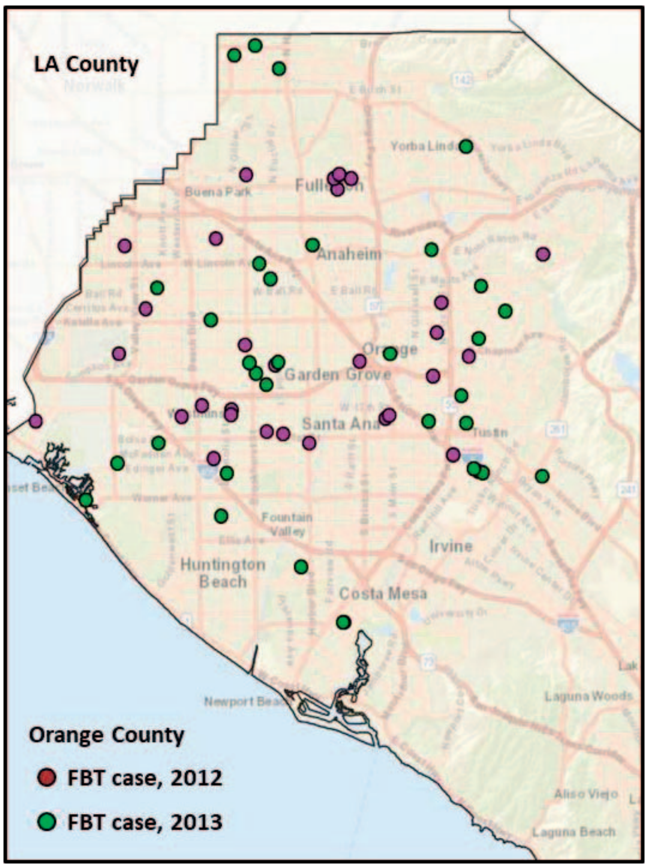

Figure 3.—Distribution of flea-borne typhus cases during 2012 (green) and 2013 (purple) in Orange County. Flea-borne typhus cases were randomly offset by 0.5 km (0.3 mi) for display per HIPAA and OC Health Care/OC Vector MOU.

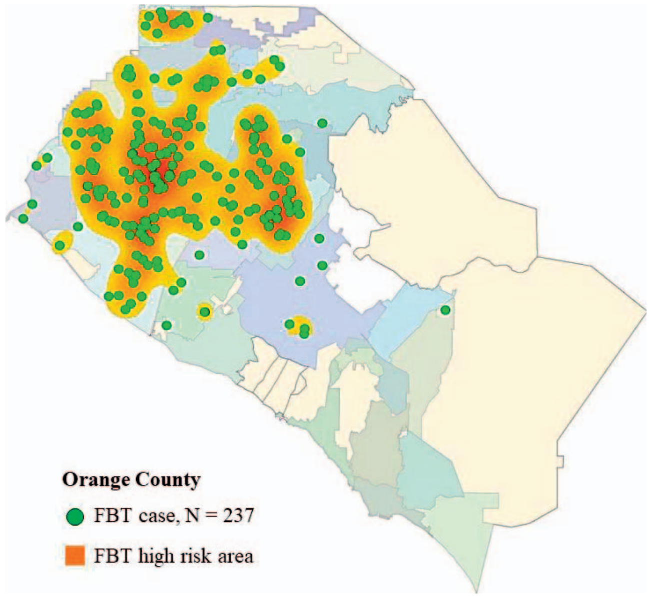

Figure 4.—Hotspot map of flea-borne typhus cases (green circles) in Orange County, 2006 – 2021. FBT cases were randomly offset by 0.5 km (0.3 mi) for display per HIPAA and OC Health Care/ OC Vector MOU. Some cases overlap because of their close proximity to one another. ESRI Spatial Statistics Tool, Hotspot Analysis/Getti-Ord GI*.

al. 2014). In 2006, the city of Long Beach (an L.A. County city adjacent to Orange County) recorded its first FBT cases (6) (LB HHS 2021), and Orange County recorded its first FBT case since 1993 (resident lived on the Long Beach-L.A. County/Orange County border, Figure 2). Within seven years, both the numbers and distributions of FBT cases increased, reaching highs of 31 and 34 cases, respectively, over wide portions of central and northern Orange County during 2012 and 2013 (Figs. 1, 3).

With FBT case addresses provided by OC Health Care, OC Vector has performed annual hotspot analysis (ESRI, ArcMap 10.2, Spatial Statistics Toolset, Hotspot Analysis 2013) to identify high risk FBT areas in the county. Figure 4 shows significant clustering among 198 of the plotted 237 FBT cases (79.7%). For the hotspot areas (shading), FBT case densities ranged from 0.5 – 1.7 cases/km^2 (1.2 – 4.4 cases/mi^2) ($p < 0.05$, Z-score 1.90 – 8.16, Getis-Ord GI*), a significant 7.1% increase in case clustering (99/135, 72.6%, $p < 0.05$, Z-score 3.6 – 11.5), since a similar analysis was done in 2016 (Cummings et al. 2016). (For epidemiological and hotspot mapping purposes, it is assumed that rickettsial transmission occurred at or near the reported case address). Since 2011, FBT cases have averaged 20.5/year (range 12 – 34) and are heavily concentrated in north-central areas of the county (Figs. 1, 4).

After a public information request, OC Animal Care provided OC Vector with the addresses of TNR cat release sites (N = 8,160 cats) for 2013 – 2018. These addresses were assigned geolocation coordinates and plotted on a map; next, a hotspot map depicting a FBT case density analysis for 191 cases from 2006 – 2018 was projected over

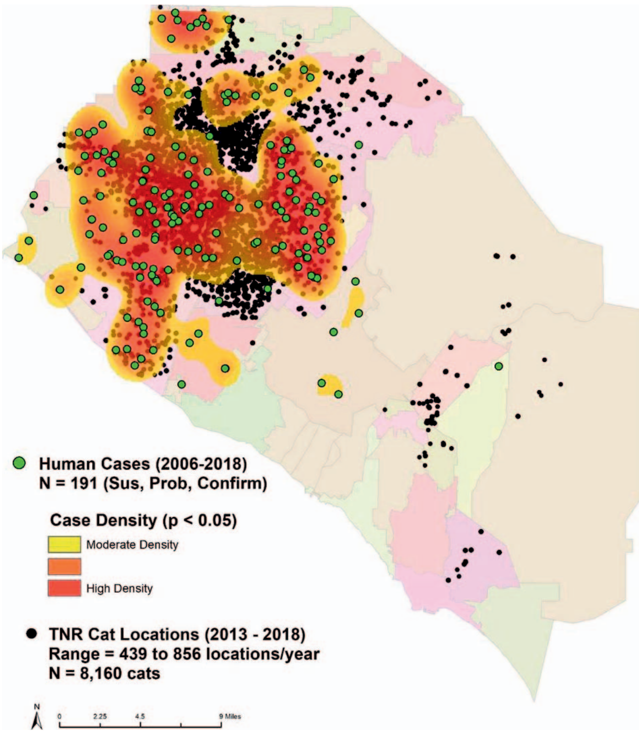

Figure 5.—Hotspot Analysis of flea-borne typhus cases (green circles) (2006 – 2018) and TNR feral cat release sites (black dots), 2013 – 2018. Flea-borne typhus cases were randomly offset by 0.5 km (0.3 mi) for display per HIPAA and OC Health Care/OC Vector MOU. ESRI Spatial Statistics Tool, Hotspot Analysis/ Getti-Ord GI*.

the TNR cat placements (Fig. 5). Although this hotspot analysis of human FBT cases demonstrated the clustering of cases (134/183, 73.2%, $p < 0.05$, Z-score 3.1 – 7.2), it did not indicate a causal relationship between placement of TNR cats and FBT transmission. However, it does show that the majority of Orange County FBT victims live in cities that received TNR cats as part of OC Animal Care's Feral FREE program (Fig. 5), thereby augmenting the flea populations that become vectors of FBT among cats, wildlife, and humans. (For Figs. 3–5, FBT cases addresses were randomly offset by 0.5 km (0.3 mi) for display to comply with the Health Insurance Portability and Accountability Act (HIPAA) and the OC Health Care/OC Vector MOU for patient confidentiality).

Challenge: Lack of local testing for FBT rickettsia in fleas and animal specimens

Cooperative research programs and development of an in-house FBT testing program

At the start of the FBT outbreak in Orange County, no county, state, or university laboratory in California were prepared to conduct routine testing of fleas and animal tissues for FBT bacteria. Because of this lack of local testing capability, OC Vector initiated a multi-agency FBT research study with the Los Angeles County Department of Public Health (LAC DPH), CDPH, and the Center for Disease Control and Prevention (CDC) in 2008. Following the completion of the CDC project (Eremeeva et al. 2012), OC Vector commenced three projects with the U.S. Naval Medical Research Center (NMRC) (Silver Spring, MD) and other interested entities. In Orange County, Maina et al. (2016) examined the prevalence of *R. typhi*/*R.felis* and other *R. felis*-like organisms (RFLOs) in fleas, and Krueger et al. (2016) documented these disease agents in urban opossums; finally, Mullins et al. (2018) explored rickettsial agents in euthanized cats of Riverside County. OC Vector laboratory staff received training for rickettsial testing of flea and animal specimens from NMRC while participating in these studies. After training, OC Vector implemented a singleplex real-time PCR assay in 2012 and recently developed a duplex PCR assay for simultaneous detection of *R. typhi* and *R. felis* in flea and animal specimens (Rangel et al. 2019). OC Vector has hosted several FBT educational workshops with local county/city health care agencies, school districts, vector control agencies, animal care groups, CDPH, and commercial pest control companies to discuss awareness of FBT in communities, control measures, neighborhood notifications, and coordination of responses to outbreaks.

Challenge: Conflicts with cat advocacy groups on FBT control actions

Refocus of animal control agencies to animal care agencies

In the U.S., feral cat advocates have gained political strength and influenced state and local laws regarding the funding and actions of organizations dealing with animals (Longcore 2009). Today, most publically sponsored animal care services focus on animal rehabilitation, adoption and rescue through partnerships with rescue groups, rather than providing animal control through density reduction. Increasingly, many public shelters have defined themselves as agencies designed to save animal lives, not to end them (Kortis 2014). One nationwide effort, the Million Cat Challenge, is a joint project of the UC Davis Koret Shelter Medicine Program and the University of Florida Maddie's Shelter Medicine Program advocating "return-to-field" as one of its five key initiatives to improve feral cat lives (Million Cat Challenge 2022).

With the requirements of the Hayden Law becoming effective in 2010, OC Animal Control was re-named as OC Animal Care and was transferred from the OC Health Care Agency to the OC Community Resources Department in 2008 for better outreach to the public and to emphasize the agency's role as an animal welfare organization. OC Animal Care started a pilot feral cat TNR program in 2009. The Program's objective was to reduce cat overpopulation by increasing sterilization of feral/stray cats and to decrease cat euthanization by adoption and returning impounded, unadoptable cats to the field.

In April, 2012, a teenage child in the city of Santa Ana, who often played at a nearby intermediate school, became ill with FBT. A subsequent ecologic investigation by OC

Vector in May found that the middle school in question had feral cats on the campus. Although some students, staff and public fed and cared for the cats, others at the school complained about cat feces and fleas. With support from the school administration, OC Vector and the Santa Ana Police Department worked together to trap and remove the cats from school property and transfer them to the city's Animal Services. Against OC Vector's advice, the Santa Ana Police Department announced their action plan to the public in a press release. A nationwide outcry quickly ensued against the removal and prospective euthanization of the feral cats. Several cat advocacy groups, *Alley Cat Allies* (2012), *Animal People* (2012), and *Catster* (Kelly 2012), hosted online forums for protesters, resulting in thousands of calls and emails to OC Vector and the Santa Ana Police Department opposing the removal of the cats; deployed traps were found to have been triggered prematurely (Anstead 2020). In response, the traps were withdrawn and a scheduled protest by *Alley Cat Allies* at the school on Saturday, June 2, 2012, was canceled. Instead, OC Vector's Manager and Communications staff met with representatives of *Alley Cat Allies* and held an outreach event on FBT prevention at the school and also provided topical flea medication to pet owners.

As TNR cats have become more abundant in the county, some cities have attempted to regulate the feeding of feral cats. In 2015, the city of Anaheim passed an ordinance restricting cat feeding only to approved cat feeders (Anaheim Municipal Code Section §6.44.1301 2015 2015). This prohibition provoked an outcry from cat advocates and led to a petition called "*Stop the Starvation of Cats of Disneyland and Anaheim*," which supposedly generated 33,430 signatures (Cunningham 2015). In response to the protesters, Anaheim kept the ordinance on file but directed cat feeders to assigned TNR sites (OC Community Cats 2015). In most situations, feral cats are unclassified as neither pet nor wildlife, and city code enforcement entities have no authority to regulate free-roaming cats.

For TNR advocates, program success is defined not by the elimination of feral cats in an area, but rather by the welfare of the cats (Longcore et al. 2009). *Alley Cat Allies* (2016) dismissed the FBT risk from feral cats, and even suggested that feral cats and the public make for a "healthy relationship". Furthermore, cat advocates have often promoted the use of feral cats as "working cats" in communities in a common misconception that feral cats will reduce rat numbers and should be used in urban rat control programs (Parsons et al. 2018).

Challenge: Contrasting views between OC Vector and OC Animal Care's TNR program

Engagement with OC Animal Care regarding FBT and its TNR program

After OC Animal Care started its TNR program in 2009, OC Vector met with OC Animal Care several times during the program's initial years to express concerns about the potential negative public health consequences associated with the proliferation of feral cats (NASPHV 1996, Jessup 2004, Gerhold and Jessup 2012, Roebling et al. 2013). Within the organization, OC Animal Care's veterinarians advised against the TNR program, calling it a potential health hazard, ineffective, and a waste of tax dollars (OC Grand Jury 2015a). Separately, OC Vector and the OC Health Care Agency both requested that the program be subjected to a full environmental impact analysis through a California Environmental Quality Act (CEQA) review (OC Grand Jury 2015a). OC Vector also expressed concerns about OC Animal Care's legal liabilities posed by releasing thousands of animals capable of harboring and transporting *Rickettsia*-infected fleas from areas where FBT disease is present to areas where the disease had not been found and requested that OC Animal Care prepare a TNR procedural manual with harm reduction provisions for FBT.

OC Animal Care rejected OC Vector's request for a CEQA review and never produced a guidance document on its TNR practices. However, after requests by OC Vector, OC Animal Care agreed to not place TNR cats within 0.48 km (0.3 mi) of hospitals, schools, parks, geographic clusters of FBT cases, and mobile home parks; OC Animal Care also agreed to assist OC Vector in its public awareness campaign by distributing flea-borne typhus education materials to veterinarians and the public (Cummings et al. 2014).

In 2014, OC Vector Manager Michael Hearst formally stated OC Vector's opposition to OC Animal Care's TNR program in a letter addressed to the OC Vector Board (OC Vector 2014). In a OC Grand Jury investigation of OC Animal Care (2014-2015), the Grand Jury questioned the effectiveness of the TNR Program at reducing cat overpopulation and identified it as a possible contributor to the spread of zoonotic disease agents, including FBT; they also recommended an immediate evaluation of the program (OC Grand Jury 2015a), which was never done. In a dissenting response, OC Animal Care maintained that the Feral FREE Program was a humane approach to cat population reduction, without creating undue risk of FBT pathogen transmission to the public (OC Grand Jury 2015b).

Challenge: Implementing existing State and local laws and regulations to prevent FBT

FBT at mobile home communities in Los Angeles and Orange counties

Animal rights groups and permissive public attitudes toward free-ranging cats have played a role in limiting the ability of public health agencies to mitigate FBT outbreaks when animal removal and/or depredation measures have been required, in addition to flea control. Within the last decade, FBT outbreaks (i.e., two or more cases at a site) have occurred at a number of mobile home communities in Los Angeles and Orange counties (LAC DPH 2015, Nelson et al. 2018, pers. comm., L. Krueger 2022). Inspections of these sites has revealed widespread cat feeding stations with unattended, surplus cat food for "community cats," a lack of sanitation associated with the stations (spilled food, animal feces, and uncovered trash bins), flea infestations, and an abundance of cats (owned and unowned), opossums, and other wildlife. Both the San Gabriel Valley Mosquito and Vector Control District (SG Vector) and OC Vector took similar pathways to resolving the animal, feces, and flea problems at these mobile home communities by enforcing State codes [CA Mobilehome Residency Law Civil Code §798.33 (2001) and CA Mobile Home Parks, CCR Title 25, Article 2, Section 1114 (a, b) (2004)] and the communities' own rules and regulations. State and community stipulations limit the number of animals allowed and prohibit free-roaming animals (pet, stray, feral) and "community" feeding of animals at mobile home parks. SG Vector also partnered with the Los Angeles County Department of Public Health (LAC DPH) and issued an abatement warrant under authority of the CA Health and Safety Code (CA HSC, Ch. 1, Div. 3, Sec §2000-2093, 2002) for the removal of feral cats and peridomestic wildlife and for flea control (LAC DPH 2015, Nelson et al. 2016, Wekesa et al. 2016b).

These agency enforcement actions were met with opposition by some residents. In the SG Vector enforcement, the local animal care responsible for the mobile home community refused to remove the animals, and a wildlife trapper authorized for animal depredation had to be used (Nelson et al. 2018). Once completed, no additional FBT cases were reported from the mobile home communities.

DISCUSSION

FBT is an example of a zoonotic disease that is difficult to manage from a public health perspective, because transmission is so closely associated with human behavior, pet cats and dogs, stray/feral cats, and peridomestic wildlife. The ecology of fleas and host animals in urban/suburban environments and the lack of area-wide interventions targeting peridomestic wildlife limit the effectiveness of many of the potential tactics for FBT disease control, both area-wide and at single residential properties (Krueger et al. 2022). Although CDFW regulations prohibit the relocation of wildlife (CDFW CCR Title 14 Sect. 465.5g(1) 2021), many residents and rescue groups relocate nuisance opossums and feral cats to other neighborhoods as a matter of convenience. These relocations by well-meaning individuals or groups may have contributed to the spread and amplification of these pathogens in Los Angeles County (LAC DPH 2016).

Once released, cats managed by TNR/RTF programs are not treated with flea control medication as part of routine colony maintenance and can support large flea infestations throughout their lifetimes. In addition, feral cat feeding stations provide supplemental food for wildlife and act as sites for interspecies exchange of fleas and pathogens among cats, wildlife, and humans (Akucewich et al. 2002, LAC DPH 2009, Hernandez et al. 2018). Current flea control medications are available only as topical or oral formulations to be applied directly to a single cat, and no formulations of oral flea medications are available that can be applied in food to the entire colony. The LA County DPH reports widespread flea infestations and public complaints in areas where cat colonies have been established (LAC DPH 2016, LAC CCP 2020a).

Mapping feral cat releases and where FBT cases occur is important to understanding threats to public health and where to focus mitigation measures. OC Vector's annual hotspot analysis has helped identify areas of high FBT disease occurrence and a greater relative risk for rickettsial transmission compared to other areas of Orange County. With this understanding, OC Vector's Communications Department has conducted targeted FBT informational programs for disease prevention in these affected neighborhoods (Krueger et al. 2022).

Although none of the recommendations from the 2015 Grand Jury were implemented to reduce zoonotic risks, OC Animal Care suspended its TNR program in 2021. OC Animal Care's new Director also informed OC Vector recently (Dec. 2021, personal comm., Laura Krueger) that TNR/RTF is a form of animal abandonment, in violation of CA State law, Cal. Penal Code §597s (2000). Nonetheless, TNR advocates continue to push to re-instate the program in Orange County (Paw Protectors Rescue 2022).

Feral cats have become a common feature in many urban/suburban landscapes in the U.S. and pose serious threats to public health and wildlife (Dutcher et al. 2021). Free-ranging, unowned cat numbers continue to increase in Los Angeles and Orange counties (LAC CCP 2020b, OC Animal Allies 2022), despite attempts to limit their numbers through sterilization efforts. Although OC Animal Care is no longer performing TNR/RTF programs, non-profit and volunteer organizations are providing TNR and other services for feral/stray cats. OC Animal Care's website (OC Animal Care 2022) directs people to animal rescue groups that offer vouchers for cat spay/neuter, but the demand for services is beyond the capacity of these groups (OC Animal Allies 2022). Altogether, 21 groups care for animals in Orange County (OC Animal Care 2021), with many private groups practicing TNR under no County oversight.

The public health obligation for which governmental animal control agencies were created and their role in animal management must be re-emphasized as one way to prevent outbreaks of zoonotic diseases. Furthermore, TNR and RTF cat programs are inconsistent with an all-encompassing "One Health Approach" that emphasizes prevention over pesticide control measures to mitigate zoonotic disease (UC Davis 2022). No TNR programs have been shown to work at the municipal level, as they spay/neuter fewer than 5% of the feral cats in a community, falling far short of the >70% sterilization rate required for effective population reduction (Jessup 2004, Foley et al. 2005, Longcore et al. 2009). Multi-agency enforcement of existing California State and local city/county codes and regulations regarding unsanitary conditions associated with feral cat colonies (feces accumulation, fleas) by county agencies (e.g., LAC Code 1959 & 1964), city ordinances (e.g., Anaheim Municipal Code Section 2015), vector control districts (CA HSC Secs. 2060-2067, 2002), California Department of Fish and Wildlife's restrictions on feeding of free-roaming cats (CDFW CCR Title 14, § 251.1 2019), and informed animal control agencies (Cal. Penal Code §597s 2000) would be instrumental in abating and limiting the spread of FBT.

CONCLUSION

A comprehensive flea-borne typhus program includes both public education and management of fleas and vertebrate hosts. OC Vector views FBT as a preventable disease but has encountered many challenges in developing a comprehensive FBT prevention program. Over the past 15 years, OC Vector has participated in studies with other agencies and universities, resulting in many publications; hosted FBT educational workshops for stakeholders; developed its own in-house FBT testing capability (PCR and ELISA); identified FBT high risk areas and developed focused educational outreach efforts; engaged local animal care groups to modify their feral cat TNR/RTF programs to reduce FBT risk; exercised its abatement powers to stop FBT outbreaks over the objections of cat advocates; and implemented a tiered FBT Response Plan.

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Spatial patterns in the density of free-roaming cats in the City of Los Angeles

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Abstract

Free-roaming cats are hosts for fleas and associated disease-causing pathogens. Recent outbreaks of typhus in southern California have been associated with free-roaming cats and the abundance of fleas collected from free-roaming cats is substantial (Maina et al. 2016, Nelson et al. 2018). Describing the distribution and temporal trends in free-roaming cat density is difficult and expensive using traditional field survey methods. Such knowledge is, however, important to understanding threats to public health and where to focus abatement activities to prevent the spread of flea-borne typhus and to inform programs to mitigate such risks.

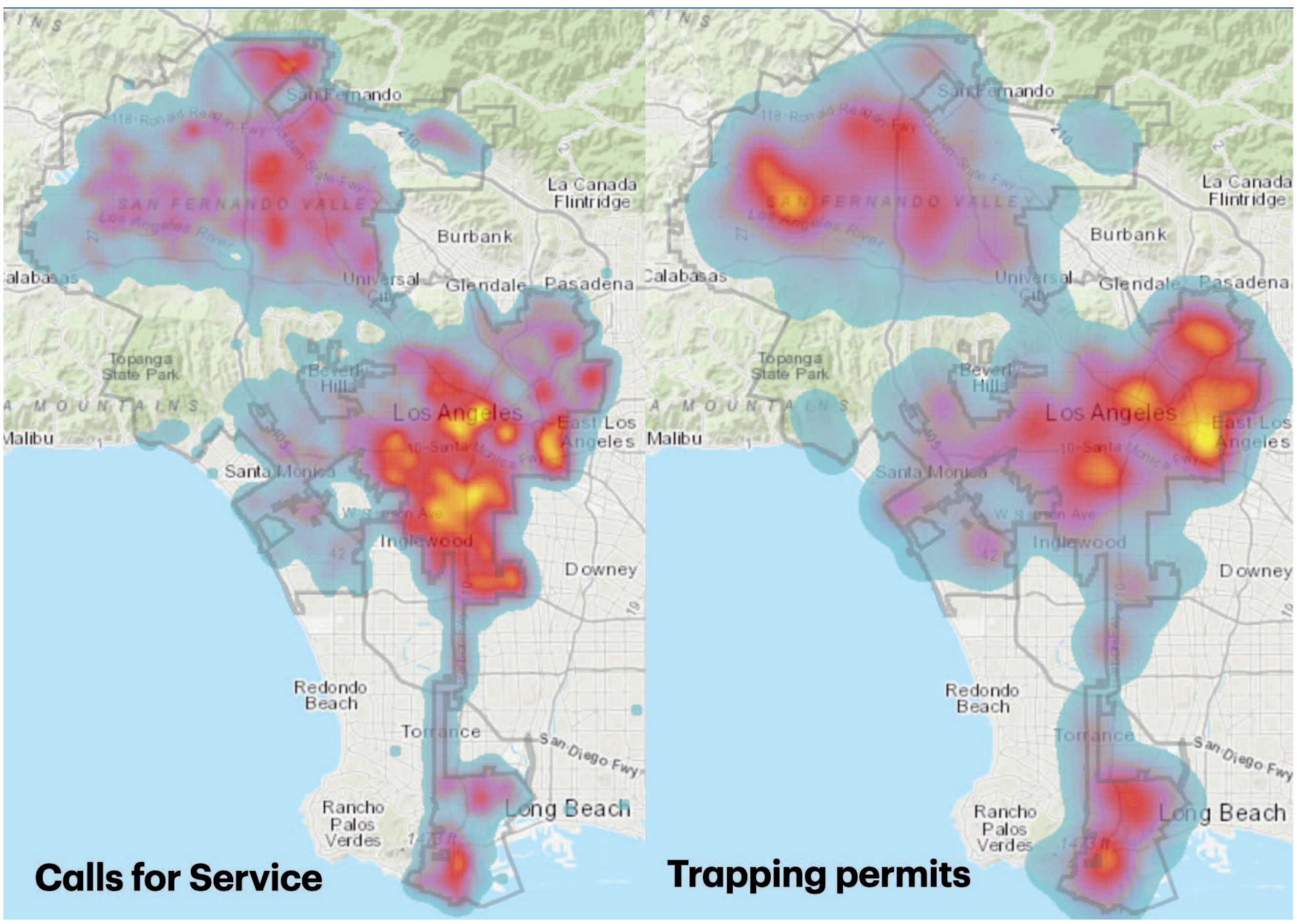

Figure 1.—Comparison of density of calls to the City of Los Angeles regarding unowned cats (left; n=20,214 records coded as "stray" cats 2009–2019) with cat trapping permits for unowned cats (right; n=2,514 applications for unowned cats, 2005–2019).

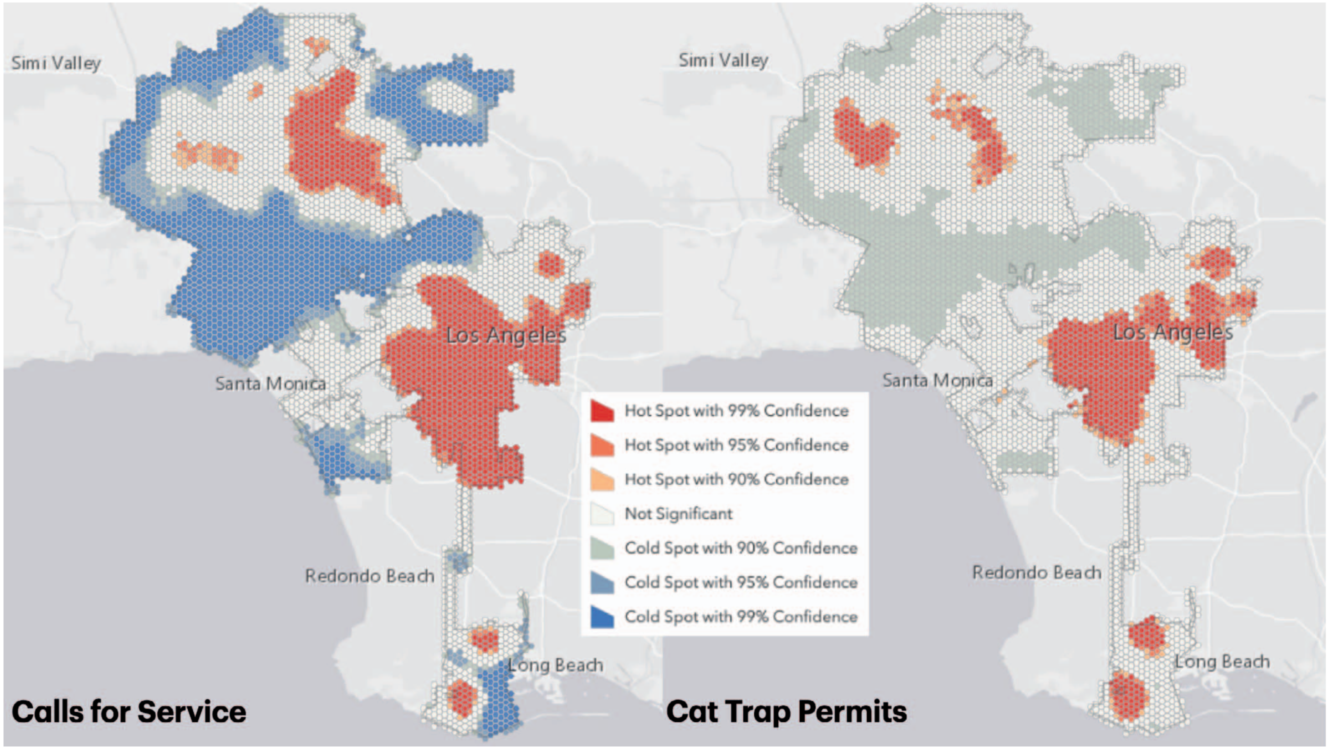

Figure 2.—Hotspots of calls for service pertaining to stray cats (left), and permits issued to trap stray cats (right), using raw data. Hotspots and statistical tests use Getis Ord G_i* statistic.

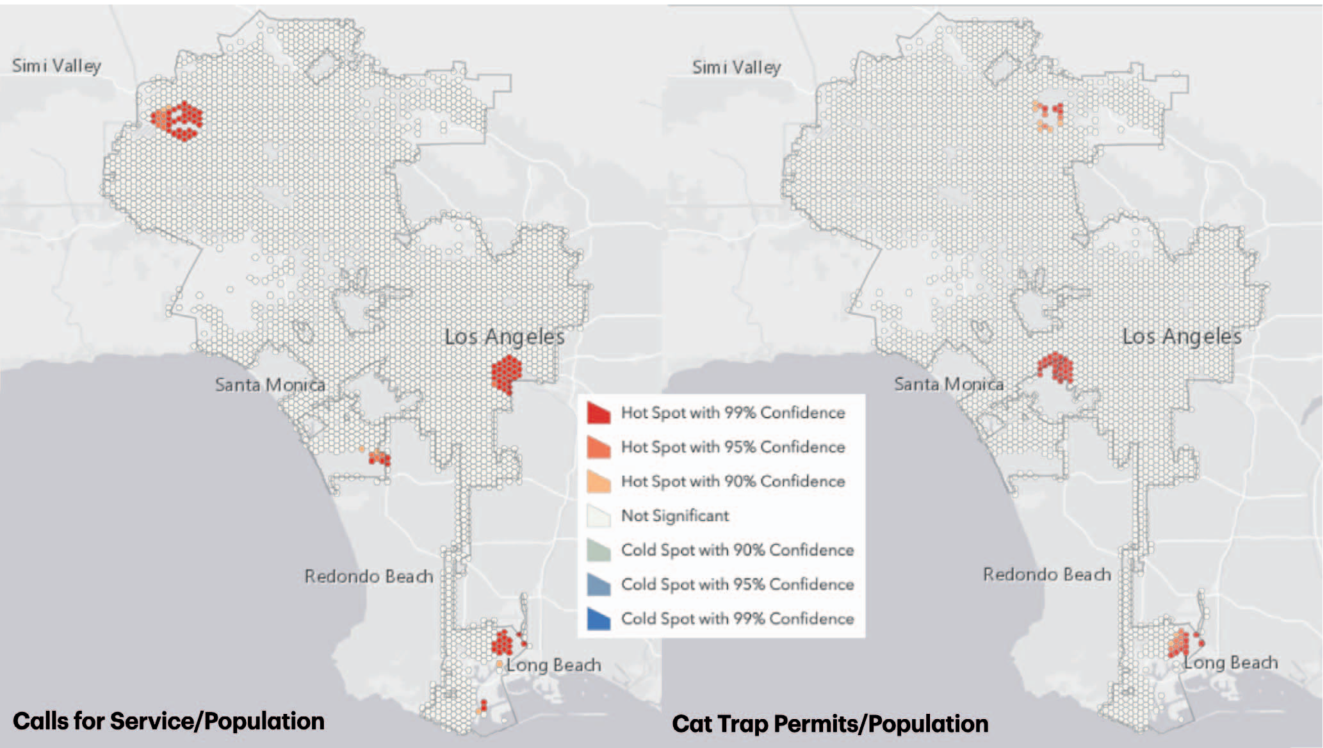

Figure 3.—Hotspots of calls for service pertaining to stray cats (left), and permits issued to trap stray cats (right), adjusted for residential population density. Hotspots and statistical tests use Getis-Ord G_i* statistic.

To understand the dynamics of free-roaming cat populations in the City of Los Angeles, two novel data sources were obtained from the City's Department of Animal Services through a Public Records Act request: 1) digital records of calls received asking for service and 2) paper records of all applications for permits to trap free-roaming cats, both over the last decade. These records were processed and transcribed into digital format and then georeferenced to locate each call for service and permit location within a geographic information system. We then visualized the data and calculated spatial hotspots of both calls for service about stray cats (n=20,214) and permit applications (n=2,513) to trap stray cats using the G_i* statistic (Getis and Ord 2010) on an equal area hexagonal grid (0.27 km^2) across the entire City on the raw data and data normalized for the residential population.

Patterns that emerged show differences between the distribution of calls for service for stray cats and those for cat trapping permits (Figure 1; Figure 2), with areas having lower median household income calling about stray cats but not securing permits to trap them (which require a $50 deposit for a trap if needed). It is likely that these areas also have lower pet cat sterilization rates because of expense (Chu et al. 2009). When normalized for population, the service requests and trapping hotspots correlated with understandable features (Figure 3). Calls for service have hotspots around industrial and port areas with larger cat numbers but fewer residents and in areas where median income shifts quickly. For trapping permits, the port areas are indicated as hotspots, along with industrial areas and urban open spaces with known feral cat colonies.

Data such as these could be used to target management actions to reduce free-roaming cat populations and associated risks by the City of Los Angeles, but they are not. The current City approach to free-roaming cats is to subsidize spay-neuter by nonprofit and individual partners, release cats from shelters, and discourage intake at shelters as part of a new Citywide Cat Program.

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Flea-Borne typhus: A cause of organ failure

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Introduction

Texas is currently experiencing an epidemiologic resurgence of flea-borne typhus and it is thus increasingly recognized as the cause of the sepsis syndrome (Murray et al. 2017). Usually perceived as a self-resolving febrile illness, flea-borne typhus actually encompasses a wide spectrum of disease severity including fulminant sepsis with multi-organ failure (Pieracci et al. 2017). Clinicians generally remain unfamiliar with severe septic presentations of flea-borne typhus with its associated dysfunction of various organs that may require care in the intensive care unit with organ support (Tsioutis et al. 2017).

Methods

In the current presentation, we review two suspected cases of fulminant sepsis due to flea-borne typhus with resultant organ failure. These cases demonstrate a diversity of end-organ effects associated with flea-borne typhus.

Results and Discussion

Case #1 was a 53-year-old man who presented with a 1-week history of nausea, vomiting and diarrhea. He was found with mild hyponatremia, elevated liver function tests, elevated creatinine, and thrombocytopenia. On physical exam he had findings of petechial rash to the lower extremities and bilateral subconjunctival hemorrhages. He was started empirically on broad spectrum antibiotics including doxycycline. *R. typhi* serology was sent. The patient's course was complicated by gastrointestinal hemorrhage, transient hypotension, and pancreatitis. He developed transient acute cognitive dysfunction associated with facial weakness and dysarthria. Imaging workup for central nervous system pathology including stroke was negative. Further in his course the patient had worsening hematologic, renal and hepatic organ dysfunction. The patient continued on treatment with doxycycline after other microbiologic workup returned negative. After hospital day #8 organ dysfunction improved gradually towards normalization. The patient was seen after discharge where diagnosis of flea-borne typhus was confirmed with positive convalescent serology for *R. typhi*.

Case #2 was a 71-year-old woman with a history of diabetes mellitus type 2, hypertension, and high cholesterol who presented to an outpatient clinic with a 1-week history of myalgias, generalized weakness and vomiting. She was found with evidence of anion gap metabolic acidosis on screening laboratory testing consistent with diabetic ketoacidosis and was referred to the emergency department for further evaluation and treatment. Further laboratory workup also discovered hyponatremia, elevated liver function tests, and thrombocytopenia. She was started on intravenous fluid resuscitation, insulin drip and ceftriaxone empirically for suspected infection. She was admitted to the medical ward. Though during her early admission her diabetic ketoacidosis improved, her condition worsened by hospital day #5 with bandemia, worsened thrombocytopenia, encephalopathy, hypotension unresponsive to intravenous fluid resuscitation and lactic acidosis, the latter consistent with septic shock. Antibiotics were broadened empirically including the addition of doxycycline. *R. typhi* serology was sent. She was transferred to intensive care unit. Her course further worsened with progressive organ dysfunction including obtundation, renal failure, coagulopathy, respiratory failure requiring mechanical ventilation, worsening acute liver injury, and shock unresponsive to multiple vasopressors. Her blood, urine, and bronchoalveolar lavage cultures as well as viral studies for HIV, respiratory, and hepatitis were negative. Unfortunately, the patient's multi-organ failure continued to worsen despite maximal life support and the patient's family decided to withdraw life support on hospital day #7. Serology sent during the acute illness returned positive for *R. typhi* IgM with a titer of 1:256. In this consistent presentation lacking another more likely cause of illness, this case was classified as a probable case of flea-borne typhus and was listed as a cause of death.

Conclusions

These two cases provide examples of the diverse multi-organ dysfunction that flea-borne typhus can precipitate. In endemic areas, it is important to suspect and empirically

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treat flea-borne typhus infection early when patients present with sepsis and organ failure without another clear source of infection.

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Delta Mosquito and Vector Control District collaboration with Oxitec on the planning and preparation for the Friendly *Aedes aegypti* Field Project in Visalia during the 2022 mosquito season

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Abstract

The invasive yellow fever mosquito, *Aedes aegypti* (L.), was first identified in California nine years ago, is now prevalent in the Central Valley, and poses a significant public health threat to its residents. Delta Mosquito and Vector Control District (DMVCD) which serves northern Tulare County is partnering and collaborating with Oxitec on a field pilot project during the 2022 mosquito season after Oxitec receives regulatory approval from the U.S. Environmental Protection Agency (EPA) and the California Department of Pesticides Regulation (DPR). Oxitec will be releasing male mosquitoes that carry a self-limiting gene which prevents their female offspring from surviving and allowing for mosquito male-only production in defined plots within the DMVCD's service area. Oxitec's self-limiting non-biting male mosquitoes will only mate with *Ae. aegypti* females and this specificity doesn't affect non-target species such as bees, butterflies, and other wildlife. In addition, the presentation discussed the planning and preparation of the proposed field pilot project and site selections which include single-point release on a household scale, multi-point release in a small neighborhood, mark/release recapture, community education, outreach, and public engagement in the DMVCD.

Advances in automated production of sterile male mosquitoes for adult control on large scale

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Abstract

The sterile insect technique is gaining evidence as a promising tool for the control and suppression of local mosquito populations by releasing large numbers of sterile male mosquitoes. One of the limiting factors of the technique is the amount of labor required, mainly to manually sex sort and package millions of male only mosquitoes. Typically, mosquitoes are sorted at their pupae level, and require good differentiation in the male and female sizes. In this presentation, our second-generation automated sex separator for high volumes will be discussed. Instead of separating the pupae, they emerge into adults, manipulated on conveying systems, providing flexibility in shipping and rearing. The novel solution, leverage automation and deep learning to provide a simple, affordable and accessible solution for quality control, sorting and packaging of high volumes of male mosquitoes. The presentation will discuss the integration of the solution within a complete mosquito production line, to provide a streamline production process with minimal intervention and disturbances to the fragile insects, targeting a cost-effective solution for city wide scale operations.

Measuring the impact of sunlight on the viability and efficacy of liquid suspension concentrate applications of Spinosad

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INTRODUCTION

Utilizing air blast sprayers for wide area larviciding has become an increasingly common form of control for urban container breeding mosquitoes. Fine and very fine droplets are blown up and over homes to deposit into cryptic breeding sources that are not easily reached. Liquid larvicides like Natular SC, a 22.5% spinosad suspension concentrate, are the preferred formulation for this kind of larval source management.

To determine how long droplets of Natular SC remain viable after depositing onto a surface and drying, a semi-field trial was conducted on common container materials. The substrates were sprayed with a dilution of Natular SC and water, then left in direct sunlight for up to a month. The substrates then were placed into deli cups filled with larvae and the ensuing mortality was observed. Our study could inform vector control districts on how and when to pre-treat known mosquito breeding sites before an area's wet season begins or mosquito emergence occurs.

MATERIALS AND METHODS

For sprayer calibration, a mixture of RD&C Red #40 dye and water were added to a Stihl handheld sprayer. Pressure of the sprayer was determined by the number of 'pumps' applied before each spray. Kromekote cards deployed parallel to a 10 ft track then were sprayed with the red dye mixture and droplet disposition was analyzed using DropVision® AG (Leading Edge, New Smyrna Beach, FL). This process was repeated until the desired droplet characteristics were acheived.

The substrates used were a sheet of rubber, a sheet of aluminum, terracotta from a pot, and steel from a roof gutter. A 6 oz. clear polystyrene jar (US Plastics, Lima, OH), the most common way of evaluating wide-area applications, was used in each replicate for comparison. Each substrate was cut into 5.4 × 6cm rectangles to have the same surface area as the polystyrene jar. Each time sample included the one of each substrate (4 total), a polystyrene jar and a water sensitive card. These were placed in a line parallel to the walking track. A 6% mixture of Natular SC and water was added to the sprayer. For application, the sprayer was held at the same height and distance from test subjects while walking at a consistent pace along the track. Five sprays were done to provide enough test materials for our 5 different drying intervals. Test materials then were collected and placed in an area outdoors that received direct sunlight until tested. The water sensitive cards were analyzed on DropVision Ag for droplet data for each spray.

After each drying interval, all test materials (substrates and jars) were collected and placed in a 16 oz. polypropylene deli cup. The deli cups and the polystyrene jars were filled with 180mL of distilled water. A total of 15-20 locally caught *Culex quinquefasciatus* larvae were added to each cup, and the ability of the larvae to make coordinated versus uncoordinated movements were observed after 24 hrs.

RESULTS AND DISCUSSION

Substantial larval mortality was observed in all cups for the first 3 drying intervals (24 hrs, 3 days, and 7 days) after application (Fig. 1). After a week post-application, levels of control varied dependent on the substrate on which the product dried (Fig. 2). Terracotta showed the longest control among the 4 substrates tested, with 14 days of control (above 75% larval mortality). Heightened control of Terracatta maybe the result of the material's porous nature, which better absorbed and retained the product than less permeable substrates such as rubber.

CONCLUSION

Direct UV exposure of substrates treated with liquid Natular affected the length of control provided when used

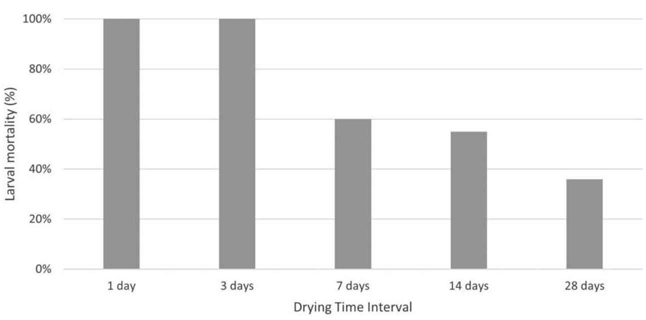

Fig. 1.—Overall larval mortality of all test materials at each drying interval.

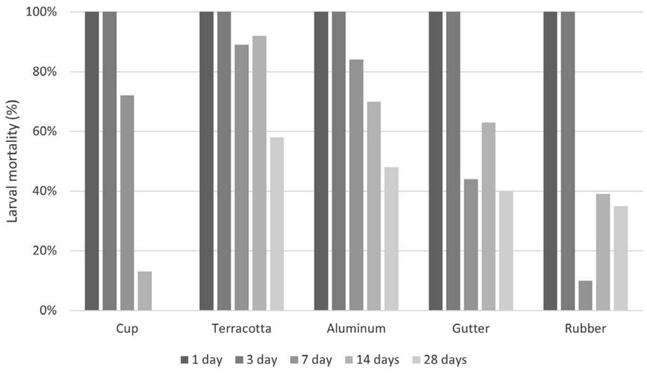

Fig. 2.—Larval mortality per substrate over 5 drying intervals.

for larval source management of container breeding mosquitoes. Dried droplets withstood exposure to direct sunlight for multiple days before degrading, but this varied with the type of substrate to which the product was applied. This information may help optimize container breeding larval control in urban environments.

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Innovation and technology application in the Anastasia Mosquito Control District, St. Augustine, Florida

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Abstract

Anastasia Mosquito Control District (AMCD), St. Augustine, Florida has collaborated with universities, institutes, and industries to investigate and develop new methods/techniques to improve customer service, arbovirus and mosquito population surveillance, and operational control. These collaborative projects and their accomplishments have benefited AMCD's operational programs. AMCD has collaborated with GeoWorld Outdoors company to develop a customized database management software and Application. The use of this new database technology has reduced the volume of service requests taken by phone by more than 50% and decreased our response time to provide service requests. AMCD collaborated with industry partners to evaluate, modify, and improve the efficacy of several different surveillance and control traps, attractants, repellents, insecticides, and equipment. The district built a wind tunnel / olfactormeter laboratory, a droplet laboratory with a laser machine to characterize insecticide sprays, 24 field larvicide evaluation pools, three large outdoor enclosures, a quarantine laboratory with two green houses, bioassay laboratory, and a molecular laboratory for applied research. These laboratory conditions and facilitates promote and benefit applied research and allowed the district to be competitive for several grants from federal, state, and industry partners. The district trains 6-7 visiting scientists worldwide,7-8 intern students, and generates 10-15 applied research publications annually. Currently, AMCD has 5 Ph.D. Scientists, 5 M.S. Biotechnicians and 1 Ph.D. student (through the University of Florida) in the team with 2 Federal grants, 3 State grants, and 5 industry funded projects. In addition, the Disease Vector Education Center (6,000 s.f.) and the sterile insect technology (SIT) mass rearing facility (6,000 s.f.) are under construction and expected to be completed by the end of 2022. Since 2004, the district began an applied research program, enhanced employee training and public education, and adopted new technology and innovation that have improved operational efficiency and saved millions of tax payers' money.

Introduction

Anastasia Mosquito Control District (AMCD) of St. Johns County, Florida was established in 1948 to cover 18 square miles. Since 1956, AMCD has continually expanded from a small portion of Anastasia Island to its present coverage of the entire 609 square miles of St. Johns County to serve more than 300,000 residents. St. Johns County is located in northeastern Florida, is bordered by the Atlantic ocean, and includes 47 miles of intercoastal waterways (salt marsh) and the St. Johns River (fresh water) (Weaver et al. 2013). More than 46 species of mosquitoes have been identified in St. Johns County. There were 4 substations (Anastasia Island, Ponte Vedra, Northwest, and Hastings) established to provide services. Since 2012, the District has gradually centralized from 4 substations to one central location of St. Johns County where a new complex was built in 2016. Currently there are 32 full time employees, 6-8 seasonal employees, 7-8 intern students, and 6-7 visiting scientists with an annual budget of \$6-\$7 millions.

Program

The district has three major programs (operations, applied research, and education) to guide the daily operations. The operational program includes answering service requests and providing customer service, surveillance (arbovirus, mosquito population, environmental parameter, and insecticide resistant monitoring), and mosquito control (ground and aerial operations). Ground operations include source reduction, larval control using *Bacillus thuringiensis* subsp. *israelensis* and insect growth regulators (methoprene), and adult mosquito control using pyrethroid insecticides (80% permethrin products, 10-20% sumithrin and other products) for ground application, and naled for aerial spraying. Applied research started in 2004 and has continued to expand at the district with a current staff of 5 Ph.D. Scientists and 5 Biotechnicians with Master Degree in Public Health, Microbiology, and Biology, and 5-6 visiting scientists worldwide and 6-7 intern students annually (Xue et al. 2015). The research ideas are generated through interaction with our residents, constituents, industry partners, and observations from employees.

Table 1.—Recognitions and achievements of the AMCD since 2004

Year	Subject	Organization
2018-present	A site to train intern students	CDC Southeastern Center of Excellence in Vector-borne Diseases
2017 & 2018	Train/certify other mosquito control educators (108) for Integrated Mosquito Management	American Mosquito Control Association (AMCA)
2019 & 2020	Mentors to train other mosquito control persons	NACCHO
2012-present	MoUs for train interns with several universities (trained 72 interns, 18 Visiting Scientists, and 4 Ph. D. through Univ. of Florida)	Univ. of North Florida, Univ. of Miami, Univ. of Florida
2006-present	Partner of the EPA & AMCA pesticide environmental stewardship program (PESP)	AMCA & EPA
2004-present	Annual Arbovirus Surveillance and Mosquito Control Workshop	AMCD, USDA/Center for Medical, Agricultural, and Veterinary Entomology, Florida Mosquito Control Association
2005	AMCD staff received 13 national awards and 12 state awards	American Mosquito Control Association, Society of Vector Ecology, AT&T Governmental Technology, Florida Mosquito Control Association, Florida Entomology Society
2005-present	AMCD staff authored and Coauthored about 200 publications & book chapters	Different professional Journals

The applied research interests enhance service techniques, surveillance technology and tools such as attractants, traps, trapping methods, repellents and personal protection, and operational control through the evaluation of new techniques including new insecticide formulations and new equipment or tools. Our applied research has been conducted mostly through collaboration with the Universities / Institutes, Federal and State governmental agencies, and industries both nationally and internationally (Li et al. 2015, Xue et al. 2016). When problems arise during our operational control, our applied research program tries to address the problem, and if successful, then promotes the solutions through employee training and continuing education. AMCD organizes and hosts an annual workshop on arbovirus surveillance and mosquito control (Xue et al. 2014), and also provides employees with the opportunity to attend many state and national professional association meetings. The district also has enhanced local collaboration and school programs to provide education through lectures, hands-on training, social media, and frequent updates of the district website.

Since 2017, the AMCD's Board of Commissioners voted and approved to collaborate with the GeoWorld Outdoors company to develop customized software for mapping and service requests which includes a phone application operated both through Apple and Android smart phones and tablets (New GeoMosquito Service App). Customers enter their service requests on their smart phone or I-Pad which then go to the AMCD database, from which it is directly assigned to the appropriate mosquito control technician in real time via their smart phone. The mosquito control technician in the service request area can immediately answer that service request. The number of service requests taken by phone has been reduced by more than 50% and the time to answer the service request has been reduced to 1-1.5 days. The district received the citizen service award for this application from the Special Districts / Technology Innovation from AT&T Governmental Technology Program in November 2021.

Collaboration

A number of applied research projects related to surveillance and control techniques have been conducted through collaboration with Federal, State, Industry, and International organizations. Major collaborators are the University of Florida (UF), USDA, Center for Medical, Agricultural, and Veterinary Entomology (CMAVE), Navy Center Entomology in Excellence (NECE), University of Miami, and Hebrew University, Israel, University of Bamako, Mali (Traore et al. 2020), and China CDC (Table 1). AMCD collaborated with the University of Florida (UF)'s Emerging Pathogen Institute to receive funds from the Florida Department of Agriculture and Consumer Service (FDACS) which resulted in the isolation of Keystone virus from several pools of adult mosquitoes collected in St. Johns County. AMCD collaborated with the UF/ Florida Medical Entomology Laboratory (FMEL) in training a Ph.D. student who researched virus-infected mosquito behavior and its impact on repellents. Also AMCD received a collaborative grant with UF/FMEL to detect arboviruses in mosquitoes through use of honey cards (Burkett-Cadena, et al. 2016). Several grant funded collaborations with the UF/ Department of Entomology and Nematology (DEN)'s and the UF Urban Entomology Laboratory have evaluated the non-target impacts of attractive toxic sugar baits (ATSB) (Fiorenzano et al. 2017), mosquito adulticides (Sanchez-Arroyo et al. 2022), mixture insecticides (Qualls et al. 2022), and nanotechnology against mosquitoes. Two Ph.D. students, 3 visiting scientists, and 3 intern students have received their degrees and training through these projects and a new student has started a doctoral program in 2021. AMCD collaborated with the UF/DEN to have received two FDACS grants evaluating spatial repellents and related toxicity (Bibbs et

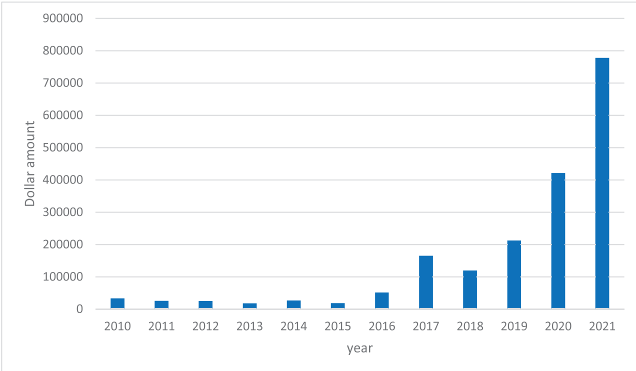

Figure 1.—Amount grand funds received from Federal, State, and industry agencies since 2010. The grant funds have increased since AMCD moved to the new complex in 2016.

