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Plenary Session

President's Opening Remarks

Stephen Abshier 1

Historical and personal reflections on mosquito ecology and control

L. Philip Lounibos 3

Generative AI in mosquito control: Opportunities and challenges

Robert Ferdan, Eric Haas-Stapleton, and Ryan Clausnitzer 4

Finding the right axis for access: Overcoming property inspection barriers in dengue control

Gilbert Holguin 5

Dengue in the Golden State: An operations approach to balancing fresh strategies, limited resources, and the risk of running on empty!

Gilbert Holguin 6

Poster Session

Small But Mite-y: An often-Overlooked Aspect of Rodent Control

Wade Lee 7

Bugs, Bytes and Budgets: A Deep Dive into Vector Control IT Costs

Eric Haas-Stapleton with the MVCAC Laboratory Technologies and Information Technology Committees 8

Clearing 724 Pools in 9 Weeks!

Brian Reisinger, Michelle Brown, Kecia Pitts-Love, and Alfonso Melgoza 10

Effects of microplastics (MPs) on the emergence rate, developmental time, and wing length of *Culex quinquefasciatus*

Anurag Joshi 11

Lookin' for Ticks (*Dermacentor*) in all the wrong places

Angie Nakano, Arielle Crews, Tara Roth, and Theresa Shelton 13

Mark-release-recapture of packed and shipped *Aedes aegypti* with *Wolbachia*: implications for conducting remote Incompatible Insect Technique programs

Johanna R. Ohm 16

Profiling mosquito kdr allele L1014F in San Diego County

Roxana Flores, Scott Vo, Nathan McConnell, Allison Bray, Emily Ferrill, and Nikos Gurfield 17

Evaluation of a multiplex qPCR assay for arbovirus detection in mosquitoes and birds: Improving consistency in results

Miguel Barretto, Ryan Clausnitzer, and Eric Haas-Stapleton 20

Innovative 3D Printed Traps: A cost-effective solution for mosquito surveillance

Kelly Furey 22

Evaluating insecticide resistance of adult *Culex tarsalis* to pyrethroids and malathion in Colusa County, 2022–2024

Kim Douglass, Hannah Romo, and Dan Kiely 24

Mosquito Abatement Through Empowerment (MATE) tractor farming

Dereje Alemayehu, Carolyn Baysdorfer, Helen Beyena, Eric Haas-Stapleton, and Ryan Clausnitzer 25

William Walton Poster Competition

William E. Walton Poster Competition

Sarah Wheeler 27

Addressing challenges in invasive *Aedes* surveillance by a Code Enforcement Program outside of a mosquito abatement district

Nader Shatara 28

At the larval stage – Identifying and processing samples

Eric Ortiz, Gabriela Perezchica-Harvey, and Jennifer A. Henke 29

Bloodfeeding patterns of <i>Aedes aegypti</i> in San Joaquin County, California	
<i>Lemya Mohamed, Tara Thiemann, and Shaoming Huang</i>	33
Control efforts in agriculture areas in the Coachella Valley	
<i>Michael Chylik, Salvador Becerra, Antonio Molina, and Gregorio Alvarado</i>	35
Determinants of immature <i>Aedes dorsalis</i> distribution in a dynamic environment	
<i>Annika Olson, Miguel Barretto, Sarah Lawton, Erick Gaona, Ryan Clausnitzer, and Eric Haas-Stapleton</i>	36
Diel Dynamics of <i>Aedes aegypti</i> in San Joaquin County, California	
<i>Noah Moral, Sumiko De La Vega, Omar Khweiss, John Fritz, and Shaoming Huang</i>	38
Effects of scales on age-grading <i>Culex tarsalis</i> using near infrared spectroscopy	
<i>Jewel Henry, Tara Thiemann, and Michael A. Riehle</i>	39
Electrifying drones: Ford F-150 Lightning powers a greener mosquito control future	
<i>Mark Wieland and Eric Haas-Stapleton</i>	41
Evaluating a drone application of VectoBac FG+ in a duck club pond	
<i>Alex Echenberg, Kim Y. Hung, Rick Ortiz, Iver Romero, and Jennifer A. Henke</i>	42
Evaluating pyrethroid resistance in the invasive mosquito, <i>Aedes aegypti</i>, in Madera, CA	
<i>Matthew Hernandez, Abraham Velazquez, and Brian Tsukimura</i>	47
Investigating the prevalence and ecology of a novel SFG <i>Rickettsia</i> in the San Francisco Bay Area	
<i>Naomi Putrika</i>	48
Optimizing <i>Culex quinquefasciatus</i> CQ1 Colony Supply: Quantitative analysis of key developmental parameters	
<i>Kendra Angel-Adkinson</i>	49
Optimizing urban mosquito management: A Mapping System for efficient source control in the Coachella Valley	
<i>Jeffery Rushing, Salvador Becerra, Antonio Molina, Gregorio Alvarado, Edward Prendez, and Marko Petrovic</i>	50
Rest in Peace, Not in Puddles: How sand is helping to solve cemetery mosquito problems	
<i>Erick Arriaga, Andrea Troupin, and Conlin Reis</i>	56
Testing efficacy of capturing gravid mosquitoes with CO₂ producing yeast encephalitis vector survey traps	
<i>Eric Moyung, Ryan Clausnitzer, and Eric Haas-Stapleton</i>	57
U.A.S product characterization	
<i>Iver Romero, Richard Ortiz, Kim Hung, and Alexandra Echenberg</i>	59
Why calibrate? Ensuring accuracy in mosquito control	
<i>Marisa Kelling, Gonzalo Valadez, and Vincent Valenzuela</i>	62
Yeast vs dry ice baited EVS trap efficacy comparison	
<i>Emma McDonough, Jack Mills, Antonio Cuevas, Ivy Munnerlyn, Stephen Bowling, Michael Pini, Nader Sidhom, and Amanda Poulsen</i>	66