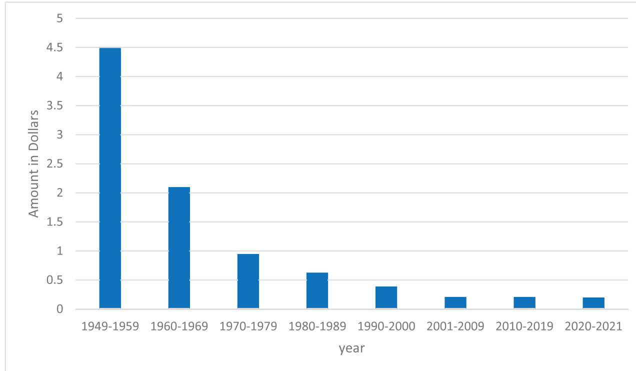

Figure 2.—AMCD's millage (property tax rate per $1,000 value) history since 1949 to present.

al. 2018) and trained a Ph.D. student and several intern students. Over the past few years AMCD has collaborated with UF/DEN and USDA/CMAVE to receive 3 FDACS grants and a CDC/DOH to fund studies on the operational control of mosquitoes using the Sterile Insect Technology (SIT) in St. Augustine (Sypes et al. 2021). The SIT project allowed training of two visiting scientists and six intern students. Since 2017 AMCD has collaborated with the MosquitoMate to evaluate the efficacy of releasing *Wolbachia*-infected male *Aedes* mosquitoes and evaluate its potential impact with other control methods (Aryaprema et al. 2022) in St. Augustine. Two intern students and a visiting scientist received their related training.

AMCD collaborated with USDA/CMAVE to evaluate new attractants and traps such as granular CO2 and Mose Hole trap (Xue et al. 2015), repellent-treated uniforms and clothing (Bernier et al. 2021), and a new control method, attractive toxic or target sugar baits (Kline et al. 2018), and SIT. The attractant comparison study enhanced the AMCD surveillance program by switching from using dry ice as attractant with the CDC light traps to octenol as the attractant for our regular surveillance traps to monitor nuisance mosquito populations, thereby reducing operational costs. For arbovirus surveillance sentinel chickens and mosquito pooling collected by 10 CDC gravid traps, 12 BG sentinel traps for container-inhabiting mosquitoes, and 10 CDC light traps baited with dry ice were used to cover the whole county. Serval modifications of the CDC-autocidal gravid ovitrap (AGO), CDC-gravid traps (Xue et al. 2021), Biogents traps, LongRay rotator traps (Shi et al. 2019), and a commercial larval trap have been evaluated and these modified traps have improved surveillance and control efficiency (Talbalaghi et al. 2020). AGO traps baited with attractants (BG lure) have increased the collection of host-seeking mosquitoes (Liu et al. 2019). Adding a sucking fan to AGO traps has increased the collection of mosquitoes (Zhu et al. 2019). The 00ZZZero commercial gravid traps have increased the collection of adult female mosquitoes after adding a sticky paper and lure (Khater et al. 2019). A commercial larval control trap increased the efficacy of the control of larval and adult mosquitoes after lowing water level in the container and adding a sticky paper on the top part (Talbalaghi et al. 2020). Collaboration with the Navy Entomology Center for Excellence (NECE) evaluated new truck larvicide applications (Doud et al. 2014), several fogging machines (Fulcher et al. 2015), and factors impacting droplet size and control efficacy. These findings benefited and improved AMCD control operations. Recent collaboration with the Armed Force Pest Management Board surveyed the action threshold for decision making in different areas to benefit military training and mosquito control operations in deployed settings (Aryaprema et al. 2022). The three year funded grant supported three scientists and two intern students. Other collaborative projects with the University of Florida allowed us to receive a two year grant through the CDC to develop and evaluate a smart sensor for bioassay cages for evaluation of mosquito adulticides. This grant supports two biological technicians and two graduate students.

A number of industry collaborations have resulted in the improvement and evaluation of new larvicides, adulticides, repellents, traps, and control methods. These collaborations are with MosquitoMate, MGK, ADAPCO, ThermCell, Central Life Sciences, Light Farmer, DNW Global, BigShot, and many more. The evaluation of three formulations of adulticides (Amoo et al. 2012) has saved AMCD over a million dollars in the past decade.

Accomplishment

The applied research and collaboration have generated more than $2 million grant funds since 2016 (Figure 1). The application of innovation and technology has gradually reduced the collection of property tax for mosquito control (Figure 2). Also, multiple funding sources has allowed AMCD to train many intern students and visiting scientists over the last 15 years. AMCD has been recognized as one of the high citation and leading districts in the field of biology and control of mosquitoes (Table 1).

AMCD has built the State-of-the-Art facility and has been under process for Good Laboratory Practice (GLP). The Disease Vector Education Center (6,000 s.f. Figure 3)

Figure 3.—AMCD's disease vector education building (6,000 s.f. with 7 sections).

Figure 4.—AMCD's sterile insect technology (SIT) building (6,000 s.f. under construction).

and the sterile insect technology (SIT) mass rearing facility (6,000 s.f. Figure 4) are under construction and expected to be completed by the end of 2022. AMCD will be the Northeastern Florida Mosquito Control, Applied Research, and Education Center after the completion of the projects mentioned above. Overall, innovation and technology applications benefit Anastasia Mosquito Control District's programs.

Acknowledgements

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Surveillance for mosquito-borne encephalitis virus activity in California, 2021

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Abstract

In 2021, the California surveillance program for mosquito-borne encephalitis virus activity tested humans, horses, dead birds, mosquitoes, and sentinel chickens.. West Nile virus (WNV) activity was reported from 39 of 58 counties in California, and St. Louis encephalitis virus (SLEV) activity was reported from 10 counties. A total of 147 human WNV infections were reported, and non-human WNV activity was detected among horses, dead birds, mosquitoes, and sentinel chickens. Four human cases of SLEV disease were identified in four counties, and enzootic SLEV activity was detected in mosquitoes and sentinel chickens from eight counties.

INTRODUCTION

The California Arbovirus Surveillance program is a cooperative effort between the California Department of Public Health (CDPH), the University of California Davis Arbovirus Research and Training (DART) laboratory, the Mosquito and Vector Control Association of California (MVCAC), local mosquito abatement and vector control agencies, county and local public health departments, and physicians and veterinarians throughout California. Additional local, state, and federal agencies collaborated on, and contributed to, the West Nile virus (WNV) component of the arbovirus surveillance program.

In 2021, the surveillance program included the following:

(1) Diagnostic testing of specimens from human patients who exhibited symptoms compatible with WNV disease as well as blood bank and organ donor screening for WNV infection.
(2) Monitoring mosquito abundance and testing mosquitoes for the presence of WNV, St. Louis encephalitis virus (SLEV), western equine encephalitis virus (WEEV), and other arboviruses as appropriate.
(3) Serological monitoring of sentinel chickens for WNV, SLEV, and WEEV antibodies.
(4) Reporting and testing of dead birds for WNV.
(5) Weekly reporting of arbovirus test results to ArboNET, the national arbovirus surveillance system.
(6) Weekly reporting of arbovirus activity in the CDPH Arbovirus Surveillance Bulletin and on the California WNV website: https://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 39 (67%) of 58 counties in California (Table 1), while SLEV activity was reported from 10 (17%) counties (Table 2).

HUMAN DISEASE SURVEILLANCE

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

In 2021, a total of 129 symptomatic and 19 asymptomatic infections with WNV were reported (Tables 1 and 3). Of the 129 symptomatic cases, 97 (75%) were classified as West Nile neuroinvasive disease (WNND) (e.g., encephalitis, meningitis, acute flaccid paralysis, or other neurologic dysfunction) and 32 (25%) were classified as non-neuroinvasive disease; 12 (9.4%) cases were fatal. Cases were residents of 28 counties and 81 (63%) were male. In 2021, WNV incidence in California was 0.3 cases per 100,000 persons. Incidence was highest (6.7 cases per 100,000 persons) in Glenn County, whereas

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Table 1.—West Nile virus activity in California by county, 2021. Humans include asymptomatic infections detected through blood bank and organ donor screening. NT = None tested

County	Humans	Horses	Dead Birds	Mosquito Pools	Sentinel Chickens
Alameda	0	0	2	0	0
Alpine	0	0	NT	NT	NT
Amador	0	1	NT	NT	NT
Butte	15	0	2	80	27
Calaveras	0	0	NT	NT	0
Colusa	0	0	NT	NT	1
Contra Costa	2	0	4	8	0
Del Norte	0	0	NT	NT	NT
El Dorado	1	0	0	NT	NT
Fresno	17	2	0	219	NT
Glenn	2	0	NT	0	0
Humboldt	0	0	NT	NT	NT
Imperial	0	0	NT	0	NT
Inyo	0	0	NT	0	NT
Kern	10	0	0	103	NT
Kings	10	1	NT	34	NT
Lake	0	0	0	4	4
Lassen	0	0	NT	NT	NT
Los Angeles	19	0	43	260	3
Madera	4	0	0	131	NT
Marin	0	0	0	0	NT
Mariposa	0	0	NT	NT	NT
Mendocino	0	0	NT	NT	NT
Merced	7	1	1	10	19
Modoc	0	0	NT	NT	NT
Mono	0	0	NT	0	NT
Monterey	0	0	0	NT	NT
Napa	0	0	0	1	NT
Nevada	0	0	2	NT	0
Orange	3	0	7	51	NT
Placer	2	0	5	63	NT
Plumas	0	0	NT	NT	NT
Riverside	3	0	0	112	NT
Sacramento	6	2	100	120	1
San Benito	0	0	0	0	0
San Bernardino	2	0	1	9	NT
San Diego	3	0	1	0	NT
San Francisco	1	0	0	0	NT
San Joaquin	8	3	17	389	NT
San Luis Obispo	2	0	0	0	NT
San Mateo	1	0	0	0	0
Santa Barbara	2	0	0	0	0
Santa Clara	3	0	1	4	NT
Santa Cruz	1	0	0	0	0
Shasta	3	0	0	28	3
Sierra	0	0	NT	NT	NT
Siskiyou	0	0	NT	NT	NT
Solano	2	0	2	22	0
Sonoma	0	0	1	2	NT
Stanislaus	6	1	2	152	NT
Sutter	0	0	1	18	11
Tehama	0	0	NT	NT	5
Trinity	0	0	NT	NT	NT
Tulare	9	0	7	375	10
Tuolumne	0	0	0	NT	NT
Ventura	0	0	1	0	0
Yolo	4	0	10	56	4
Yuba	0	2	0	12	2
State Totals	**148**	**13**	**210**	**2,263**	**90**

Table 2.—St. Louis encephalitis virus activity in California by county, 2021. NT = None tested

County	Humans	Mosquito Pools[1]	Sentinel Chickens
Fresno	1	7	NT
Imperial	0	3	NT
Kern	1	0	NT
Kings	0	1	NT
Madera	0	3	NT
Marin	1	0	NT
Merced	0	2	2
Riverside	0	24	NT
Stanislaus	1	2	NT
Tulare	0	4	0
Totals	**4**	**46**	**2**

[1] Positive mosquito pools included *Cx. tarsalis* (34 pools), *Cx. quinquefasciatus* (8 pools), and *Cx. pipiens* (4 pools)

Los Angeles County reported the most cases (16, 13% of total) (Figure 1, Table 3). The median age of those with WNND was 65 years (range, 4 to 85 years), and among cases with non-neuroinvasive disease the median age was 59 years (range, 13 to 85 years). The median age of the 12 WNV-associated fatalities was 74 years (range, 52 to 85 years). Dates of symptom onset ranged from March 31 to November 27, with the peak occurring during epidemiological week 36 (September 5 - September 10), when 17 (13%) symptomatic infections were reported.

Four symptomatic cases of SLEV infection also were identified in 2021. All cases presented with neuroinvasive disease and no fatalities were reported. Cases were residents of four counties (Table 2) and two (50%) were

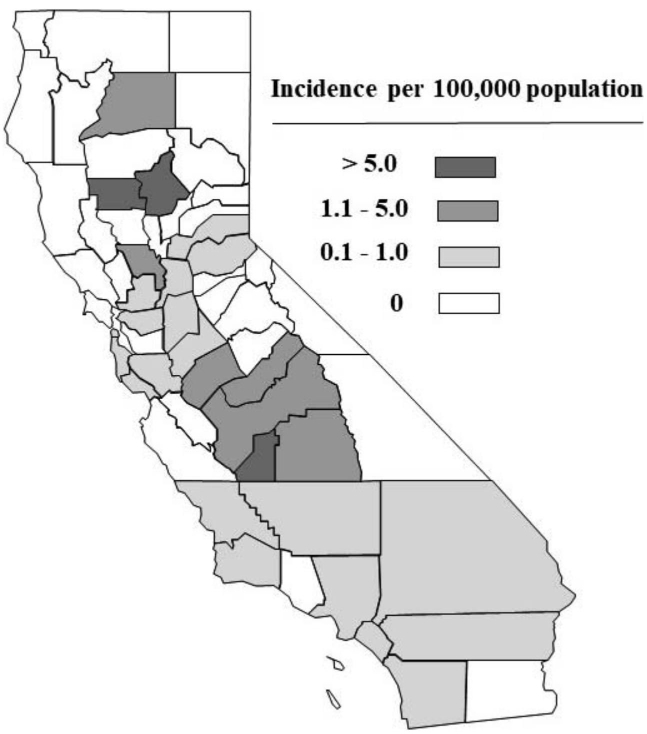

Figure 1.—Incidence of human cases of West Nile virus in California, 2021.

Table 3.—Reported West Nile virus human cases by county of residence and year, California, 2012 – 2021.

County	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2021 incidence per 100,000 person-years	Ten-year incidence per 100,000 person-years
Alameda	2	0	1	0	0	1	0	1	0	0	0.00	0.03
Alpine	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Amador	0	0	0	0	1	0	1	1	0	0	0.00	0.80
Butte	10	24	24	53	21	4	12	5	4	13	6.41	8.39
Calaveras	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Colusa	3	2	3	1	2	0	0	1	0	0	0.00	5.39
Contra Costa	4	5	5	1	4	4	4	1	4	2	0.17	0.29
Del Norte	0	0	0	0	0	0	0	0	0	0	0.00	0.00
El Dorado	0	1	0	0	1	0	0	0	1	1	0.51	0.20
Fresno	24	8	43	8	14	13	14	51	10	14	1.36	1.94
Glenn	7	9	10	19	6	0	2	0	1	2	6.74	18.87
Humboldt	0	0	0	0	0	0	1	0	0	0	0.00	0.08
Imperial	1	0	1	1	0	3	0	3	1	0	0.00	0.54
Inyo	0	0	0	0	0	4	0	0	0	0	0.00	2.15
Kern	25	25	11	11	17	30	13	28	8	8	0.88	1.93
Kings	3	1	4	0	8	5	0	3	2	8	5.24	2.23
Lake	1	0	1	2	1	0	1	0	2	0	0.00	1.25
Lassen	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Los Angeles	163	151	253	286	151	277	43	31	90	16	0.16	1.45
Madera	3	3	3	4	6	2	4	3	6	3	1.89	2.33
Marin	0	2	0	1	0	0	0	0	0	0	0.00	0.12
Mariposa	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Mendocino	0	0	1	2	0	0	0	0	0	0	0.00	0.35
Merced	13	0	1	1	0	10	2	10	12	6	2.11	1.93
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	0	1	0	1	0	0	0	0.00	0.07
Napa	0	1	0	0	0	0	1	0	0	0	0.00	0.15
Nevada	0	0	0	2	0	0	1	0	0	0	0.00	0.31
Orange	42	10	263	92	32	33	9	5	17	3	0.10	1.60
Placer	12	6	7	0	7	0	9	1	2	2	0.49	1.14
Plumas	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Riverside	19	35	14	127	11	32	15	12	10	3	0.12	1.13
Sacramento	29	11	10	4	25	6	15	4	7	6	0.38	0.75
San Benito	0	0	0	0	0	0	0	0	0	0	0.00	0.00
San Bernardino	33	13	21	54	8	57	9	7	3	1	0.05	0.95
San Diego	1	0	11	42	20	2	2	3	1	3	0.09	0.26
San Francisco	1	1	0	0	0	1	0	0	0	1	0.11	0.05
San Joaquin	13	8	9	2	13	14	14	7	2	7	0.89	1.14
San Luis Obispo	0	0	0	0	0	0	0	2	0	2	0.74	0.15
San Mateo	0	0	0	0	0	0	0	0	0	1	0.13	0.01
Santa Barbara	0	1	0	0	0	0	0	0	0	2	0.45	0.07
Santa Clara	0	2	10	8	1	0	1	1	0	3	0.16	0.14
Santa Cruz	0	0	0	0	0	0	0	0	0	1	0.38	0.04
Shasta	1	1	2	3	1	1	1	0	2	3	1.69	0.84
Sierra	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Siskiyou	0	0	0	1	0	0	0	0	0	0	0.00	0.23
Solano	2	1	5	1	4	1	0	1	1	2	0.46	0.41
Sonoma	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Stanislaus	26	17	33	13	26	28	15	16	35	5	0.90	3.85
Sutter	8	10	8	2	12	3	1	1	1	0	0.00	4.54
Tehama	4	5	4	5	5	2	2	0	2	0	0.00	4.44
Trinity	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Tulare	7	5	21	13	10	12	8	24	7	8	1.66	2.39
Tuolumne	0	0	0	0	0	0	1	0	0	0	0.00	0.19
Ventura	7	2	1	6	7	1	2	2	0	0	0.00	0.34
Yolo	10	6	15	8	16	6	11	1	4	3	1.38	3.63
Yuba	4	13	6	10	11	1	2	0	0	0	0.00	5.92
Total WNV Cases	**479**	**379**	**801**	**783**	**442**	**553**	**217**	**225**	**235**	**129**	**0.32**	**1.07**
Asymptomatic Infections	48	54	91	77	41	47	26	18	28	19		
Total WNV infections	**527**	**433**	**892**	**860**	**483**	**600**	**243**	**243**	**263**	**148**	**0.37**	**1.19**

Table 4.—Results of mosquito and sentinel chicken testing for West Nile virus, California, 2021.

County	No. mosquitoes tested	No. mosquito pools tested	WNV + pools	No. flocks	No. chickens	No. WNV positive flocks	WNV + sera
Alameda	8,601	450	0	2	8	0	0
Butte	19,105	405	80	7	54	6	27
Calaveras	0			1	10	0	0
Colusa	0			1	9	1	1
Contra Costa	14,415	396	8	4	24	0	0
Fresno	46,232	1,477	219	0			
Glenn	1,500	30	0	1	10	0	0
Imperial	2,049	140	0	0			
Inyo	760	16	0	0			
Kern	20,934	636	103	0			
Kings	6,558	181	34	0			
Lake	5,159	291	4	2	12	1	4
Los Angeles	133,650	3,805	260	22	125	1	3
Madera	22,746	579	131	0			
Marin	2,528	93	0	0			
Merced	9,311	520	10	8	48	7	19
Napa	3,072	138	1	0			
Nevada	0			1	4	0	0
Orange	133,765	4,962	51	0			
Placer	31,062	2,073	63	0			
Riverside	198,116	5,988	112	0			
Sacramento	55,404	4,883	120	3	15	1	1
San Benito	247	19	0	1	8	0	0
San Bernardino	36,201	2,168	9	0			
San Diego	19,317	1,283	0	0			
San Francisco	0			0			
San Joaquin	84,207	2,537	389	0			
San Luis Obispo	1,771	59	0	0			
San Mateo	3,414	127	0	2	14	0	0
Santa Barbara	2,402	94	0	4	26	0	0
Santa Clara	20,304	2,734	4	0			
Santa Cruz	1,904	150	0	2	12	0	0
Shasta	16,631	635	28	6	41	2	3
Solano	15,180	424	22	3	21	0	0
Sonoma	11,811	420	2	0			
Stanislaus	50,551	1,612	152	0			
Sutter	8,552	251	18	5	35	3	11
Tehama	0			3	30	3	5
Tulare	131,364	3,420	375	1	10	1	10
Ventura	3,164	65	0	3	28	0	0
Yolo	34,933	1,826	56	2	10	2	4
Yuba	5,144	154	12	2	14	1	2
Total	**1,162,064**	**45,041**	**2,263**	**86**	**568**	**29**	**90**

male. The median age was 68 years (range, 56 to 80 years) and dates of symptom onset ranged from August 3 to November 15.

MOSQUITO SURVEILLANCE

In 2021, mosquito testing was performed at DART and 12 local mosquito and vector control agencies. A total of 1,162,064 mosquitoes (45,041 pools) collected in 37 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* mosquitoes also were tested for chikungunya, dengue, and Zika viruses at DART by a separate RT-qPCR.

West Nile virus was detected in 2,263 mosquito pools from 25 counties (Tables 1 and 4), and SLEV was detected in 46 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 1.9. During California's peak transmission period (July – September) the statewide MIR in *Culex* mosquitoes was 3.0 and six counties reported MIRs greater than 5.0, the epidemic threshold value (Figures 2 and 5) (California Department of Public Health, 2021).

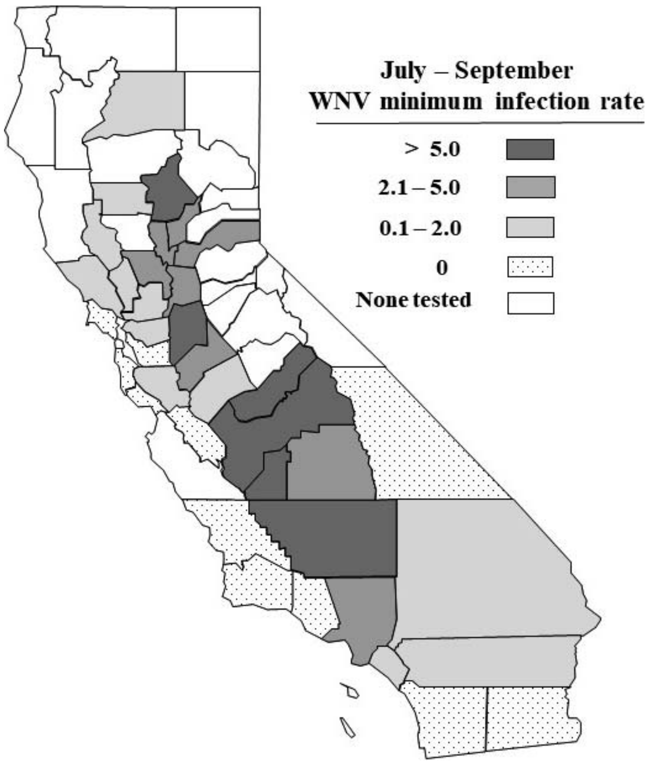

Figure 2.—West Nile virus minimum infection rate in *Culex* mosquitoes, by county, California, July – September, 2021. Minimum infection rate is defined as the minimum number of infected female mosquitoes per 1,000 tested.

Table 5.—Mosquito species tested for West Nile virus, California, 2021.

Culex species	No. Pools	No. mosquitoes	WNV +	MIR
Cx. erythrothorax	1,613	57,473	1	< 0.1
Cx. pipiens	9,426	143,622	362	2.5
Cx. quinquefasciatus	17,611	518,977	881	1.7
Cx. restuans	2	3	0	0.0
Cx. stigmatosoma	611	6,984	25	3.6
Cx. tarsalis	15,048	428,042	993	2.3
Cx. thriambus	94	207	0	0.0
All *Culex*	**44,405**	**1,155,308**	**2,262**	**2.0**
Anopheles species	Pools	No. mosquitoes	WNV +	MIR
An. franciscanus	1	2	0	0.0
An. freeborni	1	1	0	0.0
An. hermsi	6	28	0	0.0
An. punctipennis	5	135	0	0.0
All *Anopheles*	**13**	**166**	**0**	**0.0**
Aedes species	Pools	No. mosquitoes	WNV +	MIR
Ae. aegypti	371	2,291	0	0.0
Ae. albopictus	1	11	0	0.0
Ae. melanimon	18	357	0	0.0
Ae. nigromaculis	3	111	0	0.0
Ae. vexans	19	825	0	0.0
All *Aedes*	**412**	**3,595**	**0**	**0.0**
Other species	Pools	No. mosquitoes	WNV +	MIR
Culiseta incidens	134	1,414	0	0.0
Culiseta inornata	21	233	0	0.0
Culiseta particeps	22	480	0	0.0
Unknown	34	868	1	0.0
All other	**211**	**2,995**	**1**	**0.3**

West Nile virus was identified from five different *Culex* species (*Cx. erythrothorax*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. stigmatosoma*, and *Cx. tarsalis)* (Table 5) and positive pools were collected from May 19 to November 23, with the peak occurring during epidemiological week 33 (August 15 – August 21). St. Louis encephalitis virus also was identified from three *Culex* species (*Cx. pipiens*, *Cx. quinquefasciatus*, and *Cx. tarsalis)* collected from July 13 to November 9.

A total of 19,711 *Ae. aegypti* were tested for chikungunya, dengue, and Zika viruses; all were negative.

CHICKEN SEROSURVEILLANCE

In 2021, 24 local mosquito and vector control agencies in 23 counties maintained 86 sentinel chicken flocks (Table 4). Blood samples were collected from chickens every other week and tested for antibodies to WNV, SLEV, and WEEV by an EIA at the CDPH Vector-Borne Disease Section laboratory and one local agency. Presumptive positive samples were confirmed by IFA or western blot. Samples with inconclusive results were tested by PRNT at the VRDL.

Of 5,930 chicken blood samples tested, 90 seroconversions to WNV were detected among 29 (34%) flocks in 12 counties (Tables 1 and 4). Seroconversions to WNV occurred between July 20 and October 12, with the peak occurring during epidemiological week 35 (August 29 – September 4). In addition, two SLEV seroconversions were detected from two flocks located in Merced County on September 2.

DEAD BIRD SURVEILLANCE

In 2021, the WNV Dead Bird Call Center and website received a total of 5,224 dead bird reports from the public from 52 counties (Table 6). Oral swabs or tissue samples from dead bird carcasses were tested at DART or at one of 12 local agencies by RT-qPCR. Of the 1,755 bird carcasses that were deemed suitable for testing, WNV was detected in 210 (12%) carcasses from 20 counties (Tables 1 and 6). Twenty-one different bird species tested positive for WNV: 41% were American crows, 20% were California scrub-jays, 14% were other corvids, and 25% were non-corvid or unknown species. Positive birds were detected from March 9 to December 27, with the peak occurring during epidemiological week 29 (July 18 – July 24).

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 2021, WNV infection was confirmed in 13 horses from 8

Table 6.—Dead birds reported, tested, and positive for West Nile virus, California, 2021.

County	Reported	Tested	Positive	Percent
Alameda	290	77	2	3
Alpine	0			
Amador	6	0		
Butte	32	11	2	18
Calaveras	4	0		
Colusa	2	0		
Contra Costa	392	37	4	11
Del Norte	1	0		
El Dorado	37	8	0	0
Fresno	91	8	0	0
Glenn	4	0		
Humboldt	1	0		
Imperial	1	0		
Inyo	1	0		
Kern	27	4	0	0
Kings	9	0		
Lake	6	4	0	0
Lassen	0			
Los Angeles	762	133	43	32
Madera	6	2	0	0
Marin	39	2	0	0
Mariposa	1	0		
Mendocino	5	0		
Merced	35	2	1	50
Modoc	0			
Mono	0			
Monterey	13	7	0	0
Napa	26	8	0	0
Nevada	20	7	2	29
Orange	553	435	7	2
Placer	120	52	5	10
Plumas	0			
Riverside	108	26	0	0
Sacramento	712	403	100	25
San Benito	5	1	0	0
San Bernardino	110	26	1	4
San Diego	137	76	1	1
San Francisco	37	8	0	0
San Joaquin	133	54	17	31
San Luis Obispo	26	8	0	0
San Mateo	302	78	0	0
Santa Barbara	36	10	0	0
Santa Clara	453	93	1	1
Santa Cruz	66	18	0	0
Shasta	19	1	0	0
Sierra	0			
Siskiyou	1	0		
Solano	65	24	2	8
Sonoma	96	9	1	11
Stanislaus	124	14	2	14
Sutter	27	7	1	14
Tehama	2	0		
Trinity	1	0		
Tulare	48	14	7	50
Tuolumne	2	1	0	0
Ventura	48	14	1	7
Yolo	162	70	10	14
Yuba	20	3	0	0
Totals	**5,224**	**1,755**	**210**	**12**

counties (Table 1). Two (15%) of the horses died or were euthanized as a result of their infection.

DISCUSSION

In 2021, 39 (67%) of 58 counties reported WNV activity. A total of 128 human cases were reported from 28 counties, which was the lowest number of cases reported in California since 2010 (Figure 3). Los Angeles County reported the most cases (N=16), but the incidence was highest in Glenn County (Table 3). Non-human WNV activity was reported from 32 counties, and similar to the number of human cases, the environmental indicators were also lower compared to previous years (Figures 4, 5 and 6; California Department of Public Health). Surveillance results documented WNV activity throughout most of the year, but the vast majority of detections occurred from June through October, with peak activity occurring in August.

Following the re-emergence of SLEV in California in 2015, SLEV has continued to co-circulate with WNV in many areas of the state, complicating human diagnostics. Outreach to local health departments was conducted in areas with enzootic detections of SLEV and medical providers were encouraged to include SLEV testing for suspect WNV cases. This resulted in the identification of four human SLEV cases from four counties. A total of 46 SLEV-positive mosquito pools were reported from 8 counties, including two of the counties with reported SLEV cases (Table 2). Sentinel flocks were absent from almost all counties where SLEV was detected in mosquitoes (Table 2), but two SLEV seroconversions were detected in two flocks from Merced County, notably before SLEV was detected in mosquitoes collected within the county, highlighting the importance of utilizing all surveillance tools to detect arbovirus activity.

Although WEEV has not been detected in California since 2007, routine testing of mosquitoes and sentinel chickens for WEEV has continued in the event this historically endemic arbovirus reemerges. Additionally, many of the collected invasive *Aedes* mosquitoes, *Ae. aegypti* and *Ae. albopictus*, continued to be tested for chikungunya, dengue, and Zika viruses to maintain vigilance for the possible introduction of these exotic arboviruses into California.

CONCLUSIONS

Although WNV activity was lower in 2021 compared to many previous years, WNV remains the greatest vector-borne disease threat in California, with over 7,000 cases and more than 300 fatalities reported since 2003. Human cases of both WNV and SLEV disease were identified in 2021, highlighting the importance for ongoing surveillance and awareness of potential human disease risk. Environmental detections of both viruses often preceded the incidence of human cases, supporting the value of environmental surveillance to direct mosquito control

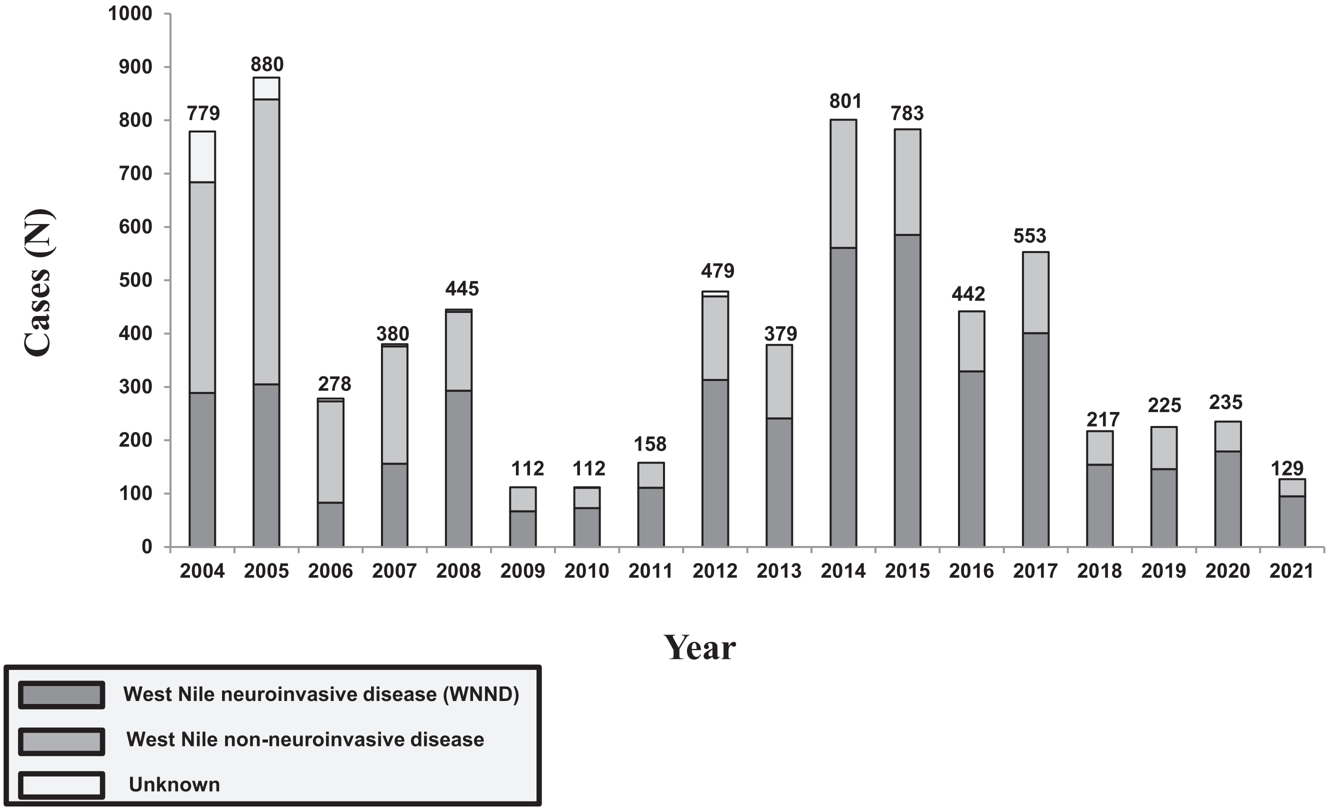

Figure 3.—Human cases of West Nile virus in California, by year, 2004 – 2021.

efforts and decrease the risk arboviral diseases in California.

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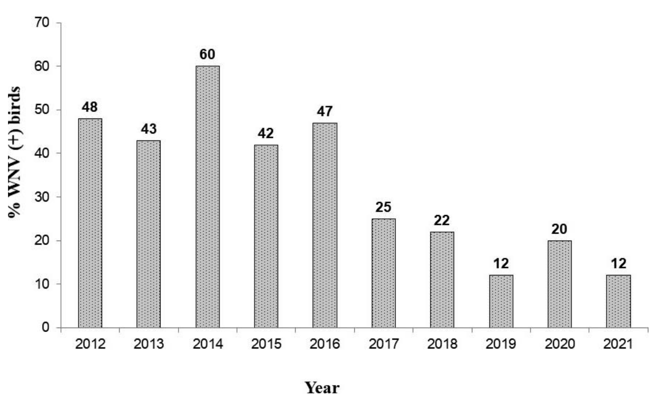

Figure 4.—Percentage of dead birds positive for West Nile virus in California, 2012 – 2021.

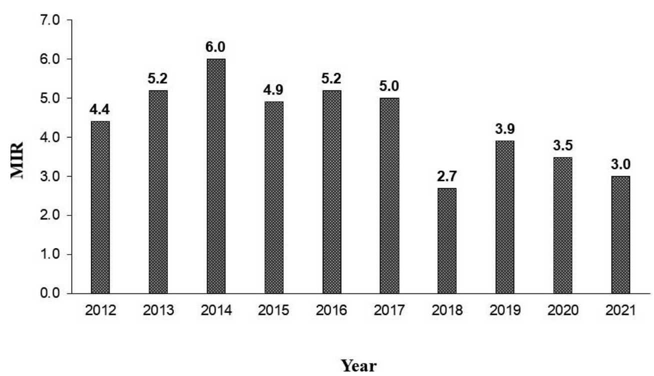

Figure 5.—Minimum infection rate (MIR) in females per 1,000 tested for West Nile virus in *Culex* mosquitoes in California, July – September, 2012–2021.

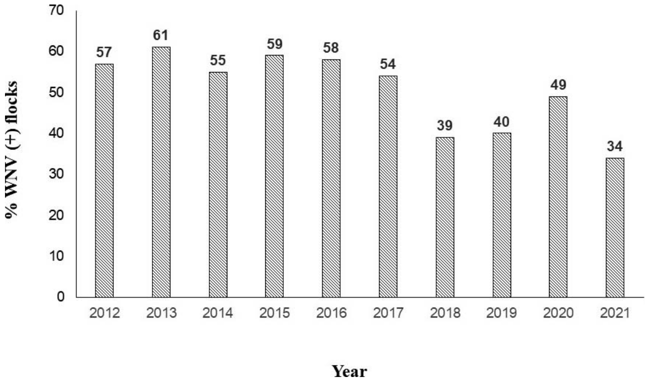

Figure 6.—Percentage of sentinel chicken flocks in California with one or more birds positive for antibodies to West Nile virus, 2012–2021.

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Human and environmental detections of St. Louis encephalitis virus in California, 2015-2020

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Abstract

St. Louis encephalitis virus (SLEV) is an endemic flavivirus in the southeastern and western United States, including California. From 1938 to 2003, the virus was detected annually in the state. However, after West Nile virus (WNV) arrived in 2003, SLEV was not detected again before re-emerging in Riverside County in 2015. From 2015 to 2020, SLEV was detected in 1,650 mosquito pools and 26 sentinel chickens in 16 (28%) of California's 58 counties. There also were 24 reported human infections in 10 California counties, including two fatalities (case fatality rate: 8.3%). Because human surveillance for SLEV relies on an environmental detection to trigger SLEV screening, which is not uniform throughout the state, and because human infections with SLEV are difficult to distinguish from more prevalent WNV with current serological diagnostic tests, the incidence of SLEV disease in California may be underestimated.

West Nile virus dead bird surveillance in California: Early versus recent years

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Abstract

Data from the early years (2004-2006) of the West Nile virus (WNV) dead bird surveillance program were compared to later years (2018-2020). Dead birds were reported approximately ten times more frequently in 2004-2006 compared to 2018-2020. Although fewer agencies collected dead birds in later years, most vector control agencies with annual WNV activity used dead birds as a component of their WNV surveillance program. Refinements to the bird species and carcass age acceptance criteria for testing helped agencies maximize the number of positive dead birds relative to the total number of birds collected. Finally, the seasonal upsurge in positive dead birds occurred earlier in years 2004-2006 than in years 2018-2020.

Thermal preferences of *Aedes aegypti* mosquitoes in California: laboratory and field studies

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Introduction

Aedes aegypti transmit a range of viruses, including Zika, dengue, and yellow fever, that cause a major burden on human health worldwide. Transmission of these viruses is closely linked to environmental conditions, including temperature, yet little is known about their thermal preferences. Current pathogen transmission models primarily use temperature data from weather stations as a proxy for the temperatures mosquitoes experience. However, temperatures from these sources have been shown to be an inadequate representation of the temperatures of local environments, or microclimates, available to adult mosquitoes. Here, we assess the thermal preferences of *Ae. aegypti* under laboratory and field conditions to improve viral transmission models that guide preventative control strategies.

Methods

Laboratory thermal preference assay

Thermal preferences of various California *Ae. aegypti* populations were assessed using an aluminum thermal gradient bar with a 5-sided Plexiglas arena attached to the gradient. Trials were conducted in a temperature and humidity controlled chamber at 23°C and 60% humidity and temperatures along the gradient were monitored continuously with thermocouple sensors (Omega). Gloves were worn when interacting with the arena to avoid interference of human scent. Mosquitoes were reared under multiple temperature conditions to assess the effect of larval rearing temperature on adult thermal preference. Cohorts of 15 mosquitoes were added to the arena from either the cold end, hot end, or middle, and were left in the arena for 25 minutes. At the end of each trial time, mosquitoes were anesthetized with CO_2 and discarded; each mosquito was used only once. Mosquitoes were monitored remotely using time-lapse photography with three c920 Logitech HD Pro webcams and VideoVelocity software. Mosquito resting locations and temperatures were determined using ImageJ and the Figure Calibration plugin.

Resting Boxes: field study in Madera, CA

Thirty 13”x13”x16” cardboard resting boxes covered on the inside with black fabric were placed in the front or backyard of 10 homes for six weeks during the season of peak *Ae. aegypti* abundance in Madera, California from August 25, 2021 until September 30, 2021. Boxes were placed to represent a variety of microhabitats (i.e. full shade, afternoon sun, under covered patio, near or under bushes or wall, fully exposed, next to sitting area, etc.). A temperature sensor was placed in each box along with a dog bowl with water to increase humidity. Resting boxes were aspirated two times a day during peak mosquito resting periods and mosquitoes were identified to species (*Aedes* and *Culex*) or genus (*Anopheles*) using microscopy.

Results and Discussion

Laboratory thermal preference assay

In preliminary trials with laboratory-adapted *Ae. aegypti* from Clovis, CA (F_{15}) exposed to a gradient of 17°C to 36°C, 76% of mosquitoes landed on a surface where their temperature could be recorded. The highest density of landing on the gradient occurred around 25°C and the lowest density occurred at the extreme temperatures at the edges of the arena, whereas mosquitoes showed a preference for the edges of the arena in control trials when there was no thermal gradient (constant 25°C). This presentation included results from trials with F_1 generation *Ae. aegypti* from Madera reared at different temperatures.

Resting Boxes: field study in Madera, CA

A total of 1,008 box aspirations were done, resulting in 1,479 total mosquitoes collected, of which 205 (13.9%) were female *Ae. aegypti*. The boxes spanned a wide range of temperatures and microhabitats. A model of *Ae. aegypti* thermal preferences in Madera was compared with the laboratory behavioral thermoregulation results.

Acknowledgements

The authors thank Sunny An for help rearing mosquitoes and preparing materials for the study, Dr. Anthony Cornel for providing the field-collected *Aedes aegypti* from Clovis, CA, Istvan Menyhay and Teresa Hamilton from the Madera County Mosquito and Vector Control District, and the residents of Madera that allowed us to place resting boxes in their yards. Financial support for this study was provided by the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement U01CK000516). OCW also acknowledges support from the National Science Foundation Graduate Research Fellowship (Grant No. 1650042).

Aedes aegypti population expansion and associated potential disease risk in large portions of Los Angeles County, California

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Introduction

Aedes aegypti is a species of serious public health concern as it is the principal vector of several arboviruses, including dengue, chikungunya, and Zika (Black IV et al. 2002, Roundy et al. 2017). It is widely distributed globally, especially in tropical and subtropical regions, and continues to expand its range into temperate regions (Kamal et al. 2018).

The first detection of *Ae. aegypti* within the boundaries of the Greater Los Angeles County Vector Control District (District) was in the city of Commerce in October 2014. By 2021 *Ae. aegypti* had been detected in nearly all areas of the District. Not only has this invasive species rapidly expanded its distribution over the past seven years, but adult abundance has steadily increased.

California reports among the highest travel-associated *Aedes*-borne disease cases in the United States (CDC 2021). Being the largest metropolitan area and tourist destination in California, Los Angeles County accounted for 20% of California's cases since 2015 (CDPH 2022). Despite imported cases of dengue, chikungunya, and Zika reported annually, Los Angeles County has not yet detected any evidence of local transmission of these arboviruses.

Our study provides a visualization of the distribution of *Ae. aegypti* over time throughout the District's service area since *Ae. aegypti* introduction in 2014. Furthermore, *Ae. aegypti* females were mapped alongside the distribution of imported *Aedes*-borne disease cases in the same area to identify overlaps and the potential for local disease transmission.

Methods

Mosquito abundance data were gathered from the California Vectorborne Disease Surveillance Gateway and associated with U.S. Census Bureau ZIP Codes. The dataset was filtered to include only adult *Ae. aegypti* mosquitoes. Human disease records provided by the Los Angeles County Department of Public Health and kept by the District were grouped into ZIP Codes and a timeline of viremic individuals was created based on symptom onset for dengue, chikungunya, and Zika virus cases. Cases were only included if they had an associated ZIP Code and a date of symptom onset.

Viremia was assumed to last two weeks post symptom onset. This assumption was to approximate the maximum observed viremic period for all three viruses. Viremia been observed to last up to 12 days in dengue infections, 13 days in chikungunya infections, and 16 days in Zika virus infections (Gubler et al. 1981, Riswari et al. 2016, Fontaine et al. 2018).

The data were mapped in QGIS version 3.22.1 (http://qgis.org). A time lapse animation was generated to illustrate the spread of *Ae. aegypti* throughout the District's service area, with cumulative numbers of detections shading in ZIP Codes over time. This visualization included detections of all adult *Ae. aegypti* regardless of sex. Time lapse animations mapping active cases of dengue, chikungunya and Zika virus also were generated, with *Ae. aegypti* detections lasting on the same map for two weeks to show overlap between detected vector populations and active disease cases. For the *Aedes*-borne disease time lapse animations, only female *Ae. aegypti* detections were included to better visualize transmission risk.

Results and Discussion

By leveraging the mapping software to create an animated time lapse, the rate at which *Ae. aegypti*

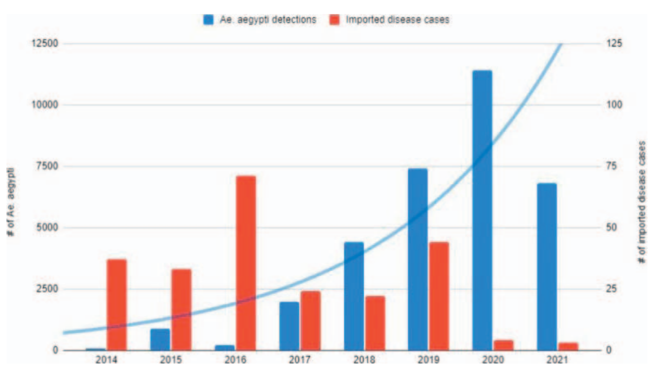

Figure 1.—Annual *Ae. aegypti* detections and imported *Aedes*-borne disease cases in Greater Los Angeles County Vector Control District

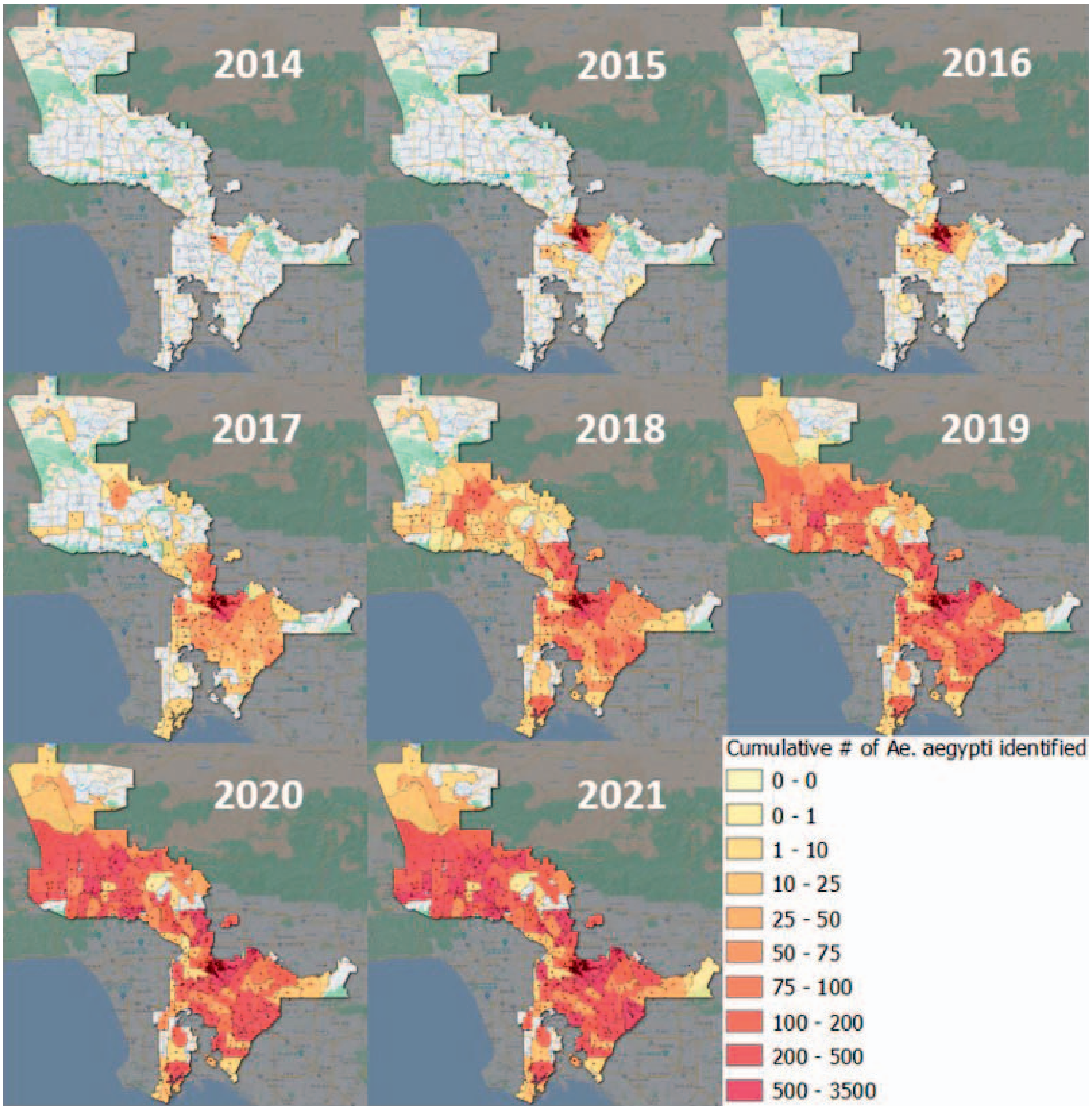

Figure 2.—Cumulative *Ae. aegypti* detections and abundance since initial detection in Greater Los Angeles County Vector Control District (generated yearly in November).

populations established themselves in different areas of the District could be clearly visualized. Between 2014 and 2016, detections were localized mostly southeast of Los Angeles. In 2017, *Ae. aegypti* spread through the southern areas of the District's service area, and also was detected in the San Fernando Valley. Between 2018 and 2021, *Ae. aegypti* detections expanded to nearly all areas of the District. Abundance trended upwards since the initial detection in 2014, albeit dipping slightly in 2021 (Figure 1). The examination of yearly snapshots of cumulative *Ae. aegypti* detections illustrated how widespread this mosquito has become throughout the District's service area (Figure 2).

Imported *Aedes*-borne disease cases identified within the District's service area varied widely in the number of new cases per year (Figure 3). The highest number of chikungunya cases were identified in 2014 and 2015, declining sharply in subsequent years. Coinciding with the 2015-2016 Zika virus epidemic, a spike of Zika virus cases was observed in 2016. Furthermore, a relatively high

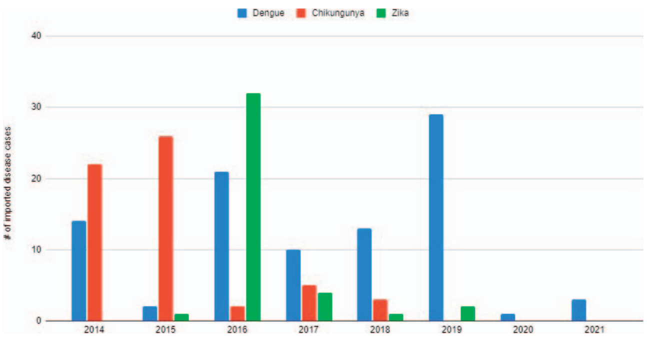

Figure 3.—Imported *Aedes*-borne disease cases in Greater Los Angeles County Vector Control District. (Actual cases may be higher, as data only includes cases with a known symptom onset date and an associated Zip Code).

number of dengue cases was identified in 2016 and 2019. The number of *Aedes*-borne disease cases in 2020 and 2021 decreased dramatically during the COVID-19 pandemic, when strict travel restrictions were in place.

Due to the relatively low distribution and abundance of *Ae. aegypti* mosquitoes within the District's service area during the first three years following their introduction, there were few intersects of imported *Aedes*-borne disease cases and *Ae. aegypti* females between 2014 and 2016. Despite a significant population expansion of *Ae. aegypti* throughout the District's service area during 2017, human disease case numbers were low (Figure 1). The most significant spatiotemporal intersects of imported *Aedes*-borne disease cases in the District's service area consisted of six instances between 2018 and 2019, with 15 imported dengue cases in areas with elevated female *Ae. aegypti* detections (Figure 4).

Although there was evidence that viremic individuals were in areas in the District abundant with *Ae. aegypti* females on numerous occasions between 2018 and 2019, there has been no evidence of any locally transmitted cases. A multitude of studies suggest that presence of the vector alongside imported cases are not the only factors in a local outbreak. There are demographic factors such as higher poverty levels and population density that are associated with dengue outbreaks (Morgan et. al 2021, Hsu et. al. 2017). However, in these respects, the District's service area seems to have similar demographic factors to areas in Florida where the local transmission of *Aedes*-borne viruses have occurred regularly (U.S. Census Bureau 2020). One hypothesis suggests that other demographic factors such as local lifestyle and the prevalence of air-conditioning and window screening affect the opportunity for mosquito-human contact (Reiter et al. 2003). These demographic factors need to be evaluated in the context of the District's

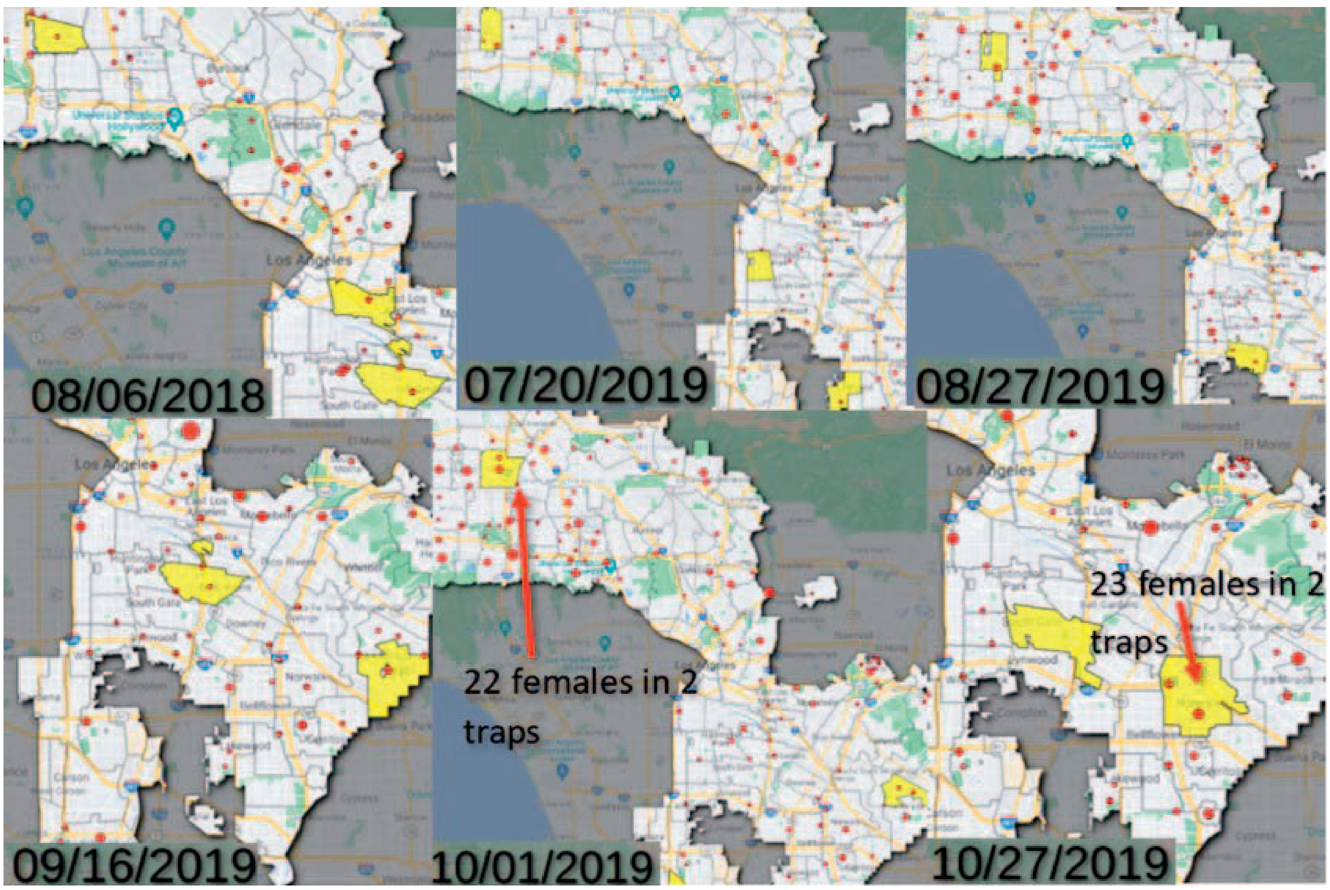

Figure 4.—Spatiotemporal intersects of *Ae. aegypti* detections and imported dengue cases in Greater Los Angeles County Vector Control District. Shaded ZIP Codes indicate presence of a viremic dengue case, and red symbols indicate detections of female *Ae. aegypti*.

service to determine if there is evidence that they contribute to the lack of local transmission of *Aedes*-borne viruses.