Monday Afternoon Session A. How Messaging and Outreach can Reduce the Risk of Vector-borne Disease

Buzzing into action: Navigating dengue with dynamic outreach and rapid response	
<i>Anais Medina Diaz</i>	68
Changing behavior at cemeteries to reduce the risk of mosquitoes through messaging	
<i>Heidi Budge, Paula Macedo, Steve Schutz, David Wexler, and Nola Woods</i>	69
Amplifying hosts: developing community leaders to spread prevention information	
<i>Kriztian Luna Corona</i>	71
Not just another tool in the social media toolbox: How Nextdoor can target your communication	
<i>Andrew Pierce, Tim Mann, David Wexler, and Nola Woods</i>	74
Where shall we start? How to start building a classroom-based Mosquito and Vector Control curriculum	
<i>Judith Pierce and Rachel Curtis-Robles</i>	76
Neighbors love free stuff: Using Nextdoor as an outreach platform	
<i>Rachel Curtis-Robles, Kacie Ring, Tara Roth, Angie Nakano, Arielle Crews, and Theresa Shelton</i>	78

Monday Afternoon Session B. Administrative and Managerial Topics

Would you like I.C.E. with that? An all-encompassing guide to living with the new mandates of EV compliance	
<i>David Wexler and Casey Stevenson</i>	79
California CLASS: A Local Government Investment Pool option for mosquito abatement districts	
<i>Ryan Clausnitzer</i>	80
Cybersecurity readiness: Safeguarding your network infrastructure	
<i>Wayne Shieh</i>	81
Empowering improvement: Building growth through supportive field evaluations	
<i>Marco Gaytan</i>	82

Monday Afternoon Session B. Mosquitofish

Indoor Fish Rearing: A sustainable shift from traditional outdoor storage	
<i>Mark Wieland</i>	83
Delta MVCD Mosquitofish Program: Current and future directions	
<i>Mark Nakata</i>	84
Origin and evolution of the Lake County Mosquitofish Program over time	
<i>Sandi Courcier</i>	85
Maintaining tanks and earthen and lined ponds for fish culture	
<i>Darrell Jew</i>	89
Sacramento-Yolo Mosquito & Vector Control District program overview for culturing <i>Gambusia affinis</i>	
<i>Ken Harris</i>	90
Standard Operating Procedure essentials for mosquitofish program success	
<i>Michael V. Saba</i>	91

Tuesday Morning Session A. Mosquito and Vector Surveillance

Controlling mosquitoes in large complex sewage-contaminated flood area	
<i>Jason Mendoza, Ray Valerio, Ray Rivera, Gillian McDivitt, Stacey Mesler, Ralph Marquez, Nathan McConnell, Wa Yang, Daniel Ramsey, Dominique Edwards, Sarah Grewal, John-Ross Glueck, and Nikos Gurfield</i>	92
An evolving approach to neglected swimming pools	
<i>Jeremy Shannon, David Wexler, Terry Davis, Wayne Shieh, Nola Woods, and Andrew Pierce</i>	93
Evaluation of RT-qPCR reagents for mosquito pool and dead bird testing	
<i>Kara Kelley, Mario Novelo, and Sarah S. Wheeler</i>	94

Tuesday Morning Session B. Mosquito Resistance and Control

Exploring resistance in <i>Culex tarsalis</i>: linking bottle bioassays to sentinel cages to field trials	
<i>Tara C. Thiemann, Sarah Wheeler, Mario Novelo Canto, Debbie Dritz, John Mayberry, Natalie Linker, and Leen Yousef</i>	97
Resisting resistance: Identifying biochemical biomarkers for pyrethroid resistance in <i>Aedes aegypti</i> mosquitoes	
<i>Carla-Cristina “CC” Edwards</i>	98
West Nile virus and Mosquito Control at East Side Mosquito Abatement District, Modesto, California	
<i>Rachel Mendoza</i>	99

Tuesday Morning Session C. Novel Mosquito Control Trials

Restricting mosquito use of underground storm drain systems in the Coachella Valley by application of botanical repellents	
<i>Elsbeth Cannell, David Popko, Alec C. Gerry, and Karthikeyan Chandrasegaran</i>	100

Suppression of <i>Aedes aegypti</i> using Incompatible Insect Technique in the British Virgin Islands	
<i>Johanna R. Ohm</i>	106
Fine-scale modeling of West Nile virus vector dynamics to inform vector control trial design	
<i>Talia S. Wong and Christopher M. Barker</i>	107
First year of targeted sterile insect releases to control <i>Aedes aegypti</i> in the West Valley region of southern California	
<i>Solomon K. Birhanie, Jennifer Thieme Castellon, Jacob Hans, Ale Macias, Rubi Casas, and Michelle Q. Brown</i>	109
Facilitating California District-led Sterile- and Incompatible-insect programs (SIT & IIT) against <i>Aedes aegypti</i> and <i>Aedes albopictus</i>	
<i>Stephen Dobson</i>	110