Climactic factors within the District's service area differ from other areas in the U.S. with *Aedes*-borne disease transmission. Los Angeles County is quite arid, with warm and dry summers, and mild winters (Bruno et. al 2000). Increasing ambient temperatures (up to a maximum of 27.5° C) and humidity are associated with lower mortality of *Ae. aegypti* (Schmidt et al. 2018). Longevity of female *Ae. aegypti* is very important for the transmission of these arboviruses. In Mexico, low dengue incidence was observed in areas with younger *Ae. aegypti* age structures (Ernst et. al. 2017). The extrinsic incubation period (EIP) of the virus must be attained for local transmission to occur. In the case of dengue, the EIP for *Ae. aegypti* increases with decreasing temperature, from roughly seven days in warmer weather to approximately 12 days in cooler weather (Watts et. al 1987). Another hypothesis as to why local transmission has not occurred in the District's service area is that the climate is not hospitable to a long-lived *Ae. aegypti* population, and that only a low proportion of *Ae. aegypti* females can reach the EIP within their lifespan. However, there does not appear to be any information about the age structure of the local *Ae. aegypti* population within the District's service area and further investigation is needed.

As post-pandemic travel resumes and if the pattern of increasing *Ae. aegypti* abundance continues, the risk of local disease outbreaks may increase as well. The District plans to continue to monitor these developments to be prepared to act on potential disease outbreaks.

Acknowledgements

We would like to thank our colleagues in the Invasive *Aedes* Team and Scientific-Technical Department for their hard work responding to the spread of *Ae. aegypti* across the District's service area and for collecting data for this project.

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Are below-ground public utility vaults harboring *Aedes aegypti*?

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Abstract

Since *Aedes aegypti* were first detected in Madera in 2013, this highly invasive species has continued to expand across central and southern California. *Ae. aegypti* is found primarily in close association with human dwellings and is both a severe nuisance biter and a competent vector of several arboviruses of human health concern. The species exploits a diverse range of water-holding containers in and around houses, including many that are hidden or difficult to access. Public utility vaults are ubiquitous below-ground structures that are found throughout California's residential neighborhoods, yet their potential for mosquito production has not been thoroughly studied, in part, due to the difficulty of obtaining access. Our study aimed to understand the significance of underground public utility vaults as a potential larval source and a harborage for overwintering adult *Ae. aegypti* females.

A survey of public utility vaults was conducted by Madera County MVCD in the cities of Madera and Chowchilla. The survey was conducted just after the annual abundance peak of *Ae. aegypti* in the early fall of 2021. Each vault's status was recorded at the time of the survey as to whether it held water, contained live larvae or pupae, or adult mosquitoes were present within the vault. Immatures were collected from vaults when possible and reared to adults in the laboratory for species identification. Emergence traps were placed over vaults with the greatest larval populations to assess egress of adults following the survey visit. Temperature data loggers were placed in vaults from fall-winter to monitor hourly temperatures , along with parallel monitoring of ambient temperatures in adjacent above-ground habitats.

This study provided a much-needed characterization of mosquito production from public utility vaults in two cities with well-established populations of *Ae. aegypti* in the Central Valley. The temperature loggers provided evidence of the potential for utility vaults to serve as refugia from otherwise lethal winter temperatures.

Comparison of species diversity capture in CO_2 baited Encephalitis Virus Surveillance and BG-Sentinel traps in Los Angeles County

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Introduction

The Greater Los Angeles County Vector Control District (GLACVCD) has traditionally used CO_2 baited Encephalitis Virus Surveillance (EVS) traps in addition to gravid traps for monitoring mosquito abundance and the presence of arboviruses among local mosquito populations. With the arrival and spread of the invasive *Aedes* species, Biogents Sentinel (BG) traps were added to nearly all routine surveillance sites in 2018. As both EVS and BG traps were baited with CO_2, deploying them in tandem required the additional investment of both time and resources, in addition to creating a significant battery waste stream. The unpredictability of dry ice availability during the COVID-19 pandemic further complicated efforts to deploy both traps in tandem at each surveillance site. In response, the collection data for both trap types were compared over a four-year period for abundance and biodiversity of collections.

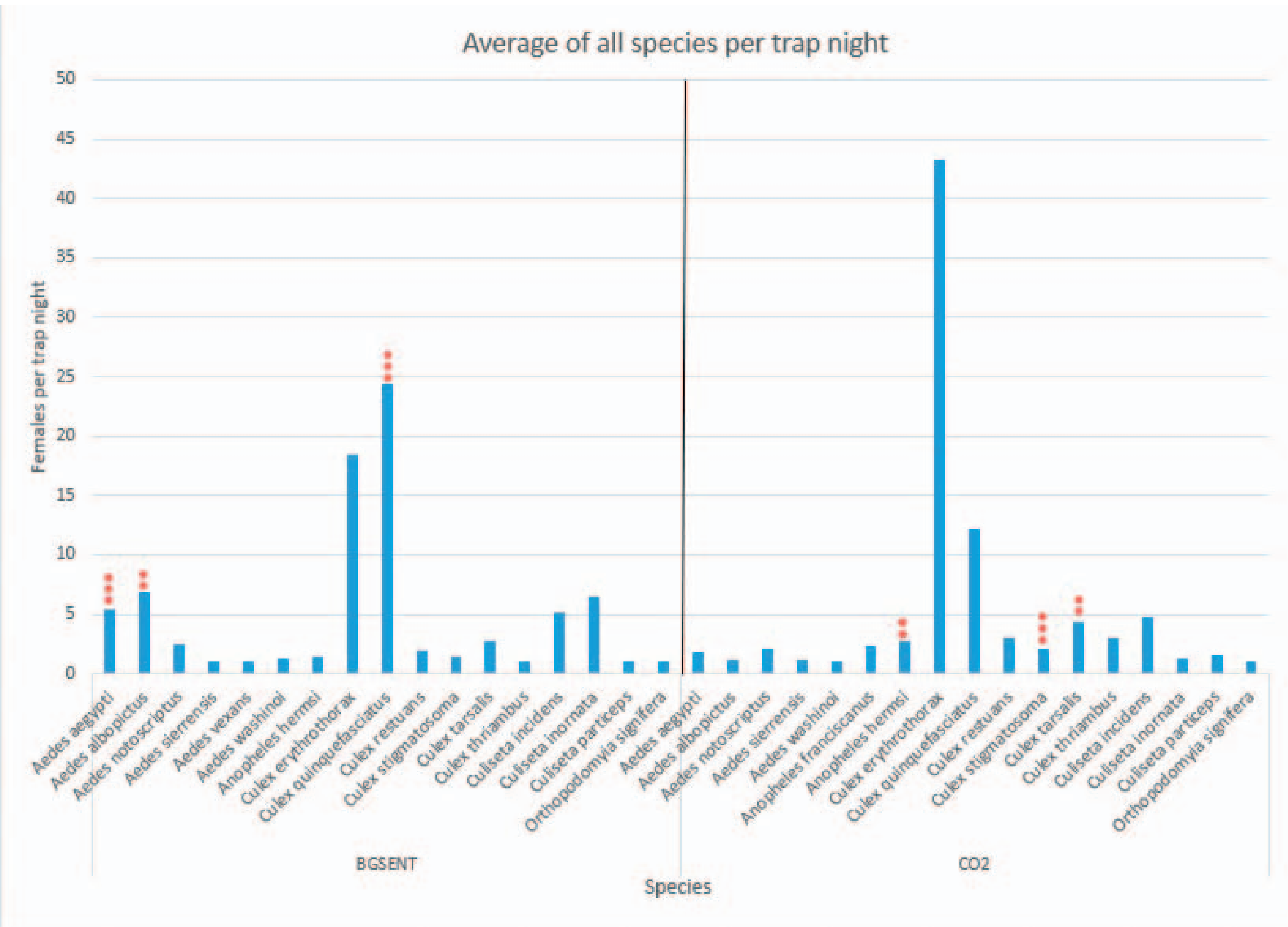

Figure.—Average of all females per trap night collected in BG and EVS traps during routine surveillance between 2018-2022.

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Table 1.—Number of mosquitoes collected from each trap type from 2018 through 2021.

	Trap Type						
	BG Sentinel			CO_2-baited EVS			
Species	*n*	Mean (SD)	Median	*n*	Mean(SD)	Median	*p-value*
Ae. aegypti	3107	5.1 (6.6)	3.0	866	1.6 (1.8)	1.0	< 0.001
Ae. albopictus	90	6.4 (8.7)	3.0	12	1.1 (0.5)	1.0	0.002
Ae. notoscriptus	135	2.5 (2.5)	1.0	41	2.0 (1.9)	1.0	0.210
Ae. sierrensis	18	0.39 (0.5)	0.0	24	0.50 (0.6)	0.0	0.587
Ae. washinoi	4	1.3 (0.5)	1.0	1	1.0	1.0	0.617
An. hermsi	20	1.5 (1.6)	1.0	87	2.7 (3.0)	1.0	0.009
Cs. incidens	1554	5.0 (12.5)	2.0	1494	4.7 (8.5)	2.0	0.616
Cs. inornata	67	5.3 (22.6)	1.0	56	1.3 (0.6)	1.0	0.516
Cs. particeps	1	1.0 (0.0)	1.0	2	1.5 (0.7)	1.5	0.480
Cx. erythrothorax	69	18.3 (39.3)	4.0	270	42.8 (133.1)	6.0	0.103
Cx. quinquefasciatus	4608	24.1 (39.3)	10.0	3531	12.2 (22.2)	5.0	< 0.001
Cx. restuans	2	2.0 (1.4)	2.0	17	2.9 (4.8)	1.0	0.644
Cx. stigmatosoma	163	1.4 (1.1)	1.0	417	2.1 (2.7)	1.0	< 0.001
Cx. tarsalis	632	2.7 (5.4)	1.0	855	4.34 (13.9)	1.0	0.005
Cx. thriambus	2	2.7 (5.4)	1.0	46	3.0 (2.6)	2.0	0.109
Or. signifera	1	1.0	1.0	1	1.0	1.0	0.500

Method

The data for these analyses were obtained through the Vectorborne Disease Surveillance Gateway (VectorSurv). Statistical analyses were conducted with the use of STATA (StataCorp, Stata Statistical Software: Release 15. College Station, TX). Preliminary examination of the distribution for the trap count data for female mosquitoes used as the dependent variable showed significant dispersion and zero-inflation. Additionally, several mosquito species were represented by small sample sizes. Hence, to assess whether mosquito collections varied significantly across trap type for each species, a series of nonparametric Wilcoxon Rank Sum Tests were conducted. Differences in mosquito collections across trap types were evaluated separately for each species. Associated probabilities of $p < 0.05$ were considered statistically significant.

Results and Conclusion

From 2018 – 2021, a total of 16 species were captured in each trap type (Figure 1). Although there was significant species overlap in the collections of each trap type, the abundance data varied (Figure. 1). The results of the analyses showed that although there were no significant differences in the number of female mosquitoes collected for a majority of species across trap types, significant differences were observed for several species, favoring either the BG Sentinel or the CO_2-baited EVS traps. BG Sentinel traps, for example, were shown to be more effective in the collection of *Ae. aegypti* ($z = 21.74$, $p<0.001$), *Ae. albopictus* ($z = 3.04$, $p<0.01$), and *Cx. quinquefasciatus* ($z = 19.49$, $p<0.001$) when compared to CO_2-baited EVS traps (Table 1). Conversely, CO_2-baited EVS traps were shown to perform better in the collection of *Cx. stigmatosoma* ($z = -4.05$, $p<0.001$), *Cx. tarsalis* ($z = -2.78$, $p<0.01$), and *An. hermsi* ($z = -2.06$, $p<0.01$) (Table 1).

Based on these findings, we conclude that the BG Sentinel trap is equally capable, if not more so, as the EVS trap for sampling mosquitoes of medical importance in a variety of habitats. Additionally, with relatively few exceptions, the use of the BG Sentinel trap does not come at the cost of reduced species diversity in trap collections. Going forward, GLACVCD has opted to phase out the use of EVS traps in most of its routine surveillance sites except for riparian and wetland habits or in areas where there are known populations of species of interest, such as *Cx. tarsalis,* which favor the EVS traps.

Host-seeking behavioral patterns of *Aedes aegypti* in Madera and Kern Counties

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Introduction

Following initial detection in 2013, *Aedes aegypti* have continued to spread across central and southern California, and they maintain high priority as surveillance and control targets due primarily to severe biting nuisance as well as their potential to transmit arboviruses such as dengue and Zika (CDPH 2019). The timing of their host seeking behavior therefore is of interest for vector control to identify periods of greater activity which could inform adulticide applications and public outreach. Although chiefly identified as daytime feeders, *Ae. aegypti* have been shown in several studies to have variable host-seeking diel patterns thought to be attributable to ambient light changes among urban and rural environments as well as differences in daily temperatures in different climates (Trpis et al. 1973, Chadee and Martinez 2000, Scott et al. 2000, Smith et al. 2018, Kawada et al. 2005). In the drier climate of the Southern Central Valley, the timing of preferable temperature and humidity conditions for *Ae. aegypti* host seeking remains unclear. Host selection patterns also have yet to be investigated in this region. We conducted pilot studies in 2019 that showed apparent increases in morning and evening activity but with noticeable differences in site-specific capture rates. Our study aimed to identify high-activity periods for host-seeking *Ae. aegypti* in Madera accounting for variability among sites and to identify blood meal sources of *Ae. aegypti* in Bakersfield and Madera.

Methods

Collection bottle rotator traps (Part #1512.5, John W. Hock Company) were set at residential sites in Madera County for six weeks in late summer 2021. Collections were divided into seven intervals of different duration over a 24-hour collection period. Two traps with staggered overlapping schedules were placed at four backyard sites. Traps were baited with dry ice and a mosquito attractant lure (BG-Lure™ cartridge, Biogents) or octenol. Mosquitoes were counted and identified to species and sex. The number of adult females collected per hour was modeled as a function of the discrete sampling intervals offset by interval length using generalized linear models with a negative binomial distribution. Coefficients for sampling intervals represent the difference between the expected capture rates of each interval and the expected capture rate of the morning activity peak, 06:00-08:00h, which was chosen as the referent group during model fitting.

Bloodfed *Ae. aegypti* were collected in bottle rotator traps, during a separate study in Madera investigating backyard microhabitats, and in routine trap collections in Madera and Bakersfield. Bloodmeal analysis was performed using PCR amplification of the cytochrome oxidase I barcoding region followed by genetic sequencing to identify the bloodmeal hosts to species.

Results and Discussion

Two activity peaks were observed, corresponding with dawn and dusk, with decreases overnight and in the afternoon. Adult females (n =246) were captured more frequently in the evening than in the morning, with the greatest difference in capture rates in the intervals immediately preceding or overlapping with sunset. The lowest capture rates occurred during overnight intervals (21:00 – 04:00h and 22:00 – 05:00h) and the afternoon interval (10:00 – 13:00h), while the highest occurred in the morning (05:00 -07:00h and 06:00 – 08:00h) and evening (17:00h – 19:00h, 18:00 – 20:00h, and 19:00 – 21:00h). The intervals corresponding with the evening peaks were the only ones with capture rates exceeding the that of the morning peak interval, with the capture rate from 19:00 to 21:00h on average over five times the morning capture rate. In addition to the evening peaks, we also observed that morning intervals generally had similar capture rates, while the capture rates in the afternoon (10:00-13:00h) and overnight (22:00-05:00h) were significantly lower in comparison, with an average rate 0.04 times that of the morning peak. Elevated activity periods were associated with changes in light cues corresponding with circadian

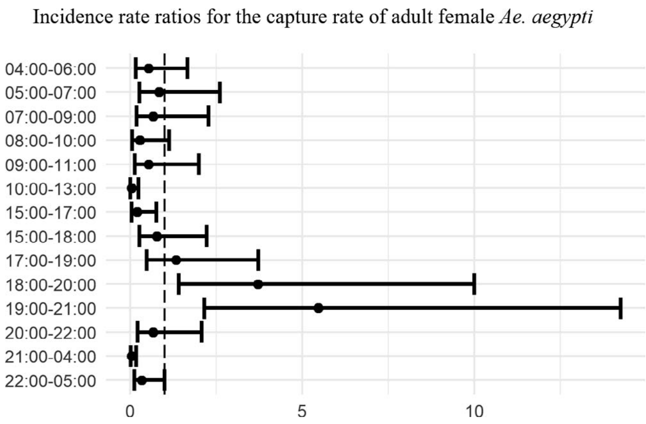

Figure 1.—Rate ratios for capture of adult female *Aedes aegypti* per sampling interval compared to the 06:00 - 08:00h interval

rhythm, so it is important to note that the precise timing of increased activity is expected to be seasonal, with activity peaks shifting in response to sunrise and sunset times rather than specific hours of the day.

Of the 38 bloodfed *Ae. aegypti* successfully tested, 15 were from Madera and 23 from Bakersfield. In Madera, most females fed on mammals (73.3%, N = 11); the rest fed on birds (26.7%, N = 4). The majority of mammal meals were from humans (40.0%, N = 6), followed by domestic cats (20%, N =3), domestic dogs (6.7%, N = 1), and a grey fox (6.7%, N =1). Avian meals were from mourning doves (20.0%, N = 3) and house finches (6.7%, N = 1). In Bakersfield, the majority also fed on mammals (87.0%, N = 20), with the remainder on birds (13.0%, N = 3). The most common mammalian host was domestic dogs (34.8%, N = 8), followed by humans (30.4%, N = 7), domestic cats (7.4%, N = 4), and horses (4.3%, N = 1). Avian meals were from chickens (8.7%, N = 2) and a green heron (4.3%, N = 1). These results showed a greater diversity of hosts than expected, as *Ae. aegypti* is well-known as an anthropophilic species, preferentially feeding on humans. We did not have measures of host availability for comparison and therefore could not make conclusions about whether this feeding behavior was opportunistic or obligate. Identifying indicators of human host availability throughout the day would be an important step in future studies characterizing *Ae. aegypti* feeding patterns in the region. Future research sampling more diverse environments also would address possible bias that arose from sampling primarily in suburban areas.

Conclusions

Host-seeking *Ae. aegypti* in Madera were primarily active during periods surrounding dawn and dusk. Seasonality of these twilight periods should therefore be taken into account in deciding the timing of adult vector control interventions as well as in public outreach materials describing personal protective measures for residents. Unexpectedly, *Ae. aegypti* fed on a diverse array of hosts in Bakersfield and Madera, although the nature of this behavior was unclear. Relative feeding frequency on human hosts is valuable information for predicting potential arbovirus transmission dynamics.

Acknowledgements

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Variation in thermal tolerance in the western tree hole mosquito, *Aedes sierrensis*

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Abstract

The western tree hole mosquito, *Aedes sierrensis*, is a major biting nuisance in western North America and a vector of the pathogen causing dog heartworm. Because vector control efforts are expensive, with annual county budgets frequently exceeding $10 million, surveillance and interventions must be tailored to the appropriate locations and seasons of mosquito activity. This requires a strong understanding of how well *Ae. sierrensis* populations are currently adapted to their local climate conditions and how they may respond to ongoing climatic changes – key questions in mosquito biology.
To address this critical gap, we investigated variation in temperature regimes and mosquito thermal tolerance for *Ae. sierrensis* populations collected across the species distribution. We reared replicates from each field-collected population under different temperature treatments, spanning the range of spring and summer temperatures experienced by the species (e.g., 10 – 30°C). For each temperature and replicate, we measured the life history traits constituting mosquito fitness (development rate, survival, fecundity, fertility) and calculated the upper thermal limits for each trait and population. We found substantial variation in the spring and summer temperatures experienced across the species range, and population-level variation in trait upper thermal limits that corresponded to the source thermal environment. These results indicated that *Ae. sierrensis* were locally thermally adapted, which may facilitate persistence under ongoing climate warming.

North American Mosquito Project: Population genetics of *Culex tarsalis* using crowdsourcing methodology

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Abstract

Culex tarsalis were collected using crowd sourcing throughout North America. The samples were sequenced using RADseq to identify 7,632 SNPs throughout the genome. The population-level allele frequencies identified six distinct populations with no association to genetic isolation-by-distance. This implies evolutionary adaptations separating the populations such as summer estivation, winter diapause, and desiccation resistance given the West to East geographic establishment pattern.

An objective method for inter-annual comparisons of historical WNV activity based on mosquito surveillance data

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Introduction

Currently, human cases are the primary basis for comparisons of West Nile virus (WNV) activity across years. This is problematic because human cases are often underreported or undetected, because most cases are asymptomatic. The goal of the current project was to create an objective method to use entomological surveillance data from 2009-2018 for measuring the intensity of WNV activity in a given year. Widely collected mosquito abundance and infection prevalence data provide a more objective indicator of WNV activity than human case incidence (Snyder et al. 2020), and comparisons of entomological risk estimates with patterns of reported human cases could reveal biases in WNV disease reporting.

Methods

Trapping effort for each mosquito control agency varies each year due to changes in surveillance strategies and needs. This variation can further increase due to adaptive sampling that intensifies surveillance in places and during times of increased WNV activity. To standardize surveillance data across years, we developed a spatial grid that allowed us to weight and standardize the data collected in each year to an effort level that represented an average year.

Weighted averages were calculated for abundance and infection prevalence data by year and hydrological region. The vector index (VI) then was calculated by multiplying abundance and infection prevalence. Recorded WNV disease incidence was compared to expected incidence predicted by the entomological data using generalized linear models.

Results and Discussion

To determine the efficacy of this method, the number of human cases that would have occurred was compared against the recorded cases that occurred in a hydrological region in a year. Deviations of observed WNV disease case counts from model-predicted WNV disease case counts based on the VI were observed. There were some years when observed cases were below VI-based expectations and other years when observed cases were above expectations. These differences have not yet been investigated to determine if they are statistically significant. These deviations could be due to variation in case detection by passive surveillance or differences in risk. Additionally, expectations based on the VI alone could have been modified by local risk factors that were not accounted for in the model, resulting in over-predictions. In particular, the degree of overlap between urbanized areas and areas where VI was high could have differed between years, resulting in under-predictions. More investigation is needed to definitively determine if the areas of high VI and dense populations do overlap.

Conclusion

The goal of this project was to provide objective estimates of the intensity of WNV activity for each year by using mosquito abundance and infection prevalence data as opposed to human case data. Results showed that observed case numbers differed by up to 20-30% from VI-based expectations. Further investigation is needed to refine the model and to explain the causes of interannual variation in differences between observed and expected case counts.

Acknowledgements

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BiTeRS: Rickettsial surveillance at the US-Mexico border to save lives, engage new partners, and expand awareness of tick-borne disease

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Introduction

Over one hundred cases of Rocky Mountain spotted fever (RMSF), many fatal, are reported annually across the US-Mexico transboundary region. In the modern era, cases originally are described on tribal lands in Arizona, although sporadic cases were identified in multiple states in Mexico more than a century ago. Over the last two decades, the epidemic has emerged in Sonora state and expanded into Baja California, Mexicali, Ensenada and Tijuana. Tracking movement through canine serology and PCR-testing of brown dog ticks indicates a high risk of further expansion into the US, particularly due to cross-border travel and climate change. Although the agent of RMSF, *Rickettsia rickettsii,* is the most highly lethal of the rickettsiae present in North America, there are numerous other species with poorly understood ecologies, including *R. parkeri* and the 364D agent, which could be associated with cross-reacting serological tests in vertebrate animals including humans. The lack of comprehensive information about the distribution of infected ticks across the entire border precludes best management and intervention practices that could allow vector control agencies to help people protect themselves and their pets.

Methods

Collaboratively with the Western Gulf Center of Excellence for Vector-Borne Disease, we established a surveillance program aimed at the entire US-Mexico border from San Diego to eastern Texas near Matamoros. Our program in California and Arizona is called Border Tick Rickettsia Surveillance, or BiTeRS. In each state (California, Arizona, New Mexico, and Texas), local leads engage partners to collect ticks, identify them to species, test them for rickettsiae, undertake training and education, and provide information about tick management, as desired by the partner. Partners in California and Arizona include Native American tribes, county vector control agencies, humane societies, US Border Patrol, state parks, wildlife rehabilitation programs, and others. We do include ticks and fleas submitted by partners or collected by our own staff, identify the arthropods with keys or using molecular tests, and then perform PCR followed by DNA sequencing of any positive samples to identify the rickettsiae to species. Data are maintained in the VectorSurv and ArboNet databases.

Results and Discussion

To date, 21 partner organizations have enrolled in BiTeRS across California and Arizona. Partners have identified education and tick collection/testing support as the most helpful elements for the program. Three interactive educational programs customized to the needs of the individual partner organization have been developed and provided in real time. Hundreds of ticks have been submitted and tested for rickettsial pathogens. The majority of ticks submitted have been either brown dog tick or American dog tick, and 10% have tested positive for rickettsial bacteria, although the majority of these are non-pathogenic species. However, approximately 2% were infected with possible human pathogens, including *Rickettsia massiliae*. Education has allowed partners to focus on the tick and disease risks that are specific to their situation, and has enhanced their ability to collect and identify ticks for ongoing surveillance. In addition, it has provided an opportunity to connect partners with resources for tick management.

Conclusions

BiTeRS has the potential to raise awareness of changing patterns of tick-borne disease to allow people to better protect themselves and their animals. Engaging partners with vector control professionals improves communication and increases trust. Our approach in which each partner chooses all details of their participation also increases satisfaction with the surveillance program and willingness to be included.

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Insights into the ecology of endemic Rocky Mountain-Spotted fever and other rickettsioses at the western US-Mexico border

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Introduction

Over one hundred cases of human rickettsiosis, many fatal, are reported annually across the US-Mexico trans-boundary region. Although cases are often attributed to *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever, multiple other *Rickettsia* pathogens are present in North America. Although dogs and brown dog ticks (*Rhipicephalus sanguineus* sensu lato) have key roles in the ecology of *R. rickettsii* at the western US-Mexico border, other rickettsiae have wild animal reservoirs and other tick, flea or mite vectors. Our goals were to investigate the diversity of rickettsiae species in domestic, synanthropic, and sylvatic mammals and their associated ticks and fleas.

Methods

The study was conducted during 2017 and 2018 in the Sonoran Desert and Sierra de Juarez in California (CA) in the USA, and Baja California (BC) in Mexico. We trapped and collected blood samples and ectoparasites from rodents, wild carnivores, and dogs, which were tested by molecular assays to determine infection rates of *Rickettsia* species.

Results and Discussion

A total of 499 mammals, including 83 dogs, 23 wild carnivores, five lagomorphs, and 388 rodents were sampled, and 413 fleas and 447 ticks belonging to 15 and 4 species, respectively, were identified and tested. We found a high diversity of seven species of rickettsiae, including five which are recognized zoonotic pathogens (*R. felis, R. massiliae, R. parkeri, R. rickettsii,* and *R. typhi*) and two of unknown pathogenic significance (*R. asembonensis* and *Rickettsia* spp). Each *Rickettsia* species partitioned to specific mammalian hosts and vectors.

Conclusions

The finding of multiple zoonotic SFG rickettsial agents in an RMSF-epidemic area suggests that at least some of the milder rickettsiosis cases attributed to *R. rickettsii* could be caused by other *Rickettsia* species. Our study strongly supports the importance of multiple-host and vector epidemiological studies and the One Health approach to better understand disease in an RMSF-epidemic region.

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Functional analysis of folate biosynthesis genes of *Rickettsia monacensis* str. Humboldt via complementation assay

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Abstract

Nutritive symbiosis between bacteria and ticks is observed across a range of microbes and hosts; however, little characterization on the molecular components responsible for these symbioses has been done. Previous studies in our laboratory demonstrated that *Rickettsia monacensis* str. Humboldt (RMSH) can synthesize folate de novo via *folA*, *folC*, *folE*, *folKP*, and *ptpS* pathways. In this study expression of RMSH folate genes, within folate gene mutant *E. coli* K-12 strain BW25113, was used to functionally characterize RMSH folate genes in vivo. RMSH folate genes were subcloned into TransBac vector and transformed into *folA, folC, folE* or *folK* knockout mutant *E. coli* K-12 strain BW25113. Each mutant containing RMSH subclone and pFE604 clone of knocked-out folate gene, was cured of pFE604. Curing of *folA* mutant was successful using acridine orange and 43.5°C, but curing *folE* and *folK* mutants was only achieved after supplementing plasmid curing media with folate end-products. Preliminary plasmid curing assay showed curing efficiency of *folA* mutant at 100%, whereas curing efficiencies for other mutants was 0% to 10%. Functional complementation was assessed by growth phenotype on minimal media with and without IPTG between RMSH *folA* and *E. coli folA* as well as RMSH *folC* and *E. coli folC* gene pairs. Large and homogenous wild-type colony growth was observed for both assayed gene pairs on minimal media with IPTG, and lack of growth or pin-point growth without IPTG. This study provided evidence substantiating the *in vivo* functionality of RMSH folate genes in producing functional gene products for folate biosynthesis.

Characterization of a novel tick and *Borrelia* spirochete pair in California

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Introduction

California is home to a wide variety of *Ixodes* species and a number of species in the *Borrelia burgdorferi* sensu lato group (Furman and Loomis 1984, Margos et al. 2017). In 2014, an *Ixodes* tick was identified on the endangered Amargosa vole (*Microtus californicus scirpensis*) in Inyo County that, based on initial genotyping, was identified it as *Ixodes minor*, which in the United States is only known to occur in the Southeast. Subsequently, the tick is being described as a novel species which is related closely to, but distinct, from *I. minor*. Simultaneously, the tick was found to carry a spirochete in the *B. burgdorferi* s.l. group, most closely identified to *B. carolinensis* based on partial sequencing of the flagellin and *calreticulin* genes; however, this was not sufficient establish species identity (Foley et al. 2014). As *B. carolinensis* has only been identified in North America in the southeast US, further investigation was warranted to determine whether this bacterium is *B. carolinensis* or a novel related species.

Methods

Ixodes ticks and tissue samples were collected from the Amargosa vole and sympatric rodents during routine trapping for health and population monitoring in the Amargosa Valley. Ticks and tissue samples were surface sterilized and placed in BSK-II culture medium. Cultures were maintained at 33° C and checked weekly for spirochete growth using phase contrast microscopy.

DNA extraction was performed on each positive culture and multi-locus sequence typing (MLST) performed using a standard set of eight housekeeping genes as previously described (Richter et al. 2006, Margos et al. 2008).

Results and Discussion

Of 55 tick and tissue samples collected, three (two samples from ticks and one from vole ear tissue) were cultured successfully without contamination. Results of MLST for seven of the eight genes demonstrated a 1.7% pairwise difference in nucleotides between the cultured samples and *B. carolinensis*; the eighth gene (*recG*) was incompletely sequenced. Based on this result, the spirochete is close to meeting the criteria for a novel species, but the determination of whether it is a novel species or subspecies will be determined by final sequencing of the eighth gene.

The origin of both the *Borrelia* spirochete and its tick vector is unclear. The marshes where the tick and vole were found are isolated and surrounded desert inhospitable to *Ixodes* ticks. Introduction by migratory birds is a possibility, though migratory routes are north-south in this region without an obvious explanation for movement from the eastern United States.

Conclusions

The finding that a tick closely related to, but distinct from, *I. minor* is a vector for a spirochete that is related closely to, but not identical to, one carried by *I. minor* suggests that the couplet found in this environment has the same origin, but has co-evolved separately. Further phylogenetic analysis of the distribution of both will help elucidate their origins. In addition, the identification of a novel tick carrying a spirochetal bacteria not previously described in California highlights that our understanding of tick species and the *B. burgdorferi* s.l. group here remains incomplete. In this particular ecological system, it is unlikely that the spirochete would be spread to humans due to limited contact between the tick and humans, but if this bacterium emerges as a pathogen elsewhere in California, there may be human health implications.

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Patterns of vector-borne disease in wild mammals of California Impacted by urban expansion

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Introduction

Vector-borne diseases include emerging zoonoses whose ranges and prevalence shift with climate and land use change (Jones et al. 2008; Rochlin 2020; Sarwar 2016; Krasnov 2008; Sonenshine 2018; Patz et al. 2003; Gage et al. 2008; Villarreal et al. 2018). Many urban-adapted mammals can host ectoparasites and pathogens of human and animal health concern (Crooks 2002; Riley et al. 2003, Gehrt et al. 2009; Koopman et al. 1998). The goal of our study was to compare patterns of arthropod-borne disease among medium-sized mammals across gradients of rural to urban landscapes in multiple regions of California.

Methods

Archived spleen, kidney, blood, and ectoparasites from coyote (*Canis latrans*), gray fox (*Urocyon cinereoargenteus*), desert kit fox (*Vulpes macrotis arsipus*), San Joaquin kit fox (*Vulpes macrotis mutica*), raccoon (*Procyon lotor*), striped skunk (*Mephitis mephitis*), spotted skunk (*Spilogale putorius*), and Virginia opossum (*Didelphis virginiana*) were collected from multiple areas in California characterized as rural, interface, or urban. Tissue and ectoparasite samples were tested for five vector-borne pathogens of public health concern (*Anaplasma phagocytophilum, Borrelia burgdorferi, Francisella tularensis,* rickettsiae, and *Yersinia pestis*) via qPCR. Samples positive for *B. burgdorferi, Rickettsia* spp., and *Y. pestis* were subjected to conventional PCR and DNA sequencing. Blood samples were evaluated via serology for antibodies to *R. rickettsii* and *A. phagocytophilum*. Statistical analysis was done with a series of logistic regression models to predict test result (positive or negative) using host species, sex of host, age class of host, urbanity, year, and season as risk factors. Location data were mapped and tested for spatial clustering.

Results and Discussion

Tissues from 860 mammals and 319 ectoparasites were evaluated. *A. phagocytophilum* DNA was found via qPCR in 5.6 % of coyotes (31/ 551), 1.6% of raccoons (2/127), and 4.5% of San Joaquin kit foxes (1/22). *B. burgdorferi* sensu lato infection was found in one coyote in Orange County and rickettsial DNA was found in two desert kit foxes in Riverside County; one of these was successfully sequenced and found to be a spotted fever group (SFG) *Rickettsia* spp. closest to *R. amblyomattis* (diverged from this species by 1.3-7.7% depending on the gene evaluated). *Y. pestis* was detected in two coyotes in Orange County via qPCR. No mammals tested positive for *F. tularensis*. There was serologic evidence of rickettsiae in coyotes (15/40), Virginia opossums (2/10), desert kit foxes (2/16), and grey foxes (1/7). Antibodies to *A. phagocytophilum* were found in coyotes (14/40), raccoons (6/15), Virginia opossums (3/10), desert kit foxes (1/16), and grey foxes (1/7). Six flea and two tick species were tested. A single *Ctenocephalides felis* flea from a raccoon was qPCR-positive for *Y. pestis;* and *Ct. felis* and *Pulex simulans* fleas from three mammal species tested positive for rickettsiae. Rickettsial DNA from 24 *Ct. felis* fleas were sequenced successfully, with 19 identified as *R. felis* and 5 as *R. senegalensis*. Nine *Ixodes pacificus* ticks from coyotes were positive for rickettsiae; all nine were sequenced successfully and were genetically closest to *R. monacensis*. One *Dermacentor similis* tick from a San Joaquin kit fox was PCR-positive for *A. phagocytophilum*. No ticks were positive for *Borrelia* or *Francisella tularensis*. Statistical analysis indicated three significant findings. First, using linear regression the fall season was a significant predictor of *A. phagocytophilum* infection (qPCR positivity) with an odds ratio of 3.53 (95% confidence interval: 1.20-12.84). Additionally, two serological findings addressed our fundamental question on the influence of urbanization on disease: using linear regression, mammalian hosts utilizing interface habitats were almost seven times (odds ratio = 6.73; 95% confidence interval: 1.16-53.48) more likely to have detectable *A. phagocytophilum* antibodies. On spatial analysis with SatScan, exposures to SFG rickettsiae were significantly more common from 2012-2015 in one northern region of the greater Los Angeles area (p=0.04).

Conclusions

The findings that interface habitat use is a significant predictor for *A. phagocytophilum* exposure in mammal hosts and the significant spatial cluster of SFG rickettsiae at an area where urban, interface and rural habitats interconnect clearly support the role of interface environments in shifting disease risk. Human-associated animals in these environments can serve as sentinels (Carrade et al. 2011; Thomas and Hughes 1992; Brown et al. 2011) and are of great concern as zoonotic vector borne diseases are present in urban centers of California. Our study provides a baseline data for disease levels while documenting the probable impacts of urban expansion. More investigation is needed to further understand risk factors and disease dynamics to mitigate disease in animal and human populations as climate change and urban expansion continue to shift disease cycles.

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CalSurv Open-Data Portal: a new avenue for data access

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Introduction

The California Vectorborne Disease Surveilance System, known as CalSurv, is overseen by a steering committee consisting of members from the Mosquito and Vector Control Association of California, the California Department of Public Health, and the University of California, Davis. CalSurv data have been provided to researchers who submitted formal data requests. These requests were distributed to all contributing vector control agencies for a two-week comment period, followed by a vote of the steering committee on whether to provide the data in response to the request. Following discussion by the CalSurv steering committee, we have launched a new open-data portal for 2022, which will give each vector control agencythe option to provide open data to anyone who wishes to download the data upon request.

Methods

The open-data portal allows users to request surveillance data within the state of California for any past period for which it is available. When making a request, users are required to submit basic details about their identity, including name, affiliation, and a valid email address, as well as a description of their intended use of the data. Data provided will be subject to the following conditions: (1) data will be aggregated by census county division and two-week period within each year, (2) open data will include only data collected $>$ 18 months prior to the date of request to allow time for error corrections by local districts or publication of research by district staff, and (3) citation of CalSurv and acknowledgement of all contributing agencies will be included with the data download and required for publication using the data.

Results and Discussion

We anticipate that CalSurv's open-data portal will accelerate research and enhance utilization of California's rich surveillance data by research partners. The existing data request process will remain for users requiring detailed data or more immediate access without the lag associated with open data.

Acknowledgements

We thank our partners, particularly the California Department of Public Health and the Mosquito and Vector Control Association of California for the long-standing support that makes the CalSurv Gateway and Maps websites possible. We also acknowledge funding support from the National Aeronautics and Space Administration's Applied Sciences Program in Health and Air Quality (Grant NNX15AF36G), the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement U01CK000516), the Mosquito and Vector Control Association of California, the State of California, and the Epidemiology and Laboratory Capacity program of the U.S. Centers for Disease Control and Prevention.

Ixodes pacificus densities increase with habitat patch area in a fragmented landscape

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INTRODUCTION

The vast majority of tick-borne pathogens are maintained by natural transmission cycles between ticks and reservoir hosts that ticks use for their bloodmeal. As a result, the spatial distribution of tick-borne pathogens in the landscape is driven by abiotic and biotic factors that affect the abundance and distribution of ticks and their vertebrate hosts. In California, fragmented landscapes restrict the movement of reservoir hosts to varying degrees, resulting in unique pathogen transmission cycles at different fragment sizes and levels of isolation. Our project investigated the impact of habitat fragmentation on the spatial distribution and risk of tick-borne diseases in oak woodland habitats. We measured the patch size of oak woodland field sites using satellite images, land cover data, and GIS tools and analyzed the relationship between habitat patch area and western blacklegged tick, *Ixodes pacificus* (Cooley and Kohls) abundance with GLMM models. *Ixodes pacificus* transmit the causal bacterium of Lyme disease, *Borrelia burgdorferi* (Johnson, Schmid, Hyde, Steigerwalt, and Brenner), as well as several other human pathogens in the western United States.

METHODS

Ixodes pacificus were collected at 14 sites in the San Francisco Bay Area between 2016 and 2020. Sites were selected to fall along a gradient of forest fragment patches ranging in size from 2.5 ha to 150,000 ha while standardizing other variables such as habitat composition, slope and aspect, but not habitat connectivity (Lawrence et al. 2018). Field surveys at each site included small mammal live trapping to determine rodent species richness and abundance, lizard mark and recapture surveys to estimate lizard abundance, and wildlife camera traps to measure richness and relative abundance of large vertebrates. Questing ticks were collected via tick drag at regular intervals across a 0.5 ha plot during the peak nymphal season each year. Densities were measured as total nymphs collected per 990 m^2 dragged at each plot across two site visits.

We used a generalized linear mixed-effects model (GLMM) with a poisson distribution and site as a random effect to test the relationship between habitat patch characteristics and density of *I. pacificus* nymphs. Year was not used as a random effect because the variance was low. Wildlife parameters for each site were associated with tick data from the following year because previous research has demonstrated that wildlife abundance drives tick populations in the following season. The best fit model was selected based on the AIC score.

RESULTS and DISCUSSION

Our GLMM analysis determined that the density of nymphal *I. pacificus* (DON) was positively correlated with habitat patch area, total rodent abundance, and *S. occidentalis* abundance (Table 1, Figure 1). These results demonstrate that habitat fragmentation is an important driver of *I. pacificus* densities in the western United States and that higher small mammal and western fence lizard densities also contribute to higher blacklegged tick nymphal densities.

CONCLUSION

Density of *I. pacificus* nymphs (DON) is an important component of Lyme disease risk, because nymphal bites account for the majority of Lyme disease infections (CDC, 2020). Understanding how habitat fragmentation impacts *I. pacificus* populations in the western United States is the first step to understanding Lyme disease risk in the context of habitat loss and isolation due to urbanization and other land use changes. These findings stand in contrast to reported patterns of higher densities of the blacklegged tick, *Ixodes scapularis* (Say), in the smallest habitat patches (Nupp and Swihart 1996, Krohne and Hoch 1999, Allan et al. 2003). In an era of increasing human-wildlife interfaces, it is imperative to track and predict how landscape modifications impact vector-borne disease risk under distinct ecological contexts. Our research contributes to the understanding of landscape factors important for tick-borne disease risk in an understudied region.

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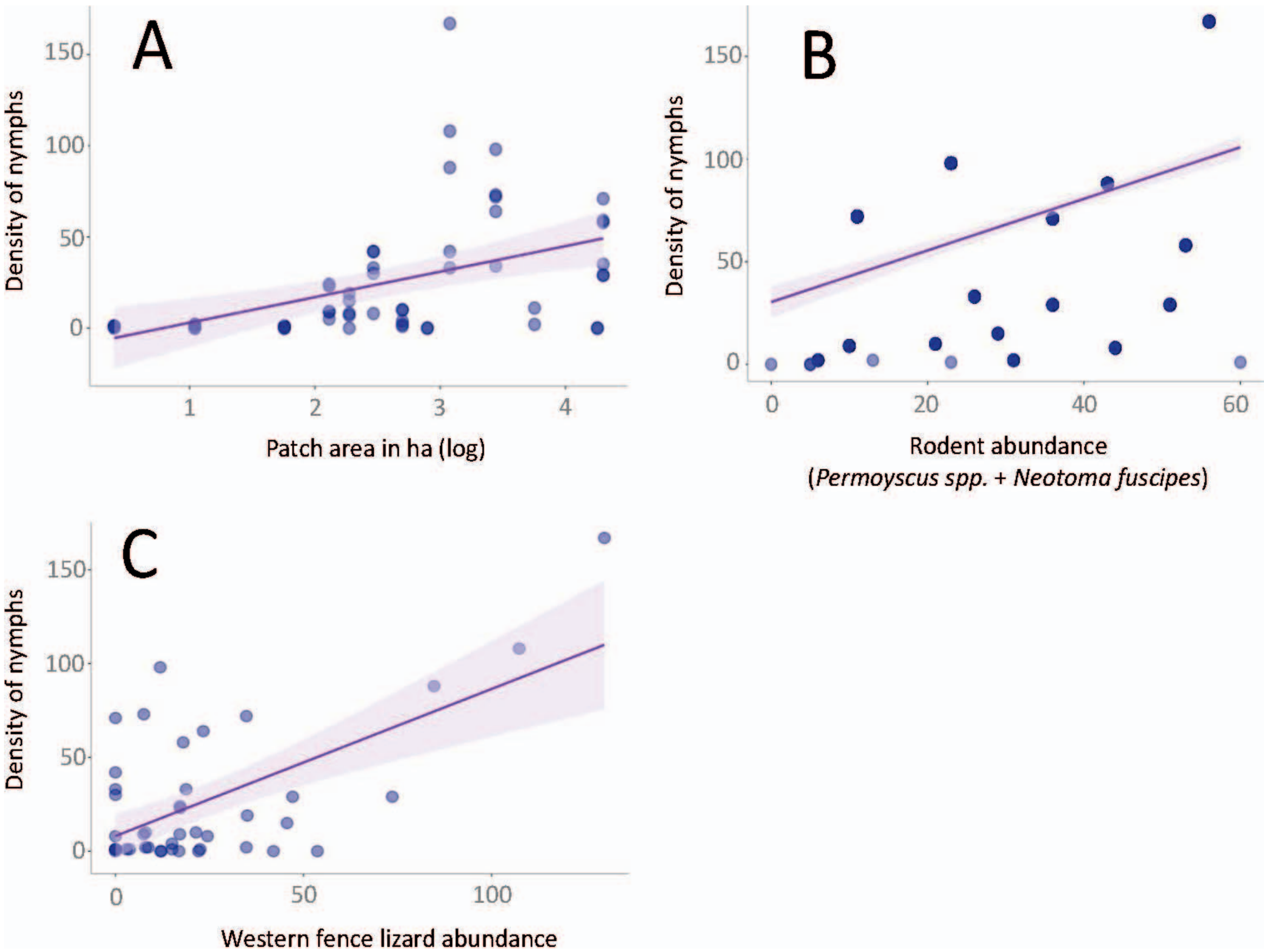

Figure 1.—Density of *Ixodes pacificus* nymphs plotted as A) a function of the log of habitat patch area, B) rodent abundance, and C) western fence lizard abundance. Density of nymphs represents the total number of *Ixodes pacificus* nymphs collected at a 0.5 ha plot across 990 m^2 of tick drag lines and includes 2 days of sampling during peak nymphal season. Rodent abundance includes the total number of individuals captured in 98 Sherman live traps across three nights of sampling. Western fence lizard abundances were estimated using data from three days of mark-recapture surveys and the R package, Rcapture. Rodent species captured in our traps included *Peromyscus* spp. and *Neotoma fuscipes*. Analysis included data from 2016 through 2020.

size calculations, and acknowledge Arielle Crews, Jordan Salomon, Samantha Sambado, Kacie Ring, Ceili Peng, Joel Villalpando, Thalia Fangon, Annabelle Cervantes, Isabella Maytorena, Nghia Tran, Monika Koczela-Stillman, Laura Hughes, and Adrienne Almarinez for their help with sample collection and processing. The authors acknowledge funding support from the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention and the National Science Foundation (Project #'s 175037 and 1900534).

Table 1.—Results of a generalized linear mixed-effects model representing habitat patch characteristics driving the density of *Ixodes pacificus* nymphs. Site was included in the model as a random effect.

Variable	Estimate	SE	z-value	P-value
Density of nymphs (DON)				
(AIC = 291.7)				
Patch area (log)	0.892	0.315	2.84	< 0.01 **
Rodent abundance	0.022	0.006	3.81	<0.001***
Western fence lizard abundance	0.008	0.003	2.76	< 0.01**
Large vertebrate diversity	0.097	0.201	0.48	0.629

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Effects of prescribed burns and wildfires on ticks and tick-borne disease in California

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Abstract

The increase in frequent, catastrophic wildfires can have significant perturbations on the complex spatial and temporal dynamics between the hosts and vectors of tick-borne diseases. As the state's management approach shifts from suppression to prescribed burning, the lack of understanding of how fires influence the complex local dynamics of tick-borne disease minimizes our ability to most effectively use prescribed burns to control tick-borne diseases. Fire influences tick-borne disease ecology in a myriad of ways. Research on the effects of fire in California suggests that ticks may survive prescribed burns (Padgett et al. 2009), but the drastic effects of wildfire can decimate competent host populations in the years immediately following the fire (Pascoe et al. 2020; MacDonald et al. 2018). Studies in other regions have demonstrated that the timing and frequency of a prescribed burn has important impacts on tick and host populations. For instance, small, singular prescribed burns may initially reduce tick populations (Polito et al. 2013), but increase the populations of highly competent hosts which are better able to recolonize the burned region (Willis et al. 2012, Adams et al 2013). The direct benefits of reducing ticks may be short-lived (Stafford et al. 1998, Wilson 1986), and even followed by significant increases in tick numbers, depending on how frequently burns are conducted (Spickett et al. 2006, Allan 2009). A cascade of changes in host populations and movement patterns due to the timing, size, severity and frequency of fires can lead to complex changes in disease dynamics (Willis et al. 2012, Gleim et al.2019, Hoch 1972). This presentation provided an overview of what is currently known about the effects of wildfires versus prescribed burning on tick-borne disease, exploring how the timing and intensity of prescribed burns versus wildfires may lead to a cascade of different effects on ticks, pathogens and hosts, and identifies areas where further research is still needed. A broad overview of multiple studies being conducted by the Foley laboratory to begin addressing these gaps in current knowledge was presented.

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The Kiss of Death: Chagas disease and triatomines in California

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Abstract

Chagas disease (also known as American trypanosomiasis) is caused by the protozoan parasite *Trypanosoma cruzi.* This parasite is most frequently transmitted by triatomine bug vectors, commonly referred to as "kissing bugs." Although Chagas disease is predominantly found in Latin American countries, *T. cruzi* exists in wildlife and vectors in the United States, including regions of California. There are four different known species of triatomines in California that are capable of transmitting Chagas disease, the most common of which is *Triatoma protracta*. At least one study has shown that 28% of triatomines collected in southern California and 55% in northern California were infected with *T. cruzi*. Within the United States, occasional cases of locally acquired Chagas disease have been reported, and recent serological surveys indicate that *T. cruzi* exposure may be occurring more frequently than previously thought. Chagas disease can be fatal if left untreated; however, most healthcare providers in the United States are unaware of the disease and are not actively screening for it. California is believed to have the highest number of people living with Chagas disease in the United States; however, it is still not a reportable disease (except in Los Angeles County). There needs to be an increase in vector surveillance and testing in California to help establish a more robust understanding of local transmission and prevention.

Host seeking behaviors of black flies (Simuliidae) in the San Gabriel Valley, California

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Abstract

Black flies are known throughout the foothills of the San Gabriel Mountains (Los Angeles County, California) for their nuisance biting behavior. Understanding when black flies are actively seeking a host can inform intervention and educational efforts to residents. The time of host-seeking was monitored weekly using a rotating bottle collecting carbon-dioxide baited trap set for 8 time periods (3 hours each). Two time periods showed an increase in host-seeking activity that was then separated into hourly trapping intervals. Preliminary results showed focused host-seeking activity, although additional research is necessary to ensure that these collections agree with highly active times of biting nuisance in the San Gabriel Valley.

Gateway Check-in: an overview of new developments, support options, and future plans for the CalSurv Gateway

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Introduction

The CalSurv Gateway provides a wide range of tools used by vector control agencies to enter, manage, analyze, and report surveillance and control data. Thanks to continued support and collaboration from the Mosquito and Vector Control Association of California, California Department of Health, the State of California and the CDC, several enhancements have been added over the past 12 months. Our presentation provides an overview of some of the Gateway tools and help options available to vector control agencies.

Methods

The CalSurv Gateway provides a wide variety of forms, calculators, reports, and data visualizations to support its growing user base. The development team strives to provide tools that are both useful and intuitive, but with > 1,300 registered users, > 100 pages of content and the regular release of new deliverables, regular updates on changes and support options are needed. Areas of the Gateway discussed include the following:

- Methods for keeping up to date with new Gateway features
- Development of a new look and added functionality for the front end of our website
- Tools and reports developed for the new Tick Module

Results and Discussion

The CalSurv Gateway provides multiple ways to obtain help or track new developments. By exploring the system's documentation pages, users can see written explanations and screenshots of most Gateway web pages. This can be done navigating to the URL (https://vectorsurv.org/docs/) or help for individual pages can be accessed directly by clicking on the "Help" link found on most of the Gateway pages and data entry forms. VectorSurv also provides a YouTube channel with detailed tutorials, and the development team is available to answer email questions anytime at help@vectorsurv.org. Also, all users now see the "Changelog" page, which gives a chronological listing of changes to the Gateway. The changelog appears immediately after a user logs into the system.