Tuesday Morning Session D. Vertebrate and Non-Vertebrate Control Measures

From the sewers to the streets: Investigating Norway rat activity above and below ground in the City of Oakland	
<i>Michelle-Ann Meas</i>	111
Improvements to the Contra Costa Mosquito and Vector Control District Rodent inspection and monitoring program	
<i>Terry Davis, David Wexler, Tim Mann, and Jeremy Shannon</i>	112
Surveillance of <i>Rickettsia</i> species in Alameda County associated with wildlife and commensal rodents	
<i>Adena Why</i>	114
Investigating the effects of prescribed fire on tick abundance in Santa Clara County grasslands	
<i>Victoria Phillips, Dana Page, Jared Bond, Noor Tietze, and Nayer Zahiri</i>	115

Tuesday Morning Session E. Data Visualization Tools

What's new with VectorSurv	
<i>Tim Valdepena, Lincoln Wells, Kylie Pace, Jody Simpson, Kurt Johnson, Shawn Ranck, Alice Chang, Christina De Cesaris, Olivia Winokur, Aynaz Lotfata, and Christopher M. Barker</i>	120
VectorSurv tools for tick and tick-borne pathogen surveillance	
<i>Lincoln Wells, Kurt Johnson, Alice Chang, Christina Marie De Cesaris, Aynaz Lotfata, Kylie Pace, Shawn Ranck, Jody Simpson, Olivia Winokur, Tim Valdepena, and Christopher M. Barker</i>	122
Beyond the Gateway: Making decisions with your VectorSurv data	
<i>Olivia Winokur, Tim Valdepena, Christina De Cesaris, Kylie Pace, Jody Simpson, Kurt Johnson, Shawn Ranck, Lincoln Wells, Alice Chang, and Christopher M. Barker</i>	124
Real-Time Dengue risk mapping Using VectorSurv	
<i>Aynaz Lotfata, Jody Simpson, Olivia Winokur, and Christopher M. Barker</i>	126
Generating Tableau reports with VectorSurv data	
<i>Kim Y. Hung, Melissa Snelling, and Jennifer A. Henke</i>	127
Creating Reports in Microsoft Power BI using the VectorSurv Gateway	
<i>Jung Ma</i>	128
Using R to design Mosquito Trapping Routes, Visualize Data, and Run Statistical Analysis	
<i>E. Taylor Kelly, Katrina Snyder, Noor Tietze, and Nayer Zahiri</i>	129
Evaluating Vetech IDX™: AI-driven identification for enhanced vector management	
<i>Casey Hubble, Mary Sorensen, Keiko Parker, Marissa Utterback, Tyler Stuhaan, Melissa Cooke, and Jake Hartle</i>	130
A GIS protocol for high-resolution mapping of mosquito abundance using mosquito trapping data and land use variables	
<i>Karen E. Click, Isik Unlu, and Christopher M. Barker</i>	136
Tracking Heat Illness	
<i>Tim Mann, Wayne Shieh, and Jeremy Shannon</i>	138

Tuesday Morning Session F. Vector-borne Disease in CA

Epidemiological trends of dengue in California	
<i>Mary Danforth, Andrea Lund, and Vicki Kramer</i>	139
Out for Blood: Standardizing local dengue case monitoring	
<i>Tristan Hallum</i>	140
MVCAC Managers vs. Biologists: who can predict West Nile virus disease?	
<i>Christopher Barker, Aynaz Lotfata, Hector Sanchez-Castellanos, and Tomás León</i>	141
2024 West Nile Virus California Forecasting Challenge	
<i>Héctor M. Sánchez C., Mary Beth Danforth, Andrea Lund, Vicki Kramer, and Tomás León</i>	142
Low risk for locally acquired Chagas disease in California: A review of human cases and triatomine submissions, 2013–2023	
<i>Andrea J. Lund, Marco E. Metzger, Vicki L. Kramer, and Anne M. Kjemtrup</i>	143
Evaluation of different approaches to detect the flea borne disease, <i>Rickettsia typhi</i>	
<i>S. Hofer and N.S. Tietze</i>	144
Recent plague activity in California: A winter domestic cat plague case and other surveillance oddities	
<i>Gregory M. Hacker and Christian Irian</i>	146
A Treehouse Tale of Soft Tick Relapsing Fever	
<i>Kim Douglass, Tony Kovach, Christian Irian, and Anne Kjemtrup</i>	147
Investigation of a novel spotted fever group rickettsiosis in California	
<i>Megan Saunders</i>	148
Enhancing <i>Dermacentor</i> tick surveillance	
<i>Gillian A McDivitt, Scott Vo, Jessica Dieckmann, Raymond Rivera, Saran Grewal, and Nikos Gurfield</i>	149
<i>Rhipicephalus sanguineus</i> infestation in South Sacramento - A case study	
<i>Mario Novelo, Whitney Clack, Megan Siefker, Andrew Cha, Ron Burkhouse, Janet Foley, Francesca Rubino, and Sarah Wheeler</i>	152
Surveillance for mosquito-borne virus activity in California, 2024	
<i>Hannah Romo, Tina Feiszli, Mary Beth Danforth, Andrea Lund, Marie Cerda, Anil Singapuri, Tim L. Valdepena, Jody Simpson, Christopher M. Barker, Sharon Messenger, and Vicki Kramer</i>	153