One of the more noteworthy changes in the last year was the introduction of the new Tick Module in 2021. The module provides forms and tables for recording and viewing tick collections and pools submitted for pathogen testing. It is also the first example of the new website design that will be replicated across the entire Gateway, and it is the first module to utilize the new RESTful API, which will soon provide new connection options for 3rd party software.

If users like the new look of the tick module, they can apply the same look to other CalSurv Gateway pages by navigating to the user preference menu and choosing "V5" (for version 5) as their preferred theme (Settings → My Account → Account Preferences → General Settings).

The addition of tick forms and tables to the CalSurv Gateway necessitated the addition of import and export tools for tick data. Users will now see choices for tick collections, pools, and test results in the data import/export tools. One thing to note is that tick pool test results can be imported into the CalSurv Gateway using the already existing Arthropod Pool Test Results import feature. Tick pool test results can be exported separately.

CalSurv will continue growing during 2022-2023. Future plans include the expansion of features on the RESTful API, a user-friendly spreadsheet template generator to facilitate collection imports, expansion of the new-look website to include all data modules, and addition of new data visualizations for ticks and pesticide usage.

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We thank our partners including the Mosquito and Vector Control Association of California, California Department of Public Health, and the State of California for the long-standing support that makes the CalSurv Gateway and Maps websites possible. We also acknowledge funding support from the National Aeronautics and Space Administration's Applied Sciences Program in Health and Air Quality (Grant NNX15AF36G) and the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement U01CK000516).

A panoply of drone uses for mosquito control

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INTRODUCTION

Aerial drones, otherwise known as unmanned aircraft systems (UAS), are being used with increasing frequency by mosquito control professionals to search for sites where mosquitoes reproduce, apply insecticides and monitor environmental conditions. Marshes with tidal water flow are challenging for mosquito control as the habitat is suitable for high density mosquito production when the influx of water is great enough to flood the landscape. San Francisco Bay (CA, USA) is ringed with tidal marsh habitats, although much has been degraded or destroyed as people develop grey infrastructure ever closer to the shoreline. Brackish tidal habitats with *Schoenoplectus spp*. (e.g., bulrush and tule vegetation) can support the development of several *Culex spp*., including those that can transmit arboviruses such as West Nile virus to people (e.g. *Culex tarsalis* and *Culex erythrothorax*). Salt marshes in the region with shallow elevation gradients can support the growth of *Salicornia pacifica* (i.e., pickleweed; Figure 1), which provide a habitat suitable for several aggressive mosquito species that can fly tens of miles inland in search of a bloodmeal (e.g., *Aedes dorsalis* and *Aedes squamiger*). Sustained saltmarsh flooding and mosquito production can be limited if water circulation channels are placed in the salt marsh that quickly draw out the tidal flow. Such channels were placed in saltmarshes throughout the Greater San Francisco Bay Area during the early 1900's and many have been maintained by mosquito and vector control districts ever since. However, some have accumulated silt and no longer function effectively or are inapparent from a ground-level vantagepoint. We used UAS to locate nonfunctioning or dilapidated water circulation channels so they could be restored to service for mosquito control in marsh habitats. US Fish and Wildlife Services (USFWS) in the San Francisco Bay Area are concerned that UAS may disturb wildlife and forbid flights at elevations lower than 150 ft. above ground level (AGL). Therefore, we assessed two airframes and three cameras to determine which would produce imagery that was best suited for planning mosquito control work.

MATERIALS AND METHODS

To assess water circulation channels, a Mavic 2 Zoom UAS (DJI, Shenzhen, China) was piloted autonomously 50 ft. AGL in a saltmarsh (GPS coordinates: 37.564116, -122.089190) at a speed of 6 mph using DroneDeploy software (San Francisco, CA), capturing imagery from angles that were perpendicular and oblige to the horizontal axis of the UAS. The UAS flights occurred when tide levels were low so that the base of water circulation channels could be visualized. To assess the impact of UAS altitude and lens choice on image resolution, a DJI Matrice 210RTK (DJI, Shenzhen, China) fitted with a DJI Zenmuse X5S camera body and DJI MFT 15 mm f/1.7 or Olympus M.Zuiko 45 mm f/1.8 lens (Central Valley, PA USA) was piloted autonomously above a bulrush marsh (GPS coordinates: 37.582890, -122.094350) at a speed of 8 mph using DroneDeploy software, capturing images that were perpendicular to the UAS airframe. Orthomosaic and elevation maps were produced using DroneDeploy software. Google Maps (Mountain View, CA USA) imagery was used for comparison.

RESULTS

To assess the depth of water circulation channels, over 1,100 images that were collected using the Mavic 2 Zoom UAS were combined using DroneDeploy software to produce a single orthomosaic map (Figure 2). The resulting map was analyzed using DroneDeploy software to quantify differences in elevation. The most prominent ditches were apparent from Google Maps imagery (Figure 1A). However, the smaller overgrown ditches were better visualized using the drone imagery (Figure 1B, orange arrowheads). In the computed elevation map (Figure 1C), depressions in the landscape were easy to identify as dark blue relative to higher elevations that were indicated by red. Potholes that could accumulate water and support mosquito reproduction were obvious in the elevation map (Figure 1C, white arrowheads), but were not visible in the Google Maps image (Figure 1A). The ditches in the red box of Figure 1C show low areas that would not drain properly and were not obvious in the Google Maps image or the photographs captured by the drone (Figures 1D, 1E), but could be easily seen as regions of dark blue in the elevation map (Figure 1F, pink arrowheads).

The camera that was permanently affixed to the Mavic 2 Zoom UAS has a relatively small imaging sensor (1/

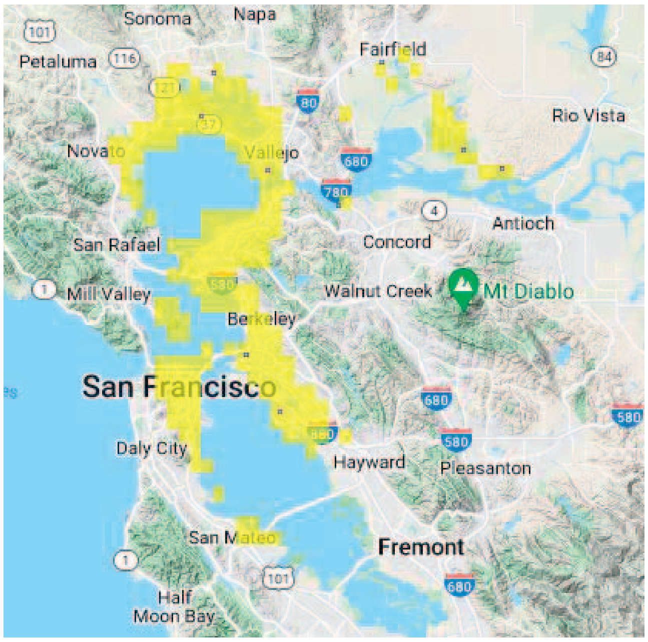

Figure 1.— Map of San Francisco Bay Area with locations where pickleweed was present in saltmarshes indicated by yellow polygons (image credit: https://calscape.org).

2.3″ CMOS, 12 megapixels) and a small lens that was equivalent to a 24 mm f/2.8 lens on a standard digital camera. The images that were captured at 150 AGL using the camera on the Mavic 2 Zoom UAS were not high enough resolution to generate orthomosaic maps that showed small water circulation channels. Additionally, the flight path of the lightweight Mavic 2 Zoom (2.4 lb.) was perturbed by coastal winds that predominate in the region, resulting in imagery that could not be aligned by the DroneDeploy software to produce complete orthomosaic maps, or was blurred. To overcome the camera resolution and flight path issues, we assessed the resolution of images that were collected using a DJI Matrice 210RTK (11.5 lb. takeoff weight) that was fitted with a DJI Zenmuse X5S camera body (4/3" CMOS, 20.8 megapixel). The resolution of images captured using the DJI MFT 15 mm f/1.7 lens at 75, 100, 120 and 150 feet AGL were all sufficient to show individual bulrush leaves (Figure 3A, 3B, 3C, 3D, respectively). To determine if higher resolution images could be captured at 150 AGL, the minimum elevation allowed by USFWS in the San Francisco Bay Area, we imaged a bulrush wetland using an Olympus M.Zuiko 45 mm f/1.8 lens and DJI Zenmus X5S camera body that was affixed to a DJI Matrice 210RTK (Figure 4A). Compared to imagery captured at the same site using the DJI MFT 15 mm f/1.7 lens (Figure 4B), there did not appear to be a substantial difference in the resolution. However, when the images were enlarged (see regions bounded by red boxes in Figure 4A, 4B), the resolution was clearly lower for the 15 mm DJI lens (Figure 4C) relative to the 45 mm Olympus lens (Figure 4D).

DISCUSSION

The results of our studies demonstrated that a heavier weight airframe (2.4 vs 11.5 lb.) and longer lens (15 vs 45 mm lens) may be better suited when using a drone to image

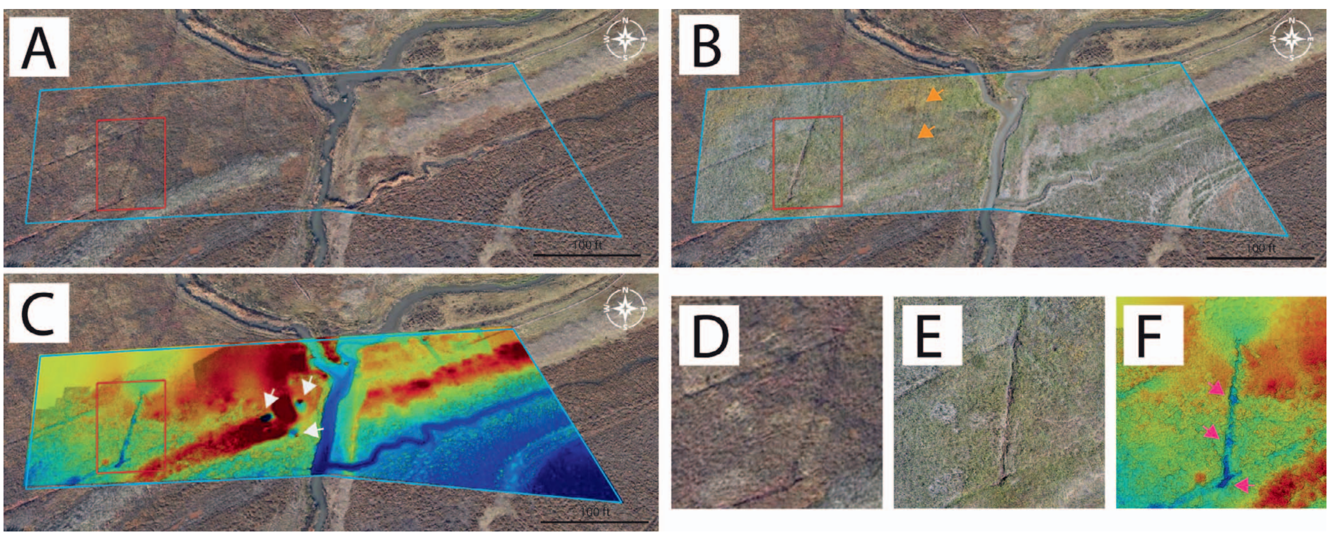

Figure 2.—Imagery collected using a DJI Mavic 2 Zoom UAS that was piloted autonomously above a saltmarsh in the area shown in the blue polygon. **(A)** Google Map image of the site that was assessed with the drone. **(B)** Orthomosaic image captured using the drone shows much higher resolution relative to the Google Maps image (orange arrowheads show a ditch that is not obvious in the Google Map image). **(C)** Computed orthomosaic elevation map captured using the drone shows areas with low elevation in dark blue and high elevation in red. White arrowheads denote potholes that could support mosquito breeding and are not apparent in Figures 2A or 2B. **(D)** Google Maps image, **(E)** orthomosaic drone image, and **(F)** computed orthomosaic elevation image of the area shown in the red box of Figure 2C (pink arrowheads show low elevation areas that may not drain properly).

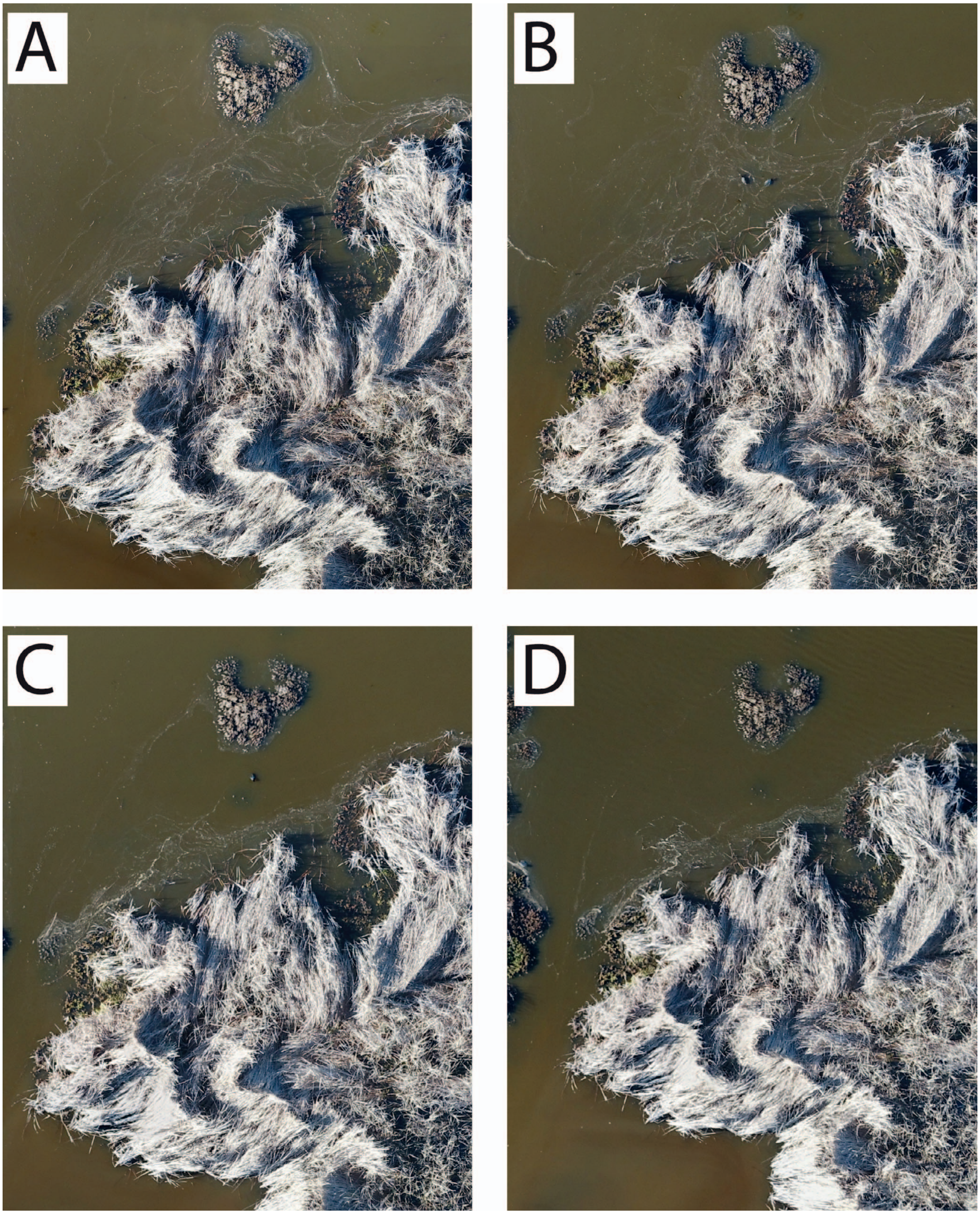

Figure 3.—Wetlands imaged using a DJI 210 RTK drone with a DJI Zenmuse X5S camera body and DJI MFT 15 mm f/1.7 lens at 75 ft (A), 100 ft. (B), 120 ft. (C) and 150 ft. (D) above ground level.

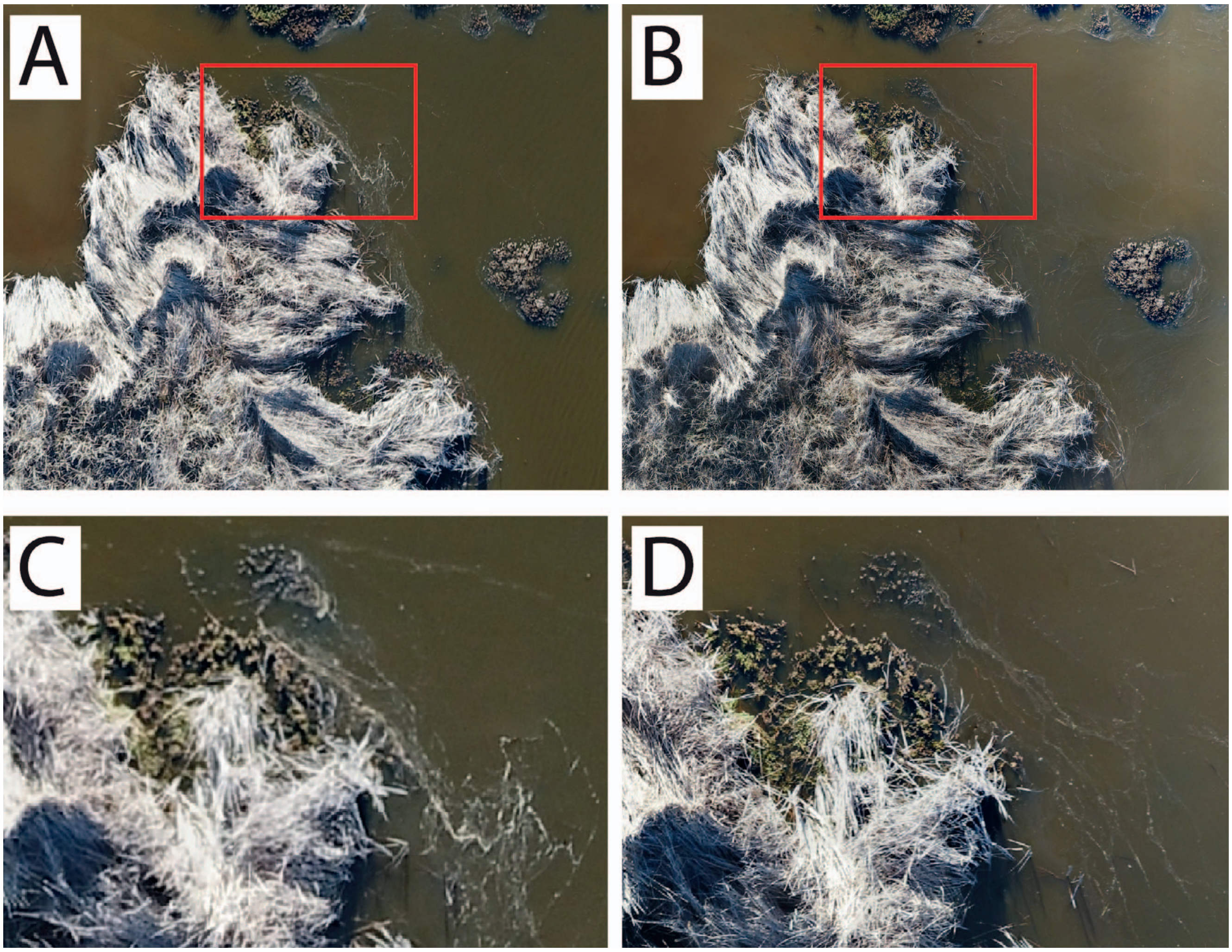

Figure 4.—Wetlands imaged using a DJI 210 RTK drone with a DJI Zenmuse X5S camera body and DJI MFT 15 mm f/1.7 lens (A, C) or Olympus M.Zuiko 45 mm f/1.8 lens (B, D) at 150 ft above ground level. Red boxes indicate areas that were magnified and shown in panels C (MFT 15 mm lens) and D (Olympus 45 mm lens).

wetlands where the minimum flight height is limited to 150 AGL. Although the aim of this study was to determine the hardware that was best suited for imaging the landscape over land managed by USFWS, determining the actual impact of UAS on wildlife based on flight parameters (e.g. elevation, speed, and airframe type) may enable more effective use of this burgeoning technology for mosquito control.

Angry Birds? (or Not) Impacts and benefits of unmanned aircraft on avian populations

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Abstract

As more unmanned aircraft systems (UAS) are integrated into mosquito control operations, the impacts, both positive and negative, on wildlife, particularly birds, need to be monitored and documented. Historically, one of the most devastating events that can occur during aerial procedures is bird collisions with aircraft. However, even minor disturbances of nesting birds and bird habitat can be considered "take" including Incidental Take, by the Federal Endangered Species Act. Surveillance and treatments for mosquito control work are often conducted within or near habitats that may contain endangered and threatened avian species. Therefore, it is imperative that these activities minimize the negative effects on wildlife populations. Fortunately, unmanned aircraft offer a unique opportunity to complete applications within these locations with minimal environmental disturbances, whether temporary (lower noise levels from spray equipment) or long term such as reducing aquatic invasive weeds. Our field observations document the bird species and their behavior before, during, and after mosquito control/surveillance efforts. Finally, recommendations for safe piloting procedures when encountering birds were presented as well as a proactive approach with protected habitat stakeholders and the public for continued support of UAS for mosquito control activities in the future.

Novel ultra-low weight high-capacity drone release system for sterile mosquitoes

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Abstract

Sterile mosquitoes are a promising technology for the suppression of local mosquito populations. Due to short flying distances of *Aedes albopictus* and *Ae. aegypti*, the target of current sterile insect technique programs, the dispersal of the sterile mosquitoes as close as possible to the local wild population is preferred to ensure sterile males are able to find the wild females. Although high density packaging of chilled insects is possible, regulations limit the payload that drones can carry, making it challenging to use conventional cooling systems. Our novel drone sterile male release system represents a breakthrough in combining an ultra-low weight chilled payload able to carry and controllably release more than 100,000 mosquitoes per flight. The new technology expands the option to utilize the sterile insect technique over large populated areas using low cost, low weight drones to make applications more accessible and more efficient.

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Best practices for adding sand to liquid suspension concentrate formulations for UAS applications

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INTRODUCTION

The mixing of liquid suspension concentrate larvicides on sand granules is one technique mosquito management districts have used to optimize larvicide applications, particularly in treatment areas with heavy vegetation and dense canopy. Mixing liquid larvicides with sand can help increase bulk density and allow for a wider swath during treatment. Benefits such as these can increase treatment efficiency by decreasing the amount of time and number of passes required for one area. This is particularly beneficial with Unmanned Aircraft System (UAS) applications which can be limited by hopper size and battery life. The purpose of this current study is to examine the viability of Natular SC®, a new 22.5% Spinosad single brood liquid suspension concentrate, as the liquid component of this method for drone applications.

METHODS

The mixing process began by calculating the amount of sand required to mix with the liquid product to achieve the suggested label treatment rate of 2.8oz of Natular SC. The concentrate was diluted by 25% with water and then mixed with 16-30 grade sand in a Harbor Freight Cement mixer and processed for 10 minutes. The ratio used was 2.8oz of Natular SC for every 10lbs of sand which can be scaled up or down based on the size of the treatment area. Then drying agent HiSil 233 was added to the mixer. The mixer was covered and processed for another 10 minutes. After the full 20-minute mixing interval was complete, the sand granules were effectively coated in the liquid product.

The Matrice 600 drone (DJI, Shenzhen Dajiang Baiwang Technology Co., Ltd.) was calibrated to deliver of 5.6lbs of coated sand granules per minute. Swath determination was conducted using trampoline-like structures called "collectagons". A 100ft transect was created with 11 of these structures. The drone was flown 80 ft. above ground, perpendicular to the transect while releasing product, and the granules collected were then weighed and converted to pounds per acre. Three replicates of this process were completed. The effective swath was determined to be 40 ft. The flight speed was set at 7 mph to achieve an application

Figure 1.—Polygons outlining the three sites chosen for this trial, each representing a seasonal pond in Madison County, Idaho surrounded by dense vegetation. Evaluation was conducted in late June.

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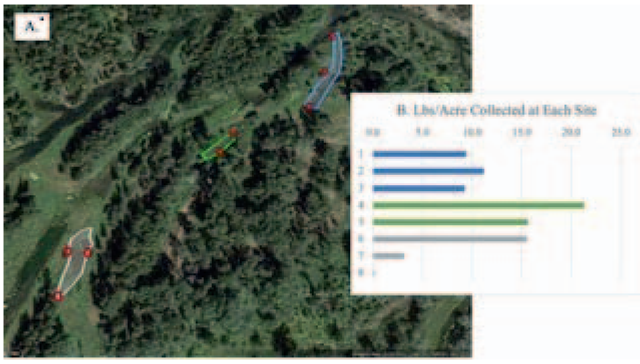

Figure 2.—A. Polygons outlining the three sites after treatment with red circles depicting the placement of a "collectagon" structure. B. The application rate observed at each 'collectagon' site.

rate of 10 lbs. of coated sand granules per acre at the determined flow rate and swath.

Three ponds surrounded by dense vegetation with sufficient pre-treatment larval dip counts were chosen as the treatment areas (Figure 1). The primary larval species found in these areas were *Culex tarsalis* and *Anopheles freeborni*. After treatment was completed, 10 dips were conducted at multiple locations within each area where larvae were found starting at 48 hours post-treatment. Following this interval, varying numbers of dips were conducted each day up to 7 days post-treatment. All values were ultimately calculated to reflect the average number of larvae of both species collected per dip for each day at each site.

RESULTS AND DISCUSSION

Granule collections utilizing the same method as calibration were conducted during treatment as well to evaluate the accuracy and evenness of the treatment. At sites 1 and 2 the granule collections were within expected ranges given the application rate. At site 3, two of the three granule sampling locations collected less than half of the intended application rate (Figure 2). The reduced collections at site 3 are likely the result of the trees that lined the perimeter of the site, which prevented the drone from centering its flight path over the pond.

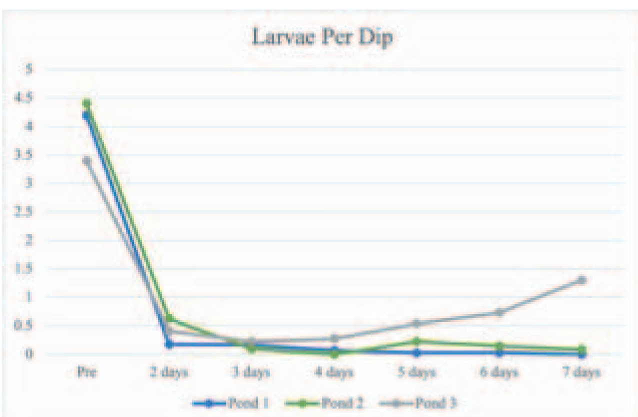

Figure 3.—The average number of both *Culex* and *Anopheles* larvae per dip collected for up to seven days post-treatment.

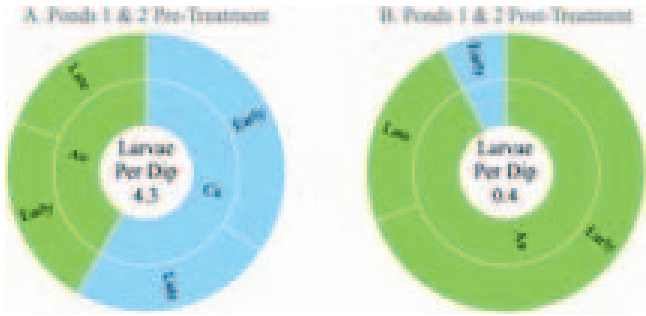

Figure 4.—A. Pre-treatment and B. 48 h post-treatment larval age structure at Sites 1 and 2.

The post treatment dip counts showed a marked decrease in larvae at 48 hours post treatment for all sites (Figure 3). These dip counts remained low at sites 1 and 2 for the one-week interval of expected control. At the third site recovery began on the 3rd day post-treatment because the granules did not effectively hit the outer edge of the pond (Figure 3).

Additionally, a noticeable shift occurred in the population age structure at sites one and two. Pre-treatment larval dips depicted a relatively even proportion of early and late instar larvae (Figure 4A). However, post-treatment larval dips resulted in a greater proportion of early instar larvae (Figure 4B). A Pearson's Chi-squared test was conducted and a significant shift in the age structure was found (p=0.0025). The presence of early instar larvae is consistent with the ecology of both *Culex* and *Anopheles* mosquitoes which produce continuous broods while water is present (Crans 2004). Hence, Natular SC on sand was effective in eliminating the larvae that were present during the time of treatment, as indicated by the shift in larval age structure and decrease in dip counts.

CONCLUSIONS

Natular SC mixed on sand for drone application was effective at controlling larval populations for a full week post-treatment. This technique is an easy and effective method for targeted larvicide applications by drone and can be customized to suit a wide range of treatment sites and habitats.

ACKNOWLEDGEMENTS

Thank you to Madison County Mosquito Abatement District for inviting Clarke to conduct this trial at their district in Idaho, and to Kings County Mosquito Abatement District for their guidance on the mixing process methods.

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UAS swath characterization derived from biological efficacy results

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Abstract

This presentation highlights operational field research performed on the PrecisionVision 35X as funded by a 3-year Deployed Warfighter Program (DWFP) grant, with the second year being completed in 2021. The presentation documents effective swath widths with biological controls (larvae and adult mosquitoes) for compounds applied by unmanned aircraft systems (UAS). This biological data is paired with droplet deposition and droplet flux data to quantitatively capture active adulticide and larvicide spray cloud characteristics.

Mosquito control use cases for UAS based LiDAR imagery and modeling

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Introduction

The Sacramento-Yolo Mosquito and Vector Control District (District) has operated an Unmanned Aircraft System (UAS) program since early 2016. The District routinely utilizes UAS collected imagery and photogrammetry to find standing water and to delineate mosquito treatment areas, but is still unable to accurately locate the water footprint under dense vegetation.

Recently the District began actively researching the current capabilities of UAS based Light Detection and Ranging (LiDAR) imagery. Over the years LiDAR sensors have become more affordable and small enough to fit on common industrial UAS platforms. LiDAR is designed to send approximately 770,000 light pulses to the ground per second and count all returns that have hit reflective objects, providing a 3-dimensional view of the landscape below. Water is not reflective, and typically will not provide any return data, so all blank spots in the processed data can be interpreted as water. Although LiDAR historically has been utilized by traditional aircraft to survey large areas, the use of UAS platforms and control software have made this technology more easily attainable to be utilized on smaller blocks more usable to the District.

The District has had a series of freshwater tidal areas restored with limited access and dense vegetation growth. Nearby adult mosquito surveillance traps contain high numbers of adult mosquitoes approximately a week after a seasonal high tide. The District is interested if LiDAR can be utilized to help locate the extent of slow draining seasonal or tidal flooding under this dense vegetation in a tidal wetland setting.

Methods

The District was approached by Aeroscn, a local UAS contractor, to experiment with a high resolution LiDAR sensor to begin the process of evaluating the usefulness of LiDAR in an extreme vegetated condition. A representative 150 acre block was chosen that contained vegetation and standing water similar to that of the inaccessible tidally flooded areas during the summer season. The goal was to see if the sensor was able to see standing water through the small gaps between the grassy vegetation and provide a method for delineating a polygon of standing water.

The area was flown utilizing a DJI Matrice 300 Quadcopter at an elevation of 150′ Above Ground Level(AGL) with a forward speed of 15 MPH, outfitted with a GreenValley International LiDAR sensor. The pre-determined flight paths were controlled by UGCS flight control and processing software. Each pathway within the polygon was slightly overlapped to prevent missed areas

Results and Discussion

The goal of the flight was to determine if UAS based LiDAR under the correct conditions can provide a clear indication of the extent of the standing water remaining after a seasonal high tide. After the data points were processed and analyzed as a Digital Elevation Model (DEM), some patterns of possible standing water emerged. The imagery provided varying degrees of shading, indicating that areas under dense vegetation were most probably water when compared to vegetated areas that were verified to be dry.

It was noted that by changing some of the mission parameters may ensure better success and future flights will need to be conducted to further evaluate this technology for widescale use. Parameters to be changed include: provide ground control points (GCPs) to aid in vertical accuracy, fly at a slower pace, and fly closer to the ground. Also flights should be completed at peak flooding to provide the greatest amount of standing water for the sensor to provide the clearest view of vegetation/soil vs water.

Conclusion

UAS based LiDAR has the capability to provide the location of standing water if the proper conditions are met. Further trials need to be conducted to fine tune the process, but even if no improvements are made, the District would be able roughly determine the extent of widespread standing water from seasonal high tidal flooding in these restored tidal wetlands.

Acknowledgements

Steve Kristoff of Aeroscn for providing the opportunity to demonstrate the potential benefits of utilizing LiDAR for finding standing water under dense vegetation, and Modus-AI for providing the UAS, LiDAR sensor, initial processing as well as piloting the mission.

Coyote control in Alameda County

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INTRODUCTION

Few sounds are a more iconic representation of the American west than the call of *Canis latrans*. The Maidu people have a story that in the beginning of time, the earth maker was floating in infinite water when a coyote calls out to him. Together they sing the world into existence. Coyote vows to spoil the world, and the earth maker orders the people to destroy the coyote. Coyote uses supernatural trickery to outwit the people, and in the end, the earth maker must acknowledge coyote as his equal. Too often when we talk about human/wildlife conflict, we are so fascinated with the organisms, we forget the human side. Listening to people is an important source of information, including indigenous knowledge. The success of *C. latrans*, and the success of management plans must account for this trickster's spectacular adaptability.

Alameda County Vector Control Services District (A.C.V.C.S.D.) operates district services for wildlife complaints within Alameda County that is facilitated through a memorandum of understanding with the United States Department of Agriculture (U.S.D.A.) Wildlife services. Alameda County is located along the eastern shoreline of the San Francisco Bay, encompassing 738 square miles (United States Census Bureau 2021) and housing 1,682,353 residents in 2021(Alameda County 2021). Alameda County Vector Control conducts routine disease surveillance and responds to requests for service from the public for insects, rodents, wildlife and nuisance complaints. Coyote complaints have dramatically increased since 2010 (Fig. 2). To better enhance and assess our wildlife management procedures, input from residents involved in coyote/human conflicts concerning our wildlife management policy was gathered using a questionnaire survey.

Coyote Biology

Canis latrans is a member of the family Canidae and is found throughout much of North and Central America (Timm 2007). There are 19 subspecies of coyote (United States Forrest Service 2022) that produce fertile hybrids with wolves and dogs (Cook County Urban Coyote Research Project 2021). Coyotes are extremely adaptable opportunistic omnivores (Tesky 1995) with an appearance that resembles a small German shepherd. Coyotes can be differentiated from dogs by the long snout, high chest, and down turned tail. Coyote tracks have a gap between the paws and the toes (Fig. 1) (Timm 2007). The species exhibits sexual dimorphism with females weighing 18-25 pounds and males 20-35 pounds, standing about 18 inches at shoulder. (Timm 2007) One litter is produced per year in spring averaging 4-7 pups depending on resource availability (Timm 2007), and the litter remains with the adults for 6 months. Dens can be excavated burrows or other structural features in the built environment offering shelter, such as drainpipes (Timm 2007). During the California Department of Fish and Wildlife Urban Coyote Workshop in 2021, Dr. Niamh Quinn discussed some of her findings regarding urban coyote ecology. In that lecture she explained that coyotes can exhibit both pack and transient behavior, and switch between the two. Dr. Quinn also discussed the range of coyote vocalizations, and it is theorized that vocalization patterns may be performed to produce the perception of exaggerated pack size to other animals including humans.

Disease Risk

Coyotes are known to bite humans, although this occurs rarely (Timm 2004). Coyotes also are known to serve as a reservoir for canine heartworm transmitted by the Western Tree hole Mosquito *Aedes sierrensis* among other species. Coyotes have been documented suffering from sarcoptic mange (Cook County Urban Coyote Research Project 2021) caused by *Sarcoptes scabiei* mites which can also affect species like the endangered San Joaquin kit fox (*Vulpes macrotis mutica*) (Rudd 2020). Coyotes have been documented with rabies in southern Texas (Cook County Urban), streptococcus (Sugden 2020), echinococcosis in Alberta (Catalano 2012) and an altered microbiome in urban coyotes (Sugden 2020). In Alameda County, the District primarily responds to public requests from people feeling unsafe based on seeing coyotes, missing pets, and more recently incidents of people being bitten.

METHODS

Following public complaint either a district biologist, U.S.D.A. wildlife service specialist, or both, consults with the reporting party over the phone. This is usually followed by a site inspection to answer questions, assess public

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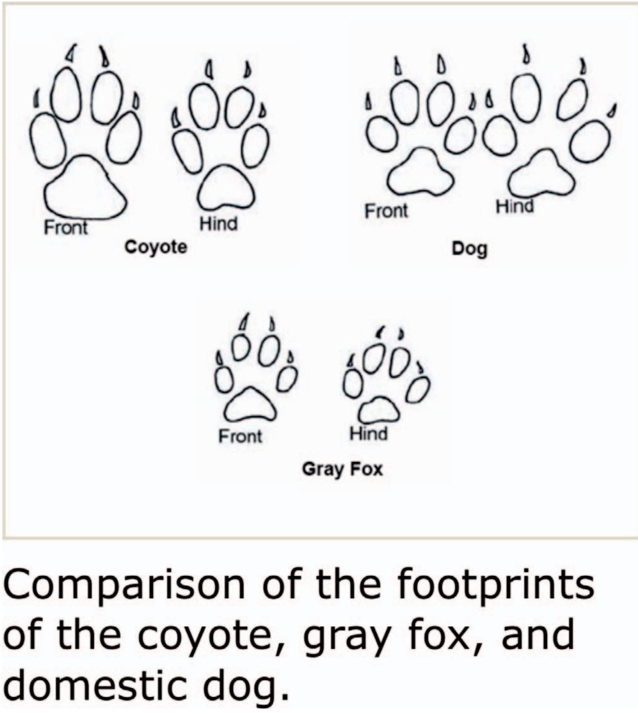

Figure 1.—Track comparison. Courtesy of U.C.A.N.R. pest notes webpage

safety, provide recommendations, and document findings. These services are offered to all County residents and institutions (agencies, businesses, H.O.A.'s, etc.). Based on conditions at the location, the biologist may recommend online education resources, such as U.C.I.P.M. pest notes or reporting platforms, such as Coyote Cacher (University of California Division of Agriculture and Natural Resources 2022), and local information sharing sites like Nextdoor to promote a concerted local effort. The biologist also offers advice about behavioral modifications the resident can undertake such as calling police for an emergency, keeping pets on leash, adjusting walking times, or carrying a defense device such as a walking stick, pepper spray, or a whistle. Habitat modification such as vegetation removal, rodent control, and food or water reduction is often suggested. The use of exclusion with fencing, electric fencing, fence rollers, and gates, or the use of motion activated frightening devices may be appropriate as well. Because coyotes are intelligent, frequently relocating frighting devices may help prevent habituation.

Based on these recommendations, the authors used Google forms to conduct a survey. Questions were compiled via consultation with district management and U.S.D.A. wildlife services. Participants were generated from reporting parties who contacted the district during the years 2019-2021. Those who had email addresses on file were sent a link and brief explanation of the survey, whereas those who had only provided phone numbers were contacted via telephone. All reporting parties were contacted via email or telephone twice. Questions were

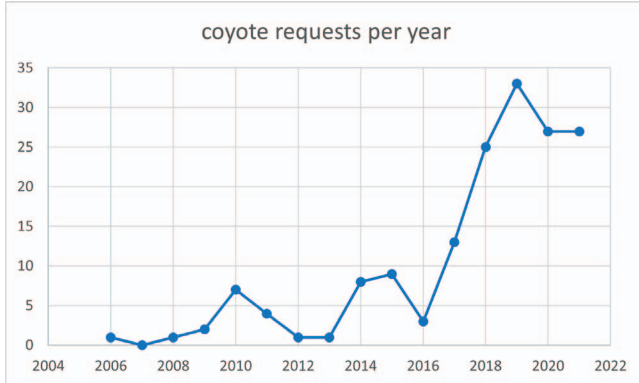

Figure 2.—Coyote service requests by year, 2004-2022

designed to verify the extent of service, satisfaction, implementation of suggestions, and results.

Questions were as follows:

1) Did you have an inspection performed by an Alameda County Vector Control Biologist or U.S.D.A. wildlife service?
2) How satisfied were you with the experience?
3) How much experience do you have with wildlife?
4) How long have you lived in Alameda County?
5) How frequently did you see coyotes prior to contacting Alameda County Vector Control?
6) Did you see coyote behavior that caused you or a pet to feel threatened or suffer injury?
7) Did you implement the suggestions provided by district representatives?
8) If so, which suggestions? Examples: Food reduction, habitat modification, fencing, electric fencing, guard dog, automatic deterrents (sprinkler, startle device), hazing, noise, carrying self-defense device?
9) How effective were the deterrents?
10) Do you feel safer as a result of speaking with a representative or implementing deterrents?
11) What would be useful, realistic services you would like to see implemented regarding coyotes?
12) Were you directed to online resources, if so which ones?
13) Did you read about or post about coyotes on social media? If so which platforms?

RESULTS

As of 8 April 2022, there have been 21 responses from 82 participants. One number was non-working, and one person declined to participate. For questions on a 1 to 10 scale, 10 represented the greatest degree of experience or satisfaction.

Overall, 42% of respondents did not have an inspection, 21% had a joint inspection with U.S.D.A and A.C.V.C.S.D., and 31% had an inspection from a district biologist. 33% ranked the experience a 10 and were very satisfied, whereas 22% ranked the experience a 5. Most

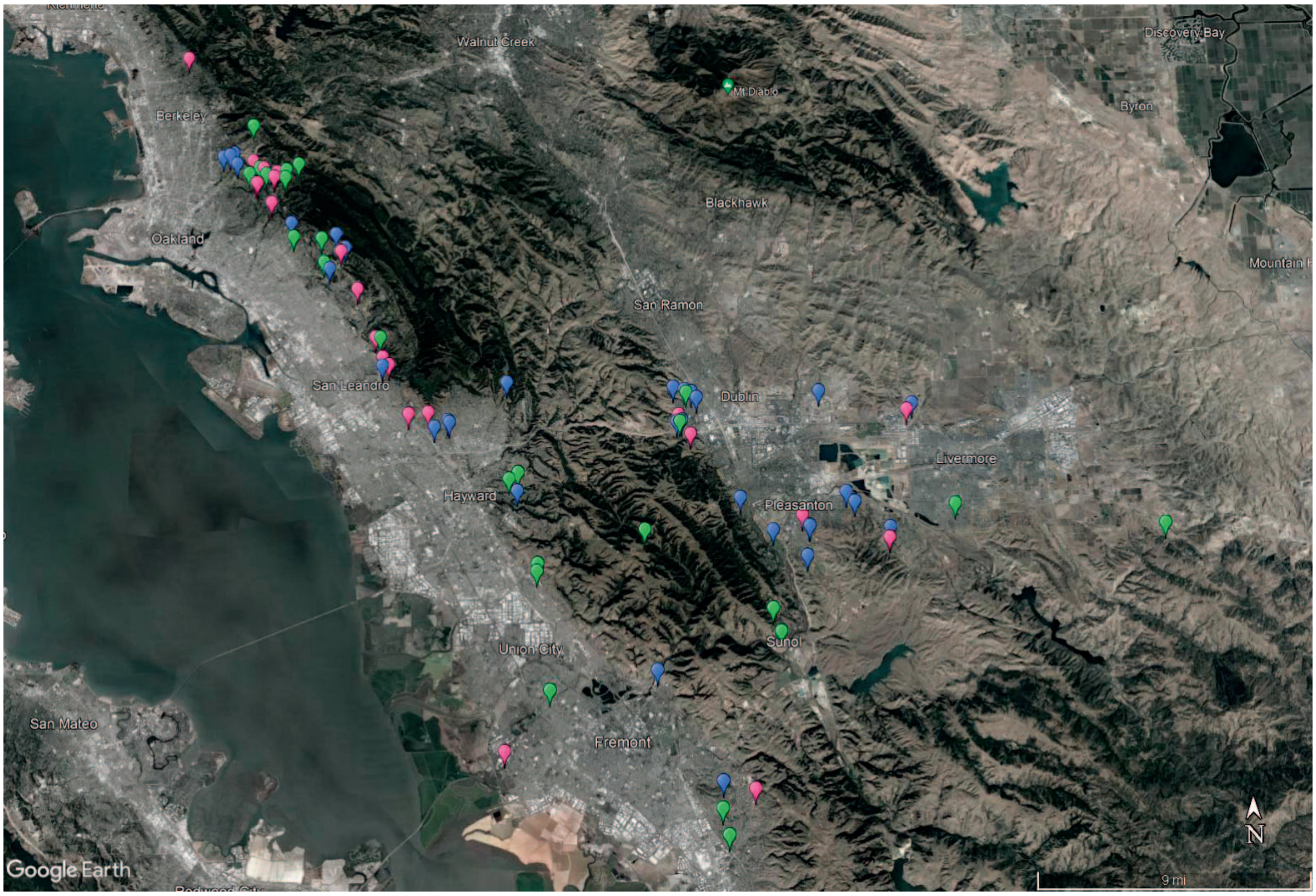

Figure 3.—Distribution of coyote complaints by year: 2019 blue, 2020 green, 2021 pink

respondents identified their experience with urban wildlife as either a 3 or a 7, with 15% ranking their experience a 10, most satisfied. There was an even split of 47.4% having been Alameda County residents for either 5 to 15 years, or greater than 15 years. The frequency of coyote encounter distribution also was evenly split: 26% seeing coyotes more than once a week, 21% seeing the animal 1 to 3 times a month, 31% seeing coyotes less than once a month, and 26% having never seen a coyote. 57.9% of respondents experienced behavior that caused them to feel unsafe, whereas 31% did not. 57.9% implemented suggestions provided by the district, whereas 31% did not. Of these suggestions, noise, hazing, and carrying a stick were most frequently implemented (by 8, 6, and 4 respondents, respectively) with other suggestion being implemented once except electric fencing; fencing and guard dogs were implemented by 3 and 2 respondents. Suggestions were overwhelmingly found to be ineffective, with half of the respondents rating them a 1 of 10, whereas the rest rated them 5 to 10 for effectiveness. 52% of respondents did not feel safer as a result of speaking with representatives or implementing deterrents, whereas 26% felt safer after speaking to a biologist and 21% felt safer from the use of deterrents. The location of encounters within the county that generated complaints was mapped (Fig. 3)

DISCUSSION

The survey response rate was about 25% which is slightly below average (Cleave 2020). The sample set also was focused on people who contacted Alameda County Vector Control, and people who took the time to respond to this survey. This sample may not be representative, and further outreach by the District could generate more information about public attitude toward our program. Suggestions for improvement of district services elicited by the survey included having biologist patrol this area, relocating animals, increased lethal control, better exclusion, and signage warning residents of the dangers of coyotes in the area. Residents who contacted the District with coyote complaints live near the suburban/wildland interface (Fig. 3). Most of the residents had lived in Alameda County longer than 5 years. In the authors' experience working with the public and other agencies, there is a perception that coyote conflict primarily occurs with people who are new to the area or reside in areas recently developed. These beliefs were not supported by the survey results, although the results may not be representative.

Based on the reported lack of effectiveness, it may be that more effective methods are required if the responding demographic is taken as representative residents. It is interesting that although most people were satisfied with the experience of consulting with a biologist, most people still felt threatened. Although most people implemented suggestions, they did not find them effective or feel safer as a result. This may indicate a need for more research and education related to coyote risk assessment and effective hazing methods. As an organization operating at the County level with limited resources to address complaints of this nature, the survey indicated a high degree of satisfaction with the outreach, inspection, and educational services provided by the District.

Agency Roles

Local police are usually the most aware of problem animals in an area. Residents feeling unsafe should always contact the police in emergencies. In Pleasanton and San Francisco signs informing the public of coyote activity in the area are often used when reports occur. Municipalities can advise residents to contact local vector districts, U.C. Cooperative extension specialist, or file a wildlife incident report with California Department of Fish and Wildlife (D.F.W.). Public safety assessment for lethal control is made by D.F.W. based on their own criteria and usually occurs in our county following human injury. Apprehending the offending animal is performed through the expertise of U.S.D.A. wildlife services. Leg hold traps were outlawed in California in 2019, but still may be used with a tension device once a public safety threat has been declared by D.F.W. Because Alameda County is in the range of the endangered San Joaquin kit fox, neck snares, leg snares, and collarum traps cannot be used. When performing coyote control in Alameda County the only tools available to U.S.D.A. are shooting or cage style traps.

Eastern Alameda County staff collect blood and ectoparasites from mesocarnivors killed by vehicle strike. Local animal control agencies will contact district biologists who may collect samples of blood, tissue, or ectoparasites from animals outside of our normal wildlife calls. Nobuto strips are sent to California Department of Public Health Vector-Borne Disease when blood from an appropriate species is collected in a timely manner. Coyotes are included in this program. Since 2016 we have collected, sampled and/or processed North American river otter (*Lontra canadensis*), American badger (*Taxidea taxus*), puma (*Puma concolor*), wild pig (S*us scrofa*), bobcat (*Lynx rufus*), grey fox (*Urocyon cinereoargenteus*) and Columbian blacktail deer (*Odocoileus hemionus columbianus*) as well as various local bats, rodents, and more common wildlife.

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Tree squirrels and ground squirrels: Is there a difference in behavior and control?

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Introduction

Alameda County Vector Control Services District (ACVCSD) serves the 1.4 million residents of Alameda County, which encompasses 821 square miles. The County is comprised of a mixture of highly urbanized centers, where a large numbers of residents live and work, as well as rural areas and agricultural lands. ACVCSD helps residents concerned with wildlife that may have taken up residence inside a home or may be causing damage to their property. Residents can submit a Request for Service (RFS) by calling our office or submitting a request online. The RFS is routed to the biologist assigned to that area of the County, and who will then visit the residence and conduct an investigation.

ACVCSD receives calls every year related to Red fox squirrels (*Sciurus niger* L.) and California ground squirrels (*Otospermophilus beecheyi* Richardson). The methods used to remove and deter these animals from homes, businesses and parks differ by species. ACVCSD biologists are trained in utilizing several different methods to help deter the animals from residential, commercial and public lands. The methods utilized to deter each squirrel species differs because the two species exhibit different behaviors and occupy different niches in the environment. We help implement these methods at locations county-wide in conjunction with educating the citizens on effective deterrence and control measures.

Red fox squirrels are the largest tree squirrel in North America. Originally native to the eastern parts of North America, they are considered invasive in California and Europe (Thorington 2005; European Commission 2022). They forage for food on the ground but spend the most of their time in trees, on fences and climbing on roofs and man-made structures. They are well adapted to finding food and shelter in urban and rural environments.

California ground squirrels are native to California and spend all of their lives on or below ground. They are gregarious and tend to live in large colonies. They construct large underground burrow complexes and can cause damage to lawns, irrigation systems, undermine sheds and foundations, and cause livestock to trip and break their legs in fields and pastures (Jameson and Peeters 2004).

Materials, Methods and Results

ACVCSD responds to Red fox squirrel and California ground squirrel calls differently. After one of the Vector Control Biologists (VCB) performs an inspection and assesses the situation, they will introduce the resident to a number of control and deterrence measures.

Red Fox Squirrels

If the animals have been chewing on a structure (e.g., fence/gate, siding, fruit trees, etc.) non-lethal deterrence methods can be employed. These include spraying the animals with a water hose, spraying the area they are actively chewing on with a chili pepper oil-based product, and removing food and vegetables that are attracting the animals to an area. If someone has been feeding the animals, ACVCSD will explain that it is best not to feed wildlife at all. However, if none of these methods work in stopping property damage, ACVCSD can remove the animals by trapping. All animals that are trapped must be euthanized in accordance with the California Department of Fish and Wildlife regulations.

Fox squirrels may chew their way into attic spaces and roof junctures and removing them may be difficult, taking several days/weeks. Entry point(s) are monitored to discern if they are actively used, and if active, then either a Pro Rat Tomahawk Live Trap baited with peanut butter and nuts (Tomahawk Live Traps, WI) or a one-way door will be set. It may take several attempts to trap the animal and the resident is asked to assist by monitoring the trap to determine if an animal has been captured. ACVCSD staff will remove the live animal from the property. If it is determined that a one-way door can be used, it is secured over the entry point so that the animals cannot re-enter the area. As the name implies, a one-way door allows the animals to exit an area but does not allow them to re-enter (Fig. 1). If there are multiple entry points in an attic/roof area, all but one of these areas must be sealed with either sheet metal, ¼ inch hardware cloth, or equivalent material prior to the one-way door being installed. One-way doors should be left in place for at least a week to ensure that the animal has left, and we often set up a wildlife camera in conjunction with the traps to monitor for activity and ensure that all animals have been removed prior to closing up the remaining access point. Once the one-way door trap

Figure 1.—Caption: One-way door installed in a gutter to remove a red fox squirrel from an attic area. Photo by Kimberly Daum, ACVCSD.

is removed, the resident must close the access hole immediately so that the animal(s) do not re-enter.

California Ground Squirrels

Residents call when ground squirrels start to cause damage in and around their property. The burrowing caused by ground squirrels can be extensive and cause damage to infrastructure, including undermining foundations, walkways, levies, sidewalks, irrigation systems, etc. If the population of ground squirrels in an area has been determined to be small, live-traps baited with peanut butter and oats can be used to remove the animals. Other baits can be used such as cereal, granola bars, etc. Traps should be placed near evidence of current activity: active burrowing, burrow entrances open and clear of debris, food outside burrow entrances, or droppings.

If the population is large, baiting is the most cost-effective means of control. This involves placing bait stations into the ground where the squirrels can access the bait. There are different bait station designs, but most are variations of the basic 't-bait station', which is composed of PVC piping that allows the animals to enter a "tunnel" into the main chamber where the bait is held. The bait station is buried in the ground and rocks (or other) material piled on top to hide their location. The entrances must be well hidden in such a way that the animals will enter the bait stations. Once set, the stations can be left in place indefinitely and the bait refilled on a regular basis to maintain control.

Discussion

Long-term control measures for squirrels can be difficult to implement and maintain, but if left un-checked, these animals can cause damage to residential and commercial properties/infrastructure. ACVCSD tries to educate the public on the types of damage both species can cause. We strongly recommend that the public not feed these animals, not only due to the property damage they cause, but both species also harbor fleas that may transmit zoonoses to humans and pets.

ACVCSD is continuing to monitor the flea species and pathogens associated with both the Red fox squirrel and the California ground squirrel as part of our surveillance efforts county-wide. One of the biggest issues we face is in deterring the public from actively feeding the animals in parks and public spaces. This leads to spikes in the populations of both species and subsequent control measures may need to be undertaken to prevent damage to property and infrastructure.

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Avian pests of Alameda County

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Introduction

In Alameda County, residents and businesses contact the Alameda County Vector Control Services District (ACVCSD) about different species of avian pests, most commonly feral pigeons, wild turkeys, crows, and chickens. Avian pests are a public health concern because they can transmit zoonotic pathogens to humans. (CDC 2019, Hicks 2008). Besides diseases, birds also cause a great nuisance, including flies and odor from backyard chicken coops, rats and wildlife animals being attracted to bird seed feeders, crows swooping to attack during nesting season, pigeon droppings causing property damage, noise from cawing crows and crowing roosters, and bird mites. Nuisances caused by domestic birds such as backyard chickens can easily be resolved by increasing sanitation conditions and rodent-proofing the enclosure. For wild birds, however, there are several control techniques that may help to harass or deter them.

Methods

Knowing the species of bird is important, because many wild species are protected under federal and state regulations, including the Migratory Bird Treaty Act (MBTA) that protects migratory birds internationally. For species protected under the MBTA, it is "illegal for any person to intentionally take, possess, transport, sell, or purchase them or their parts, i.e. eggs, nests, feathers, without a permit" (USFWS 2022). Permits are only authorized by California Department of Fish and Wildlife (CDFW) or United States Fish and Wildlife Services (USFWS). For example, CDFW issues depredation permits for wild turkeys (CDFW 2022). Another factor to consider is the biological time of year. There are different nesting and breeding seasons depending on the species of wild bird. During turkey breeding season in the springtime, male turkeys may become more aggressive. (Dolkas 2022). For crows, nesting season occurs in the summer months and parent crows that feel threatened tend to swoop down from their nest to protect their young. For swallows, nests can only be knocked down after CDFW's designated swallow season of February 15 through September 1 (Salmon and Gorenzel 2005). Becoming more familiar with the bird species and their biology will not only help with controlling the avian pests, but also prevent legal issues.

Harassment and deterrent devices may be used to prevent birds from remaining around unwanted locations (Seamans and Gosser 2016). Perching birds, such as feral pigeons, are adapted to urban infrastructure utilizing ledges to roost and awnings to make nests. Tactile repellents installed on ledges such as sticky material or thin wire can be uncomfortable for birds to land on. Physical barriers such as spikes and netting also will deter perching birds from landing. Chemical avicide may be used by licensed professionals as a frightening agent. Other harassment devices include lasers, distress calls, predator eye balloons, and reflectors.

Many people enjoy feeding wild birds to become closer to nature and animals. Roof rats (*Rattus rattus*) and other unintended wildlife often scavenge on leftover bird seed from feeders. Cleaning up spilled bird seed is common advice for people with bird seed feeders. Sometimes well-meaning people will intentionally feed bread and cracked corn to waterfowl at lakes, parking lots, and parks. It can be a difficult task to convince people to stop feeding wild birds. Some methods to use are informing them that feeding can be detrimental to the health and wellbeing of the animals. Wild birds can lose their natural fear of humans and an unnatural diet can cause a metabolic bone disease called "angel wing" (Rickard 2015). Other urban food attractants include food scraps in trash cans and ripe fruits on trees. Making sure trash cans are closed and harvesting ripe fruit will help reduce food availability, thereby reducing the likelihood of birds causing a nuisance.

Case Studies

Over the last couple years, ACVCSD has been called for several unique avian pest issues. In November 2021, a resident in Pleasanton, California, described woodpeckers making holes in the exterior walls of their home. The location in Pleasanton was surrounded by oak trees full of acorns. It was determined that acorn woodpeckers (*Melanerpes formicivorus*) were using the Styrofoam trim detail of the home as a granary to store their acorns. The long-term advice was to replace the Styrofoam with a sturdier material for exclusion.

In April 2021, residents in Alameda, California, complained that their backyard was unusable due to many bird droppings splattered everywhere. Upon inspection, it was determined that double crested cormorants (*Phalacrocorax auratus*) were nesting in the nearby tall redwood tree. The residents were advised to remove the nests and trim the tree after all the cormorants left at the end of nesting season around September to prevent their return the next year.

In March through October of 2020, ACVCSD received calls about Gerald the wild turkey (*Meleagris gallopavo*) terrorizing Morcom Rose Garden visitors in Oakland, California. Although relocating wildlife isn't an ideal solution, CDFW eventually relocated Gerald to a farther location (Martichoux 2020). Solutions to avian pest issues are unique and dependent on a case-by-case basis.

Conclusion

Avian pests will continue to be problem in Alameda County. With plentiful food sources and infrastructure providing shelter in urban neighborhoods, birds will easily adapt. By educating the public about modifications to the environment that can deter avian pests, ACVCSD can make a positive impact in preventing avian related pathogen transmission and reducing avian nuisances.

Acknowledgements

I would like to thank all my colleagues at ACVCSD for their contributions in expanding our collective knowledge of avian pests and their control. I would especially like to thank Biologist Michael Mooney for allowing me to observe how he helped residents resolve turkey, egret, crow, and woodpecker issues. Thank you to USDA Wildlife Specialist Dave Hammett for his knowledge and expertise with several pigeon, turkey, and cormorant issues. Lastly, thank you to Supervisor Paul Cooper who provided general advice and guidance.

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Evicting mother raccoons and their kits from residential properties in Alameda County

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Introduction

Raccoons (*Procyon lotor* Linnaeus 1758) are a native species in California and a common part of urban wildlife in Alameda County. In 2020, Alameda County Vector Control Services District (ACVCSD) received 489 raccoon related requests for service, 26% of which involved raccoons harboring in and around human dwellings. Of those "harboring" requests, 29% involved mother raccoons and their kits, which is about 8% of ACVCSD's total raccoon service calls in 2020. An "urban" raccoon reproduction season in Alameda County is highly variable, ranging from March through November. Preferred urban den locations consist of any area that can provide the mother raccoon an enclosed and protected space. Raccoon mothers seek out areas in residential neighborhoods such as attics, subfloors, decks, and sheds to raise their young. Mothers will stay in this den location until their offspring are fully weaned at about 8-10 weeks. After the kits are weaned, the mother raccoon will leave with her kits to find another home. To reduce the risks a raccoon family poses living so close to humans, eviction is recommended. Evicting a mother raccoon and kits can be challenging and time-consuming for ACVCSD Biologists, and stressful for the residents.

Methods

To avoid the risk of separating a mother raccoon and her kits, some eviction tools such as one-way doors and cage traps are not appropriate, if kits are suspected. To confirm the presence of kits it is essential to monitor the residence where the raccoon is living. Young raccoons make loud and distinctive chattering noises, which can commonly be heard during an inspection of the property or by the residents. Strategic placement of motion activated trail cameras, located inside the den space or near the opening of the den, also can confirm the presence of kits or a lactating female raccoon. After confirming the presence of kits, ACVCSD uses humane harassment techniques, such as the use of loud noises and raccoon eviction fluid to successfully and safely evict the mother and kits from a property. Loud noises, such as a leaf blower, placed inside the den location and used periodically during the day can harass a mother raccoon while she is sleeping. Raccoon eviction fluid is a by-product of the male raccoon scent and can be placed inside the den or at the den opening (WCS 2022). Male raccoons can pose a danger to kits, and mother raccoons will often move their offspring to another den location if a male's presence is sensed nearby.

Results and Discussion

Raccoons living in close proximity to humans increase the risk of human-wildlife conflict, property damage, and disease. Raccoons generally avoid humans and domestic pets, but bites and attacks can occur. Raccoons can do extensive damage to buildings while accessing and living in their chosen den location. A family of raccoons living on a property can make loud noises and the build-up of urine and feces can create strong odors and staining in the surround area. The presence of raccoon feces on a property increases the risk of the transmission of the raccoon roundworm, *Baylisascaris procyonis*, to humans and domestic pets (CDC 2018). The cat flea (*Ctenocephalides felis* Bouché 1835) is found commonly on raccoons in Alameda County. The suburban transmission cycle of murine typhus, *Rickettsia typhi*, transmitted by *C. felis* is of particular concern to the ACVCSD as the interaction between humans, feral cats, and wildlife can lead to the spread of fleas and the pathogens they carry (Anstead 2021). There has not been a reported case of a rabid raccoon in California to date (CDC 2020).

Selecting the appropriate eviction technique is dependent on the cooperation of the residents and the wellbeing of the raccoon family. ACVCSD has had success using both noise harassment and raccoon eviction fluid to evict mother raccoons and kits. However, these techniques will not work immediately and are not feasible in every situation. A mother raccoon is generally resistant to leave her den, and she will need to find another safe location to move her young. As a result, evicting a raccoon family may take multiple days, or longer, and residents may become impatient or stressed in the process. The use of noise to harass a mother raccoon does require the participation of the resident to implement the sound, which is not possible if the resident is either unwilling or unable to do so. Eviction fluid can be applied by ACVCSD Biologists, but our ability to apply it is reliant on the den location being accessible. In cases where the harassment methods are not feasible, or the raccoon mother is undeterred by the harassment, waiting for her to move out, after the kits are fully weaned, is recommended. In situations where the resident wants immediate removal of the raccoon family, hiring a professional wildlife company is an option.

Conclusion

Raccoons will always be a part of our urban environment, and their presence alone does not constitute a cause for concern. However, conflict can arise when raccoons choose residential homes and properties for their den locations. ACVCSD has found the use of noise and raccoon eviction fluid to be effective methods for raccoon eviction. However, these methods may not work in all cases. Raccoons are highly adaptable and intelligent animals, so utilizing multiple humane harassment methods is recommended. ACVCSD Biologists are motivated to discover new and creative methods to humanely evict mother raccoons and their kits from properties while addressing the public health, nuisance and animal welfare concerns.

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Bobcat eviction in suburban Fremont California. **

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**A complete manuscript was published elsewhere (Mooney and Wannamaker 2020).

Introduction

Alameda County Vector Control Services District (ACVCSD) received almost 2,000 wildlife related requests for service in 2019. This district has been keeping request for service records for 20 years; however, it wasn't until May of 2019 that it received its first request involving a bobcat (*Lynx rufus*) denning at a property. In this case, a bobcat queen and her four kittens were observed denning under the backyard wooden deck of a home that had been uninhabited for 6 months. The home is located in suburban Fremont at the interface of suburban housing and undeveloped open space. Although ACVCSD biologists and the USDA wildlife specialist who joined them have vast experience with evicting other forms of wildlife, this was their first opportunity to observe and record the behaviors of bobcats living in a suburban environment and to test various humane harassment techniques on a denning bobcat queen and her four kittens.

Methods

The biologists and wildlife specialist team set up game cameras to monitor activity and began applying humane harassment techniques at the property on May 14, 2019. Over the next 7 days, the team continued to observe signs of bobcat activity and denning. During this time, they tried multiple eviction strategies. The strategies included noise harassment (activating a leaf blower with a timer and installing a bat emitter), water harassment (spraying the deck with a hose), smell harassment (applying bobcat and raccoon urine to the deck), human presence (2 or more people coming to the backyard every weekday and spending time near the den) and removing sections of the deck to expose the inside of the den. The camera footage revealed no further signs of bobcat denning at the property after May 21, 2019; however, they were briefly seen at the property on June 2, 2019.

Results and Discussion

The bobcats were first reported to ACVCSD on May 9, 2019. After various harassment strategies were tried, the bobcats were last seen utilizing the den on May 21, 2019, and last spotted visiting the location on June 2, 2019. The various humane harassment techniques employed by the biologists and wildlife specialist were noise, water, smell, human presence, and den alteration. Although the eviction was successful, it was impossible to know if the causative factor was a specific technique, the combination of techniques, or if the bobcat queen would have decided to move on with or without the eviction efforts. During the eviction process, a different bobcat was seen by the biologists on a nearby street, indicating that the population in the neighborhood was larger than previously known. In October of 2019, three bobcats were sighted on a security camera about a mile away from this location. It is possible that this was the same group of bobcats traveling in the area, or it could have been an entirely separate group. Whether this was a different group of bobcats or not, bobcats are being sighted and reported to ACVCSD more frequently than in the past.

Conclusion

Multiple humane harassment techniques were used to evict a bobcat queen and 4 kittens. Even though this was the first bobcat eviction request in Alameda County, it will very likely not be the last, especially as houses continue to be built in and near open space. Learning how to co-exist with wildlife will continue to be important and include further explorations of effective wildlife eviction methods.

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Raccoon latrines and *Baylisascaris procyonis*

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Abstract

Each year, Alameda County Vector Control receives a number of requests for service for raccoon latrines. Raccoon latrines present both a nuisance and public health risk to County residents. In addition to the nuisance posed by the presence of raccoons and feces, latrines may harbor *Baylisascaris procyonis* eggs, which can be infectious to humans if ingested. Although the number of documented cases of *B. procyonis* in the United States is low, the prevalence of *B. procyonis* eggs in latrines can be quite high. This presentation provides an overview of *B. procyonis*, latrine management and cleanup, and recommendations frequently given to Alameda County residents for raccoon management.

A brief introduction to *Rattus rattus* (roof rats) and their commonly associated diseases, a perspective from Alameda County.

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Introduction

Rattus rattus (roof rats) are a common commensal pest of concern within urban and sylvatic areas of Alameda County. Knowledge of associated ectoparasites and disease is not readily available. Surveillance and ectoparasite collection from captured rodents can provide insight for future targeted measures of intervention.

Methods

Rats were captured in urban and sylvatic areas of the county with live-traps. Surveillance areas were determined using request for service (RFS) calls from residents of the county as well as observations from Alameda County Vector Control Biologists. Captured rats were identified, ectoparasites collected, ear and tail snips taken, and pertinent information recorded. The primary identification key used was H. E. Stark's key to North American Fleas (Furman and Catts 1982).

Results and Discussion

There was a distinct difference between ectoparasites collected on *Rattus rattus* captured from sylvatic areas (parks and other green areas bordering residential communities) and urban areas (residential areas not adjacent to parks or green areas). A total of 620 roof rats were captured: 526 from urban areas and 94 from sylvatic areas. A total of 239 fleas were collected and identified: 115 from urban rats and 124 from sylvatic rats. Within urban areas, 4 different flea species were represented: *Hoplopsyllus anomalous, Leptopsylla segnis, Nosopsyllus fasciatus,* and *Ctenocephalides felis*. In comparison, 8 flea species were found on roof rats collected from sylvatic areas: *Malaraeus telchinus, Opisodasys keeni, Orchopeas sexdentatus, Etielloides longipalpus, Hystrichopsylla occidentalis, H. anomalous, L. segnis,* and *N. fasciatus*. The most abundant flea on urban *Rattus rattus* was *N. fasciatus*. The two most abundant fleas from sylvatic *Rattus rattus* were *M. telchinus* and *O. keeni*.

Conclusions

Rattus rattus captured from sylvatic areas within Alameda County generally were infested with a greater number and diversity of fleas compared to those captured in urban areas. Knowledge of specific ectoparasite species, abundance, and distribution can facilitate efficient use of resources for targeted control and disease mitigation.

Acknowledgements

I will acknowledge all my colleagues at Alameda County Vector Control Services District for their contributions and input. Vector Control Biologists S. Kurniawan, K. Daum, and H. Burroughs especially provided great insight and visuals for the presentation.

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Skunks in Alameda County, California

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Introduction

Skunks and skunk-related nuisances comprise a high percentage of wildlife-related calls to the Alameda County Vector Control Services District. Skunks are an important host of the rabies virus, and additionally pose a nuisance to Alameda County residents due to their potent malodorous spray. The skunk population in Alameda County is composed primarily of striped skunks (*Mephitis mephitis*) in the family Mephitidae, which includes skunks and stink badgers.

Alameda County residents can call the Alameda County Vector Control Services District (ACVCSD) to request assistance regarding health-hazard related animals such as skunks. Skunks are considered important vectors of rabies and other diseases. In California, the most common rabies variants are bat and skunk rabies variants. These rabies variants are better adapted and contagious to their host animals than other variants; however, they have the potential to infect other species. ACVCSD regularly submits suspect wildlife to the Alameda County Public Health laboratory for rabies testing.

Materials and Methods

Residents of Alameda County can submit a request for service (RFS) for vector-related issues to the ACVCSD via phone, website form, or email. Residents submit their contact information, location, species of animal involved (if available), and other information. A biologist will then respond to the request and assist the resident with their issue. These service requests are stored in an online database. Temporal and spatial data can be extracted in batches from this database. Diagrams were created using Microsoft Excel and ArcGIS Pro. The National Land Cover Database shape file is from the U.S. Geological Survey (USGS 2019). Skunk rabies information was taken from CDPH's Annual Rabies Report and Alameda County Vector Control's rabies records.

Results & Discussion

Skunk-Related Requests for Service

In Alameda County, most wildlife-related requests for service involved skunks, raccoons and opossums. In 2019, there were a total of 5693 requests for service, of which 10.1% were skunk-related (Fig.1). While this is a small proportion of total calls to ACVCSD, skunks are the most commonly reported wildlife species overall.

Skunk Distribution

In Alameda County, skunk requests for service are most frequent at interfaces between suburban/urban areas and undeveloped areas (Fig. 2). The highest density of skunk-related requests were from residents in the southwestern part of the county, mostly around the cities of Union City, Newark, and the northern part of Fremont (Fig. 2,3). Unlike skunk-related requests for service in other cities, these requests covered a widespread suburban area and did not consist of interfaces of suburban/urban areas and undeveloped areas.

One hypothesis for the large number of requests in this region is that there are abundant food sources and shelter. Skunks are opportunistic omnivores and unlike their rural counterparts, suburban skunks also forage for urban food sources such as pet food, fallen fruit, bird seed, garbage, and lawn grubs. Alameda County residents often call after seeing a skunk eating pet food from their pets' bowls or after they dig up their lawn for beetle larvae. Another possibility is the large number of sheds, decks, and accessible crawl spaces under homes that skunks can use as dens. Residents often call after a skunk has dug a burrow under a structure or has accessed the subarea of a home via an open vent screen. Both factors lead to increased

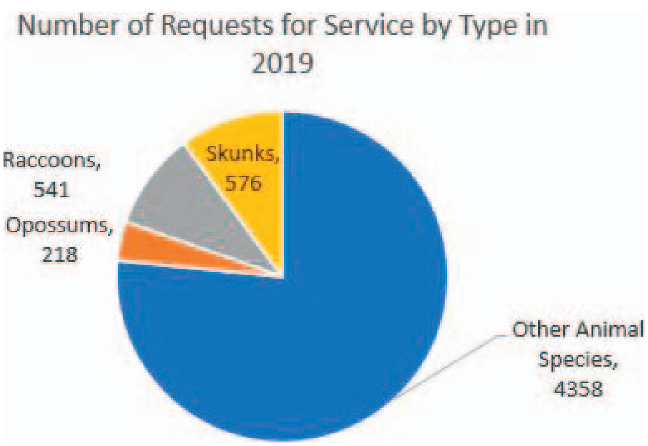

Figure 1.—Opossum, raccoon, and skunk-related service requests in Alameda County in 2019 in proportion to other types of service requests.

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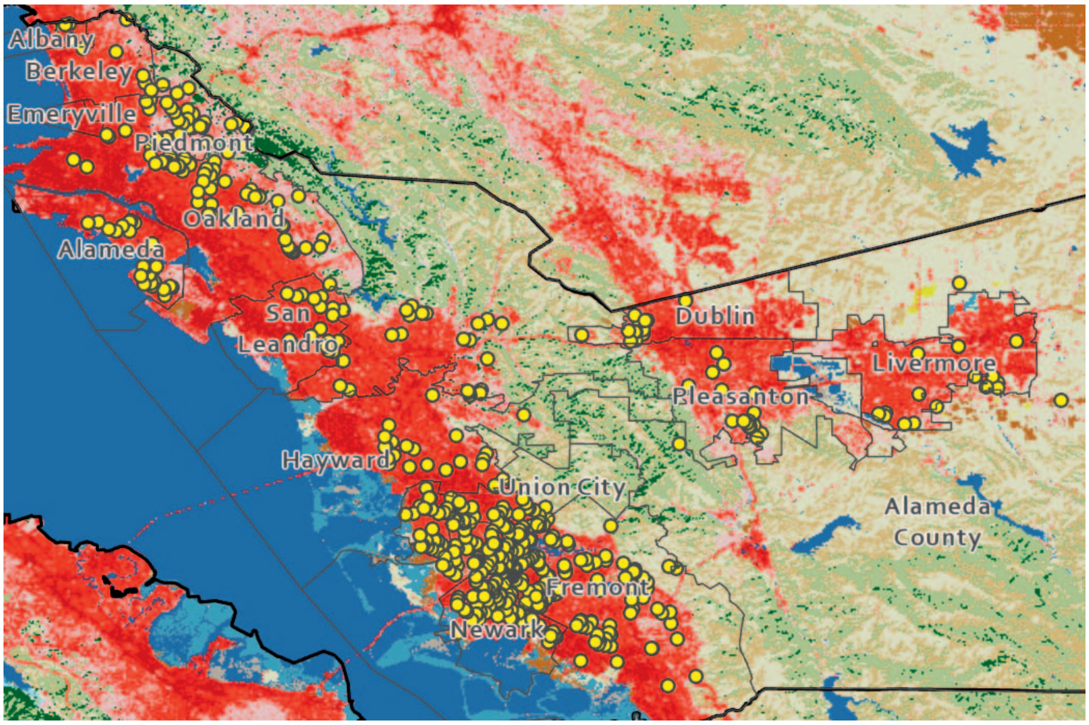

Figure 2.—Distribution of skunk-related requests for service in 2021. Circles indicate skunk-related requests for service. Red areas indicate areas of urban development according to the National Land Cover Database.

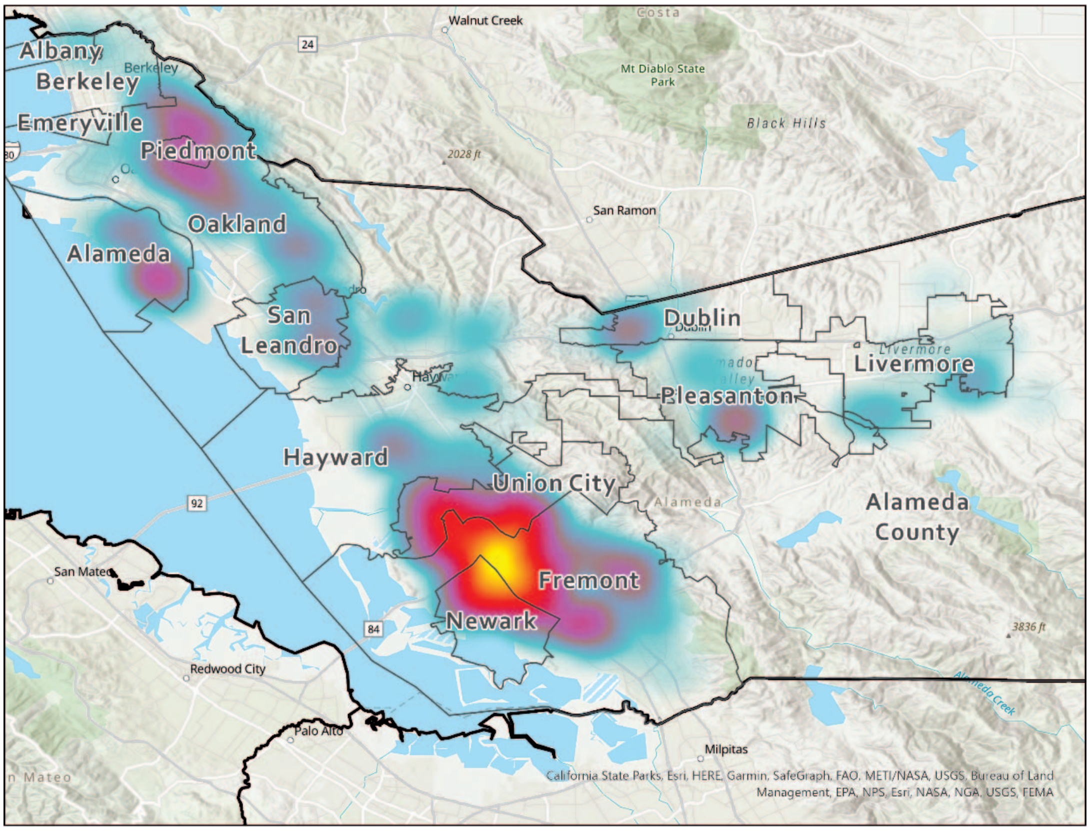

Figure 3.—Heat map of the density of skunk-related requests for service in Alameda County in 2021.

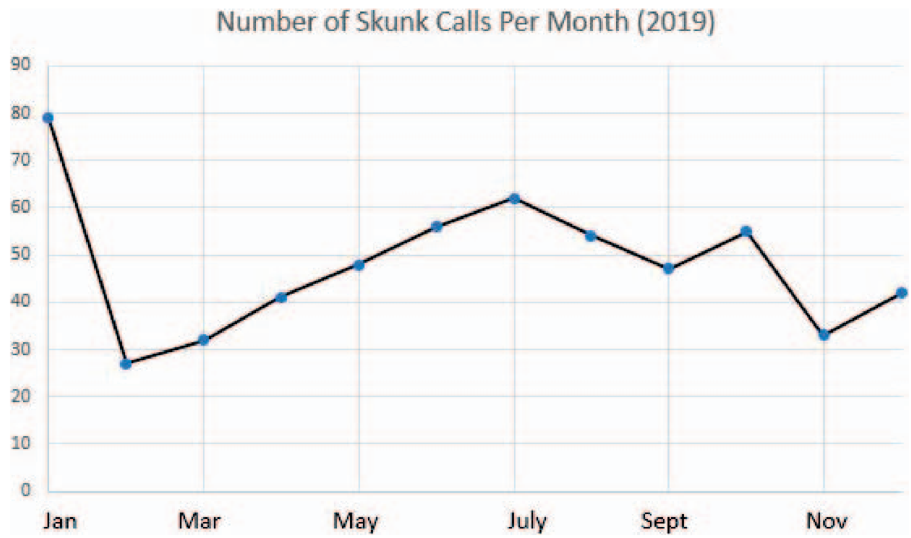

Figure 4.—Monthly total of requests for service related to skunks in 2019.

probability of human and skunk conflict. The volume of requests also may be related to the levels of tolerances of residents to these encounters.

Skunk Seasonality

Before any exclusion or harassment techniques are used on a denning skunk, it is important to note the seasonality of skunk biology. ACVCSD uses Tomahawk one-way doors (Tomahawk 2022) to allow a denning animal to exit and not re-enter the den. These one-way doors are secured over a burrow entrance and allow the exit of the animal but prevent re-entry. One-way doors come in various sizes and a door that is appropriately sized for an adult skunk may accidentally trap younger skunks, as they are not strong enough to lift open the door. This can potentially cause families to be separated resulting in the death of the young or more damage from the distressed mother attempting to retrieve her litter. To avoid such circumstances, it is not recommended to deploy one-way doors during the breeding and litter seasons. A game camera can be used to verify if the skunk is a lactating female, but this can be difficult to determine due to their long fur. Game cameras also can verify if the litter is strong enough to use a one-way door.

Increases in skunk-related requests for service likely correspond with the breeding and litter season. During the breeding season, female skunks often den under structures waiting for potential mates. Fights between rival males and between the female and unwanted suitors can result in spraying. The strong odor often upsets residents, especially if the fight occurs under a house. Although many sources state that the skunk breeding season spans from February through late March (Kiiskila et. al 2014, VTFW 2022, MassWildlife 2022), this schedule differs from anecdotal experience in Alameda County. Large numbers of skunk related requests, often related to skunk spray and denning, start rising in December, then peak in January. A decrease in calls happens shortly after, likely due to the skunks denning and giving birth, which results in less spraying and less visible skunk activity. This suggests that the breeding season starts earlier in Alameda County (Fig. 4). Lactating female skunks have been spotted in Alameda County as early as March (ACVCSD, unpublished). Requests regarding skunks slow down during the likely gestation and birth of litters and increase during the summer as mother and juvenile skunks venture outside of their den and are spotted by residents. Juvenile skunks start to be spotted outside dens around early April (ACVCSD, data unpublished). This seasonality appears to be different than other regions, where young are said to be born in late April to early June (Kiiskila et. al 2014, VTFW 2022, MassWildlife 2022).

Disease Concern

According to CDPH, rabid skunks are reported in California every year (Fig. 5). ACVCSD regularly reports rabies-positive bats, but the last rabies-positive skunk in Alameda County was reported in 2005 (Fig.6). Skunks with rabies-like symptoms have tested negative for rabies and are thought to be suffering from canine distemper. There are reports of potential canine distemper outbreaks in the California Bay Area (Simons 2022). These reports, however, are suspect and most are not confirmed by laboratory testing. Anecdotally, local animal control agencies and wildlife rehabilitation centers have reported increased numbers of wildlife suffering from canine distemper symptoms during 2019-2021.

Skunks can harbor leptospirosis, which has similar symptoms and has been reported in Alameda County dog parks in Fremont and Union City by local news media (Geha 2022). These dog parks are located near high densities of skunk reports (Fig. 2, 3).

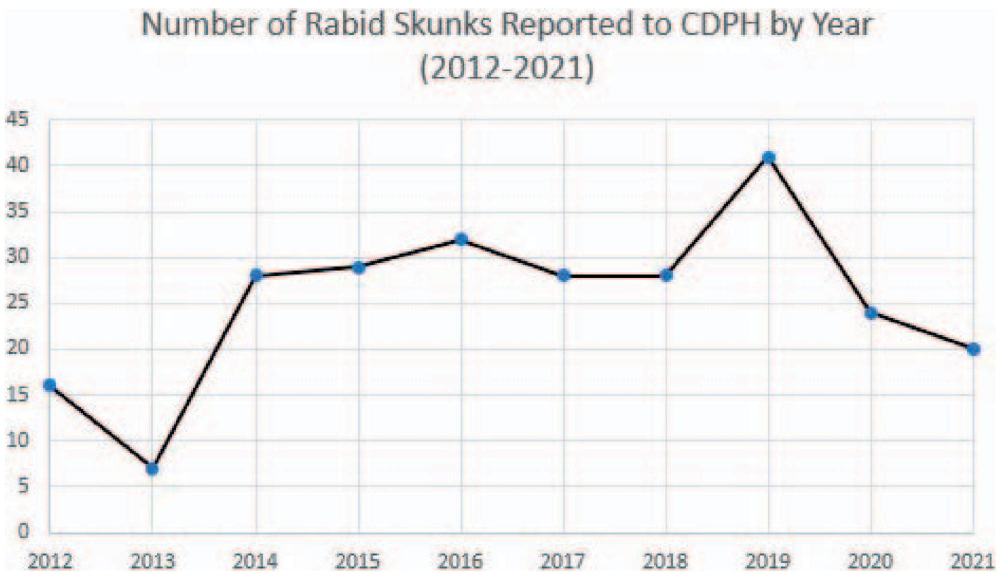

Figure 5.—Yearly total of rabid skunks reported in California to the CDPH from 2012-2021.

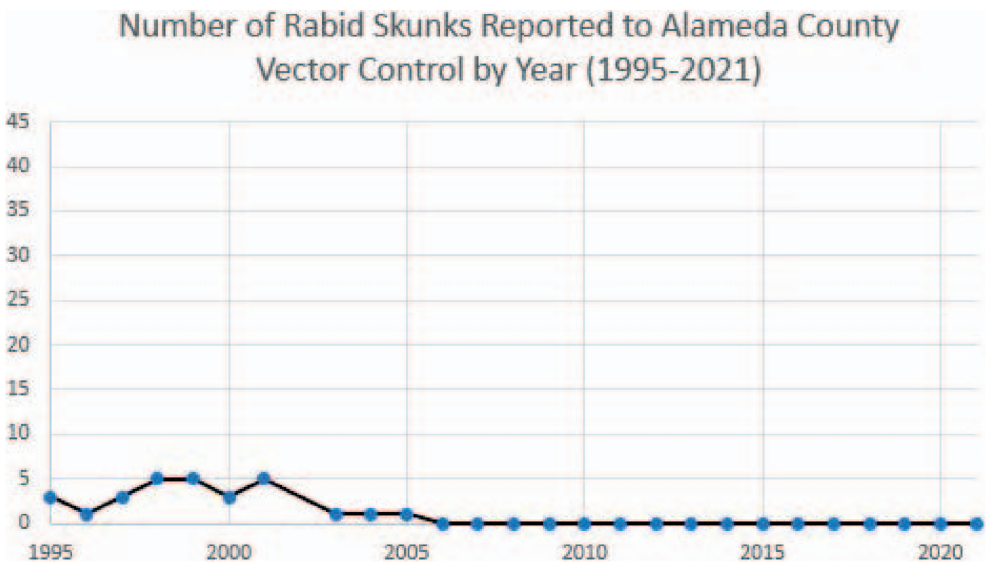

Figure 6.—Yearly total of rabid skunks reported by Alameda County Vector Control Services District from 1995 to 2021

Conclusions

Most skunk-related issues can be managed by educating the public, employing harassment, and/ or installing exclusion; however, it is important to note the seasonality and condition of the skunk before implementing any tactics. Although there have not been rabid skunks reported in Alameda County since 2005, skunks remain a potentially important rabies vector. Understanding the spatial and temporal distribution of skunks in Alameda is important in determining appropriate methods of skunk management.

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The curious tale of the Covina cluster – An integrated approach

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Abstract

West Nile virus (WNV) is endemic to Los Angeles County; however, in recent years, evidence of transmission was thought to be scattered and isolated, especially in the San Gabriel Valley. Beginning in May 2021, a cluster of West Nile virus activity was first identified through mapping the increased number of dead crows found in the city of Covina. Testing of birds and mosquitoes in the area confirmed West Nile virus was circulating, triggering an interdepartmental and collaborative response to help mitigate the potential threat to residents. These actions consisted of increased mosquito sampling, door-to-door neighborhood notifications, and wide area treatments via truck mounted sprayers. Although the number of WNV positive samples in 2021 was high in comparison to recent years, in a historical context, this level of activity had been previously observed.

Taking technology to the field to reduce resistance

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Abstract

Managing an effective pesticide resistance program involves several types of testing and monitoring practices. The most commonly known types of testing methods are for determining ***technical resistance*** by utilizing biological, molecular, or biochemical assays. These assays are widely accepted because laboratory conditions are easily controlled, and resistance mechanisms/levels can be detected fairly quickly in local mosquito populations. The technical tests provide concise data for evidence-based decision making if control efforts and products need to be adjusted.

Practical resistance testing is equally critical when monitoring for resistance, although not as frequently performed. Field testing for efficacy begins with proper tools for equipment calibration and ends with geospatial data integration for operational staff. Droplet analysis (size, density, deposition), exposure dosages, environmental conditions, maintaining rotational product inventory, detailed spray equipment records, and integrated surveillance data all contribute to reducing practical resistance issues in the field. This presentation covers technological tools for mosquito control that enhance field performance of control activities while conserving financial resources for the districts that utilize them.

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Detection of methoprene and spinosad resistance in immature *Culex pipiens* in Woodland, California

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Introduction

In 2008 the Sacramento-Yolo Mosquito and Vector Control District (Sac-Yolo MVCD) detected resistance to the larvicide methoprene in immature populations of *Culex pipiens* collected from catch basin habitats in Woodland, CA. Resistance initially was noted due to control failures, and was subsequently confirmed using cup bioassays. As a result of detecting resistant populations, Sac-Yolo MVCD discontinued use of larvicides containing methoprene within the town of Woodland. Mosquitoes in catch basins were controlled with a rotation of VectoMax FG (active ingredients: *Bacillus sphaericus* and *Bacillus thuringiensis* subsp. *israelensis*) and Natular XRT (active ingredient: spinosad).

In 2019 control failures of Natular XRT were reported by mosquito control technicians working in Woodland. These control failures prompted a wide-scale investigation into spinosad and methoprene resistance in *Culex pipiens* in Woodland. The goal of the investigation was to quantify levels of resistance to each larvicide and to determine how, or whether, the two larvicides should be used in that area.

Methods

Sampling density in Woodland was based on a *Cx. pipiens* dispersal range of 2.1 km (Hamer et al. 2014). Overlapping dispersal radii (sectors) were positioned across the town of Woodland, with the goal of collecting *Cx. pipiens* for resistance testing within each sector. Mosquitoes were collected using gravid traps baited with a fermented hay and hog chow infusion. Gravid trap collections were sorted and gravid *Cx. pipiens* were presented with oviposition cups. The target sample size for each population was 36 eggs rafts from each sector. Three rafts were reared per pan across 12 pans.

Cup bioassays protocols followed previously published methods (Su and Mulla 2004). To test for resistance to spinosad and methoprene Natular 2EC (Clarke; Roselle, IL) and Altosid Liquid Larvicide (Zoecon; Schaumburg, IL) were used, respectively. Larvae were tested for spinosad resistance as early third-instars and methoprene resistance as fourth-instars. A susceptible laboratory colony of *Culex quinquefasciatus* (CQ1) was included as a control with each population and was used to calculate resistance ratios (RR).

The bioassay response data were analyzed using a probit analysis calculator (Mekapogu 2021) to obtain median lethal concentration (LC_{50}) and inhibition of emergence estimates for 50% of the population (IE_{50}). Data quality was assessed using 95% confidence intervals, data heterogeneity (X^2) and coefficient of determination (R^2). To quantify levels of resistance a resistance ratio (RR) was calculated using the following formula RR = LC_{50} field population / LC_{50} CQ1 (susceptible colony). The resistance ratios were interpreted as < 5 = susceptible, 5-20 = low-level resistance, 21-100 = mid-level resistance, and >100 = high-level resistance.

Results and Discussion

Overall, there were 20 sectors designated for *Cx. pipiens* collection in Woodland. Although collection was attempted in each location not all trapping attempts yielded sufficient numbers for testing. In addition, cup assays did not always pass quality control due to excessive mortality or high deviation from the slope of the fitted line ($R^2 < 0.8$). For the current report only the LC_{50}/IE_{50} values were considered.

Of the 20 sectors, 14 were evaluated for methoprene resistance. Following a twelve-year cessation of methoprene-based larvicide applications in Woodland it was of note to find resistant populations in 7 out of 14 of the sectors assessed. Of the resistant populations detected 5 collections exhibited low-level resistance ratios and 2 collections exhibited mid-level resistance ratios (Fig 1). For the spinosad evaluation 15 collections of *Cx. pipiens* were tested. Of these populations only 3 were considered susceptible, 8 collections exhibited low-level resistance and 4 populations exhibited mid-level resistance (Fig 1).

The detection of resistance to methoprene and spinosad throughout the town of Woodland presents challenges for controlling larval mosquitoes in catch basins, a productive habitat for *Cx. pipiens,* a primary vector for West Nile virus. Catch basins may be predisposed to evolving resistance due to repeat treatments with residual larvicides, continual flushing with urban runoff, and introduction of other industrial and residential pesticides in runoff water. Mosquito control agencies may combat resistance by rotation of larvicide active ingredients. It is likely that the

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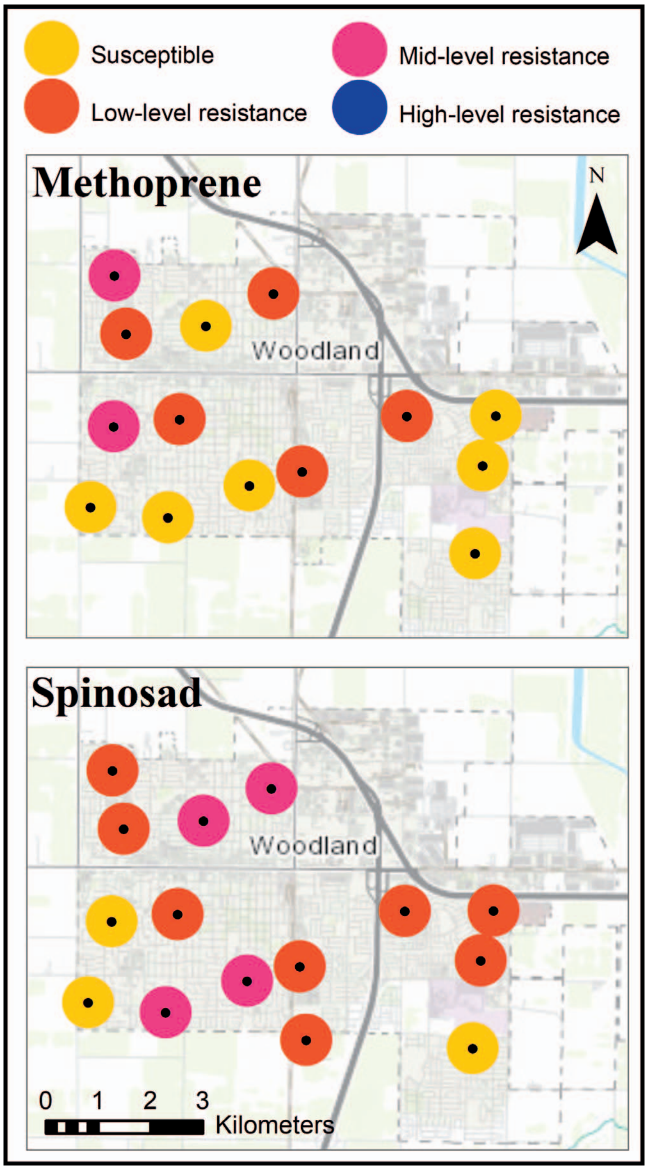

Fig. 1.—Map showing the collection location of populations of *Cx. pipiens* tested for methoprene or Spinosad resistance in Woodland, CA. The color of the dot indicates the level of resistance based on resistance ratios.

withdrawal of methoprene from Woodland and increased reliance on spinosad led to the widespread development of spinosad resistance. Future work will expand larvicide resistance testing and will be coupled with product efficacy testing throughout catch basin habitats in Sacramento and Yolo counties.

Acknowledgments

We would like to thank the technicians and supervisors at Sacramento-Yolo Mosquito and Vector Control District who helped with the detection of control failures, collection of field populations, maintenance of mosquito colonies, and the conducting of larval resistance assays. Without their hard work this project never would have come to fruition.

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Larvicide resistance: The *pipiens* strike back

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Abstract

After many years of repeated use of VectoLex WSP in the bicycle catch basin treatment program at Salt Lake City Mosquito Abatement District (SLCMAD), resistance to the active ingredient, *Bacillus sphaericus,* was detected in 2016 in *Culex pipiens*. For the next two years SLCMAD did not use products with *B. sphaericus,* but rotated between larvicides with the active ingredients of Spinosad and Methoprene. The *Cx. pipiens* population was then tested for susceptibility to *B. sphaericus*. The mosquitoes did not show resistance, so larvicide products with *B. sphaericus* were re-inserted into rotation along with other active ingredients. In 2021, resistance was again suspected from follow-up inspections to catch basin treatments. Further follow-up inspections were conducted along with laboratory assays which showed that resistance was once again present in the urban *Cx. pipiens* population. SLCMAD has new strategies going forward to prevent and monitor this larvicide resistance.

Artificial selection with larvicides and strategies to reduce mosquito numbers without increasing selection pressure

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Abstract

Continued selection pressure over long periods of time by a single control agent may result in the development of resistance by any organism. Mosquitoes have been around for millions of years and have evolved strategies that enable them to survive even in the most difficult of environments. Humans have been combating mosquitoes extensively only for the past couple of centuries. Although the spectrum of larvicides has evolved, these agents continue to propagate resistance. Some of these induced population level changes have huge fitness costs, but others do not. This presentation focuses on known resistance examples to the different larvicide mode of actions and on suggested best practices to alleviate selection pressure.

Benchmark pyrethroid resistance levels for *Culex pipiens* in Sacramento and Yolo Counties

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Introduction

In recent years, there has been increased evidence of pyrethroid resistance in *Culex pipiens* complex populations in California (McAbee et al. 2004; Samra et al. 2012). Pyrethroids remain an important means for control of the mosquitoes that transmit West Nile virus, and resistance to pyrethroids can reduce the ability of mosquito control programs to suppress vector biting and WNV transmission. The extent of resistance in Sacramento and Yolo Counties in northern California has not been well characterized. Our research aims to fill this gap and to measure resistance levels in these counties. Characterizing resistance levels within Sacramento and Yolo counties will provide a baseline that can inform mosquito control decisions to select the most effective insecticides and to understand and manage insecticide resistance.

Methods

The data utilized in this study were compiled during 2018, 2019, and 2021 from both the field and laboratory at Sacramento-Yolo Mosquito and Vector Control District. Field data included weekly collections of adult female mosquitoes from gravid traps operated in urbanized areas throughout Sacramento and Yolo Counties. When gravid traps captured a sufficient number of mosquitoes (>50 per trap per site), the trap location was marked as a potential location for resistance testing. All collection habitats were suburban or urban parks and residential neighborhoods within the cities of Dunnigan, Woodland, Davis, Winters, Sacramento, Florin, Elk Grove, Carmichael, Orangevale, and Folsom. To collect mosquitoes for resistance testing, six to eight gravid traps (Bioquip; Rancho Dominguez, California) were set per location. Traps were baited with a fermented alfalfa and hog chow infusion and were run overnight for one or two nights until a sufficient number of mosquitoes was collected for testing. Collections were returned to the laboratory where they were sorted to species, labeled, egg rafts collected and the larvae reared to adults. For each trapping site, up to 36 egg rafts were reared at a density of three rafts per pan (24 cm x 30 cm, with a water depth of 4 cm) and fed a mixture of 1 part ground TetraMin fish food (Spectrum Brands Pet; Blacksburg, Virginia), 1 part ground alfalfa pellets, 0.05 parts liver powder, and 0.05 parts brewer's yeast. Pupae were sorted by collection date so that susceptible colony mosquitoes and field-collected populations could be age-matched for bottle bioassays.

For each population, 20-25 adult female mosquitoes, aged 5-13 days after emergence, were used in each of three replicate CDC bottle bioassays (CDC 2022). The specific combination of insecticides tested differed among the populations tested, due, in part, to variation in the number of mosquitoes available for testing from each location. Most populations were tested for resistance against the following pyrethroids (dose): deltamethrin (22μg), permethrin (43μg), and permethrin + piperonyl butoxide (PBO; 400μg). Additional populations also were tested for resistance to sumithrin (22μg) and the organophosphates, malathion (400μg) and Naled (10μg). Each field-collected population was compared concurrently to a susceptible laboratory colony of *Cx. quinquefasciatus* (CQ1). Three acetone-only control bottles were included with each population. At 15-minute intervals for 3 hours, bottles were checked to observe knock down, and the numbers were recorded. After the last reading, bottles were placed in a freezer to kill any surviving mosquitoes and the total number per bottle was confirmed. Mortality at diagnostic time was recorded for all populations. Resistance level was determined using CDC recommendations; for each active ingredient's diagnostic time, if a population's percent mortality was < 90%, they were considered resistant (CDC 2022).

Results and Discussion

Ninety assays were conducted to test *Cx. pipiens* for resistance. Overall, field-collected populations tested against deltamethrin or permethrin were found to be more resistant than paired susceptible colony mosquitoes, indicating that resistance to both pyrethroid products was widespread. All populations tested against sumithrin also were found to be resistant, but this product was used in only six assays, which limited our conclusions about how widespread the resistance may be. In general, greater susceptibility was observed for the organophosphates, Naled and malathion, compared to pyrethroids. Mortality

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also varied among geographic locations, implying possible differences in susceptibility among populations within the study area and suggesting the need for sustained monitoring to determine the stability of the spatial patterns in resistance over time. Pyrethrum was tested for two populations in paired assays with and without the synergist, piperonyl butoxide (PBO). For both populations tested, the addition of PBO resulted in increased mortality.

Conclusion

Culex pipiens resistance to pyrethroids is a growing issue in Sacramento and Yolo Counties. This study found that there was widespread resistance to pyrethroids in both counties based on bioassays, and that *Cx. pipiens* were generally more susceptible to the organophosphates tested. Resistance was also geographically variable, indicating that ongoing monitoring is needed to ensure that insecticides used for mosquito control are effective against their target populations.

Acknowledgements

We would like to thank the Chester and Patricia Moore Experiential Learning in Entomology Fund, which enabled Gehena Girish to perform this research and attend the MVCAC conference to present the results. CMB acknowledges funding support from the Pacific Southwest Center of Excellence in Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516). We also thank Deborah Dritz and Marcia Reed at Sacramento-Yolo Mosquito & Vector Control District for assisting with bioassays and analyses.

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Pyrethroid occurrence across Los Angeles County storm drain structures

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Abstract

Pyrethroid insecticides are used widely in agricultural and urban settings for controlling pests, including adult mosquitoes. Residues of pyrethroids, such as bifenthrin, may remain in the environment for an extended period, potentially contributing to inadvertent exposure and resistance development. To better understand the extent of contamination in urban settings, we assessed the presence and concentration of multiple pyrethroids in various media across storm drain structures in Los Angeles County. In summer 2021, samples such as water, algae, and sediments were collected from multiple structures, including stormwater outfalls and flood control channels. Samples were extracted with solvents and analyzed via gas chromatography-mass spectrometry. Bifenthrin was detected at 100% of sites, whereas other pyrethroid analytes were detected at 0 to 60% of sites. Median total pyrethroid concentrations in water and suspended solids were 5 ng/L and 1,377 ng/g, respectively. Total pyrethroid concentrations in suspended solids were significantly greater than in algae or sediments, and whole water concentrations of bifenthrin were significantly greater in sumps than in channels. No other significant trends were seen, and sample availability was limited by site dryness and accessibility. Although bioavailable pyrethroid residues were likely low, further research is needed to determine potential sublethal effects of exposure, including resistance development.

Introduction

Pyrethroid insecticides have been detected in many water bodies, with the primary contributor often being urban runoff.(Amweg et al. 2006, Hladik and Kuivila 2012, Kuivila et al. 2012) In addition to concentrations often being above aquatic toxicity benchmarks, sublethal exposure risks resistance development in populations of urban mosquitoes. Although downstream concentrations are well-documented, little is known about pyrethroids in urban underground storm drain system (USDS) structures, which are common sources for the larval development of species such as *Culex quinquefasciatus* (Su et al. 2003, Popko et al. 2018). Thus, the current study sought to assess the extent of pyrethroid contamination in an urbanized USDS.

Methods

Sites and Sampling

Sites selected included catch basins/sumps, open channels, and outfalls in Los Angeles County. The county has over 7,409 km of sewer pipes and 1,343 km of surface channels, with the city of Los Angeles alone having over 38,000 catch basins (Porse 2018). Sites were selected based on the presence of water. Through June and July 2021, unglazed ceramic tiles were placed at each site to allow for the colonization of biofilms. After 60 days, samples collected from each site and included water, sediment, algae, and/or biofilms. All samples were stored in glass containers and transported in containers chilled with ice packs, after which they were stored at 4 °C and extracted within 90 days.

Sample Processing

Water samples were filtered using 0.4 µm glass fiber filters to separate suspended solids. Before extraction, bifenthrin-d_5 was added to each sample as a surrogate recovery standard. Afterwards, water samples were extracted using liquid-liquid extraction with dichloromethane, concentrated using a rotary evaporator, and transferred to hexane solvent. Suspended solids, sediments, algae, and biofilms were dried and weighed before extraction. Extraction consisted of the addition of hexane and acetone, ultrasonication, cleanup with Florisil, and transfer to hexane solvent.

GC-MS Analysis

Processed extracts were injected into an Agilent 6890N-5973 GC-MS system for quantitation of 8 pyrethroid analytes: bifenthrin, fenpropathrin, lambda-cyhalothrin, permethrin, cyfluthrin, cypermethrin, esfenvalerate, and deltamethrin. Quality control measures included an 8-point external calibration curve which was run every 12 samples to assure relative standard deviation was within tolerable limits. Detection frequencies and concentrations were acquired for each analyte in each sample. Paired *t*-tests were used to determine statistically significant trends between USDS structures and environmental media.

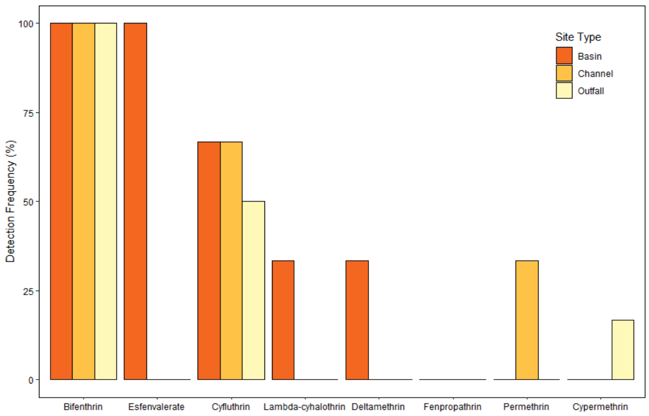

Figure 1.—Detection frequencies of each pyrethroid by site type.

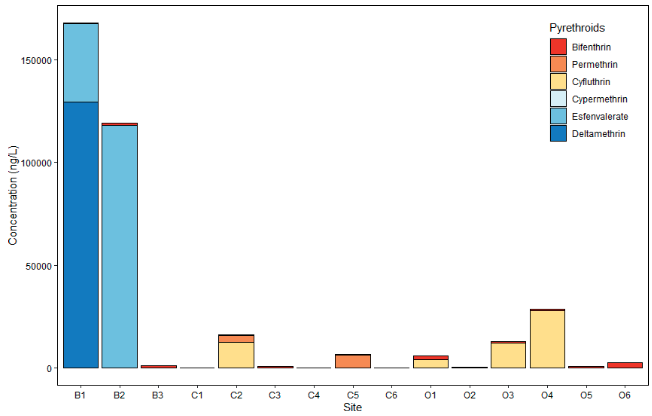

Figure 2.—Whole water pyrethroid concentrations at each site. Each column is separated to show contributions from individual pyrethroids.

Results and Discussion

A total of 3 sumps, 6 open channels, and 6 outfalls were sampled. Though catch basins with water were identified earlier in the year, all of them, along with multiple other sites, were dry during the summer when they were visited for sampling.

Detection Frequencies

By far, the most frequently detected pyrethroid was bifenthrin, which was found at every site. Cyfluthrin was the next most frequently observed, detected at 2 basins, 4 channels, and 3 outfalls. Esfenvalerate, lambda-cyhalothrin, deltamethrin, permethrin, and cypermethrin were detected infrequently, and fenpropathrin was not detected at all (Fig. 1). The widespread presence of bifenthrin was in agreement with previous literature that implicated it as the pyrethroid of greatest concern for ecological toxicity due to its use and persistence in the environment (Amweg et al. 2006, Hladik and Kuivila 2012, Kuivila et al. 2012, Weston and Lydy 2012). Though pyrethroids have broad toxicity towards insects, bifenthrin is not generally used for mosquito control in the United States, and its effects on larval mosquitoes may be somewhat different from permethrin, another type I pyrethroid.