Tuesday Afternoon Session A. Application Equipment

A decade of truck mounted larviciding	
<i>David Lopez</i>	161
Mist blower rotary atomizer adjustments and droplet data collection	
<i>Marty Scholl</i>	162
Which Way to WALs? A new method for setting up WALs transect trials	
<i>Sarah S. Wheeler, Mario Novelo, David Smith, and Marty Scholl</i>	165
Trending techniques for analyzing aerial and ground-based mosquito control applications	
<i>Bill Reynolds</i>	168
Optimizing Unmanned Aircraft System operations at Placer Mosquito and Vector Control District with AirData	
<i>Scott Schon, Ev Ortiz, and Joel Buettner</i>	169
AMCA Drone Program Update 2025	
<i>Joel Buettner</i>	170

Tuesday Afternoon Session B. Invasive *Aedes* Successes and Failures

Spectroscopic age grading of <i>Aedes aegypti</i>: Techniques and Confounding factors	
<i>Dalton Manbeck-Mosig, Christopher Barker, Sunny An, Steve Vetrone, Tanya Posey, Nicolas Tremblay, Ryan Amick, and Zili Gao</i>	171

Integrating eDNA and qPCR for early detection and surveillance of mosquito larval habitats <i>Miguel Barretto</i>	173
Battle of the BG's: A comparative analysis of Biogents sentinel and BG Pro traps for <i>Aedes aegypti</i> surveillance in Sacramento, California <i>Whitney Clack, Mario Novelo, David Smith, and Sarah S. Wheeler</i>	174
Contra Costa Mosquito & Vector Control District versus <i>Aedes aegypti</i>, a tale of two cities <i>Steve Schutz, Eric Ghilarducci, Damien Clauson, and Olivia Zaragoza</i>	178
Evaluation of A1 efficacy on larval breeding cups in front and back yards following <i>Aedes aegypti</i> detections in San Jose <i>E. Taylor Kelly</i>	182
Assessing the efficacy of Zenivex E20 against <i>Aedes</i> and <i>Culex</i> mosquitoes: Insights from field trials and lessons learned <i>Joseph Songer</i>	183
Evaluation of larviciding as an <i>Aedes</i> control strategy <i>Jennifer A. Henke, Gabriela Perezchica-Harvey, Kim Y. Hung, Greg Alvarado, Robert C. Gaona, and Edward H. Prendez, Jr.</i>	184
In2Care stations, control, and community outreach <i>Brian Reisinger, Michelle Brown, Solomon Birhanie, and Alfonso Melgoza</i>	185
Progress and Challenges: First year of <i>Aedes aegypti</i> releases in a Sterile Insect Technique (SIT) pilot program in Los Angeles County <i>Ryan Amick, Nicolas Tremblay, Colt Bellman, Courtney Chagolla, Christie K. Miranda, Rande Gallant, Tanya Posey, and Steve Vetrone</i>	186
Dengue in our backyard: Insights and innovations from 2024 <i>Jason Farned</i>	194
Locally acquired dengue response in Panorama City, California <i>Maritza Olmos</i>	195
Submitted manuscript [not presented]	
The Scientific Legacy of Jan O. Washburn (1952–2024) <i>Ryan Clausnitzer, David Egerter, Gordon Frankie, Eric Haas-Stapleton, L. Philip Lounibos, Eric Lyons, Taro Ohkawa, Dave Wagner, and Libe Washburn</i>	196

President's Opening Remarks

Stephen Abshier*

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Let me extend a warm welcome and thank you for your attendance and participation in the 93rd MVCAC Annual Conference in Oakland, California. My name is Stephen Abshier and I am the District Manager at the Sutter-Yuba Mosquito and Vector Control District and the President of the MVCAC for 2025. It has been my privilege to work with the membership and our management firm AMG to plan Conference. I take this opportunity to welcome participants from mosquito and vector control agencies, public health, and academia from across California, many parts of the country and perhaps the world.

This conference, convened annually, gives us the opportunity to reflect on the work our members have engaged in over the last season, finding answers, forming conclusions or recognizing that more data must be collected to evaluate theories, ideas and strategies. Our keynote speaker this morning is Dr. Phillip Lounibos of the Florida Medical Entomology Laboratory. His experiences over the course of his career will certainly inspire and encourage the ongoing work at our agencies. The remainder of the plenary session this morning and the symposia over the next days will continue to educate and inform members and attendees.

I first thank the many speakers, presenters and poster makers willing to share their experiences and their results. Thank you to our moderators, volunteers, and all of our exhibitors for making this meeting possible. I take a moment to especially thank the sponsors of this conference, as we cannot have this event without their support: **Azelis, Central Life Sciences, Valent, AMGUARD, Vesperis, Five Star Bank, Clarke, Target Specialty Products, and SCI.** Please make it a priority to meet and engage with our sponsors and the many exhibitors in the hall.

During our conference, we will hear about the successes and challenges of new or reformed strategies conceptualized in the minds of those that take up the work of marginalizing the health threats posed by vectors that reach into our communities, impacting untold lives around us. I want to be inclusive and say thank you to all of you. You are the innovators, evaluators and overcomers, builders and fabricators, engineers and improvisors, planners and organizers, where you carry out work in your District, county, agency or jurisdiction. It has been an honor to plan and organize this year's conference to recognize the important work that you do.

The theme of this year's conference is "Vector Control; A path to a more hospitable world." If we take a step back and examine human history, ours has been a struggle to thrive in

the reality of a world filled with threats to our health and wellness. From violent human conflict, to dangerous encounters with terrestrial and aquatic wildlife, to microscopic pathogens, real dangers exist and affect us in our world. Throughout history, civilizations have risen and fallen, and have been shaped by the threats they faced, including the morbidity and mortality caused by vector-borne illnesses. The names given to devastating illnesses such as malaria, which means "bad air", were based on misinformed beliefs. Events like the Black Death that spread across Europe in the mid 1300's, that took as many as 30 to 50% of the population of Europe were not understood. The thought that a microscopic 'germ' could infect and cause illness or death was not proven until the late 19th century. This discovery caused a paradigm shift, opening a new world of scientific exploration to understand and mitigate these unseen threats.

There is quite a difference between understanding a cause-effect relationship to infection and building an operational strategy that effectively interferes in the transmission of disease. Pioneers led with courage and curiosity such as Jesse William Lazear (who proved mosquitoes carried yellow fever and died from the disease), Walter Reed (an Army doctor and scientist who led a team to Cuba to test the idea that mosquitoes spread yellow fever), and Clara Maass (a nurse who volunteered to be bitten by infected mosquitoes to study yellow fever and died as a result). William B. Herms was instrumental in developing malaria control programs in California, followed by his student William C. Reeves who largely discovered the relationships among *Culex tarsalis* and transmission of Western equine and St Louis encephalitis viruses.

In our present complicated world, transmission of mosquito and vector-borne illnesses have been slowed where organized control measures have been established and effectively implemented, but these pathogens still 'tear' through communities around the world, causing serious illness and mortality that approach millions annually.

At my District, in the early 1990's we occasionally detected Western equine encephalitis virus in mosquito samples. At that time, it was sobering for me to think about the risk of mosquitoes possibly infecting someone. Although we did not see human illness, this was a first awakening and close encounter for me to the reality of the serious nature of mosquito-borne illnesses and the need for control. Since the introduction of West Nile virus into the US in 1999, tens of thousands of infections have caused illness and death across

the country including 154 human cases with 7 deaths in my District. There are many more infections that go undiagnosed and unreported. From interactions we have with members of our communities, we know so many people know of someone or have had a West Nile infection themselves.

With recent local transmission of dengue in California, we are confronted with new risks to human health. We're grappling for new solutions, novel strategies and mitigation measures around an invasive species of mosquito that has been successful in establishing itself. We are learning from both failures and successes in controlling this species, but this ignites a fresh and invigorated effort with the goal to ultimately reduce human suffering. Later this afternoon, I'm pleased to have Dr Phuong Luu, Public Health Officer for Sutter and Yuba Counties, share her work in the dengue endemic areas of the Pacific Islands.

Through scientific curiosity, discovery, trials, successes and failures, improvement to human health and longevity has progressed. Humanity tends to be curious and hungry for understanding. An inherent problem-solving nature provides a path to unlock knowledge and solutions that promote both prevention and healing.

As vector control specialists, we focus on a small segment of human health threats. Guided by a scientific approach to investigate and understand disease, and the mechanisms by which they are transmitted causing illness and mortality, we are part of a group dedicated to making our world more hospitable. Our mission, in part, is to break the transmission cycle and reduce the risks of illness and suffering. We are all part of that effort, both through our state association and at a more granular level field technicians, laboratory technicians, administrative, data management and information technologies, public education and messaging, vehicle and equipment maintenance, rearing of mosquitofish and other biologicals, policy makers, oversight bodies, lobbyists, advocates, researchers, product manufacturers and suppliers are all critical elements in the chain. As a state association made up of a diverse group of committees and varied geographical regions we are a part of, we work together, learn from each other, and advocate for solutions that help in our efforts to make our world a better place.

A personal hope for me for this conference is that it would be a time to reflect on the difference we make in the lives of people in the communities we serve. Because of the efforts we make and commitments we keep, we positively

impact environmental quality, public health and reduce illness. Generally, the better we perform our jobs, and the more successful we are at eliminating annoyance and reducing vectors, the less recognition we get. We become a forgotten group until we make the news, detections of disease surface, or a surge in mosquito abundance causes the phone to ring. It may be impossible to calculate the difference we make; however, when we look backward in time, we are assured that organized mosquito and vector control reduces illnesses and deaths and improves public health.

We live in an ever-changing world, where it is essential to innovate, as rules and regulations surrounding our traditional and proven methods are challenged by competing interests. We must continue to refine and improve existing strategies as well as innovate to find new ones and develop new tools and technologies that affect positive outcomes. Exploration into the use of tools such as drones to expand our vision and reach, and the genesis and discovery of novel approaches through the application of sterile insect techniques, are examples of focusing new technologies in different ways. Adopting new technologies is often expensive, and we don't necessarily experience good returns on these investments. They are costly in terms of our time, and are often filled with disappointment when our ideas and efforts fail. Exploration is a process not unlike wandering around a dimly lit space, filled with impediments, while trying to reach the other side. Well-constructed trials provide insight into how new ideas can be applied to enhance efficacy or performance. They may require the approval of regulatory agencies and often are replete with partial successes, failures, disappointments and delays, but coming up short frequently points us in better directions, hopefully leading to future success.