Concentrations

Median total pyrethroid concentrations in water and suspended solids were 5 ng/L and 1,377 ng/g, respectively. Bifenthrin was the only individual pyrethroid with median concentrations above its detection limit (3 ng/L in water and 384 ng/g in suspended solids). Maximum total concentrations in water, suspended solids, algae, biofilms, and sediments were 1,039 ng/L, 97,433 ng/g, 2,292 ng/g, 101,853 ng/g, and 271 ng/g, respectively. Pyrethroids are highly hydrophobic (log K_{OW} 6-7) and thus partition favorably into media high in organic matter, such as sediment and algae, compared to water (Laskowski 2002).

Although high whole water concentrations were observed in basins/sumps, too few were sampled to determine a clear difference between structures. Still, whole water concentrations of bifenthrin in sumps were significantly greater than those in open channels ($p < 0.05$; Fig. 2). However, it is possible that most residues from urban applications reside in catch basin sediments, only being transported to the rest of the USDS during storm events. Periods of high rainfall are associated with high loading of pyrethroids and other particle-associated contaminants into downstream sites (Budd et al. 2020).

Although a limited number of non-water samples were collected, concentrations in suspended solids were significantly greater than those in algae and sediments ($p < 0.05$; Fig. 3). Generally, aqueous phase residues are more bioavailable than those in other media. Even if organisms come into contact with or ingest contaminated sediments or suspended solids, highly hydrophobic contaminants are unlikely to desorb from these phases and be absorbed by the organism. However, high concentrations in other phases may indicate the potential for pyrethroids to be retained in USDS structures and become resistant to degradation by processes such as hydrolysis and oxidation. These structures may then become sinks for pyrethroids, with a risk of release into future stormwater.

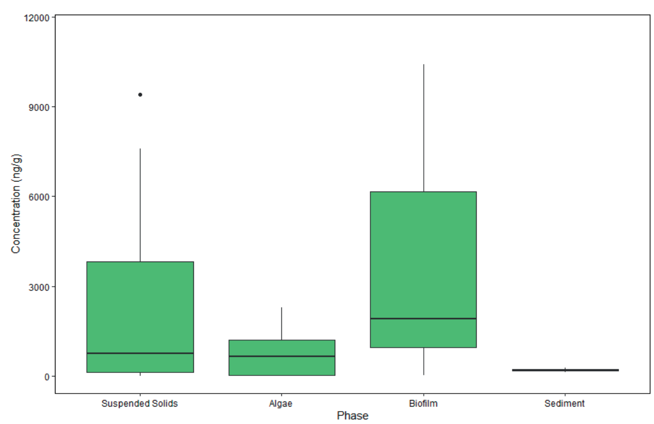

Figure 3.—Median and range of pyrethroid concentrations found in samples grouped by environmental source (phase).

Conclusions

Pyrethroids were present in many USDS structures, and it is likely that residues are present in USDSs where previous monitoring programs have detected residues in outfalls and downstream water bodies. Investigating the USDS directly can provide insights for pyrethroid fate and transport, including major sources, along with effects on organisms inside these systems, such as mosquito larvae. Additional sampling is required to better understand pyrethroid environmental behavior, but dry season study design must take into account limited site availability due to dryness.

Observed concentrations of pyrethroids were generally at sublethal levels for mosquito larvae. The prevalence of low concentrations bifenthrin may have unique implications for mosquitoes, as most studies selecting for pyrethroid resistance have used pyrethroids like permethrin and deltamethrin, often at concentrations in the μg/L range, which is 3 to 5 orders of magnitude higher than the ng/L range we observed in USDS systems in Los Angeles County (David et al. 2014, Shi et al. 2015).

Acknowledgements

This work is supported by the Pacific Southwest Center of Excellence in Vector-Borne Diseases, funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516). We thank Greater Los Angeles County Vector Control District, including Susanne Kluh and Ernie Sanchez, for assistance with site selection and access. We also thank Matthew Hall, Qingyang Shi, Yaxin Xiong, and Parminder Kaur for assistance with sampling and processing. Thanks to David Lyons and the Environmental Sciences Research Laboratory at UC Riverside for additional analyses.

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Molecular markers of metabolic pyrethroid resistance in California *Aedes aegypti*

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Abstract

Resistance to pyrethroid based insecticides has facilitated the rapid spread of *Aedes aegypti* throughout California. *Aedes aegypti* demonstrate both metabolic and target site resistance mechanisms to insecticides, but precise mechanisms of pyrethroid detoxification are not yet well described and appear to vary between and within populations. For our study, we produced F2 generation colonies representing two genetically distinct populations of *Ae. aegypti* from the San Joaquin Valley and from Southern California. These two populations were then subjected to a modified CDC bottle-assay with Deltamethrin, a pyrethroid insecticide. Following Deltamethrin exposure, we observed knock-down times and collected mosquitoes from the upper and lower knock-down quartiles, representing susceptible and resistant insects. We apportioned 10 pools of 5 adult female *Ae. aegypti* organized by population and susceptible or resistant status and submitted them to the West Coast Metabolomics Center for high-throughput metabolomic analysis. This technique offers a snapshot of the insects' metabolomes, revealing different levels of activity and demands on metabolic pathways. We analyzed the data in Metaboanalyst to look for differences between the susceptible and resistant insects and shared features between the two populations. These analyses revealed elevated levels of vitamin B5, an important co-factor in lipid metabolism, in resistant mosquitoes and multiple markers of RNA modification. We identified 5 molecules that may serve as metabolic markers of the resistance phenotype.

Pyrethroid resistance and spread of *Aedes aegypti* in California

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Abstract

Invasive *Aedes aegypti* became established in the Central Valley in 2013. Since then, it has moved across the state and has now been detected in 22 of the 58 counties in California. The high levels of pyrethroid insecticide resistance in these mosquitoes have made control difficult. Using an assay developed for the iPLEX MassARRAY system, which detects selected single nucleotide polymorphisms (SNPs), we gained insight on the resistance profiles and California origins of mosquitoes in the state. The assay includes 5 mutations in the voltage gated sodium channel (VGSC), the pyrethroid site of action, and 31 location related SNPs. Previously, using mosquitoes collected in Clovis, Sanger, Kingsburg, Dinuba, and the Greater LA area, we found wide variation in the resistance genotype and phenotype across groups. We also determined that those collected from the Greater LA area were significantly different in their location based-SNP profile than those from the Central Valley populations. Using this assay, we placed mosquitoes into one of these two general populations in California. Since this initial study, we have used this assay on mosquitoes collected in other areas of California to provide mosquito abatement districts with information on the origin of these introduced mosquitoes, as well as some information on their resistance profile. We plan to continue to collect mosquitoes from counties with recent introductions to trace their origins.

Novel automated mosquito identification and pooling solutions

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Abstract

Surveillance of local mosquito populations is one of the most important tasks in integrated mosquito management programs. To date, surveillance required experts to perform a vast of non-expert tasks, including manual mosquito separation, identification of common mosquitoes and pooling, resulting in poor optimization of the expert time. These tasks are tedious and simple, yet, experts spend substantial time on performing them. An abatement district must always have an expert on site, and such that lack sufficient man power, are limited in their ability to the extent of not having at all, a surveillance program in their area. In the presentation, a game changer technology for automated pooling and identification will be discussed, showing how artificial intelligence and automation are leveraged in order to optimize the work of experts, performing all the routine and simple tasks by a robotic system, able to continuously separate thousands of mosquitoes, image and identify the common mosquitoes using deep learning, while providing online remote access for experts to identify the more difficult mosquitoes via high resolution images, advancing mosquito surveillance operations to a whole new level.

Delta Mosquito and Vector Control District's wireless smart mosquitofish feeder system

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INTRODUCTION

One of the challenges in aquaculture is feeding mosquitofish regularly and appropriately. Mosquitofish are cannibalistic, meaning that larger adult mosquitofish will eat smaller juveniles and fry (Coykendall 1980). Previous studies have shown that "cannibalism by mosquitofish was inversely related to food supply" (Swanson et al. 1996). Also, mosquitofish require a target daily ration of 3-5% wet fish biomass for adult mosquitofish and 5-10% for juvenile mosquitofish (Swanson et al. 1996). Therefore, automatic feeders are the recommended solution to these issues, because of their efficiency and reliability in dispensing feed into contained culture systems (Swanson et al. 1996). These machines work 24/7, follow a programmable schedule for consistent and accurate feeding, and can be adapted to fit a wide variety systems. With a nourishing diet that is appropriately balanced in protein and fats, cannibalism can be minimized, while improving survival and enhancing reproduction rates (Swanson et al. 1996).

METHODS

The Pentair AES 3L vibratory feeders were chosen for their high capacity food storage and simple operational design. An open sourced web-based lawn sprinkler controller named OpenSprinkler OS3.0 with zone expansion board was chosen as the feeder controller, which is responsible for programming the feeding. Wireless RF transmitters and receivers are used to connect the feeder controller to the feeders up to a distance of 55 m. Figure 1 shows the wiring schematic for connecting all of these DC-powered components. All electrical components were placed in weatherproof PVC electrical boxes and conduit. Once assembled, the feeders and controller were mounted into place.

DISCUSSION

The feeder controller is accessible through a web browser or mobile app by establishing a local network connection through WiFi. Once connected, users can edit the default display to show all the connected feeders (Fig. 2). Custom weekly feeding schedules are created by selecting the days of the week to feed, number of feedings per day, times of feedings, and duration of each feeding down to one second. This allows each feeder to have a unique, customized feeding schedule that matches the size of mosquitofish and stocking density. Users can visually review the daily feeding schedules created for each feeder using the program preview tab (Fig. 3). All feedings are automatically recorded by the software and viewed through the past logs function. Overall, the software works well when adapted to control a complex feeder system.

CONCLUSION

Delta Mosquito and Vector Control District constructed a feeder system that fulfilled the needs of the District's mosquitofish program in a more cost-effective manner than any commercially available product. The wireless smart feeder system has been used since Spring 2021 with great success. The large capacity feeders require minimal maintenance, reduce the frequency of refills, and enable the system to be self-sufficient for a substantial amount of time. Automating the task of feeding allows the mosquitofish to be fed daily and appropriately according to their size and stocking density.

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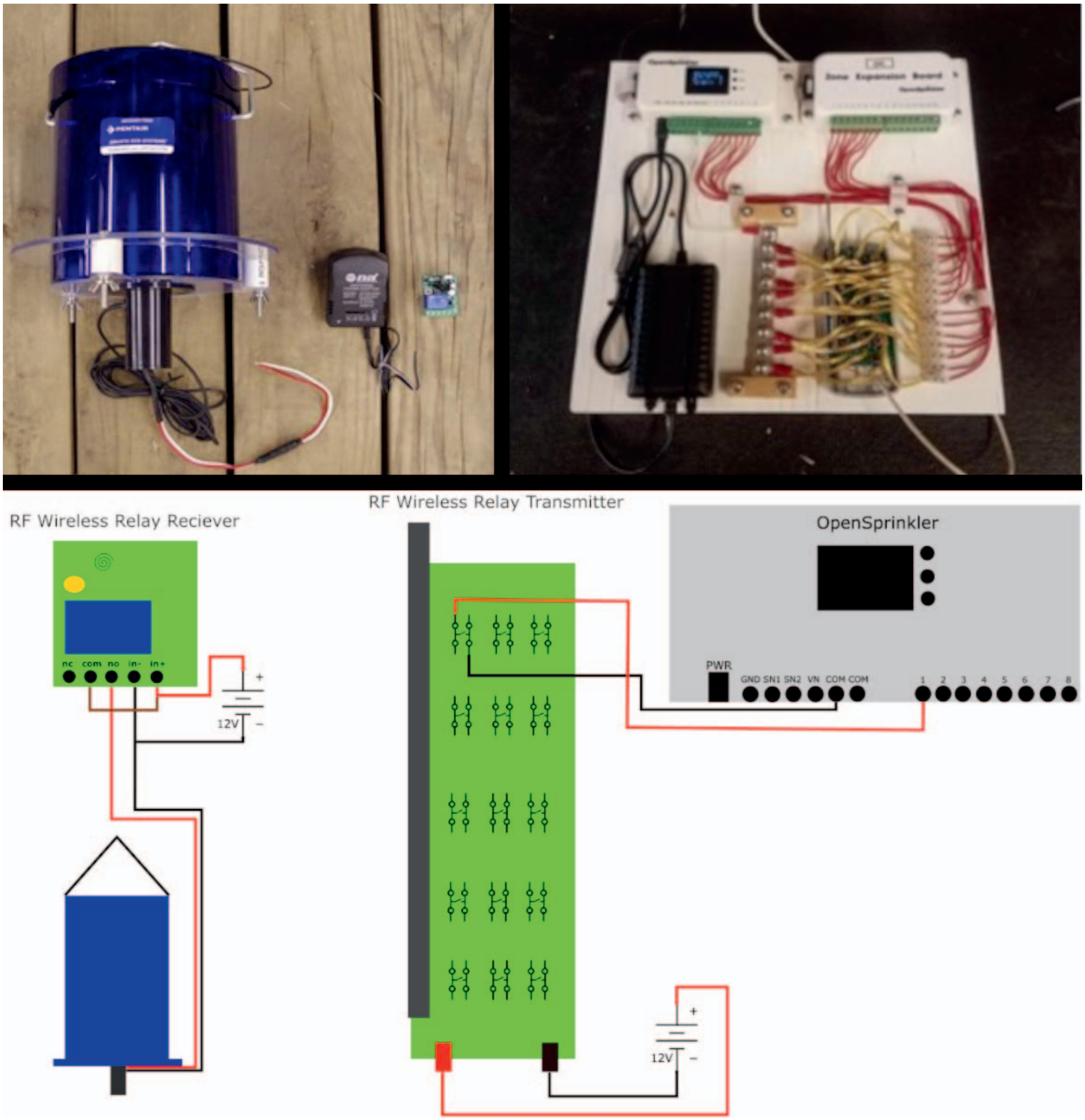

Figure 1.—Pentair AES 3L vibratory feeder and wireless RF receiver. Assembled feeder controller consisting of an OpenSprinkler OS3.0, zone expansion board, and wireless RF transmitter. Wiring schematic for assembling the feeder controller and feeder.

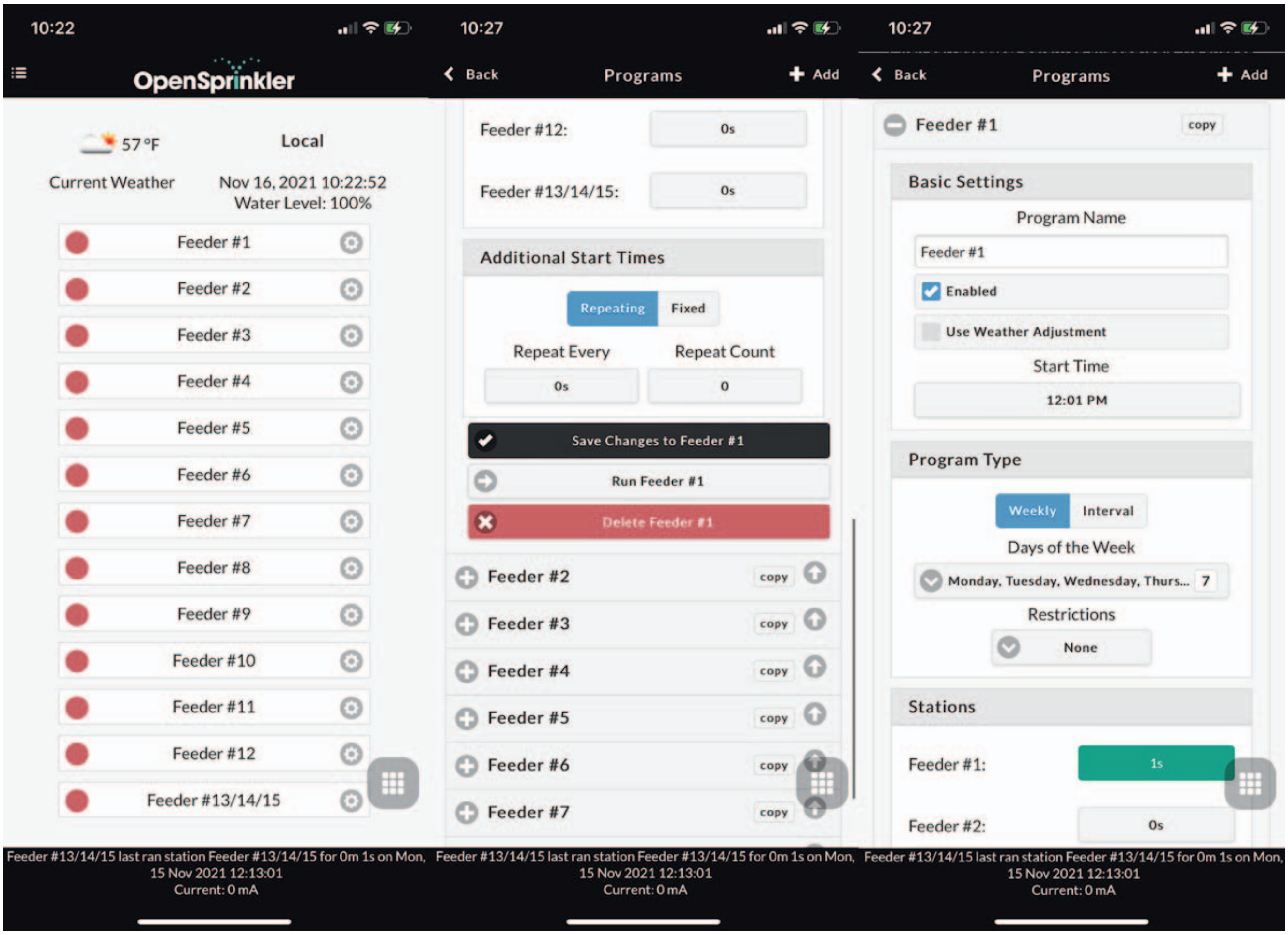

Figure 2.—Programming of feeders using the Opensprinkler user interface via mobile app. The user interface is identical if viewed on a web browser.

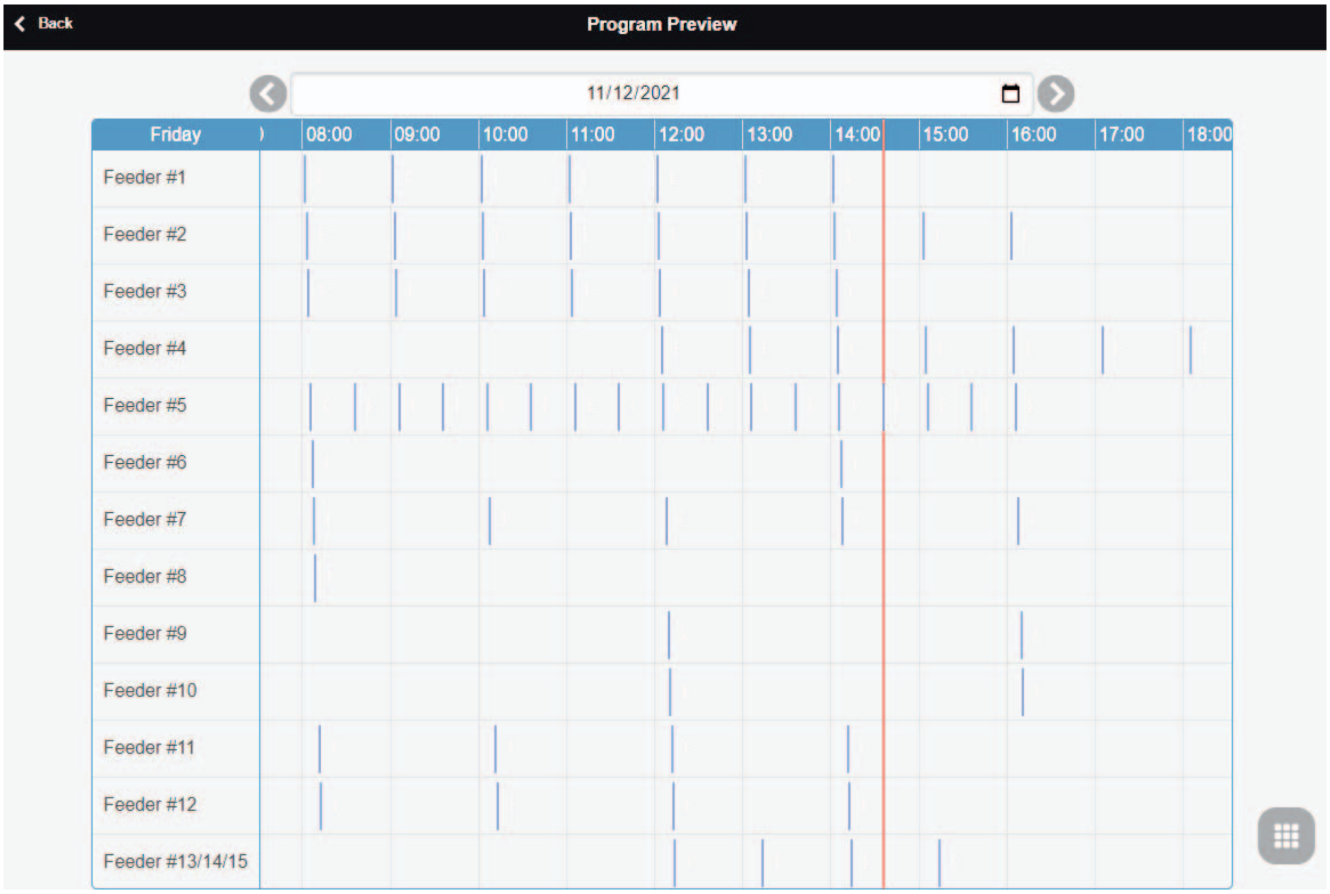

Figure 3.—Opensprinkler's program preview showcasing the daily planned feeding schedule programmed for each feeder.

Figure 4.—The installed automatic feeder system in the indoor and outdoor mosquitofish facility.

Innovation and programmatic changes to the Sacramento-Yolo Mosquito and Vector Control District Catch Basin Program

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Introduction

The Catch Basin Program at the Sacramento-Yolo Mosquito and Vector Control District (District) was established in 2005 to provide a focused control response to *Culex pipiens* production in over 300,000 storm drains within the District's service area. The proliferation of these basins for stormwater conveyance along with their close proximity to residences, schools, and businesses highlight their importance in mosquito control. "*Beginning with paper maps acquired from local municipalities, the District's Catch Basin Program undertook an ongoing project of locating and identifying basins in order to create a rotational treatment plan for managing the production of mosquitoes from these storm water utilities.*" Technicians riding in pairs in light-duty trucks annotated utility maps by hand on paper maps with information related to actions taken or edits needed. The program continued to evolve over the past decade and has since employed the use of right-hand drive (RHD) Jeep Wranglers and modified bicycles to expedite access to catch basins. To further streamline the task of maintaining a historical database of treatments, larval sampling data, and scheduled retreatment dates, the program implemented the use of a variety of digital applications leading to the creation of a custom web application for mobile devices. The evolution in equipment and technology yielded an increase in efficiency and allowed for the better allocation of manpower. This presentation highlights the changes that the program has made over the years as well as future plans to improve efficiency.

Methods

The use of paper utility maps to catalog catch basin locations and inspections created numerous logistical issues related to archiving, updating, and analyzing information. Creating a custom (not commercially-available)mobile application eliminated the need for paper maps and manual data entry while providing instant data recall and analysis. Pairing technicians in trucks to perform catch basin treatments was an inefficient use of manpower. Transitioning to RHD Jeep Wranglers afforded a better distribution of technicians and positioned a single technician directly over the source was inspecting.

Results and Discussion

By creating an in-house mobile application, the printing, copying and managing of thousands of paper .pdfs was replaced by an all-in-one navigation, treatment, and scheduling system for tablet, phone, and desktop computer. Assignment and management of tasks were reduced to a largely automated process, and data could be analyzed in real-time within the application. Coupling this transition with a new fleet of RHD Jeep Wranglers has markedly increased work efficiency within the District's jurisdiction.

Conclusion

The paradigm shift from paper maps and paired technicians to a custom mobile application and single technicians in RHD Jeeps has rapidly increased the department's daily work output while requiring less manpower. Data can be seen by users and management in real-time, reports can be easily automated, and labor-intensive data entry is avoided altogether.

Acknowledgments

Sacramento-Yolo Mosquito and Vector Control Catch Basin Program staff for collaboration in the creation and testing of the equipment and methodologies discussed herein.

Assessment of larvicidal mixtures for extended control of *Aedes aegypti* in underground storm drain systems of Los Angeles County, California

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Introduction

Los Angeles County's Underground Storm Drain Systems (USDS) consist of more than 2,650 miles of storm drains which can produce large quantities of *Culex* mosquitoes (Kluh et al. 2001). Previous surveillance, coupled with field observations, confirmed the exploitation of the USDS by *Aedes aegypti* and as a result, has forced Greater Los Angeles County Vector Control District to evaluate and modify control strategies. Herein, we describe field trials to evaluate the effectiveness of VectoBac WDG and VectoLex WDG mixtures for control of mosquito populations, including *Ae. aegypti*, within the USDS of Los Angeles County.

Methods and Materials

Nine USDS systems known to be inhabited by *Ae. aegypti* were selected for the assessment. Each site was treated using a mixture of VectoBac WDG with an application rate of 11 oz./acre and VectoLex WDG at a rate of 22 oz./acre, followed by an application of Cocobear. The sites then were trapped weekly from May to November 2021, using an un-baited EVS trap. The presence of male *Ae. aegypti* would prompt successive treatments of the system.

Results and Discussion

Surveillance data showed that the USDS *Ae. aegypti* population mirrored the above ground population abundance trends, which indicated that there was not a significant increase in either *Ae. aegypti* population until mid-August. After the initial treatment in May, sites did not need to be retreated until August and those applications were effective in providing a minimum of three weeks of control, not two weeks as anticipated.

Conclusion

As *Ae. aegypti* become established throughout the expansive USDS of Los Angeles County, time between treatments will be reduced from four weeks to three weeks within systems known to be inhabited by *Ae. aegypti*. The mixture of VectoBac WDG and VectoLex WDG was effective in providing a minimum of three weeks of *Ae. aegypti* control within the USDS, longer than the two weeks anticipated.

Acknowledgements

The authors would like to thank Yessenia Curiel, Adrian Velasco, Ryan Amick, and Nicolas Tremblay for their assistance and collaboration.

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Field evaluation of an aerially applied mosquito larvicide

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Introduction

The District uses carbon dioxide baited traps (CO_2) in the rural habitats of the Coachella Valley to monitor *Culex tarsalis* mosquitoes, the primary and most abundant vector of West Nile virus (WNV) and Saint Louis Encephalitis virus (SLEV) at the Salton Sea shoreline. In April and May 2021, larvicide applications were made by helicopter to reduce the high number of *Cx. tarsalis* mosquitoes. Making aerial applications to the remote and extensive areas at the Salton Sea shoreline permits a large area to be treated more rapidly than by ground. Standing emergent vegetation of bulrush and cattails can obscure the pilot's view of standing water as well as reduce the likelihood that the pesticide will reach the water. These evaluations were intended to ensure that the applications penetrated the vegetative canopy and treated the entire site.

Materials and Methods

Two larvicide applications of VectoBac WDG (active ingredient: *Bacillus thuringiensis israelensis*) were applied by helicopter at 0.5 lbs. per acre near the Salton Sea shoreline in April and May 2021. The applications were evaluated in two ways to determine if the product reached target locations on the ground. Empty plastic cups (16 oz., Uline) were placed on the ground either under vegetation or in the open. Water sensitive paper (Teejet Technologies, 52 × 76mm) attached to a plastic CD case and secured to a stake with Velcro was placed in the open 4 ft. above the ground at each location (Fig. 1). Applications were evaluated at two sites – Johnson Street (24 acres) and Hayes Street (30 acres). Six locations were identified at each site, although only five of the six locations were used for the April application at Hayes Street. In April, cups were placed either under vegetation or in the open, while in May all cups were placed under vegetation. Cups and papers were placed just before the treatments were made and collected one hour after the treatments. Papers and cups were returned to the District, and the cups frozen. Cups were thawed to room temperature. Approximately 100 mL of water and fifteen third-instar laboratory-reared *Cx. tarsalis* larvae (susceptible Bakersfield strain) were added to each cup. Larval mortality was counted at 4, 24, 48, 72, and 96 hours.

Results

For the April application, the water sensitive papers placed at the Johnson site had more droplets at locations 1, 2, and 3, fewer droplets at locations 4 and 6, and no droplets at location 5. This result was similar to the larval mortality – 100% larval mortality in cups at locations 1, 2, and 3, partial mortality at locations 4 and 6, and 0% mortality at location 5. The water sensitive papers placed at the Hayes site had more droplets at locations 1, 3, 4, and fewer droplets at locations 2 and 5. Similarly, 100% larval mortality was observed in the cups at locations 1, 3, and 4, partial mortality at location 5, and 0% mortality at location 2 (Fig. 2).

In May, all the water sensitive papers had droplets for both sites (Fig. 3). 100% larval mortality was observed in all the cups placed at all locations, except for location 1 at the Hayes site (Fig.3).

Discussion

Aerial larvicide applications continue to be an effective method to treat large areas of standing water quickly. This is particularly important in the spring months when birds are nesting in areas which coincide with the production of vector mosquito populations. Applications by foot and off-road vehicles are time-consuming and present opportunities for employees to get physically stuck and to potentially disturb nesting birds.

The application in April showed that the target locations were treated partially. With this information, the operational effort was adjusted for the inconsistencies of the first application, resulting in improved coverage in May when all the water sensitive papers had spray droplets and larval mortality was observed in all cup assays. The improved results in May were attributed to the onboard tracking that was used for the application as well as the guidance from the first application. Both applications were made by helicopter, using spray booms. The applicator in April had extensive experience flying and monitored the applications by sight. The applicator in May used a GPS system to track the application in real-time. This tracking of the treatments was shared with the District, which could have contributed to the improved results.

Figure 1.—Left: Cup open to the sky. Right: Cup under vegetation. Both water sensitive papers weree placed in the open.

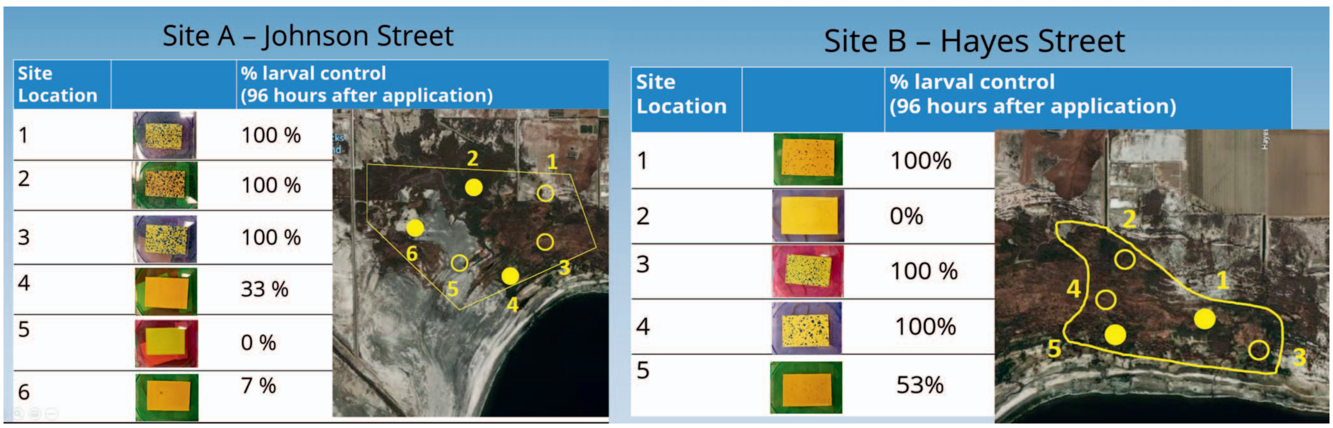

Figure 2.—Results of the April application. The locations on the table represent the placement of the papers and cups on the map. Water sensitive papers were placed in the open. A solid yellow circle indicates the cup was placed under vegetation and a clear yellow circle that the cup was placed in the open.

Figure 3.—Results of the May application. The locations on the table represent the placement of the papers and cups on the map. Water sensitive papers were placed in the open. A solid yellow circle indicates a cup placed under vegetation.

Conclusion

Evaluations of helicopter applications of VectoBac WDG larvicide at the Salton Sea shoreline demonstrated the potential to reduce the number of *Cx. tarsalis,* even in areas with vegetative canopy.

Acknowledgements

The authors thank Arturo Gutierrez for assisting with the weather station, Melissa Snelling for providing *Cx. tarsalis* larvae for the cup assays, and Vincent Valenzuela for assisting during the application process.

Evaluation of all terrain mounted ULV fogger for West Nile virus response

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Introduction

The Sacramento-Yolo Mosquito and Vector Control District (Sac-Yolo MVCD) routinely utilizes four-wheel all-terrain vehicles (ATV) to apply ultra-low volume adult mosquito control products in urban and suburban areas including parks, green belts and golf courses. However, previous efficacy trials performed by Sac-Yolo MVCD have focused on truck-mounted applications. Therefore, the goal of our study was to evaluate the effective swath width of two different products when applied by ATV in both open field and obstructed environments. The two products evaluated were DeltaGard (Bayer Environmental Science, Cary, NC) and Fyfanon EW (FMC Corporation, Philadelphia, PA).

Methods

Applications were made using Guardian 55es ultra low volume (ULV) sprayers (Adapco, Lake Mary, FL) mounted on a Polaris Sportsman 500 four-wheel ATV. Separate sprayers were set up for each product tested, and spray units were exchanged as necessary to allow for scheduled applications. A quick-release system was used that allowed for the easy exchange of spray gear on the ATVs. The spray unit for Fyfanon EW unit was calibrated for an application output of 1.5 fl oz per acre driven at a speed of 4.5 mph with a target swath width of 300 ft. The DeltaGard sprayer was calibrated for an application output of 0.67 fl oz per acre driven at a speed of 6 mph also with a target swath width of 300 ft. Both products were applied at "mid label" rates.

The open field assessment was performed on private property in Wilton, California. Weather monitoring units (Kestrel Weather Instruments USA; Boothwyn, PA) were placed at 5 and 30 ft above ground level, and were used to monitor wind speed and temperatures. Applications were performed at or after sundown and were delayed until there was a thermal inversion of at least 0.5° F (30 ft temp > 5 ft temp), and a wind speed greater than 1 mph. Two replicate parallel 300 ft transects were set with tripods stationed at 50, 100, 200 and 300 ft. Transects were set perpendicular to the spray path, in alignment with the wind direction. There was 500 ft between each transects to eliminate overspray. Each tripod held two sentinel cages (Townzen and Natvig 1973) containing 20 adult females. Two colony populations were used: CQ1 (*Culex quinquefasciatus*) pesticide-susceptible and WCP (Woodland *Culex pipiens*) resistant to pyrethrins and pyrethroids. A second tripod was set up next to each set of sentinel cages that held a droplet impinger (Leading Edge Technologies, Waynesville, NC) with two Teflon-coated slides for droplet deposition. Negative control sentinel cages and a droplet impinger were set approximately 700 ft upwind of the spray path. Fyfanon EW was applied first by driving the ATV perpendicular to the transect line upwind 50 ft from the first station using the ATV speedometer to maintain a 4.5 mph speed during application. After the Fyfanon EW application the unit containing Fyfanon EW was removed from the ATV and replaced with the sprayer containing DeltaGard for the second application. Cages and slides were reset and DeltaGard was applied approximately 1 hr after the Fyfanon EW application using the ATV speedometer to maintain a 6 mph speed during application. Approximately 30 min after each application cages and slides were collected and a 30 min knockdown count was performed in the field. Individual sentinel cages then were stored in zip-top bags, placed into a cooler, and brought back to the District for a 12 hr knockdown count the following day. Slides were read the next morning using Dropvision Basic (Leading Edge Technologies, Waynesville, NC) to obtain information about droplet size and density.

The second assessment was performed on a public golf course in Elk Grove, California. Weather monitoring units were again placed at 5 and 30 ft above ground level to monitor wind speed and temperatures. Eight individual sites were selected for sentinel cages and droplet impinger placement downwind from the ATV spray path. Sentinel cages and impingers were set as described above. The sites ranged in distance from 50 ft to 235 ft from the ATV spray path and were selected opportunistically behind heavy to medium vegetation cover or in open areas with little to no vegetation obstructing the application drift. Control sentinel cages and an impinger with slides were set approximately 450ft upwind of the ATV spray path. Fyfanon EW was applied first followed by DeltaGard. The same protocol used in the open field assessment was followed for counting and collection of the sentinel cages and slides.

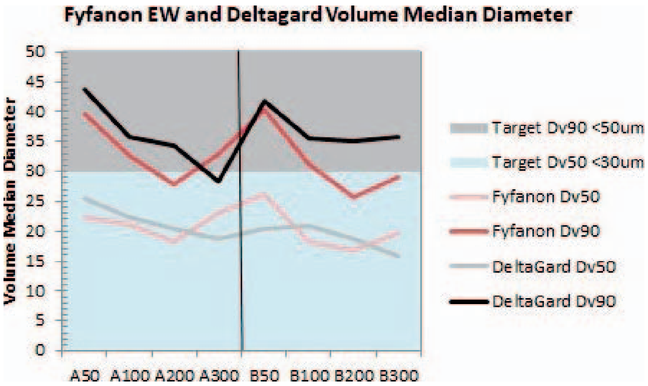

Figure 1.—Volume Median Diameter (um) observed in the open field trial; mosquito mortality at 12 h was 100% across all locations and populations.

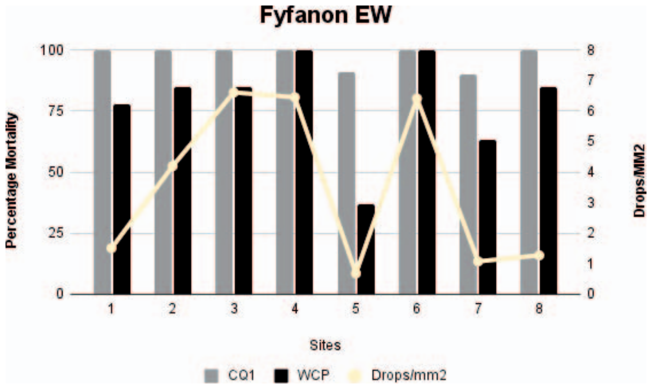

Figure 2.—Golf Course Assessment of Fyfanon EW. Percent mortality at 12 h post treatment for each population and droplet density per mm^2 were shown for each station

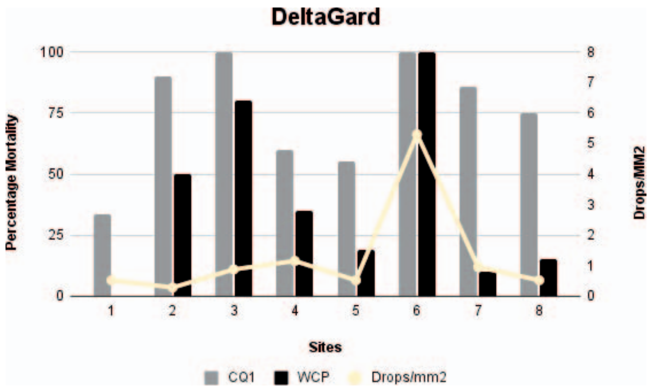

Figure 3.—Golf Course Assessment of DeltaGard. Percent mortality at 12 h post treatment for each population and droplet density per mm^2 were shown at each station.

Results and Discussion

In the open field assessment both Fyfanon EW and DeltaGard achieved 100% mortality at 12 h post treatment in all cages out to 300ft against both CQ1 and WCP populations. Two different Guardian ES55 units applying different materials produced an effective swath width up to 300ft and droplets within label specifications and acceptable density to achieve 100% mortality against two test populations in the open field assessment (Fig. 1).

The Golf Course assessment used Fyfanon EW for the first application and yielded 75 - 100% mortality against the CQ1 population at all stations and against the WCP population at six of the eight stations (Figure 2). The two stations that had mortality <75% mortality were placed within heavy vegetation. The second application at the golf course used DeltaGard. It should be noted that the weather conditions changed markedly at the start of application, with a loss of inversion and swirling winds interrupting the spray path, and diminished droplet deposition and mortality in sentinel cages (Fig. 3).

Conclusions

These assessments of the ATV mounted Guardian 55es ULV sprayer for adult mosquito control demonstrated that an effective swath width up to 300 ft was reliable and achieved, even with dense vegetation. These assessments also demonstrated that the spray parameters tested for both products resulted in label specified droplet spectra with some capacity for penetrating dense vegetation. The key finding was that the ATV sprayers were functionally comparable to truck-based applications, with the added advantages of a compact and highly maneuverable delivery vehicle.

Acknowledgements

We would like to thank the staff at Sacramento-Yolo Mosquito and Vector Control with their help in performing these trials, and Adapco for their continued support in these evaluations.

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Examining product rates for *Aedes* control

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Introduction

Since *Aedes aegypti* was first detected in the Coachella Valley in 2016, the Coachella Valley Mosquito and Vector Control District (District) has conducted a variety of large area larvicide applications to control mosquitoes on residential properties (Henke et al. 2019, 2021). A review of work completed in 2020 highlighted factors used for planning applications in 2021. Namely, the team preferred using VectoBac WDG to other products currently available (having experienced fewer issues with application equipment) and using less pesticide had resulted in acceptable reductions in adult mosquito collections. The 2020 applications found 0.5 and 0.35 lbs of VectoBac WDG per acre within the La Quinta community to provide comparable control (Henke et al. 2021). In 2021, we determined whether applications of VectoBac WDG at 0.25 lbs. per acre would produce control comparable to 0.35 lbs. per acre. Reducing the amount of pesticide used in a neighborhood would lead to a large financial savings to the District, provided that treatments were effective at reducing the mosquito population.

Methods

The District conducted area-wide larvicide applications by truck to reduce the *Aedes aegypti* populations in two cities. Applications were made using an A1 Super Duty; ground applications of VectoBac WDG were made at 0.35 lbs. per acre in Cathedral City and 0.25 lbs. per acre in the city of Coachella. Applications were made weekly in July (weeks 27, 28, 29, and 30) and biweekly in August (weeks 32 and 34).

Efficacy of applications were examined by two methods: 1) Two dry cups were placed upwind in empty lots: one in the open and one with partial coverage from weeds or overhanging bushes. Cups were placed on Friday before the Saturday application and picked up on the Monday following the application. Cups were transported to the laboratory where approximately 100 mL of water was added with 15 *Culex quinquefasciatus* larvae (susceptible CQ1 strain). Mortality was reported at 96 hours after the larvae were added to the cups. 2) Because a reduction in the adult mosquito population was the goal of these applications, BG-Sentinel traps were set weekly with dry ice and BG Lures. Adult mosquito collections in the application areas were compared with collections from other areas where mosquito control efforts continued but no area-wide larviciding was conducted in 2021.

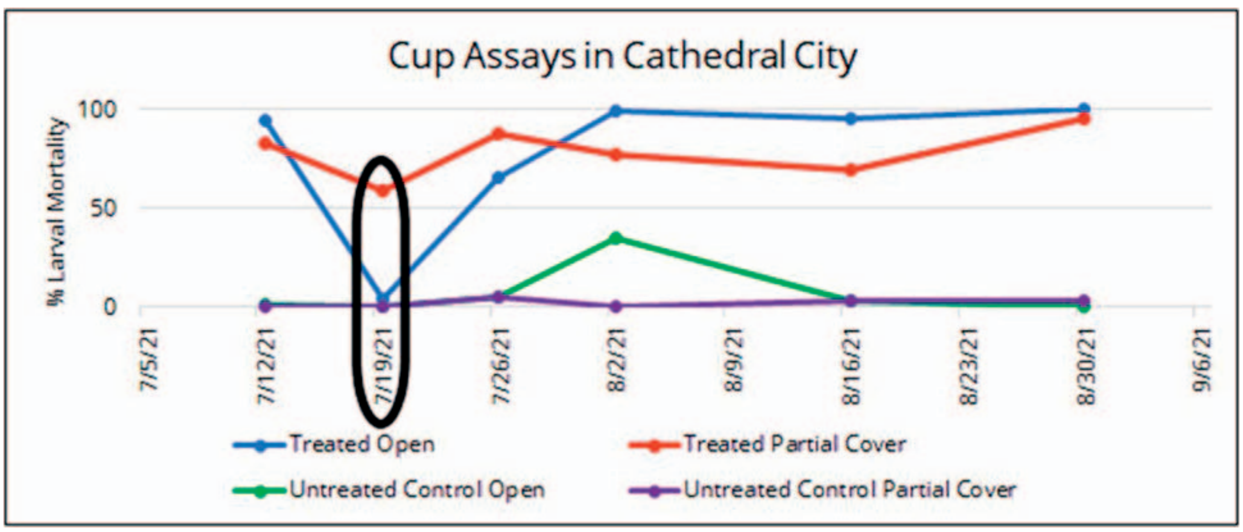

Figure. 1.—Results of weekly cup assays in Cathedral City (0.25 lbs. per acre). The application in the second week had winds of 0 mph, impacting larvicide dispersal.

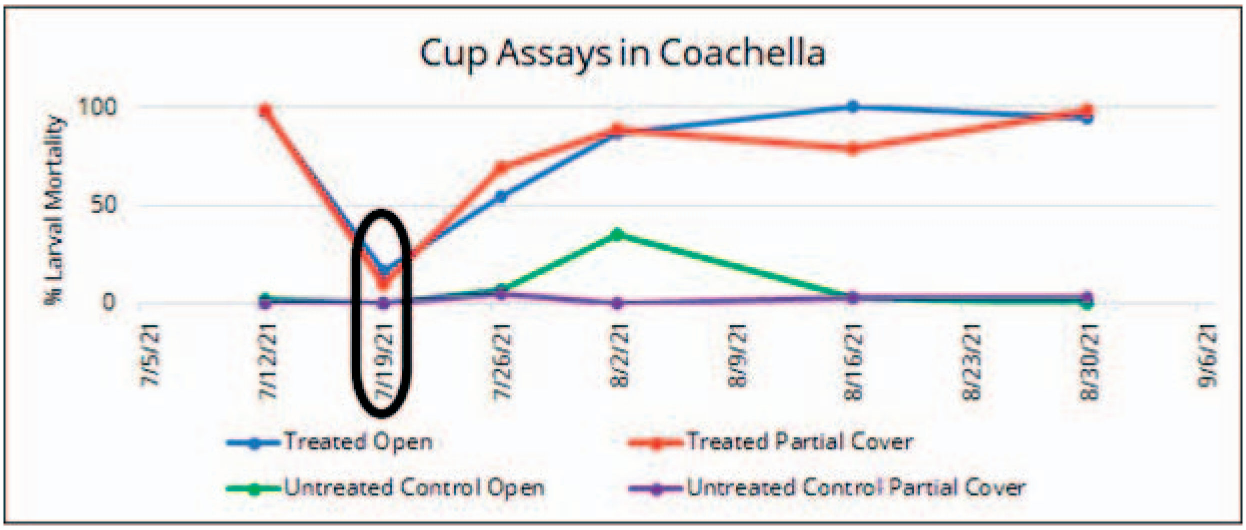

Figure. 2.—Results of weekly cup assays in Coachella (0.35 lbs. per acre). The application the second week had winds of 0 mph, impacting the larvicide dispersal.

Results

The efficacy of both applications in the cups was high, with the exception of the second week in which wind speed dropped to zero during application (circled black, Fig. 1 and 2).

Both the Cathedral City (0.35 lbs. per acre) and Coachella (0.25 lbs. per acre) routes had similar decreases in the number of adults collected per trap (Fig. 3). There was a rebound in the collections in Coachella (lower rate) when compared with Cathedral City (higher rate).

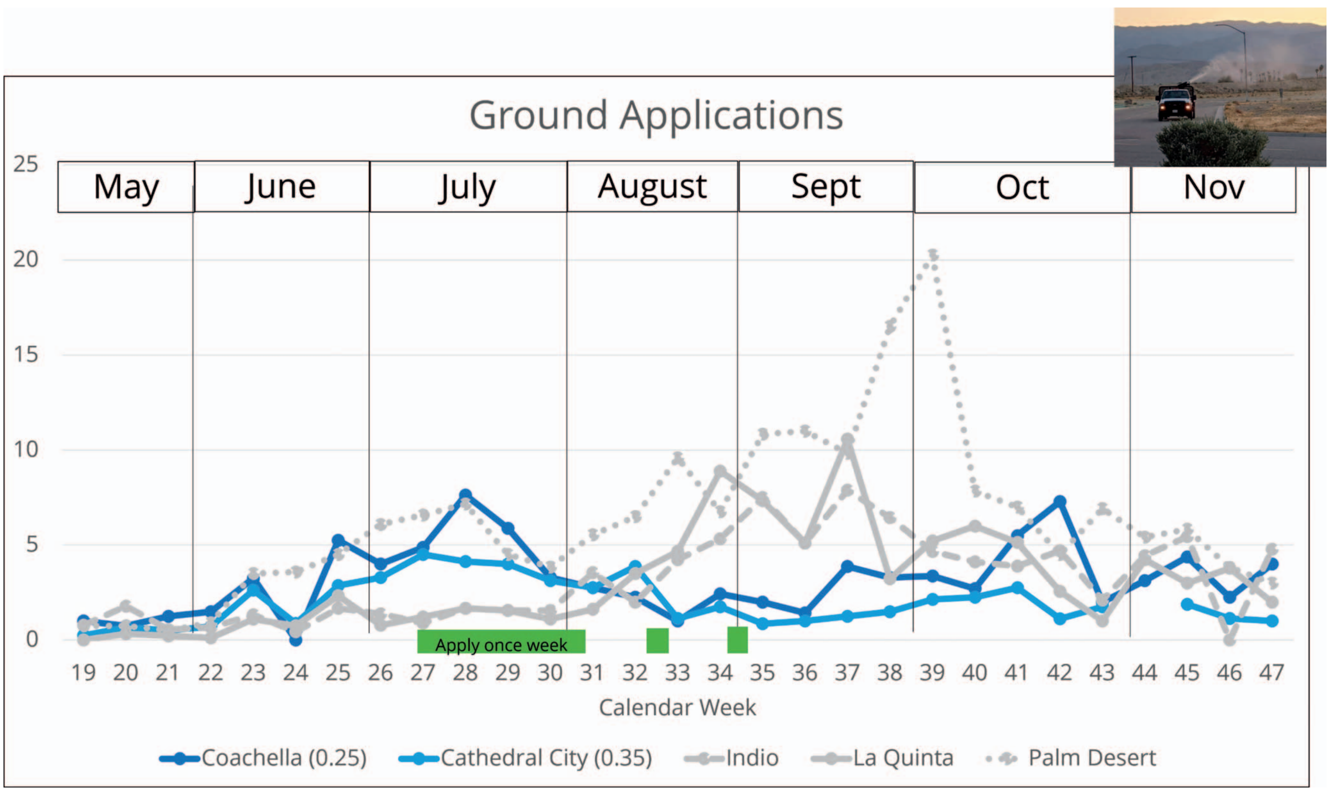

Figure. 3.—Average number of female *Aedes aegypti* per BG-Sentinel trap per day. The green boxes are the approximate times of applications, at the end of weeks 27, 28, 29, 30, 32, and 34. Gray lines indicated cities were a comparable number of traps were used to cover a similar size area as the two application areas.

Discussion

The District has continued to use the cup assay to examine whether applications are reaching their intended sources. Because the application is a larvicide, the abundance of the adult population was expected to decrease 2-3 weeks after the application. Using cups to show that the pesticide was applied correctly and reached protected parts of residential properties allowed us to highlight successful applications and, if not, make changes during a set of treatments.

Adult abundance decreased following applications at both rates, similar to applications in La Quinta in 2020. Possibly, the higher rate slightly improved reduction of the population and may have persisted longer, delaying repopulation after the applications ended. Future work should consider if adding an application at the lower rate (making it 7 applications) would be better, or if 6 applications at the slightly higher rate is more efficient and effective.

Acknowledgements

This work is not possible without the dedicated team of individuals at the District who ensure that the applications are made. We are grateful for the technicians who made these applications as well as the Call Center team who answered questions about the work being done. Further, we thank Rick Ortiz, Juan Carlos Herrera, and Tony Molina for ensuring the equipment was calibrated and operating effectively.

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Mr. Mister: Rockin' the *Aedes* of the San Francisco Bay salt marshes

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Abstract

Turbine mist sprayers (TMS) are being rapidly adopted nationwide for applying larvicides to control peridomestic *Aedes aegypti*. Because TMS can loft large quantities of larvicide over relatively long distances, we examined its efficacy in a tidal marsh habitat for controlling *Aedes dorsalis*. Liquid Vectobac 12AS larvicide was applied at 1 pt / ac using a TMS with the output nozzle directed 5 – 15 degrees below horizontal at a ground speed of 10 mph from a levy that was elevated 3 – 6 ft above the tidal marsh where surface wind speed was 10.7 mph. Cards that change color when exposed to liquid were placed perpendicular to the path of the TMS showed that the larvicide mist traveled up to 60 yd from the TMS and did not extend to 90 yd. Use of the TMS enabled a 4-fold increase in total acres treated during 2020 – 2021 relative to the prior 2 yrs (3777 and 922 ac, respectively) without a concomitant increase in staff time. There was no difference in the number of high tide events that may have affected the number of *Ae. dorsalis* egg hatches. Notably, there was an 83% reduction in the quantity of *Ae. dorsalis* larvae at 5 d post treatment. Similarly, there was a 63% reduction in adult female *Ae. dorsalis* that were collected in EVS traps from nearby communities relative to the prior 2 yrs. There were 2.3-fold fewer requests for service to address a mosquito problem from residents of cites that abut the tidal marshes, suggesting the applications had a positive impact on these communities. TMS offer an attractive alternative to hand treatments in tidal marshes where the use of unmanned aircraft or all-terrain vehicles is prohibited by national wildlife refuge managers.