We stand at the threshold of an open door, to a powerful technology known as artificial intelligence. People express both hope that AI (artificial intelligence) can unlock knowledge and remedies to complex problems like cancer, as well as fears that our privacy, security and control will be erased. Whichever way you lean, it is nearly certain that AI will become more intertwined into our lives. I remain hopeful that AI will be steered as a tool to improve the human experience. It will be fascinating to participate in and observe how AI will be leveraged to diminish vector-borne illnesses.

In summary, I want to recognize and encourage all those who are engaged in and have made it their business to make a difference and to make our world a more hospitable place. Thank you for what you do.

Historical and personal reflections on mosquito ecology and control

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After periods of fluctuating emphasis on controlling pest vs. vector mosquitoes, Mosquito Control Districts (MCDs) in the USA currently prioritize public health above nuisance control. This emphasis at the moment is motivated throughout most of the country by the prevalence of West Nile fever, but in the majority of tropical countries malaria and dengue account for most morbidity and mortality attributable to pathogens transmitted by mosquitoes.

Despite the considerable research effort devoted to the topic, Service (1983) declared (in a plenary address at an American Mosquito Control Association (AMCA) meeting) that the prospects for the biological control of mosquitoes or the diseases they transmit were not promising. Despite this pessimism, local projects in subsequent decades demonstrated that in certain circumstances (1) polyphagous predators, such as cyclopoid copepods, may control *Aedes aegypti* and (2) biological control may suppress malaria, dengue, and other mosquito-borne diseases.

From the 15-19th centuries, invasive mosquito species ‘hitch-hiked’ between continents on sailing ships, whereas ocean transport for mosquitoes in the 20th and 21st centuries favored container ships. Mosquito species traits that favor invasion success include domesticity, desiccation-resistant eggs, and preferences for ecotonal (disturbed) habitats. The invasion of *Anopheles gambiae* s.l. into NE Brazil in the early 1930s led to high mortality and emigration from that area before this African vector species was eradicated by dry-season larviciding (Soper & Wilson 1943). Although the container species *Ae. aegypti* and *Aedes albopictus* have encountered one another frequently in their invasive or native ranges, competitive displacement of the former species by the latter is known from only two areas in the New World (Lounibos & Juliano 2018). The capacity of male *Ae. albopictus* to satyriize female *Ae. aegypti* in the SE USA but not in Brazil is the likely cause of the displacement of the Yellow Fever Mosquito in the USA, which was not observed in Brazil (Honorio et al. 2018). *Anopheles stephensi* in Africa is the most dangerous mosquito invasion of the 21st century, exacerbating malaria transmission in previously less affected dry and urban zones.

Research on obscure, non-vector species may inform future strategies for mosquito control. An example is illustrated from the North American pitcher plant mosquito *Wyeomyia smithii*, which is known to have evolved from the

Gulf Coast and Atlantic Coastal Plain to post-glacial pitcher-plant bogs in the northern USA and Canada (Bradshaw & Lounibos 1977). Laboratory selection for nonbiting among facultative blood-feeding *Wy. smithii* from the southern USA produced a nonbiting line whose differential gene expression mirrored that of a naturally evolved nonbiting population of the species from the northern USA (Bradshaw et al. 2017). Understanding the genetic control of autogeny in mosquitoes has potential applications for vector control.

Current control methods that release laboratory-modified mosquitoes are directed towards either suppression or modification of field populations. In a rural Kenyan village, domestic *Ae. aegypti* were eliminated by source reduction, and recolonization from neighboring villages was monitored by weekly surveillance. Recovery of *Ae. aegypti* numbers occurred within 6-8 weeks after the cessation of source reduction (Lounibos 2004), which argues for the preferability of population modification methods over suppression.

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Generative AI in mosquito control: Opportunities and challenges

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Abstract

Generative Artificial Intelligence (AI) is transforming many industries, including public health and vector control, by offering innovative ways to address operational and research challenges. In this plenary session, we introduced the historical context of AI, examined the differences between traditional machine learning and generative models, and discussed practical applications relevant to mosquito and vector control agencies.

Our foundational overview set the stage, tracing AI's evolution from early machine learning techniques, where computers learn from existing data to classify or predict outcomes, to today's generative models, which not only process information but also create new content. These advances enable more automated, efficient, and creative problem solving, such as identifying mosquitoes from images and analyzing large-scale entomological data sets.

The presentation highlighted key strengths of several AI platforms for tasks encountered by mosquito control professionals. Emphasis was placed on exploring the trade-offs between freely available models, which often collect user input to further train their algorithms, versus proprietary or specialized solutions with heightened security measures.

Despite these advantages, the session addressed AI's limitations. We covered the importance of recognizing inherent model biases, verifying the credibility of AI-generated outputs, and protecting sensitive data from potential breaches. By showcasing real-world scenarios, from drafting staff emails to generating images and videos for public outreach, the session underscored the need for human oversight to ensure quality, accuracy, and appropriateness.

Looking ahead, the presentation offered a vision of AI's continued integration into the public health sector, including emerging uses for automated call management. Attendees were left with practical guidelines to select the right AI tools, safeguard data, and effectively collaborate with AI to enhance operations. This plenary underscored that, when deployed responsibly, generative AI can be a powerful partner in advancing mosquito control and public health initiatives.

Finding the right axis for access: Overcoming property inspection barriers in dengue control

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Abstract

Recent dengue cases in our area underscore the critical need for effective outreach and control. Restricted property access in areas with active dengue cases severely limits essential inspections and treatments, which are vital for identifying and eliminating mosquito larval sources. These efforts help curb mosquito populations and prevent further transmission of the virus. Without timely intervention, unchecked sources enable mosquito populations to grow, increasing the risk of virus spread within the community. When access barriers delay these control efforts, the potential for outbreaks rise, making property access an urgent priority in dengue intervention.

This presentation outlined strategies used by the San Gabriel Valley MVCD to address these access challenges, including securing inspection warrants to gain entry to properties where access was previously denied. It also provided data on properties with mosquito sources, demonstrating the connection between access restrictions and increased disease risk, as well as the importance of community cooperation in the fight against dengue.

Dengue in the Golden State: An operations approach to balancing fresh strategies, limited resources, and the risk of running on empty!

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Abstract

Dengue viruses have become a significant public health concern in the Golden State, driven by rising global temperatures and shifting weather patterns that facilitate its spread. With the uptick of recent imported cases, it is essential to adopt a proactive approach that addresses immediate health risks and implements innovative mosquito control strategies. Fostering employee engagement and buy-in also is crucial for the success of these initiatives. Providing training and encouraging feedback enhances their commitment and empowers them to actively participate in dengue prevention efforts. However, we must also navigate the challenges of limited staffing, budget constraints, and the persistent threat of resource exhaustion. A successful operational framework relies on efficient resource allocation to prioritize high-risk areas, while balancing these demands with the urgent need for a public health response underscores the importance of developing sustainable practices that can endure resource limitations.

This presentation discussed how we balanced resources and prioritized our efforts in combating dengue by leveraging data to inform our decision-making. By creating a prioritized list of programs and tasks, we were able to allocate resources strategically and maximize our impact. Regular check-ins with employees were crucial for ensuring their well-being and preventing burnout, allowing us to maintain a motivated and engaged team. This holistic approach not only enhanced our operational efficiency, but also fostered a supportive work environment that empowered our staff to contribute effectively to dengue control initiatives.

Small But Mite-y: An often-Overlooked Aspect of Rodent Control

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Abstract

Often, residents hire a pest control service to rid themselves of commensal rat issues but ectoparasites are overlooked. *Laelaps echidninus* (spiny rat mite) and *Ornithonyssus bacoti* (tropical rat mite) are mite species commonly found among commensal rats within Alameda County.

These hematophagous mites are parasites of *Rattus rattus* and *Rattus norvegicus*. Mite bites can reach an unbearable level, especially when their primary rat host is removed from a residence. The mites can scatter from the vacant nests in search of a bloodmeal.

Though these mites are not known as disease vectors, they can be a cause of dermatitis and mental anguish when they bite residents. Taxonomically, mites are acarids and are closely related to ticks. By identifying rodent mites, we can distinguish the offending mites from other potential biting arthropod and non-arthropod related causes.

Bugs, Bytes and Budgets: A Deep Dive into Vector Control IT Costs

Eric Haas-Stapleton^{1*} with the MVCAC Laboratory Technologies and Information Technology Committees²

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Abstract

As mosquito and vector control programs become increasingly data-driven, understanding the technological landscape across California's districts is critical for strategic planning and resource allocation. In 2024, the Laboratory Technologies and Information Technology Committees of the Mosquito and Vector Control Association of California (MVCAC) conducted a statewide survey to assess software usage, IT staffing, and associated costs among member districts. With an 31% response rate (18 districts), the survey revealed that although nearly all districts rely on CalSurv for surveillance data entry, many supplement it with platforms such as ArcGIS, Tableau, Power BI, and custom tools for data analysis and visualization.

Most districts use software to record mature vector populations, insect pathogens and service requests, with fewer tracking immature vectors or dead birds. Software costs were typically under \$25,000 annually, and hardware expenses remained below \$10,000 for most districts. Notably, the number of users did not correlate with IT costs per user, which varied widely, highlighting diverse approaches to infrastructure investment. The median staffing allocation for IT support was 0.4 full-time equivalents (FTE), with most districts replacing hardware and software on a roughly two-year cycle.

These findings provide a snapshot of current practices and challenges in IT management for vector control, emphasizing the need for scalable, cost-effective solutions and opportunities for shared resources or training across districts.



Bugs, Bytes and Budgets: A Deep Dive into Vector Control IT Costs

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Objectives

Determine what vector data is recorded by vector control districts, the software used, and overall costs.

Project Design

- MVCAC Laboratory Technologies and IT Committees collaborated to design survey questions.
- Advertised through MVCAC News Briefs and Regional Representatives.
- Available from March – May of 2024 through Google Forms.
- 31% (18) districts responded.



MVCAC: Software used by districts for vector surveillance and control

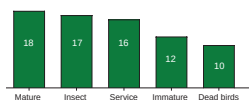
The MVCAC Information Technology and Laboratory Technologies Committees are seeking to understand the software and hardware that is used by districts to record, analyze and visualize data that is collected for monitoring and controlling vectors.