INTRODUCTION

The San Francisco Bay is home to 7.8 million people, with over 20 % residing in Alameda County (2020 US Census). The highest population densities occur in communities that abut the San Francisco Bay (https://statisticalatlas.com/county/California/Alameda-County/Population) where marsh habitats provide wildlife habitat, recreation for people, and mosquito production in the absence of control measures. The flight radius of several mosquito species from salt and bulrush marshes extends tens of miles, putting people living in the most populace regions of Alameda County at risk for bites and arbovirus infection. *Aedes dorsalis*, the summer salt marsh mosquito, reproduces exclusively in salt marshes and may contribute to the transmission of Jamestown Canyon virus in coastal California (Fulhorst et al. 1996). However, it is a particularly aggressive day-time and crepuscular biter that may fly upwards of 5 miles in search of a blood meal (Verdonschot and Besse-Lototskaya 2014) before returning the salt marsh to oviposit on *Salicornia pacifica* (*i.e.,* pickleweed). An exceptionally high tide (*i.e.,* king tide) triggers the eggs to hatch, resulting in upwards of a 16-fold increase in the abundance of *Ae. dorsalis* larvae (Kramer et al. 1995). While much of the land that rings the San Francisco Bay has environmental conditions that are suitable for pickleweed, much of the habitat has been degraded or lost due to the construction of grey infrastructure such as roads and rooftops. Approximately 10,000 acres of salt marsh remain around the San Francisco Bay. Water circulation channels were placed in salt marshes during the early 1900s to promote the outflow of tidal waters from the marsh and limit mosquito production. Land subsidence can lead to depressions where tidal water can accumulate to creating areas for mosquito production. Environmental regulations limit the extension of water circulation channels, leaving the application of insecticides as the sole alternative if salt marsh mosquitoes are to be controlled after an exceptionally high tide. The application of insecticides that target the adult mosquito stage (*i.e.,* adulticide) over water is limited by some state regulations and may not be effective if the adult mosquitoes have already dispersed from the salt marsh in search of a blood meal. Instead, insecticides that target the larval stage (*i.e.,* larvicide) are typically applied by mosquito control agencies shortly before or after an exceptionally high tide by foot, amphibious vehicle or aircraft. Although the latter can cover hundreds of acres in a day, the cost of purchasing and maintaining a manned aircraft or hiring one as often as needed is greater than what many mosquito control agencies can support. Truck-mounted turbine mist sprayers (TMS) that can loft a large quantity of larvicide several hundreds of feet have recently been adopted by mosquito control agencies to control non-native mosquitoes such as *Aedes aegypti* that reproduce residential back yards. We sought to determine if a TMS could be repurposed from our

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urban mosquito control program for use in a salt marsh after an exceptionally high tide to control *Ae. dorsalis*.

MATERIALS AND METHODS

Study site

The study was conducted at a salt marsh in Fremont, CA (USA; Figure 1A; GPS coordinates: 37.497395, -122.010363) that is bounded by a hard packed levee and inundated with seawater from the San Francisco Bay via Coyote Creek. The frequency and magnitude of tides were determined by measurements provided by the National Oceanic and Atmospheric Administration at that San Francisco Gate (https://tidesandcurrents.noaa.gov/).

Larvicide application

A Super Duty TMS (A1 Mist Sprayers; Ponca, New England USA) that was mounted to the bed of a Ford F150 truck was used throughout the study. The TMS was equipped with the Micronair AU5000 atomizer (Micron Sprayers Limited, United Kingdom). Calibrations were set with an operating bypass pressure of 50 psi, engine speed at full throttle (3500 RPM) yielding a flow rate of 1.5 gal per min. Any adjustments to the throttle or bypass pressure directly affected the flow rate. Wind speed and direction was measured using a Kestrel 550 Pocket Weather Meter (Boothwyn, PA USA). Vectobac 12AS larvicide (Valent BioSciences, Libertyville, IL USA) that was mixed with red dye FD&C Red #40 (Novact Corporation, Miami FL USA) was applied at 1160 ml / hectare (1 pint / acre) using a TMS with the output nozzle directed 5 – 15 degrees below horizontal at a ground speed of 24 kph (15 mph) from a levy that was elevated approximately 1.5 m (5 ft.) above the tidal marsh where surface wind speed was 17.2 kph (10.7 mph). The TMS wind that propels the Vectobac 12AS mist was 158 kph (98.3 mph) at the mister output atomizer.

Larvicide droplet assessments

Water-sensitive spray cards (TeeJet, Springfield, IL USA) that change color from yellow to blue when exposed to liquid or white 7.6 × 12.7 cm (3 × 5 inch) index cards that showed the red dyed droplets were affixed to wooden stakes and placed perpendicular to the path of the mister 0.5 m (1.5 ft.) above the pickleweed canopy to capture the larvicide mist. The water-sensitive and white cards were placed next to each other at distances of 3, 7, 15, 30, 60 and 90 m (10 – 300 feet) from the levee edge at 4 sites along the drive path of the TMS, with each group separated by approximately 0.6 km (0.4 mi.) from one another. The cards were collected within 15 min of the 12AS application and each placed in a CD jewel case to prevent additional moisture to contacting the card or loss of the red-dyed Vectobac 12AS from the card. Cards were imaged using a Nikon D700 DSLR camera that was fitted with a Nikon AF-S Micro Nikkor 105mm 1:2.8G lens (Tokyo, Japan), and the droplet characteristics analyzed using ImageJ software (Schneider et al. 2012). The droplet size was calibrated in the ImageJ software by placing a microruler scale bar (https://www.eeob.iastate.edu/faculty/DrewesC/htdocs/microruler-links.htm) on each card prior to imaging (Figure 1B).

Mosquito abundance assessments

A dipper (BioQuip Products, Rancho Dominquez, CA USA) was used to collect mosquito larvae 1 – 3 days prior and 2 – 5 days after larvicide applications, as described previously (Hagstrum 1971). Encephalitis virus survey (EVS) traps (BioQuip Products, Rancho Dominquez, CA USA) were baited with dry ice and a BG-Lure (Biogents, Regensburg, Germany), and placed over night at sites within and adjacent to the study area to collect adult mosquitoes (N = 285 EVS traps during 2018 – 2019; N = 297 EVS traps during 2020 – 2021). Mosquitoes were enumerated and identified to species using a dissection microscope.

Service requests

Public perception of mosquito abundance was assessed by measuring the number of calls for biting mosquitoes from people that lived within 4 km (2.5 mi.) of the study area during the two years prior to and after the TMS was used for mosquito control.

RESULTS

The Vectobac 12AS mist traveled up to 60 m from the mister, but did not extend substantially beyond 30 m (Figure 1C; 1 m is approximately 1 yard). Droplet density was greatest 7 m from the mister. Although a greater droplet density was observed using the water sensitive cards compared to those captured by the red dye droplets, the difference was not significant (27.9 and 16.0 droplets / cm^2, respectively; paired t-test, P = 0.1514; Figure 1C). Average droplet diameter measurements were largest on the red dye cards at 15 m from the mister (123.8 microns (μm)), and at 30 m for the water sensitive cards (118.1 μm; Figure 1D). The larger droplet diameter on the red dye cards may have resulted from the red-dyed Vectobac 12AS becoming flattened when the cards were collected. Many vector control laboratories report mean droplet diameter as mean droplet volume (DV 0.5). However, it may be of greater value to report the distribution of droplet area using a violin plot (Figure 1E). The calculated volume of Vectobac 12AS that was applied was measured at 3 – 60 m from the mister using the radius of droplets on red dye cards and the formula of a sphere (volume = $(4/3) * (\text{radius})^3 * \pi$). When the calculated volume was converted to units indicated on the Vectobac 12AS label (pints / acre), we found that the application rate was optimal at 7 – 15 m from the mister (1.1 ± 0.4 pints / acre; Figure 1F). Use of the TMS enabled a 3-fold increase in total acres treated during 2020 – 2021 relative to the prior 2 years (Figure 2A; unpaired t-test, P <0.0001 without a concomitant increase in staff time. Notably, there was an 84% reduction in the quantity of *Ae. dorsalis* larvae at 5 days post treatment

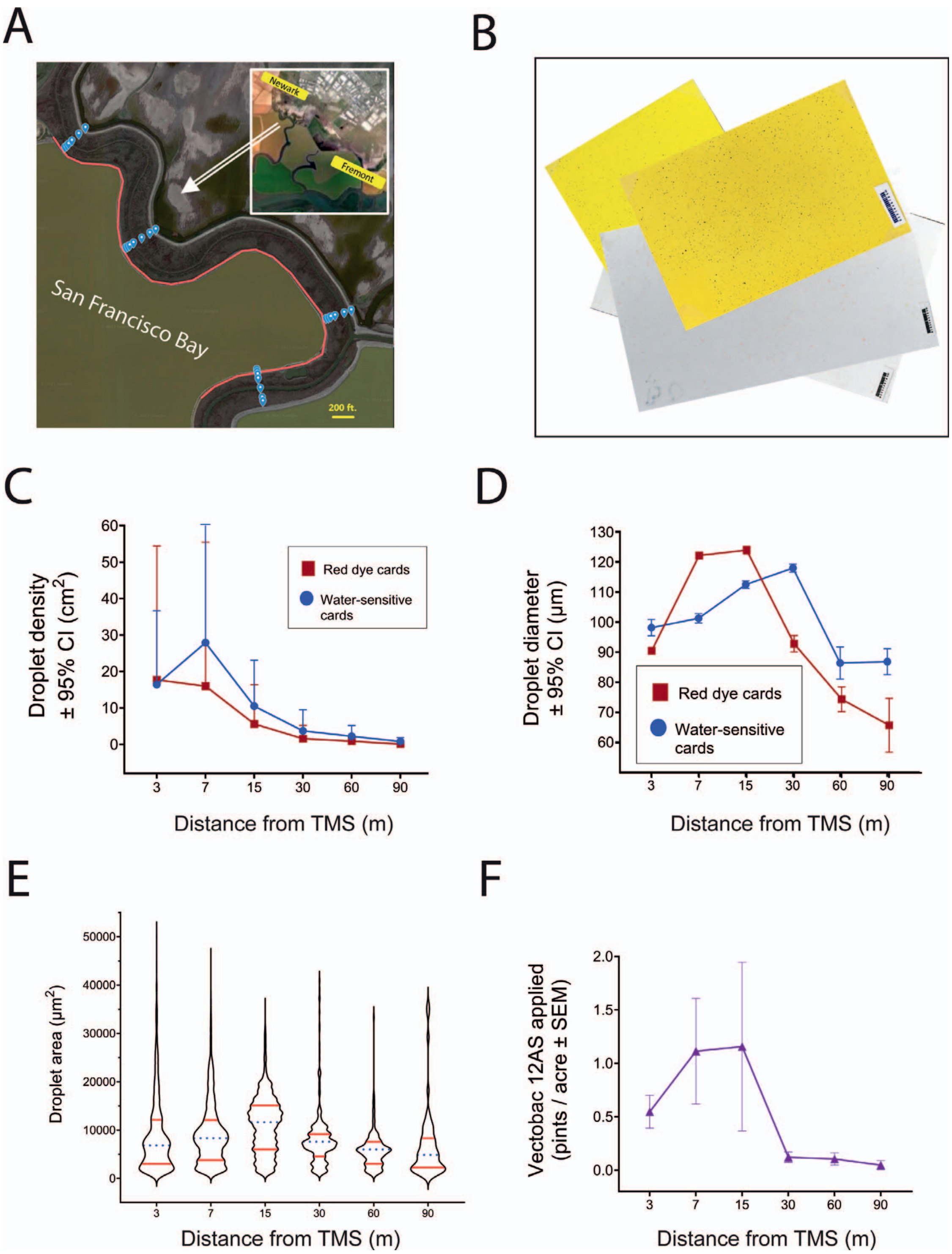

Figure 1.—Analysis of Vectobac 12AS droplets from a TMS used in a saltmarsh that abuts the San Francisco Bay. (A) Study site with the drive path of the TMS indicated by a red line and blue markers showing the location of spray cards. (B) Water-sensitive spray cards showing blue Vectobac 12AS droplets and white spray cards showing red-dyed Vectobac 12 AS. Scale bars used to calibrate the ImageJ software. (C) Droplet density, (D) diameter, (E) area and calculate volume of Vectobac 12AS at 3, 7, 15l 30, 60 and 90 m from the mister.

(Figure 2B). Similarly, there was a 63% reduction in adult female *Ae. dorsalis* that were collected in EVS traps from nearby communities relative to the prior 2 years (Figure 2C). There was no significant difference in the number or height of high tide events that may have affected the number of *Ae. dorsalis* egg hatches (Figure 2D; unpaired t-test, $P = 0.4022$). There were 2.3-fold fewer service requests to address a mosquito problem from residents of cites that abut the tidal marshes during the study period relative to the two prior years (Figure 2E), suggesting the

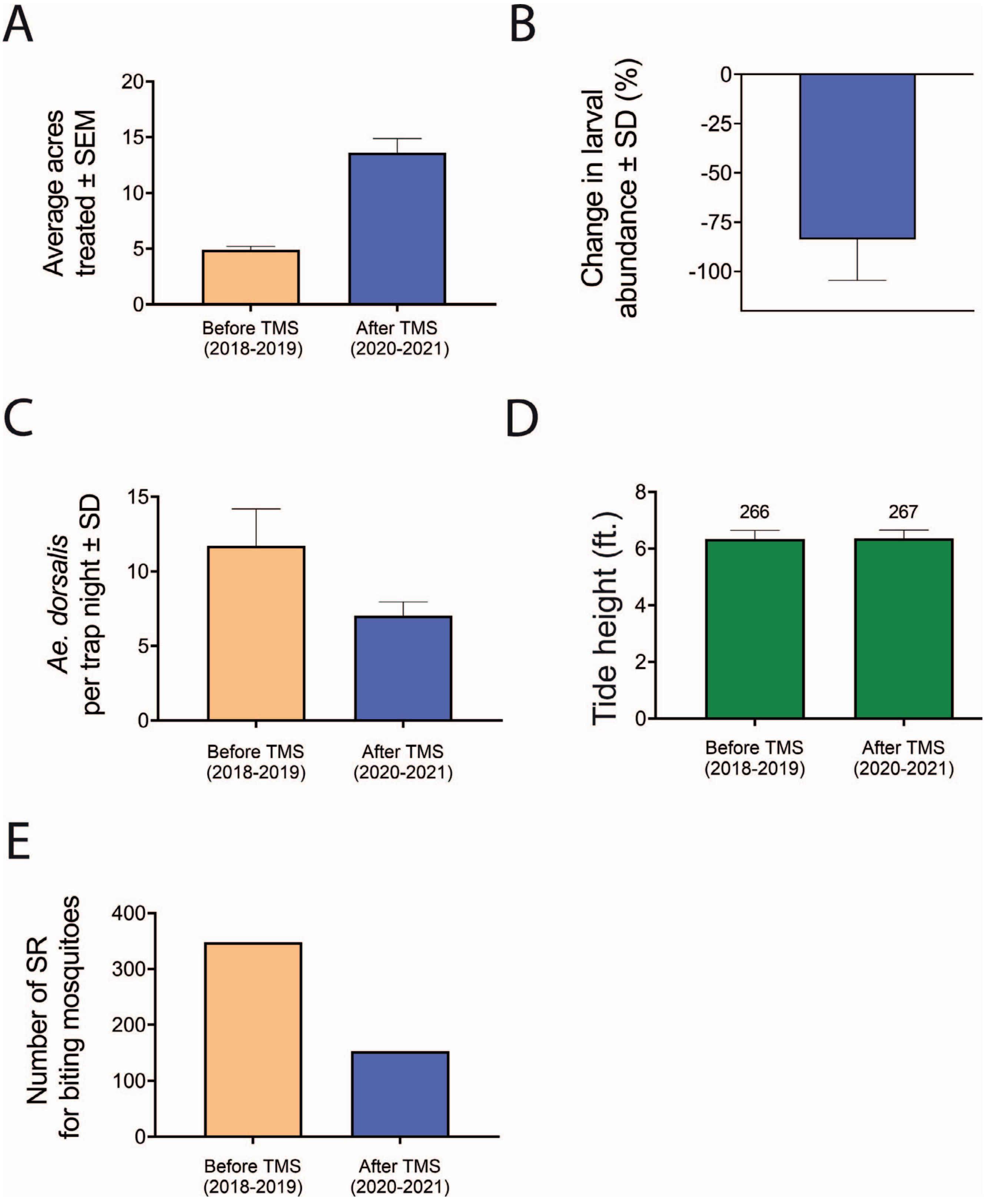

Figure 2.—Outcomes of larvicide applications using a TMS in a saltmarsh. (A) Acres of saltmarsh treated before and after a TMS was used to apply VectoBac 12AS. (B) Change in larval abundance measured using a dipper before and after Vectobac 12AS was applied to the saltmarsh. (C) Average number of Ae. dorsalis collected in EVS traps before and after TMS was used to apply larvicide in the study area. (D) Average tide height and number of high tide events at the study area. (E) Number of service requests (SR) from residents around the study area before and after the TMS was used to apply larvicide.

applications had a positive impact on these communities. Turbine misters offer an attractive alternative to hand treatments in tidal marshes where the use of unmanned aircraft or all-terrain vehicles is prohibited by national wildlife refuge managers.

DISCUSSION

Our applications of the A1 Super Duty Mist Sprayer were conducted without adjustments to the equipment's original design. The direction and angle of the nozzle output was the only modification, and this was a technical application adjustment, not mechanical. Awareness of wind speed and direction were key components of a successful application. These directly influenced the nozzle angle and vehicle speed during application. Although we were pleased with these results, there are several mechanical modifications that may increase application efficacy. Adjustments to the Micronair AU5000 blade pitch can alter the droplet size and density. Removing the Micronair AU5000 and reverting to customary flat or conical nozzles may provide a better spray pattern for our specific target area. The angle of the TMS can be adjusted while driving the truck to optimize the direction of the mist toward water that supports mosquito production and is of particular importance as wind direction changes in relationship to the direction that the TMS truck is driven. This is best achieved if a second truck follows the one with the TMS and communicates via radio or cell phone the optimal angle of the TMS.

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Pathogen detection in *Ixodes pacificus* in California, 2019-2021

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Abstract

The western blacklegged tick (*Ixodes pacificus*) transmits the human pathogens *Anaplasma phagocytophilum, Borrelia burgdorferi* s.l., and *Borrelia miyamotoi* in California. The California Department of Public Health's Vector-Borne Disease Section performs public health surveillance for these pathogens, both as routine surveillance and in response to human cases of disease. This presentation summarizes three years of western blacklegged tick surveillance and pathogen testing and the results of genetic sequencing of the *Borrelia burgdorferi* s.l. species found in California *Ixodes pacificus*.

Five years of surveillance for tularemia serovar B (*Francisella tularensis holarctica*) including two human cases at an endemic site in San Mateo County, California

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Introduction

Tularemia is a highly infectious and virulent disease caused by the intracellular bacterium *Francisella tularensis*. An average of 3.4 cases per year have been reported from California. Two pediatric cases of tularemia were reported from coastal southwestern San Mateo County, CA; one in June of 2004 and one in April of 2006. In response, tick surveillance was conducted by the San Mateo County Mosquito and Vector Control District. In 2004, 103 *Dermacentor spp*. ticks were collected, but tested negative. In 2006, 95 *D. occidentalis* and 199 *D. variabilis* were collected (there was one positive pool of each; overall minimum infection prevalence (MIP) = 0.68%). In 2007, 128 *D. occidentalis* and 102 *D. variabilis* were collected (there were five positive pools of *D. occidentalis* and three of *D. variabilis*; overall MIP = 3.5%).

Methods

A five-year inter-epizootic surveillance program for this region was initiated in 2017. *Dermacentor occidentalis, D. variabilis,* and *Haemaphysalis leporispalustris* were collected from the trail edge by flagging, separated by species, sex, date collected and trail section (east vs. west of Highway 1), and pooled. Tick pools were processed using a tissue homogenizer (OMNI International), extracted using MagMax Viral RNA Isolation Kit (2017-2019) or the MagMax CORE Kit (2020-2021) (Applied Biosystems), and screened via real-time PCR using primers and probe for the tul4 region (Versage et.al. 2003). Rodent surveillance was conducted in June of 2019 and blood was collected for serological testing by the California Department of Public Health. All statistical analyses were performed using R (R-statistical Software).

Results

A total of 3,021 *D. occidentalis* and 1,019 *D. variabilis* were collected from 2017 - 2021. Of those, 25 pools positive for *F. tularensis* were detected. Positive pools of ticks were detected every year except for 2020, when collecting was restricted due to the COVID-19 pandemic. Percent of pools positive by species were 7.12 (4.42-10.88) for *D. occidentalis* (MIP 0.73%) and 2.09 (0.37-6.82) for *D. variabilis* (MIP 0.29%). We did not detect any positive *H. leporispalustris* pools (n=36). Eighty-eight percent of all positive pools were collected from the western half of the site, nearest to the ocean ($z=2.68$; $p=0.007$). Location and species were significant factors for the probability of detecting a positive pool. There was no significant effect of season. Samples from twenty-four small mammals were tested serologically for prior *F. tularensis* infection, but none were positive. Finally, two human cases were reported during the study period: one in 2019 and one in 2021.

Conclusions

Tularemia clearly is endemic and active in this region of San Mateo County, but the extent of its range and its ecology are not well understood.

Acknowledgements

We would like to thank Mary Joyce Packingan and Melissa Yoshimisu for serotyping and providing CDPH support for this project; Warren McDonald, Elizabeth Flores, Ryan Thorndike, Maritza Leon, and Alex Flores for field support and tick collection; and the staff of the SMCMVCD including Theresa Shelton, Chindi Peavey and Brian Weber.

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The trials and tribulations of an itch mite investigation

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Introduction

In September, 2020, the San Mateo County Mosquito and Vector Control District (District) received a report that groundskeepers and a few students at a local school had experienced a number of what appeared to be insect or arthropod bites following seasonal landscape maintenance activities. This report recounts the investigation conducted over the next few months by District staff to identify, collect and learn more about the microscopic arthropods causing the bite outbreak, itch mites belonging to the *Pyemotes ventricosus* group.

Materials and Methods

District staff were notified on September 2, 2020, that a custodian mowing the El Granada School athletic field earlier in the week had developed dozens of 'bite-like itchy rashes' around his neck and chest area. Although school activities at the time were being conducted remotely due to the COVID-19 pandemic, a small number of children attending an on-site daycare center also had experienced bites after playing on the field a day after the mowing. A District technician conducted a visual inspection for rodents, fleas and mosquito sources, but had found none. District laboratory staff visited the site on September 3 and 4 to set and retrieve carbon dioxide-baited traps, survey for ticks and fleas using white flannel flags, sample for chiggers on the field and surrounding areas using black plates, and collect additional samples from the field and vegetation surrounding the field area through soil samples and plant clippings. Grass, mulch and soil samples were processed through Berlese-Tullgren funnels for small arthropods.

District laboratory staff returned several times over the ensuing weeks to re-sample the area, primarily using black plastic plates, using a modification of the biting mite collection method described in Clopton and Gold (1993). Black 1/8"-thick PVC plastic sheets were cut into 6" squares. These squares were placed in sampling areas for five-minute periods, before collection into individual zip-top plastic bags for transport back to the District laboratory. Mites were flushed off the plates using 70% isopropyl or ethyl alcohol and counted using dissecting scopes.

Given the rare opportunity to learn more about *Pyemotes* mites occurring on a school athletic field without a student population present on campus, the District laboratory spent a few weeks attempting to learn more about detecting, collecting and controlling these mites.

Research questions addressed during this investigation:

1. The District had two sets of black mite collection plates with different textures: smooth and pebbled. The average number of mites collected per plate were compared from the two different types of black plates to determine if texture influenced catch.
2. Some collection days were windy and others calm. The average number of mites collected per plate for eleven collection events over two months was compared to collection time wind speed at the Half Moon Bay, CA Weather Underground weather station (https://www.wunderground.com), to determine if wind speed affected mite activity.
3. The mite-infested athletic field was comprised of mostly dead, brown grass with scattered patches of green grass or weeds. Average number of mites per plate was compared between collections taken from brown areas versus green areas of the field to determine if plant condition affected mite catch.
4. A 10,000 square-foot area of mite-infested field was treated by Birchmeyer backpack sprayers using the botanical insecticide Essentria IC-3 diluted in water at 4 oz/gallon. Black plate samples were taken from treated and untreated areas of the field before treatment, after treatment, and for the two weeks following.

Statistical analyses were run using online tools from http://www.statskingdom.com.

Results and Discussion

The initial collection of samples taken by District laboratory staff included the following:

- One mosquito (*Culex pipiens*) collected from a carbon-dioxide-baited trap
- No biting arthropods collected from white flannel flags
- Fifteen (15) *Pyemotes* mites collected from grass, soil, and other substrate samples
- Eighty-three (83) *Pyemotes* mites collected from plant clippings
- Five-hundred and forty-one (541) *Pyemotes* mites collected from black plates

Biting mites were identified as belonging to the *Pyemotes ventricosus* group based on morphological

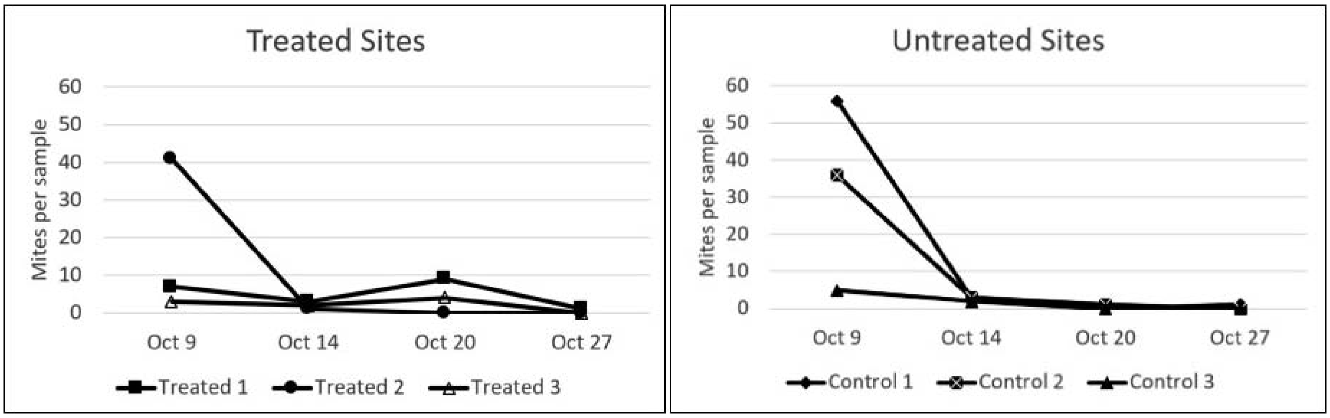

Figure 1.—Average number of *Pyemotes* mites collected per sampling plate by collection date from treated and untreated sites. Treatment with Essentria IC-3 was conducted on October 9, after the sample was taken.

features using keys from Cross and Moser (1975). Samples were sent for confirmation to mite expert Dr. H. Klompen at the Ohio State University. Identification to species was not feasible, as no male mites were collected. Based on these collection results and the biting reports consistent with *Pyemotes* mites, the District concentrated sampling efforts on *Pyemotes* mites in subsequent site visits using the black plate collection method.

Collection numbers of mites were compared using black plates with two different surface textures. A two sample t-test revealed no difference between collections from pebbled ($M = 19.0$, $SD = 55.3$) versus smooth ($M = 20.6$, $SD = 73.2$) textured plates ($p = 0.947$, $t(26) = 0.06$).

Results of the Pearson correlation indicated there was a non-significant, very small negative relationship between the average number of mites collected per plate per day and the wind speed during the sampling period ($r(df=9) = -0.312$, $p = 0.350$).

There was a non-significant medium difference in the average number of mites collected from twelve paired sets of plates deployed within green patches ($M = 50.8$, $SD = 82.4$) and brown patches ($M = 29.9$, $SD = 41.9$) of turf. The p-value of a paired t-test comparing the two sets of collection was $P = 0.148$ ($t(11) = 1.6$).

The average number of mites collected in sites treated and not treated with Essentria decreased during the weeks post-treatment (Figure 1). Due, in part, to low pre-treatment mite numbers in some samples and what may have been a natural, seasonal decline in the mite population as a whole, it was not possible to determine if the insecticide controlled mites in this experiment. Following the third post-treatment mite collection for the Essentria trial on October 27, 2020, no additional live mites were observed or collected from El Granada Elementary School. No reports of mite-like bites were reported from the school in 2021.

Conclusions

Outbreaks of biting *Pyemotes* mites have been documented to occur in conjunction with population explosions of their host arthropods, as recently demonstrated by biting mite reports associated with periodical cicada emergence events (Kritsky 2021). Although the host arthropod was not identified in the current investigation, the self-limited appearance of *Pyemotes* mites at the El Granada school was consistent with this type of event. Although the factors analyzed (sampling surface, wind speed, and turf condition), were not found to be significantly related to mite density, and the efficacy of the Essentria insecticide could not be confirmed, conducting such investigations are worth the effort when opportunities arise. These results add to the limited body of knowledge on how to detect and control these cryptic biting pests.

Acknowledgements

We would like to thank the staff at the El Granada Elementary School for allowing us to conduct this study on their campus. Dr. Hans Klompen at The Ohio State University provided invaluable support with mite identification. We would also like to thank SMCMVCD Operations Supervisor Ryan Thorndike and retired Vector Ecologist Tina Sebay for their assistance on this project.

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Adulticide resistance bottle bioassays for *Culex quinquefasciatus* and *Cx. tarsalis*

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Abstract

The Coachella Valley Mosquito and Vector Control District evaluates the status of adulticide resistance of field-collected *Culex tarsalis* and *Cx. quinquefasciatus* in comparison with susceptible laboratory strains (Bakersfield and CQ1, respectively) on an annual basis. The results of these evaluations determine whether the products can efficiently reduce adult mosquito abundance and disrupt virus transmission. Field-collected and susceptible laboratory strains of *Cx. tarsalis* and *Cx. quinquefasciatus* adults were exposed to a dilution series of the adulticides Aqua-Reslin, Duet, and Scourge 18+54 that were used to coat the inside of bottles. The District also examined resistance of *Cx. tarsalis* against Merus 3.0 and *Cx. quinquefasciatus* against DeltaGard. Results were analyzed using WHO guidelines for mosquito mortality for bottle bioassays. Field-collected *Cx. quinquefasciatus* demonstrated resistance to all the adulticide products assayed, with low knockdown rates (<80%) at diagnostic time and at the end of the assay period. *Cx. tarsalis* demonstrated resistance to Merus 3.0 with knockdown rate of 73% at diagnostic time. There was resistance developing for Aqua-Reslin and Duet with knockdown rates between 82-83%. Cx. tarsalis showed no resistance for Scourge 18+54. Details of these assays may be found in the following accepted manuscripts:

Hung, K.Y., A. Gutierrez, M. Snelling, and J. Tarango. 2022. Insecticide resistance bottle bioassay evaluation of *Culex tarsalis* mosquitoes from Coachella Valley, 2021. Arthropod Management Tests. 10.1093/amt/tsac056

Hung, K.Y., M. Snelling, and J. Tarango. 2022. Insecticide resistance bottle bioassay evaluation of *Culex quinquefasicatus* mosquitoes from Coachella Valley, 2021. Arthropod Management Tests. 10.1093/amt/tsac068

Build-A-Flavivirus

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Introduction

This document provides instructions for building a 12-inch diameter model of a West Nile virus particle using craft materials, for use in public outreach programs. The actual WNV particle has a diameter of about 50 nanometers. By enlarging it 6,096,000 times, something invisible can seem real. When vector control agencies spotlight the science involved with mosquito control and the diseases mosquito control prevents, we communicate that we are more than just pesticide applicators. Other flaviviruses, such as Zika and St. Louis enceaphalitis, are also represented by this model with small differences such as the length of the RNA; however, choosing West Nile virus to model will emphasize to the public that WNV is still of great concern in California. The model can be made with wire, yarn, poster board, a beach ball, and pipe cleaners. A poster explaining the parts of the virus would be displayed with the model. The estimated cost is about $43, and the estimated time to assemble the model is approximately four hours.

Materials

A flavivirus consists of one segment of single-stranded RNA (11 kilobases long), an icosahedral capsid made of 180 copies of the same protein, a lipid envelope derived from host membrane, and 180 copies of each of two surface proteins (Table 1).

Methods

Genetic material

1. Eleven kilobases is about 340 nm, so enlarged 6,906,000 times for a 12inch diameter virus equals about 75 feet! Cut yarn for each gene in the lengths shown in Figure 1. Tie the sections together.
2. Bend wire to form hairpin loops of the untranslated RNA regions as in Figure 1. Bend wire and tie yarn to connect each end.
3. Place a pony bead at the leading end to represent the methylated nucleotide cap if extra detail is desired.

Protein nucleocapsid

1. Follow a tutorial to make a 20-sided icosahedron on YouTube. Six-inch triangle edges were used to make a 12inch diameter virus. https://www.youtube.com/watch?v=mKxW2v6Nd2Q
2. Leave one face unglued for access to the RNA. Apply a hook and loop closure. (See Figure 2)

Lipid envelope

1. Cut a beach ball at the equator, but leave about 6inch connecting the two halves.
2. For a 12inch virus model, cut two 40inch lengths of wire. Position the wire along the edges of the two half-spheres, fold the edge of the ball over the wire, and tape or sew it into place.
3. Apply a hook and loop closure.

Surface proteins M and E

1. Cut 60 red pipe cleaners into thirds and 90 yellow pipe cleaners into halves, so there will be 180 pieces of each.
2. Arrange as in Figures 3 and 5 by poking holes through the beach ball and bending the pipe cleaner wire against the inside ball surface. Use nine pieces of each color per 1/20th of the surface.
3. Bend pipe cleaners down so they lay flat on the surface. Bend into spiral or wavy shapes.

Results and Discussion

This was a good Covid-19 work-from-home project, but Covid-19 unfortunately also madefinding people to hold and explore the model problematic. Hand sanitizer or disposable gloves should accompany the display.

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Table 1.—Materials bought at Michael's, The Dollar Tree, and Amazon.com. Cost was about $43.

COMPONANT	MATERIALS	VENDOR	COST
10 RNA genes	75 ft. of yarn in 2 to 10 colors	Michael's	$5
hairpin loops of RNA untranslated regions (UTR RNA)	18<20 gauge Wire	Dollar Tree	$1
protein capsid	White poster board 20" x 36"	Michael's	$7
capsid	Protractor and wing compass	Dollar Tree	$2
capsid	Super glue	Dollar Tree	$1
capsid and envelope	Hook and loop closure	Michael's	$5
lipid envelope	Beachball, 12 inch diameter (often sold as 20 inch deflated diameter)	Amazon	$7
180 E and 180 M surface proteins	60 red pipe cleaners 90 yellow pipe cleaners	Michael's	$7
envelope	Clear Gorilla Tape	Amazon	$7
envelope	15<19 gauge Wire		$1
capsid	Yardstick Scissors Marker, pencil		
			$43

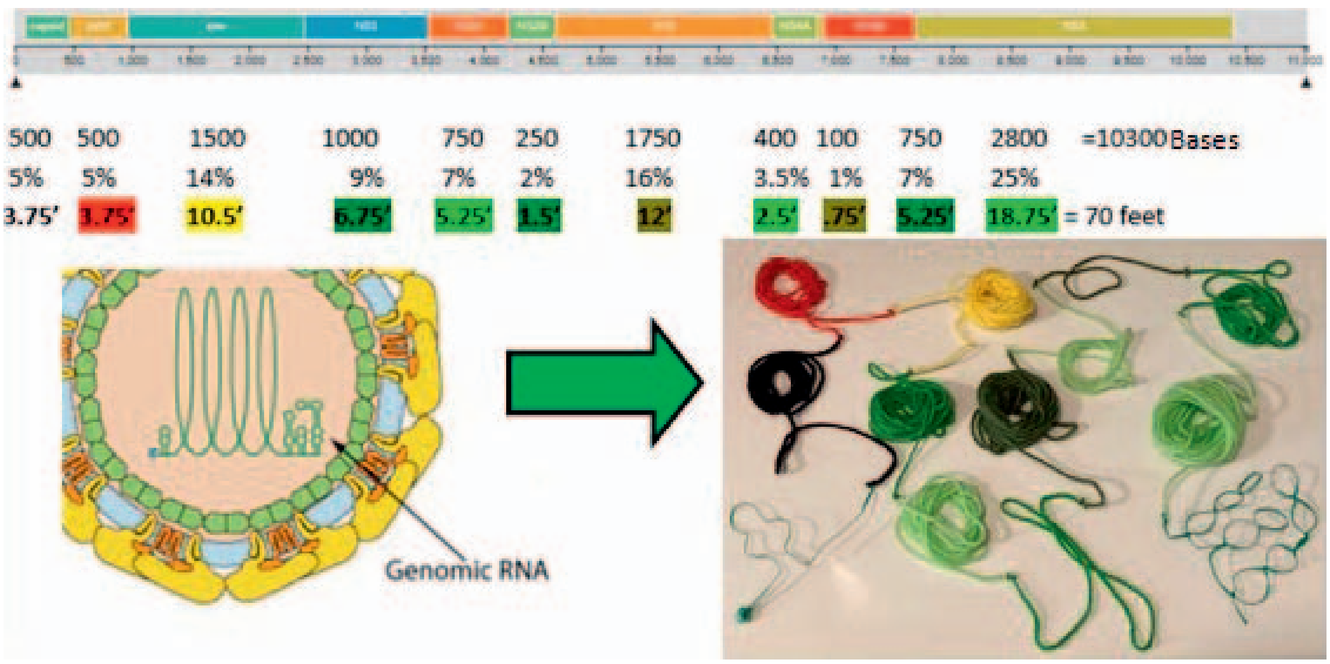

Figure 1.—WNV RNA is about 11,000 bases long. Different colors and proportional lengths of yarn are tied together to represent the ten genes. Wire bent into loops represents the non-coding regions at each end of the RNA.

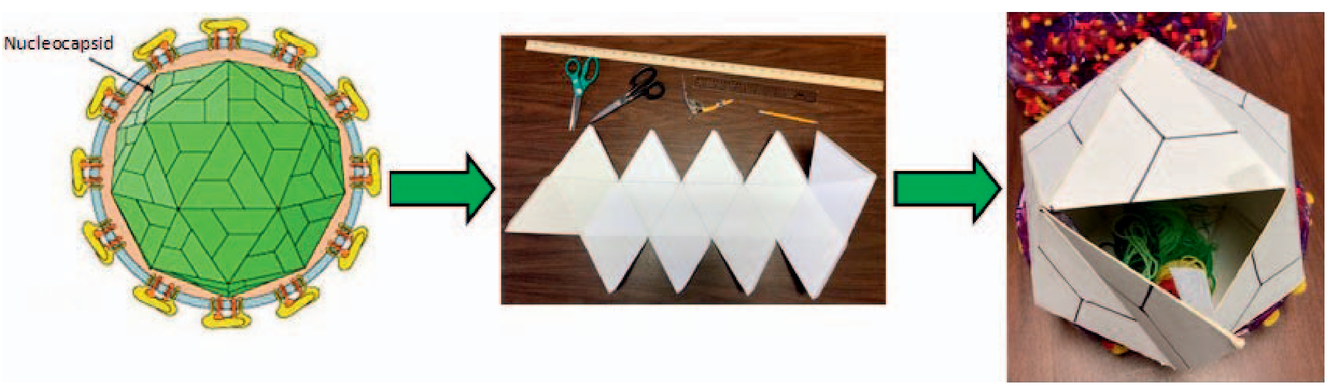

Figure 2.—A cardboard icosahedron represents the protein capsid that contains the RNA.

Figure 3.—The E surface protein (yellow) is larger and more prominent than the M surface protein (red).

Figure 4.—The lipid envelope (beach ball) surrounds the WNV nucleocapsid (cardboard).

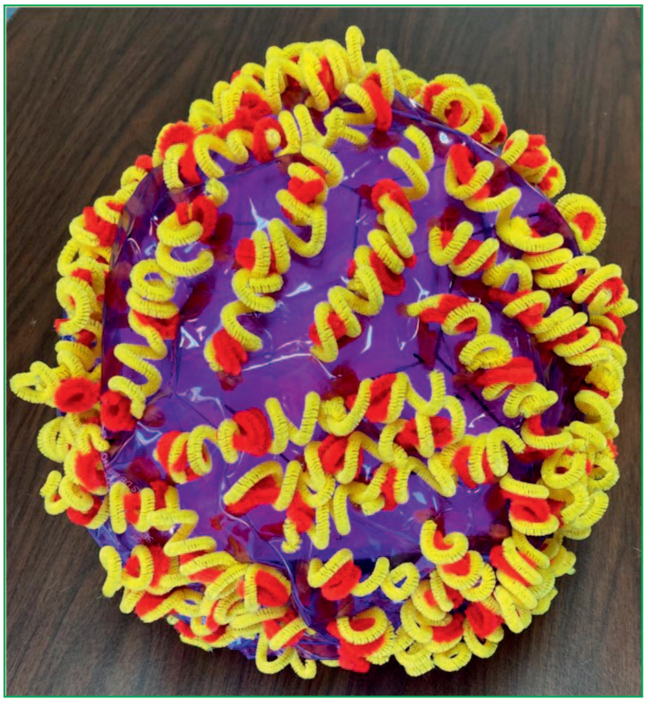

Figure 5.—Surface proteins (pipe cleaners) are embedded in the lipid envelope (beach ball).

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https://en.wikipedia.org/wiki/Capsid

YouTube 20-sided icosahedron tutorial: https://www.youtube.com/watch?v=mKxW2v6Nd2Q

Kissing bugs among us

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Abstract

Approximately, six to seven million people worldwide are infected with *Trypanosoma cruzi*, the causative agent of Chagas disease. The majority of these infections occur in Latin American countries from the bite of an infected triatomine/kissing bug, however other routes of transmission do exist. *Trypanosoma cruzi* infection is curable when treatment begins shortly after infection. However, this infection is not immediately apparent without testing that may not be offered in all areas. As climate change affects the distribution of vectors, the possibility that this disease risk disperses to non-Latin American countries increases. Vector control is considered the most useful method to prevent Chagas disease according to the World Health Organization, but vector control districts may be unaware of the threat in their districts. In 2018, Delta Mosquito and Vector Control District (DMVCD) was involved in the identification and testing of the triatomine vector, *Triatoma protracta,*from just outside the District borders. This specimenr had taken a blood meal and did test positive for *T. cruzi*. In 2021, DMVCD staff were trained on *Triatoma* surveillance. Flyers were distributed to notify residents of the potential threat, and encourage reporting of triatomines to the District, as we begin a surveillance plan for this Chagas disease vector.

Microbial composition in larval water enhances *Aedes aegypti* development but reduces transmissibility of Zika virus

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Abstract

Arthropod-borne viruses comprise a significant portion of the global disease burden. Surveillance and mitigation of arboviruses such as Zika virus (ZIKV) require accurate estimates of transmissibility by vector mosquitoes. Although *Aedes spp*. have been established as competent ZIKV vectors, differences in experimental protocols across studies prevent direct comparisons of relative transmissibility. An understudied factor complicating these comparisons is different environmental microbiota exposures, where most vector competence studies use mosquitoes reared in laboratory tap water, which does not represent the microbial complexity of environmental water where wild larvae develop. We simulated natural larval development by rearing Californian *Aedes aegypti* with microbes obtained from cemetery headstone water (containing rainwater) compared to conventional tap water. All larvae were provided sterile fish flakes and rodent chow embedded in agarose plugs to control for nutrient input. *Ae. aegypti* larvae reared in environmental cemetery water pupated 3 days faster and at higher proportions than those reared in laboratory water. Female adult mosquitoes reared in environmental water were less competent vectors of ZIKV compared to laboratory water-reared *Ae. aegypti*, as evidenced by significantly reduced infection and transmission rates. Microbiome comparisons of laboratory- and environment-water showed significantly higher bacterial diversity in environment water; however, corresponding differences in diversity were not consistently detected in adult mosquitoes reared in different water sources. We also detected greater correlations (by permutational ANOVA) between the microbial composition of adult mosquitoes and their bloodfeeding status than by the aquatic environment in which they developed. Together, these results highlight the role of transient microbes in the larval environment in modulating vector competence. Laboratory vector competence likely overestimates true transmissibility of arboviruses like ZIKV when conventional laboratory water is used for rearing.

Pyrethrum resistance in *Culex quinquefasciatus* in northern Tulare County, California

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Abstract

Adulticide applications are used to reduce the public health risk to residents by reducing large mosquito populations or targeting areas with increased West Nile virus activity. The Delta Mosquito and Vector Control District (District) has relied on the use of pyrethrum-based pesticides to reduce adult mosquitoes in areas of high disease risk for several years. The present study identified insecticide resistance in rural and urban *Culex quinquefasciatu*s Say populations within the District. Egg rafts were collected by placing Rubbermaid tote boxes (50.8 × 38.1 × 12.7cm) with 5 liters of hay infusion (275g of Kruse's Perfection Brand Rabbit food, 30g of NOW Brewer's yeast, and 227 liters of tap water) overnight in three of the District's cities: Woodlake, Visalia, and Dinuba. Larval *Cx. quinquefasciatu*s were reared under insectary conditions of 28°C and 70% humidity and fed alfalfa pellets weekly. Adults were maintained under insectary conditions of 28°C and 70% humidity, with 10% sugar water available *ad libitum*. The CDC bottle bioassay was performed on adult mosquitoes using technical grade pyrethrum at 15μg/bottle (250mL Wheaton Boston round bottle). Control adults were from the susceptible *Cx. quinquefasciatus* strain CQ1. Resistance to pyrethrum was observed in each of the District's cities, with mortality from 27-31%. Preliminary testing of other pesticides has indicated that *Cx. quinquefascitus* populations within our District are susceptible to Malathion. Identification of insecticide resistance in different regions is vital to optimizing pesticide rotation and combatting insecticide resistance.

Semi-field adulticide evaluations of *Culex* mosquitoes from the Coachella Valley

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Abstract

Semi-field evaluations are useful in determining the efficacy of a product against local mosquito populations. The Coachella Valley Mosquito and Vector Control District conducted multiple semi-field trials between May and July 2021. *Culex quinquefasciatus* were collected from La Quinta and Palm Desert, and *Cx. tarsalis* were collected from Northshore. Susceptible laboratory strains of *Cx. tarsalis* (Bakersfield) and *Cx. quinquefasciatus* (CQ1) were used to compare with the wild mosquitoes. Both species are arbovirus vectors, making them species of concern. The District regularly conducts ULV applications after detecting arbovirus infections in either species. The following adulticide products were evaluated using ground equipment: Aqua-Reslin; DeltaGard; Duet; Evergreen 5-25; Fyfanon; Merus 3.0; and Scourge 18+54. The adult mosquitoes were placed into sentinel cages and treated with two treatment passes. Once all passes were complete, the knockdown rate was monitored for 48 hours post treatment. Aqua-Reslin, Evergreen 5-25, Fyfanon, Merus 3.0, and Scourge 18+54 proved to be effective against *Cx. tarsalis*, demonstrating at least 90% knockdown. Duet was the only product that did not have a 90% knockdown rate against *Cx. tarsalis* (76.1%). DeltaGard was the only product that was effective against both wild populations of *Cx. quinquefasciatus* with 97% knockdown after 48 hours. Aqua-Reslin, Duet, and Evergreen 5-25 failed to achieve knockdown above 33.6% after 48 hours. The results from these evaluations provide the District with insight on the efficacy of several products being applied in the field. Details of these assays may be found in the following accepted manuscripts:

Hung, K.Y., A. Gutierrez, M. Kensington, M. Snelling, J. Tarango, G. Valadez, V. Valenzuela. 2022. Insecticide resistance semi-field evaluation of *Culex quinquefasciatus* mosquitoes from Coachella Valley, 2021. Arthropod Management Tests. (Accepted)

Hung, K.Y., J. Tarango, M. Kensington, A. Gutierrez, M. Snelling, G. Valadez. 2022. Insecticide sesistance semi-field evaluation of *Culex tarsalis* mosquitoes from Coachella Valley, 2021. Arthropod Management Tests. (Accepted)

Utilizing archived data for district operational decisions

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Abstract

Planning vector control operations is a key component in effectively using district resources to control vector populations and better serve residents. In 2017, the Delta Mosquito and Vector Control District (DMVCD) re-discovered *Aedes aegypti* (L.) within the District through a positive Zika travel case in the Visalia area. Since the detection of *Ae. aegypti*, the District has had to modify their best management practices, specifically trapping, inspection, and control methods. Since 2016, DMVCD expanded their surveillance program from 43 bi-weekly gravid traps to 272 sites, surveyed weekly. Site selection is based on the U.S. Public Land Survey System to allow mosquito trapping in a ¼-mile grid-based system. Analyzing historical surveillance data and control measures allows operations to predict problem areas before the peak of the vector season, thereby lowering potential vector sources and reducing mosquito abundance and associated disease risk.

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Field evaluation of an experimental fiber pot ovitrap compared with two commercial ovitraps to collect eggs of *Aedes* mosquitoes in Northeastern Florida

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Abstract

Surveillance of *Aedes aegypti* and *Aedes albopictus* is critical for preventing the spread of these mosquitoes and the viruses they transmit to new areas. Cost-effective trapping schemes, such as oviposition traps, help mosquito control and public health practitioners discover new environments invaded by *Ae. aegypti* and *Ae. albopictus*. Our study tests the effectiveness of a new oviposition trap, comprised of an experimental and environmentally friendly fiber pot, compared to commercial In2care traps and the Springstar Trap-n-kill ovitrap (TNK) containers and their attractants without any mosquitocidal activity. The results showed that the In2Care trap container performed the best, followed by the fiber pot trap, for collecting the eggs of both species of container-inhabiting *Aedes* mosquitoes. Although it collected the second highest number of eggs, the fiber pot trap had poor structural integrity and required modifications to remain functional for the test duration. Future changes to the trap should focus on making it last longer in the field.

Introduction

Aedes aegypti (L.) and *Aedes albopictus* (Skuse) are anthropophilic mosquitoes that transmit arboviruses to humans, including yellow fever, dengue, chikungunya, and Zika viruses. No effective vaccine or antiviral treatment have been developed for these diseases except yellow fever, so the best intervention is control of the vector mosquitoes. *Aedes aegypti* and *Ae. albopictus* are adaptable to multiple environments found throughout the U.S., with preferences for tropical, subtropical and temperate climates (CDC 2018). In addition, *Ae. aegypti* are adapting to urban environments and *Ae. albopictus* to vegetative environments in response to human encroachment, mosquito invasion, and global climate change (Higa 2011, Ayllón et al. 2018, Lwande et al. 2020). Both species oviposit in natural and artificial containers (bottles, buckets, bird baths, and toys) found near humans, which increases the likelihood of arbovirus transmission to humans (Lwande et al. 2020). Surveillance of *Ae. aegypti* and *Ae. albopictus* near human dwellings therefore is critical for detecting invasions into new areas increasing the risk for arbovirus transmission (Dixon et al. 2020). Cost-effective trapping schemes, such as oviposition traps, are one of several surveillance methodologies for *Ae. aegypti* and *Ae. albopictus*.

Oviposition traps take advantage of oviposition site seeking behavior by gravid females. Several factors attract gravid mosquitoes to a site for egg deposition, including water, color, surface texture, shade and smell (Day 2016). Most oviposition traps used to detect *Aedes* are black plastic cups lined with seed germination paper and filled with plant-based infusion water (Day 2016). Although this style of oviposition trap is cost-effective, others such as lethal ovitraps may perform better if modified for surveillance purposes. Also, the high number of eggs deposited in the lethal traps will increase larval control efficacy after the egg hatching. In addition, new oviposition traps made of different materials may have improved success, when compared to the current ovitraps. The new experimental fiber pot trap (EXP trap) is comprised of a brown fiber gardening pot and uses fish-food infusion water as the oviposition attractant. It requires weekly inspection to be dumped regularly to control hatching larvae. In the current study, the EXP trap was compared to two lethal ovitrap containers and their attractants, the In2Care trap (In2C) and the Springstar Trap-n-Kill ovitrap (TNK) (Buckner et al. 2017, Zeichner 2011), that have been modified to prevent mortality to ovipositing females. The materials that comprise the reservoir of the EXP trap are biodegradable which is more environmentally friendly than the plastic components found on most black ovitraps.

Materials and methods

The In2C traps (In2Care®, The Netherlands) were purchased from the Univa Solution company and the TNK (Springstar®, WA, USA) traps were purchased from Springstar LLC. The experimental fiber pot traps (EXP) were provided by the Greenhouse Megastore (Aventura,

Figure 1.—Location and layout of traps in Evergreen cemetery, St. Augustine, FL. Red circles with red labels indicate the location of each of the traps. The traps were placed in a 3 × 3 grid, A = TNK trap, B = In2C trap, and C = EXP trap.

Florida). Each trap was assembled according to the manufacturer instructions, except that the lethal components were deleted, because the EXP trap has no toxicant. Seed germination (SG) paper was added to each of the traps as an oviposition substrate. For In2Care traps, SG paper (71cm L x 9cm W) was interlaced with the center multipronged ring. For TNKs, SG paper (38 cm L x 9cm W) was cut into rectangular sheets and held in place using the trap lid. Finally, because EXP trap is essentially an open fiber pot with no lid, a large surface area (71 cm L x 18 cm W) of SG paper was used to cover the inside for egg deposition.

The three traps were tested at the Evergreen Cemetery located at 541-599 N Rodriquez Street, St. Augustine, FL 32084 (N29.893804, W81.336040) and were positioned in 3 replicate rows 7.6 m apart as shown in Figure 1. Trees, graves, and flower pots surrounded the traps. The graveyard was surrounded by bamboo, which was a major source of *Aedes* that move into the Cemetery. Traps were rotated weekly by letter row in a Latin square design. Each row contained three traps with different infusion water depending on the trap type. Based on the instructions with each trap, orchard hay infusion was used in the In2C (In2Care®, The Netherlands) and TNK (Springstar®, WA, USA) traps, whereas a fish food infusion was used in the EXP traps. All infusions were made with 24 g of substrate in 3 L of water in a 19 L container that was capped to facilitate fermentation and prevent insects from contaminating the infusion. Each infusion was diluted 10% in tap water (1.5 L into 15 L) prior to addition to the trap reservoirs. SG paper was replaced and infusion water levels were added to bring the level to previous level weekly with each rotation. In2Care, TNK, and EXP traps were filled with 2 L, 0.5 L, and 2.5 L of infusion water, respectively. While optimizing the EXP trap prior to the assay, the fiber material started to dissolve, so for support during the current experiment, the trap was placed in a black plastic pot of equal size and shape.

SG papers from each trap collection were dried at room temperature overnight, and eggs were counted under an Amscope dissecting microscope (Amscope®, CA, USA). Data was entered into Microsoft Office Excel and analyzed using the Analysis Tool pack ANOVA. For each week's collections, the three replicates of each trap type were averaged to determine weekly performance. Data is reported as the mean ± SE (standard error).

Results

A mean ± SE of 214.3 ± 68.4, 100.6 ± 25.3 and 44.9 ± 19.7 eggs per week were recovered from the In2Care, EXP, and TNK traps, respectively. The new EXP traps ranked 2nd for the mean number of *Aedes* eggs. The mean number of eggs collected by the In2Care traps with their attractant was significantly greater than the number of eggs collected by the EXP traps and THK traps ($F_{2,6} = 7.79$, $p = 0.02$). Eggs were not identified to species, so it was not known if different traps were more attractive to different *Aedes* species.

Discussion

Some of the factors that could account for variability in performance of the EXP trap include the infusion water and the trap materials. Different infusions were used for each trap because the goal was to assess the EXP trap, compared to other trap types using their recommended components. In2Care and TNK traps used orchard grass infusion as per the manufacturer's instructions, whereas the EXP trap used a fish food infusion provided by the manufacturer. Although the two infusions (orchard grass and fish food) were at equal volume (2 L), variability in nutrient density and substrate properties likely affected their performance. The TNK traps had an identical infusion to the In2Care, but the size and volume of attractive infusion may have affected the performance. For future improvements to the

EXP trap, new infusions should be tested to assess which will collect the most eggs.

Another factor that may have affected the performance of the EXP trap was degradation of the fiber. During preliminary optimization assays for the EXP trap, the fiber began to soften and break down as it absorbed water. No new materials were provided, so a black plastic planter pot was used to support the EXP trap. Based on the poor structural integrity of the EXP trap, improvements are needed for operational surveillance. Although the materials are biodegradable, it dissolved too quickly. A different biodegradable fiber substrate that can withstand the rain and humidity of Florida summers would be much more advantageous.

Conclusion

Black plastic acts as a good material for long-lasting, repeatable, and cost-effective *Aedes* mosquito surveillance traps. However, when plastic is disposed it takes far too long to degrade and is not environmentally friendly. Biodegradable materials should be considered for future ovitrap design. Future improvements should include 1) longer lasting fiber material that degrades after disposal but lasts for several surveillance seasons, 2) using environmentally friendly black paint, and 3) evaluation of different sized traps. The world is entering a new era in which "green" materials and practices are implemented to improve the impact humans have on the environment. Mosquito control should work to integrate itself into that future to better protect humans from mosquitoes using the cleanest technologies possible. Biodegradable fiber pots for mosquito surveillance represent one of many future improvements.

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Residual efficacy of copper sulphate pentahydrate against *Aedes aegypti* and *Culex quinquefasciatus* in laboratory and semi-field studies

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Abstract

Larval source management is a part of integrated vector control conducted by various chemicals or biological agents to reduce mosquito abundance. The persistent larvicidal efficacy of copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) on *Aedes aegypti* and *Culex quinquefasciatus* was evaluated under laboratory and semi-field conditions. Larval mortality at 72 h was approximately 80% at the selected concentrations of 14 ppm for *Cx. quinquefasciatus* and 14 and 15 ppm for *Ae. aegypti* in laboratory and semi-field settings. In semi-field tests, the mortality of $CuSO_4 \cdot 5H_2O$ against *Ae. aegypti* in the containers (black buckets) increased by week 2 and then decreased posttreatment, while mortality of *Cx. quinquefasciatus* in the pools decreased continuously after 48 h. The larvicidal effect of copper sulfate was more persistent in the laboratory compared to semi-field settings, especially against *Ae. aegypti*. Further studies will be needed to confirm concentration and persistence to effectively control mosquito larvae in the field.

Introduction

Current mosquito larvicides are too costly to sustain for some public health systems (Lau 2018). Alternative active ingredients consisting of metallic compounds such as copper sulphate have been revisited and proposed as insecticides to control mosquito larvae (Kennedy. 1941; Romi et al. 2000; Reza et al. 2012, 2013, 2014; Becker et al. 2015). Copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$), commercially named SAFI (DNW Global, Windermere, Florida), has been registered and used for the suppression of bacterial odors and toxic gas in standing or moving water bodies containing organic matter such as algae or bacteria. It is used worldwide as an algaecide and a fungicide in aquaculture and agriculture (Lasiene et al. 2016). In a preliminary field study in Orlando, Florida, the SAFI product showed effective control of mosquito larvae (unpublished report). DNW Global requested more detailed studies done under Good Laboratory Practice as supporting information to apply for EPA registration for additional function as amosquito larvicide

A laboratory study using metallic copper ions in water showed that 10 g/L and 20 g/L could inhibit *Aedes albopictus* (Skuse) larval development and induce high larval mortality (Romi et al. 2000). A more recent effort reported that metallic copper spray caused 100% mortality in *Aedes* spp. larvae in 14 days (Becker et al. 2015). However, there are few reports on the lasting effect of copper sulfate as a larvicide against other species of larval mosquitoes. In the current study, we investigated the persistent efficacy of $CuSO_4 \cdot 5H_2O$ as a mosquito larvicide against 3rd instar *Aedes aegypti* (L.) and *Culex quinquefasciatus* Say under laboratory and semi-field conditions.

Materials and Methods

Aedes aegypti (Orlando strain, 1952 colony) and *Cx. quinquefasciatus* (Gainesville strain, 1989 colony) were obtained as eggs from the United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, FL, and reared at 27.0±1 °C, 80.0 ± 5% relative humidity (RH), and a 14L:10D photoperiod at the Anastasia Mosquito Control District insectary. Larvae were reared in 12 cm × 35 cm × 50 cm plastic trays containing distilled water and fed a mixture of yeast and powdered fish food. Third instar larvae were used in all studies.

Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$; SAFI, DNW Global LLC, Florida, USA; 19.8 % active ingredients and 80.2 % other ingredients) was used in larval bioassays. The application rates (14 ppm for laboratory and 10 ppm for semi-field) of the copper sulfate pentahydrate for *Cx. quinquefasciatus* and 15 ppm and 18 ppm for *Ae. aegypti* in laboratory and 14 ppm for semi-field were selected and used to assess persistence based on a previous report (Miah et al. 2021). Laboratory larval bioassays were conducted using a protocol modified from WHO Bioassay Guidelines (WHO. 2005). Twenty 3rd instar larvae of each species were transferred into a clear plastic 266 mL cup containing

Table 1.—Mean percent mortality effect of copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) solution (SAFI) on 3rd instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* under laboratory conditions.

	Ae. aegypti			*Ae. aegypti*			*Cx. quinquefasciatus*		
	% mortality (15 ppm)			% mortality (18 ppm)			% mortality (14 ppm)		
Time	24h	48h	72h	24h	48h	72h	24h	48h	72h
1st week	95^{Aa}	100^{Aa}	100^{Aa}	81.7^{Aa}	88.3^{Aa}	95^{Aa}	61.7^{Aa}	100^{Ab}	100^{Ab}
2nd week	93.3^{Aa}	98.3^{Aa}	100^{Aa}	100^{Ba}	100^{Ba}	100^{Aa}	86.7^{Ba}	96.7^{Aab}	100^{Ab}
3rd week	100^{Aa}	100^{Aa}	100^{Aa}	100^{Ba}	100^{Ba}	100^{Aa}	43.3^{Aa}	73.3^{Bb}	86.7^{Bb}

Note: *Means with the same capital letter in a column are not significantly different and with the same lowercase letter in a row are not significantly different ($\alpha = 0.05$).

either 100 mL treatment solution or distilled water as control. Three (3) replicates were conducted using a completely randomized design. Cups were kept in an incubator at 27.0°±1°C, 80±10 %RH, and 12L:12D photoperiod. During the test period larvae were not provided food. Dead larvae were recorded after 24 h, 48 h, and 72 h. After 72 h all larvae were collected, and new larvae were released into the same cups the following week.

For the container-inhabiting mosquito, *Ae. aegypti*, the semi-field bioassay consisted of thirty 3rd instar larvae released into each of three replicate 25 L black plastic buckets containing 2 L of 14 ppm $CuSO_4 \cdot 5H_2O$ (Miah et al. 2021) or untreated control water and were maintained under an open tent with a roof only. The concentration for the semi-field testing was selected based on the previous laboratory results as mentioned above. Larval mortality was determined by counting the number of dead larvae at 24 h, 48 h, and 72 h. After 72 h larvae were collected, and new 3rd instar larvae were released into the buckets next week without provided food.

For *Cx. quinquefasciatus*, 200 - 3rd instar larvae each were released into three concrete test pools containing 10 ppm of $CuSO_4 \cdot 5H_2O$ (Miah et al. 2021) or untreated pools as a control. Each pool was situated in a natural environment, had 946 L capacity, 4′ x 4′ x 2′ inner dimension, and contained 757 L of treated or untreated water, respectively. Larval mortality was determined by counting the number of living larvae and subtracting from the number released. For this experiment, well water that had been treated with 10 ppm $CuSO_4 \cdot 5H_2O$ after standing for 24h in the concrete pools. No food was provided for larvae during semi-field bioassays. After 72 h larvae were collected, and new larvae were released into pools the next week.

The percentage of larval mortality for semi-field bioassays was recorded after 24h, 48h, and 72h and corrected using Abbott's formula (Abbott 1925):

$$\text{Corrected mortality (\%)} = \frac{\%\ \text{mortality in treated} - \%\ \text{mortality in control}}{100 - \%\ \text{mortality in control}} \times 100$$

Mortality data were analyzed using Chi-square tests at a 0.05 significance level in SPSS 20.0.

Results

The larvicidal effect of copper sulfate against 3rd instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* over time in laboratory bioassays are presented in Table 1. The mortality of selected 15 ppm of stock $CuSO_4 \cdot 5H_2O$ (active ingredient) against *Ae. aegypti* larvae ranged from 93.3% to 100% after exposure of 24h, 48h, and 72 h for three weeks. There were no significant differences among week or hour groups ($P > 0.05$). Larvicidal effect of the treated group was significantly different from its control group at each period, with no deaths observed in the control groups. For 24 h of exposure in the second week, the mortality in the 15 ppm $CuSO_4 \cdot 5H_2O$ (a.i.) group was statistically significant at 93.3%, compared to no death in the control group (Chi squared = 62.2, df = 1, $P < 0.001$). The mortality using 14 ppm $CuSO_4 \cdot 5H_2O$ (a.i.) against *Cx. quinquefasciatus* larvae ranged from 43.3% to 100% after 24h, 48h, and 72h of exposure for three weeks. For *Ae. aegypti* there was no significant difference in mortality at 24h, 48h, and 72h post exposure comparing the 15 to 18 ppm groups ($P > 0.05$).

The larvicidal effects of copper sulfate against 3rd instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* under semi-field conditions were presented in Table 2. The mortality of 14 ppm $CuSO_4 \cdot 5H_2O$ against *Ae. aegypti* larvae was significantly different among weeks 1 to 3 after 24h and 48h of exposure. The mortality of 10 ppm $CuSO_4 \cdot 5H_2O$ against *Cx. quinquefasciatus* larvae also differed significantly among weeks 1 to 3 after 24h and 48h of exposure. Against *Ae. aegypti*, the mortality rate decreased at 3rd week posttreatment, while against *Cx. quinquefasciatus*,

Table 2.—Mean percent Abbott-corrected mortality effect of copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) solution (SAFI) on 3rd instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* under semi-field conditions.

	Ae. aegypti			*Cx. quinquefasciatus*		
	% mortality (14 ppm)			% mortality (10 ppm)		
Time	24 h	48 h	72 h	24 h	48 h	72 h
1st week	23.3^{Aa}	55.6^{Ab}	87.8^{Ac}	68.3^{Aa}	96.7^{Ab}	98.7^{Ab}
2nd week	98.9^{Ba}	98.9^{Ba}	100^{Ba}	51.2^{Ba}	78.5^{Bb}	98.3^{Ac}
3rd week	64.4^{Ca}	74.4^{Ca}	78.9^{Aa}	39.5^{Ca}	52.2^{Cb}	61.2^{Bc}

Note: *Means with the same capital letter in a column are not significantly different and with the same lowercase letter in a row are not significantly different (α=0.05).

the mortality rate decreased continuously after the 2nd week.

In the semi-field test, mortality at 14 ppm $CuSO_4 \cdot 5H_2O$ against *Ae. aegypti* was 23.3% in the first week after 24 hours of exposure, while mortality at 24-hour during the third week was 64.4%; significantly higher than the first week ($P<0.05$). This indicated that over time the well water may have increased the toxicity of the copper sulphate in some fashion.

Discussion

Copper is one of the essential elements in organisms, and its pollution is harmful to some extent (Clements et a1.1988; Hare et a1.1992). Previous studies have shown that the relationship between insect growth and Cu^{2+} concentration was parabolic. Low Cu^{2+} concentration promoted growth in insects, whereas high Cu^{2+} concentration inhibited growth in insects. Our previous study (Miah et al. 2021) showed that $CuSO_4 \cdot 5H_2O$ solution resulted in the highest larvicidal activity at 72h after exposure against *Ae. aegypti*, *An. quadrimaculatus,* and *Cx. quinqufasciatus,* with their lowest LC_{50} values of 5.5ppm, 2.25ppm and 2.0ppm, respectively. Compared with other pesticides, copper sulfate solution has the advantages of convenience and low toxicity. Therefore, copper may be considered as an alternative mosquito larvicides.

The current exploratory study described the persistence of $CuSO_4 \cdot 5H_2O$ related mortality against mosquito larvae in laboratory and semi-field conditions. The $CuSO_4 \cdot 5H_2O$ solution showed a larvicidal effect for 2-3 weeks post treatment. Efficacy and persistence varied due to application rate, species of mosquito, and weeks post treatment. Romi (2000) reported that copper immersion at 1.2 ppm in water had no significant effect on egg hatching and oviposition of *Ae. albopicus*, because the highest concentration of copper ions obtained by immersion was less than the effective larvicidal concentration. Herein, no obviously significant difference in larval mortality was observed for *Ae. aegypti* treated with $CuSO_4 \cdot 5H_2O$ at 15ppm and 18ppm. This suggests a lower dose should be sought.

$CuSO_4 \cdot 5H_2O$ had a persistent larvicidal effect against 3rd instar larvae with 80% mortality both in laboratory and in semi-field settings for our three week experimental period. Zhang (2010) reported that 1 ppm and 10 ppm copper sulfate solution controlled mosquitoes in a one week field test, while 100 ppm copper sulfate solution yielded control for two months. In the current study, the larvicidal effect of copper sulfate persisted longer in the laboratory, compared to a semi-field evaluation. The semi-field containers and pools may have been influenced by weather conditions, sun light, debris and other organic substances that reduced copper sulfate solution gradually and thus weakened its larvicidal effect. Also, there were differences between the two species of mosquito larvae.

In summary, the $CuSO_4 \cdot 5H_2O$ had lasting larvicidal activity against the mosquito larvae of container-inhabiting *Ae. aegypti* and *Cx. quinquefasciatus* for 2-3 weeks. $CuSO_4 \cdot 5H_2O$ may be an easy, safe, and low-cost alternative larvicide recommended for mosquito larval control. $CuSO_4 \cdot 5H_2O$ may be directly used in certain larval habitats, such as discarded swarming pools, storm drains, and containers to prevent maturation of mosquito developmental stages. Further studies will be needed to confirm concentration and persistence to effectively control mosquito larvae in the field.

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Evaluating three RNA extraction kits for arbovirus testing

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INTRODUCTION

Vector control districts routinely test female mosquitoes for the presence of arboviruses that can infect people as part of their public health protection program. Although there are many mosquito-transmitted arboviruses worldwide known to infect people, in California there are currently three that are of substantial concern to public health: St. Louis encephalitis virus (SLEV), West Nile virus (WNV), and Western equine encephalitis virus (WEEV). The genome of these viruses is comprised of RNA, which is tested for using quantitative reverse transcription polymerase chain reaction (qRT-PCR). In California, there are predominantly three mosquito species that transmit these arboviruses to humans: *Culex erythrothorax*, *Culex pipiens* complex (includes *Culex quinquefasciatus*) and *Culex tarsalis*. The methods used to extract RNA have improved the speed, quantity, and quality of RNA that is retrieved from specimens. Early methods relied upon liquid-liquid extraction (LLE) that partitions the RNA into two phases: an aqueous phase that contains the RNA, an interphase with proteins, and an organic phase that contains lipids (Green and Sambrook 2012). LLE requires a great deal of technical expertise and regularly yielded RNA that contained phenol, an organic solvent that could not be removed unless another LLE step was undertaken. Minute quantities of phenol denatures enzymes, and its presence results in a failed RT-PCR assay. A solid-phase extraction (SPE) method that utilizes silica was developed for RNA extraction that does not use organic solvents (Marko et al. 1982, Boom et al. 1990), and is now one of the most frequently used methods for RNA extraction from biological specimens. Many vector control laboratories in California currently use the MagMAX Viral Isolation Kit (Thermo Fisher Scientific, Waltham, MA USA) in their arbovirus testing program. Vector control laboratories often test new products as they become available, but these evaluations are not typically shared broadly.

The Laboratory Technologies Committee (LTC) of the Mosquito and Vector Control Association of California (MVCAC) sought to compare two SPE RNA extraction kits (Quick-DNA/RNA Pathogen MagBead Kit (Zymo Research, Irvine, CA USA) and MagMAX CORE Nucleic Acid Purification Kit (Thermo Fisher Scientific)) with the MagMAX Viral Isolation Kit, and share the results broadly with MVCAC members via a poster presentation at the 2022 Annual Meeting of MVCAC and within the Proceedings of the MVCAC. Laboratories at Alameda County Mosquito Abatement District (ACMAD), Sacramento-Yolo Mosquito and Vector Control District (SYMVCD), and UC Davis Arbovirus Research and Training Laboratory (DART) independently tested each kit.

MATERIALS AND METHODS

Sample collection

SYMVCD collected mosquitoes using encephalitis virus survey (EVS) or gravid traps, identified them to species and froze them at -80°C until use. ACMAD used WNV RT-PCR positive control standards that were prepared from in activated virus cultured on Vero cells and provided by DART. Both ACMAD and SYMVCD serially diluted specimens in viral transport media prior to testing for WNV using qRT-PCR. DART used collections of mosquitoes (i.e., mosquito pools) that were provided by mosquito control districts in California and frozen at -80°C until use. Environmental specimens (i.e., from mosquitoes) were used in the current study only if prior arbovirus tests showed that they contained WNV RNA.

RNA extraction and quantitative RT-PCR assays

RNA was extracted from environmental specimens or serial dilutions of WNV RT-PCR positive controls using the MagMAX Viral Isolation Kit (MagMAX-V), Quick-DNA/RNA Pathogen MagBead Kit (Quick-NA), and MagMAX CORE Nucleic Acid Purification Kit (CORE), according to the protocols provided by the manufacturers. To increase the throughput of RNA extractions, SYMVCD and DART used a Kingfisher Flex (Thermo Fisher Scientific) whereas ACMAD used a KingFisher Duo Prime (Thermo Fisher Scientific) automated nucleic acid purification system. All specimens were extracted with each of the three RNA extraction kits. RNA extracts were tested for the presence of RNA using the TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher Scientific) with primers and

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Table 1.—Primers and probes used to amplify WNV in QRT-PCR assays

Name	Sequence (5′ → 3′)
Primers	
WNV-E 1_forward	TCA GCG ATC TCT CCA CCA AAG
WNV-E 1_reverse	GGG TCA GCA CGT TTG TCA TTG
Probe	
WNV-E 1 Probe	FAM-TGC CCG ACC ATG GGA GAA GCTC - QSY

probes specific for WNV (Table 1), with a QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific) or ABI 7500 Fast Real-Time PCR System (Thermo Fisher Scientific), as described by the manufacturer. Of note, the extractions and WNV qRT-PCR assays were performed at three different facilities using the equipment at each laboratory.

Software

CT values from the qRT-PCR assays were calculated using QuantStudio 5 or Sequence Detection Software (ABI 7500) software and the results analyzed and graphed using Prism software (GraphPad, San Diego, CA). Each point or bar on a graph represents a single CT value. Auto baseline and threshold settings were applied for all qRT-PCR assays.

RESULTS AND DISCUSSION

There was a linear increase in CT values for each series of diluted RNA (Figure 1; average slope = 3.631 ± 0.272), demonstrating that sample preparation and analysis at ACMAD and SYMVCD were internally consistent. There was no significant difference in CT values for the CORE and Quick-NA kits relative to the MagMAX kits tested at ACMAD or SYMVCD (paired t tests, $P > 0.13$) demonstrating that each kit was equally effective for extracting WNV RNA from mosquitoes or cell culture. The higher CT values from ACMAD may have resulted from small differences in the initial concentration of WNV RNA in the specimens prior to serial dilution. Paired comparisons by DART of specimens collected from birds known to contain WNV showed no significant difference in CT values for RNA that as extracted using the MagMAX or CORE kit (Figure 2; paired t tests, $P = 0.2228$).

CONCLUSION

The results indicated that the CORE, Quick-NA and MagMAX kits were equally effective for isolating RNA from environmental or laboratory-generated specimens for WNV testing using qRT-PCR.

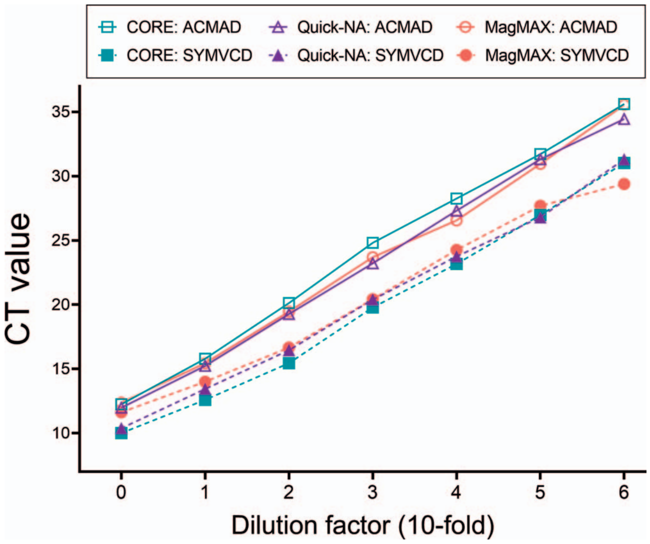

Figure 1.—CT values from serially diluted RNA extracted by ACMAD and SYMVCD using MagMAX, CORE or Quick-NA kits.

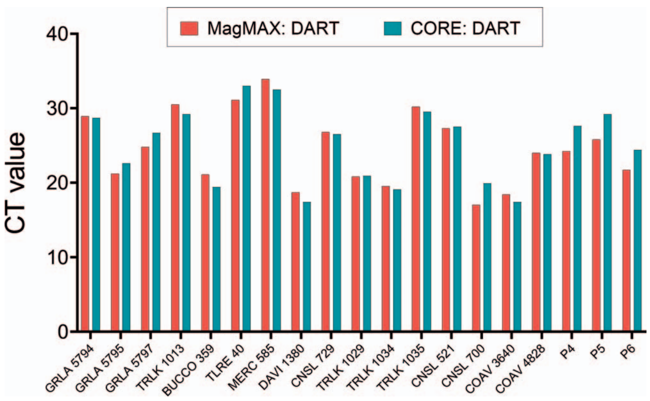

Figure 2.—CT values from RNA isolated by DART from mosquito pools known to have been infected by WNV.

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Aerial larviciding over rice: efficacy evaluation over a full growing season

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Abstract

Rice fields have long been understood as important mosquito sources, yet reducing mosquito populations in these habitats remains challenging. Placer County, California is no exception, with rice habitat creating more mosquitoes and more West Nile virus detections in mosquito pools than anywhere else in the county. The current study evaluated the efficacy of aerial larvicide treatments in rice fields across a rice growing season. From June to September of 2017, each of 14 applications of aerial *Bacillus thuringiensis israelensis* larvicide (VectoBac 12AS, 0.6 L/ha) was evaluated by collecting the treatment material in cups at water level, mid-level in the rice canopy, and at the top of the rice canopy, as well as taking samples directly from the rice-growing water before and after the application. Laboratory bioassays were performed on the samples using laboratory-reared 3rd instar *Culex tarsalis* larvae. Overall treatment efficacy was good until late in the season when a thick, tall rice canopy blocked some larvicide from reaching the water surface.

Introduction

Rice growing habitat in southwestern Placer County in the Sacramento Valley region of California produces high numbers of the West Nile virus vector *Culex tarsalis* in close proximity to high density human populations, despite an active integrated vector control program incorporating treatments with both larval and adult mosquito control products. Additionally, the rice growing areas of Placer County, and more generally of the Sacramento valley, produce a high number of West Nile virus detections in mosquito pools year after year. Treating these areas with larval and adult mosquito control products is costly, both in terms of the control products themselves as well as the cost of aerial application. The substantial public health significance of the rice growing area, combined with the high associated costs, make it critically important that mosquito control activities in and around the rice fields are as effective and efficient as possible.

In other types of mosquito habitat, the efficacy of larvicide treatments is assessed by "back-checking", or visiting a treated site after application of a larvicide material and dipping the source to look for live and dead larvae. Rice fields differ from other larval mosquito sources in that it is difficult to find larval mosquitoes by dipping. The enormous surface area, dense vegetation, and high variability of larval mosquito density make quantifying mosquito larvae in a rice field difficult, so therefore "back-checking" is not effective for measuring treatment efficacy in rice fields.

Assessment of larvicide treatment efficacy in rice is further complicated by the presence of the thick rice canopy covering and obscuring the water surface. As the rice reaches the tillering stage, each plant produces multiple stems, increasing the density of emergent vegetation and covering more of the water surface. As the stems grow taller and the seed becomes heavier, stalks may bend over, further covering the water surface. It is possible that the rice canopy may block the penetration of the larvicide material to the water surface.

The objectives of the current study were to 1) experimentally assess the efficacy of aerial *Bacillus thuringiensis israelensis* (Bti) larvicide treatment in a rice field over a growing season, and 2) to evaluate the effect of the rice canopy in blocking treatment material from reaching the water surface.

Methods

From June to September of 2017, 14 aerial applications of larvicide were evaluated. *Bacillus thuringiensis israelensis* (Bti) larvicide (VectoBac 12AS, Valent Biosciences, Chicago, IL) was applied by air (AirTractor 402, Arena Pesticide Management, Woodland, CA) from a height of around 30 feet, at a rate of 0.6 L of product per hectare (8 fl. oz./acre). The spray system installed on the aircraft consisted of a low pressure pump system and 2 wind driven rotary atomizers (Mincronair 4000, Bembridge Fort, Sandown, Isle of Wight, PO36 8QY, U.K.). To achieve the desired flow rate, the pump system was set at 15psi and the variable restrictor unit (VRU) was set at 11 on the atomizers. To achieve the correct droplet size, short fan blades were used on the atomizer and were set at an angle of 65 (degrees).

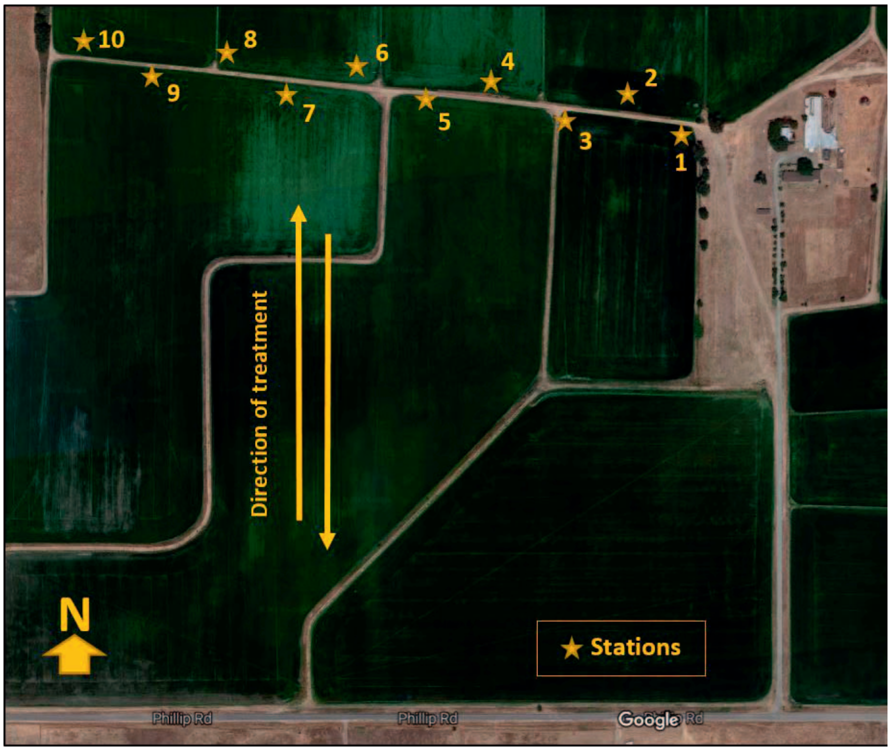

Figure 1.—Aerial view of rice fields evaluated for larvicide efficacy in Placer County, CA. Location of each sampling station is marked by stars. Arrows indicate orientation of aerial application.

All evaluations were done at the same field site (north of Phillip Road, Roseville, CA) (Figure 1). The site was chosen due to the presence of a vehicle accessible levy road closely bordered by rice fields on either side that were scheduled for treatment. The airplane treated the two rice fields by flying perpendicular to the road. Stations were placed about 200 feet apart so that stations would be treated by different passes (swath width 180′) of the airplane.

On each treatment date, water depth and height of rice canopy (from water level) were recorded at each station, 1-2m from the edge of the field. Plastic sample cups (polypropylene, 237ml, 73 mm diameter opening, VWR International LLC, Radnor, PA) were used to collect applied materials. At each of the ten stations, five samples were collected for a total of 50 samples per treatment date. Before and after the larvicide applications, water samples were taken directly from the surface of rice growing water by scooping roughly 200ml into a cup, 1-2 m from the edge of the field. Three additional cups per station were filled

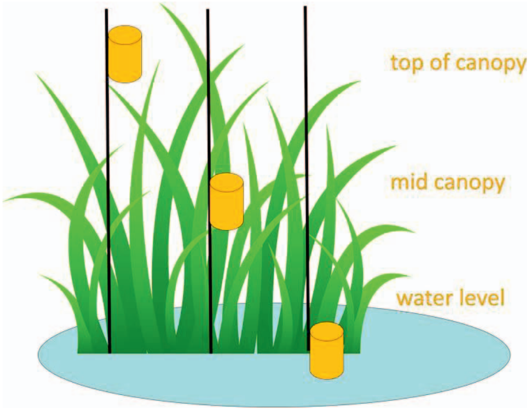

Figure 2.—Placement of 200ml sampling cups in the rice canopy to collect aerial larvicide treatment material for bioassay assessment.

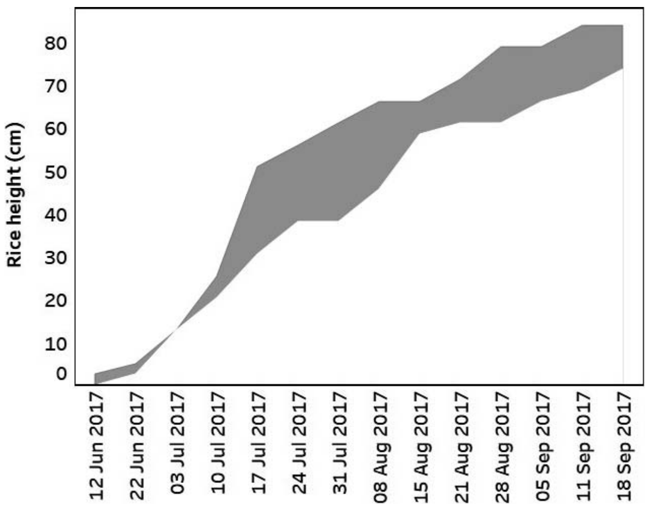

Figure 3.—Height of the emergent rice canopy at study site in Placer County, CA. Shaded area indicates range of rice heights measured 1-2m from the edge of the rice field at ten sampling stations.

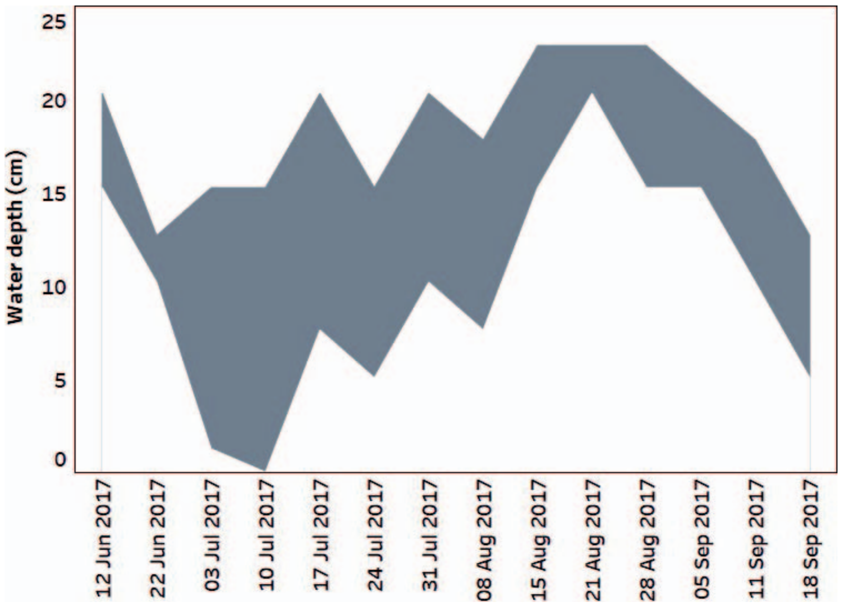

Figure 4.—Water depth in rice at a study site in Placer County, CA. Shaded area indicates range of water depths measured 1-2m from the edge of the rice field at ten sampling stations.

with 200ml of reverse-osmosis filtered tap water and positioned to sample the larvicide treatment at different heights using a stake and clip system. One cup was stationed at water level, one cup at the middle of the rice canopy, and one cup at the top of the rice canopy (Figure 2). When the rice canopy was less than 15cm, only water level cups were set. For rice 15-20cm, water level and top level cups were set. For rice more than 20cm tall, water level, mid-level, and top level cups were set. The three rice canopy cups were set before the treatment began and were left in place for 30 minutes after the application was completed.

The amount of larvicide in each sample was assessed indirectly by bioassay for samples of rice-growing water

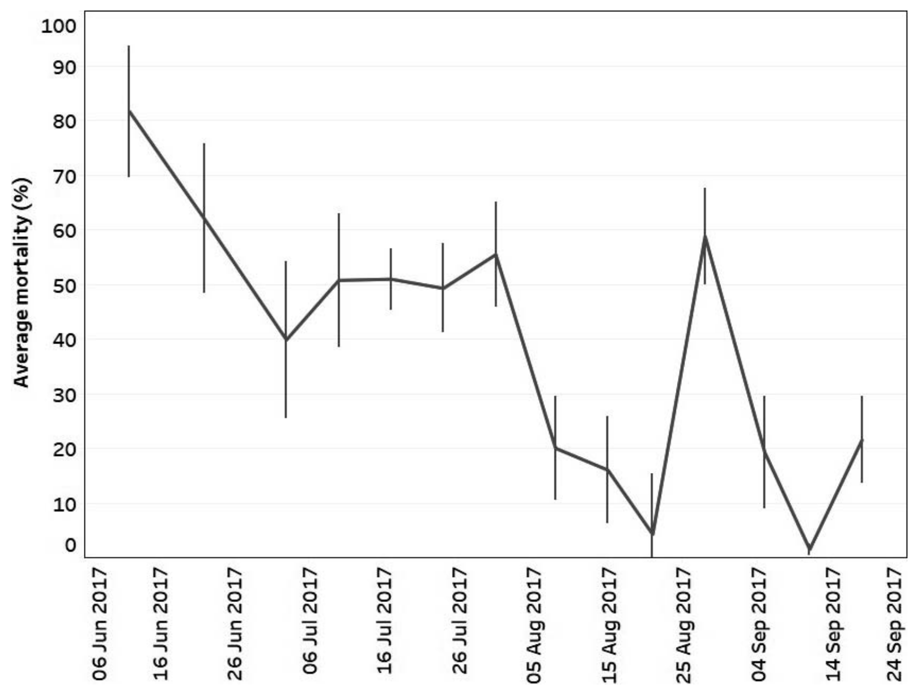

Figure 5.—Average treatment mortality (after-treatment mortality – before-treatment mortality) in bioassays of water taken from rice fields treated with aerial *Bacillus thuringiensis israelensis* larvicide (Vectobac 12AS). Samples were collected in approximately 200 ml of water and mortality assessed for 20 3rd instar laboratory-reared *Culex tarsalis* larvae. Bars represent standard error of samples from ten stations per date of treatment.

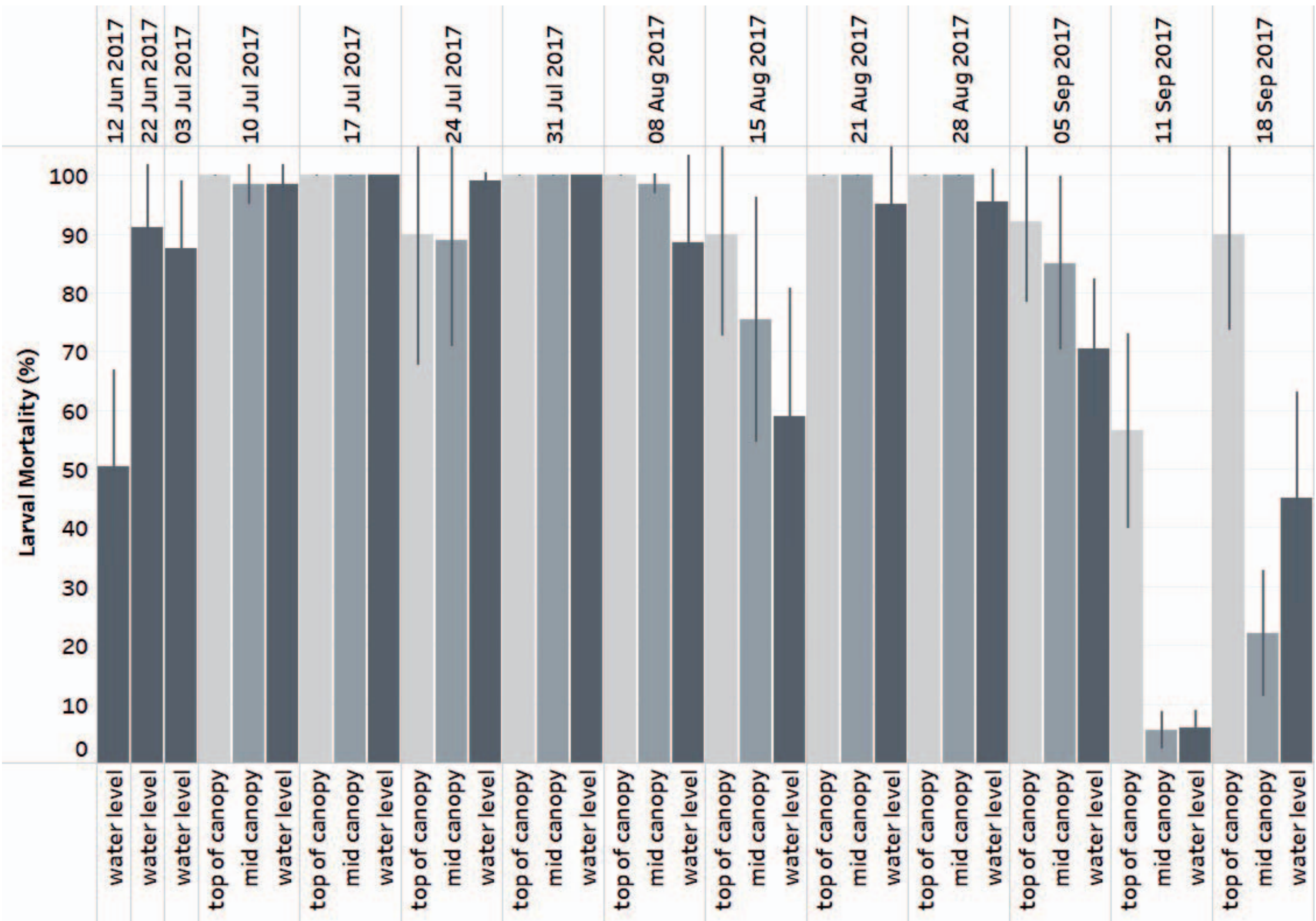

Figure 6.—Average mortality in cups of filtered water placed in a rice field during aerial treatment with *Bacillus thuringiensis israelensis* larvicide (Vectobac 12AS). Cups were placed at the top of the rice canopy, middle of the rice canopy, or at water level, then subjected to bioassay with 20 3rd instar laboratory-reared *Culex tarsalis* larvae. Bars represent standard error of samples from ten stations per date of treatment.

and for cups placed in the rice canopy. Twenty laboratory-reared 3rd instar *Cx. tarsalis* (BFS colony strain) larvae were introduced into the water sample, and live larvae were counted after 24 hours. Larvae that pupated were not included in the determination of the percentage of mortality. Two cups per treatment date were prepared with filtered water and held without treatment exposure, as bioassay controls.

Whenever possible, bioassays were carried out on water samples within 24 hours of collection. If immediate bioassay was not possible for a week or more, samples were frozen at -80° C and defrosted for 12-24 hours before bioassay. A separate trial was conducted to verify that freezing did not significantly alter the toxicity of the samples for up to one month (data not shown, but see also Boisvert and Boisvert 2001)

Treatment efficacy in the rice-growing water was determined by subtracting the percentage mortality of *Cx. tarsalis* larvae in the before-treatment samples from the percentage mortality in the after-treatment samples. The average and standard error of mortality of the ten stations was calculated.

Results

Fourteen applications of larvicide were evaluated over the course of the growing season. One additional application of granular Bti (VectoBac GR) occurred on 17 August. Rice grew steadily throughout the season, reaching its maximum height of around 80cm in mid-September, around the time of the second-to-last treatment (Figure 3). Water depth fluctuated from 0-23cm, with the shallowest water depth measured during the last treatment on 18 September, and the greatest water depth on 21 August (Figure 4).

In samples of rice-growing water, average mortality attributed to the treatment (bioassay mortality in after-treatment samples – bioassay mortality in before-treatment samples) was 7-94% (Figure 5). Starting around 8 August, treatment mortality seemed to decrease, with average mortality due to the treatment of 47-94% from 12 June to 31 July, compared to 7-59% from 8 August to 18 September. Bioassay mortality on 28 August (59%) stands out as somewhat anomalous.

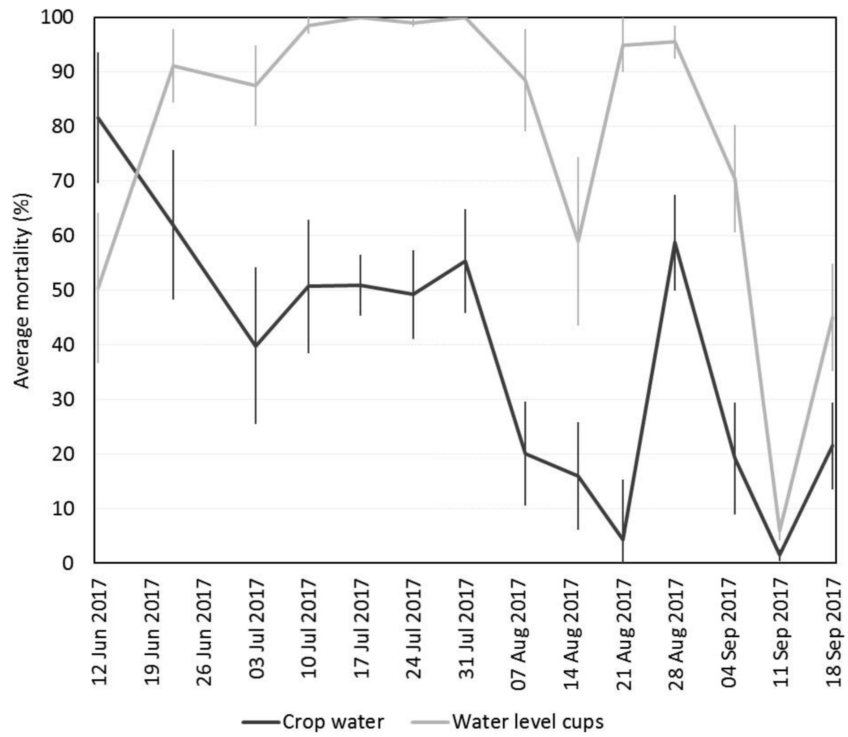

Figure 7.—Average bioassay mortality in cups of filtered water placed in a rice field during aerial treatment with *Bacillus thuringiensis israelensis* larvicide (Vectobac 12AS), compared to treatment mortality (after-treatment mortality – before-treatment mortality) in bioassays of water taken directly from the treated rice fields. Bioassays included 20 3rd instar laboratory-reared *Culex tarsalis* larvae. Bars represent standard error of samples from ten stations per date of treatment.

Cups of filtered water did not demonstrate a difference in average bioassay mortality for different heights in the rice canopy for the first 12 application dates, during which time mortality varied from around 50% to near 90%. On the last two application dates, 11 and 18 September, mortality at the mid-canopy level and water level was reduced compared to mortality in cups from the top of the rice canopy (Figure 6). Treatment mortality was not adjusted for control mortality, which was less than 10 percent.

As compared to mortality in bioassays of water level cups, samples taken directly from the rice growing water demonstrated less mortality (Figure 7).

Discussion

The current study had two objectives: 1) measure the efficacy of aerial larvicide treatments in rice, and 2) evaluate the effect of the rice canopy in blocking larvicide material from reaching the water, thereby potentially decreasing treatment efficacy. Penetration of larvicide through the canopy was assessed by collecting BTI applications in cups placed at different heights within the rice canopy and then testing samples by bioassay. Bioassays found no evidence that rice wasblocking penetration of larvicide to the water surface until the last two treatment dates. On the last two treatment dates, bioassay mortality in cups from the top of the rice canopy was greater than in cups placed at water level, underneath the rice canopy, providing strong evidence that the larvicide was not reaching the water surface. At this time in the season the rice was around 80cm tall (Figure 3) and the rice canopy had become thick and dense.

To address the broader question of efficacy of aerial Bti larvicide, this study incorporated two approaches. Samples of rice-growing water were taken directly from the rice field before and after the treatment, and additionally, cups collected treatment material at water level below the rice canopy, thereby sampling the larvicide entering the field. Both of these methods found that efficacy was variable, with water-level cups producing from 6-97% mortality in bioassays, and treatments accounting for between 7-94% of mortality in bioassays of rice growing water. On most treatment dates, mortality in water level cups was greater than treatment mortality in the rice-growing water (Figure 7), indicating factors in the rice water such as water depth, pesticide dispersal, organic material, or another property of the rice growing water may render the treatment less effective.

Throughout the season, large variation in efficacy was observed between treatments (Figure 7). Disregarding the last two treatments where rice canopy was most dense, larvicide treatment caused 50-97% mortality in water-level cups. These cups contained filtered water from the laboratory, therefore bioassay results in water-level cups reflect variability in the treatment itself and not potential variability in the rice-growing water. Applications may have been more or less successful based on atmospheric factors or equipment variables, but without efficacy data such as collected in this study, it was not possible to identify areas of possible improvement for application procedures and techniques.

The 21 August treatment had the greatest difference in efficacy as determined by water level cups compared to rice-growing water (Figure 7). On 17 August, an application of VectoBac GR occurred in between applications of VectoBac 12AS, and based on mortality in crop water samples taken before VectoBac 12AS treatments (data not shown), the VectoBac GR treatment may have had some residual efficacy in rice-growing water during the 21 August treatment.

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Seventeen years of proficiency panels: how results provided a path to current testing methods

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Background

Starting in 2006, The California Department of Public Health's Vector-Borne Disease Section (VBDS) partnered with the Davis Arbovirus Research and Training (DART) Laboratory, then known as the Center for Vectorborne Diseases, to develop proficiency panels that were administered annually to local mosquito control districts conducting in-house testing. In 2006, three different tests – reverse transcription polymerase chain reaction (RT-PCR), VecTest (Medical Analysis Systems, Inc., Camarillo, CA), and Rapid Analyte Measurement Platform (RAMP; Response Biomedical Corp., Burnaby, British Columbia, Canada) – were being used by 26 different laboratories to test for West Nile virus (WNV) in mosquito pools and dead birds. The adoption of proficiency panels for validating local testing laboratories was motivated by the growth and diversification of testing by local agencies and the resulting need to ensure consistency and accuracy of testing among laboratories and maintain confidence in statewide surveillance results.

History of arboviral testing by local mosquito control agencies

Since 2006, testing methods utilized by local mosquito control agencies have shifted toward RT-PCR (Fig. 1), which is the testing method now used by all local laboratories that report arboviral surveillance results for mosquitoes and dead birds in California. Many agencies also utilized rapid antigen tests (VecTest or RAMP) in the first few years after WNV's arrival in California, but these agencies have discontinued their broad use for surveillance, because they are much less sensitive than RT-PCR (Chiles et al. 2004, Brault et al. 2015). Acccordingly, since 2018, California's proficiency panels have not included rapid antigen testing.

A timeline of important changes in proficiency panels and local mosquito control agencies' RT-PCR testing is shown in Figure 1. From 2006-2008, local agencies that conducted RT-PCR testing utilized only singleplex assays for WNV, then beginning in 2009, some local agencies began to adopt multiplex testing for the three main endemic arboviruses of public-health importance in California: WNV, St. Louis encephalitis virus (SLEV), and western equine encephalomyelitis virus (WEEV).

History of proficiency panels

Since the inception of California's arboviral proficiency panel program in 2006, all panels have included inactivated virus samples, along with at least one negative sample. WNV has been included in panels for all years, and SLEV was added to the panel in 2009, followed by the addition of WEEV starting in 2014.

From 2006 to 2015, the panels' positive coded samples had consisted of a randomly sorted dilution series of WNV, along with a negative control and a single sample of SLEV (2009-2013) or SLEV+WEEV (2014-2015).

Starting in 2016, panels were modified to include simulated mosquito pool samples as unknowns in an effort to represent mosquito pool testing more realistically. These simulated pools consisted of mosquito slurry spiked with one or more aliquots of inactivated virus. From 2016-2021, the unknown samples included several simulated mosquito pools spiked with WNV at various titers, one simulated mosquito pool containing mixed WEEV and SLEV, and one negative control. Also, to test local laboratories' dilution technique and amplification efficiency, recent panels included one vial each of undiluted, inactivated WNV (2016-present) and SLEV (2017-present), from which district staff were asked to perform RNA extraction and prepare a 10-fold dilution series before testing the resulting samples by RT-PCR.

In 2021, the same general format of simulated mosquito pools and dilution series for WNV and SLEV was retained, but in an effort to increase the realism of unknown samples, the numbers of simulated mosquito pools that were expected to be positive or negative for each arbovirus (WNV, SLEV, or WEEV) was not revealed to local laboratories at the time of testing. This differed from previous years in which WNV was expected to be in all but two of the unknown samples (i.e., the negative sample and the WEEV+SLEV sample).

Conclusions

Throughout the program's history, California's annual proficiency panels have helped to standardize testing by local mosquito control laboratories. Most participating laboratories have been able to distinguish accurately between positive and negative unknown samples on all panels. However, in some cases, repeated testing has been required to obtain accurate results, and variation among

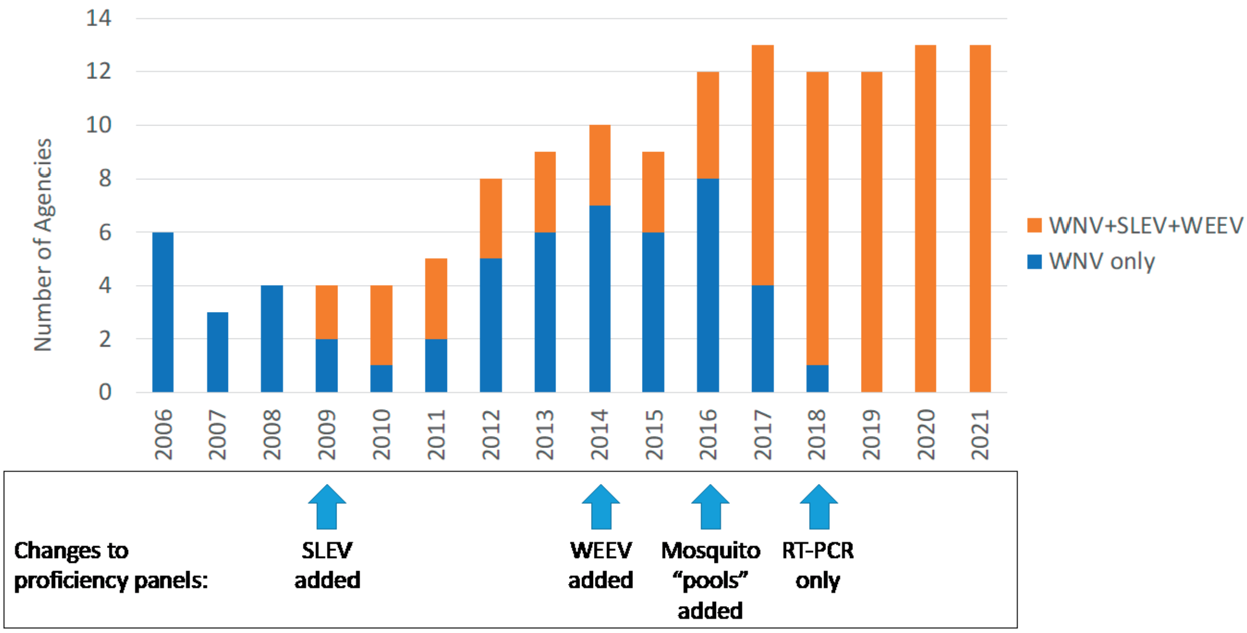

Figure 1.—Timeline of key changes in proficiency panels and local mosquito control agencies' RT-PCR testing.

laboratories remains an issue in recent years. Inconsistencies on panel results for a few agencies have been addressed through submission of early-season positive and negative samples for paired testing locally and at DART, which has allowed for local troubleshooting and quality assurance for statewide reporting. Proficiency panels have fostered productive statewide conversations among VBDS, DART, and the MVCAC Laboratory Technologies Committee each year to continue to find ways to improve California's arboviral testing and ensure consistency among reported results.

Acknowledgements

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