The purpose of this survey is to better inform the MVCAC membership of methods that agencies use to document their surveillance and operational outcomes.

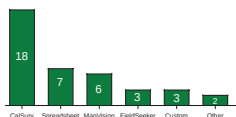
We will share the outcomes of this survey to the membership in a presentation at a quarterly or annual MVCAC meeting.

Districts Survey of Software Uses and Costs

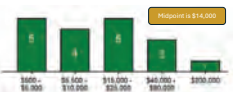
Number of districts that record each category



Recording the data: Number of districts using each software



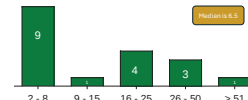
How much do districts pay for software every year?



Replacement timeline for computer hardware and software



Number of software users at each district



Analyzing the data: Number of districts using each software



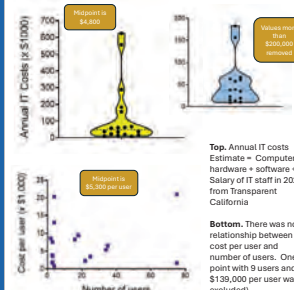
How much do districts pay for computer hardware every year?



How much staff time in full-time equivalents (FTE) do districts devote to managing computer hardware and software?



Annual IT Cost Estimate



Key Survey Insights

All districts use CalSurv. Most employ software to log mature vectors, insect pathogens, & service requests.

Most rely on multiple software systems to analyze vector data.

Annual software costs are typically less than \$25,000, and hardware costs are below \$10,000.

IT costs per user do not correlate with the number of users in a district.

Acknowledgements

We extend our gratitude to colleagues statewide for responding to the survey and making this study possible.

Clearing 724 Pools in 9 Weeks!

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Introduction

Unkempt swimming pools pose a well-known mosquito breeding risk to a neighborhood. They can also take an incredible amount of time and resources to bring back into compliance for vector control districts by traditional means, e.g. creating service requests and sending a technician to address.

Methods

In order to efficiently gain compliance, the District devised an alternate method of addressing swimming pools, using administrative staff to handle the majority of the work regarding pools. We began by vetting the pictures provided by

Aerial Services, then we generated a list and mailed residents a letter with an accompanying picture of the pool. Residents were given seven days to comply, proof of compliance was achieved by sending the District a picture via text or email. Non-compliant residents were sent a second letter, and those still non-compliant received a direct posting on their doors.

Conclusions

Most pools were handled with direct mailing with only a sparing use of direct posting and traditional service requests for non-compliant residents. This method has proven to give us quicker and more thorough compliance than traditional service requests methods and significantly reduced the man-hours allocated to pools.

Effects of microplastics (MPs) on the emergence rate, developmental time, and wing length of *Culex quinquefasciatus*

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Abstract

Microplastics (MPs) are plastic fragments that are greater than 1nm and less than 5 mm in size that are ubiquitous environmental contaminants. Worldwide, MPs are becoming prevalent and contaminating both terrestrial and marine habitats. Because the majority of research conducted thus far has focused on marine ecosystems, the effects of MP pollution on freshwater ecosystems is not well understood. Although there are many different paths for MPs to proliferate and a wide variety of animals actively consume MPs in freshwater environments, there is a dearth of research regarding the effects of MPs in these systems. Mosquitoes are aquatic insects during their larval stage and are susceptible to microplastic exposure. The present study examined how MPs impacted mosquito emergence and physiological traits such as wing length. We explored the potential effect of different concentrations (50 and 100 microplastic particles per milliliter (MPPs/ml)) of two main MPs, polystyrene (PS) and low-density polyethylene (LDPE), on the larval development and emergence of *Culex quinquefasciatus* in a controlled laboratory setting. Although there was a slight impact of concentration of both PS and LDPE on emergence rates and the wing length in female mosquitoes, we found no impact of MPs on mortality. This indicated that MPs had no impact on nutritional uptake by *Cx. quinquefasciatus*.

Effects of Microplastics (MPs) on emergence rate, developmental time, and wing length of *Culex quinquefasciatus*

By Anurag Joshi, Dr. Angela Caranci, Dr. Phillip Gedalanga, Greg Williams, Jessica Coolidge, and Jennifer Wong
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Abstract

Microplastics (MPs) are ubiquitous environmental contaminants that contaminate terrestrial and marine habitats worldwide. The effects of MPs pollution on freshwater ecosystems are still poorly understood, especially in aquatic insects like mosquitoes. This study examines the impact of different concentrations of polystyrene (PS) and low-density polyethylene (LDPE) on *Culex quinquefasciatus* larval stages and adult emergence. Results show slight impacts on emergence rates and wing length in female mosquitoes, but no impact on mortality rates. The study aims to identify knowledge gaps and future research directions in this critical area.

Introduction

- Microplastics (MPs) are a growing environmental concern, primarily consumed by filter-feeding animals like mosquitoes and zooplankton.
- To understand their ecological impacts, toxicity tests using natural exposure times and concentrations are needed.
- MPs exposure can alter mosquito development and physiological traits, potentially affecting human health and disease transmission.
- Studying MPs' effects on *Culex* mosquitoes could lead to new control methods.

Objective

- This research aimed to assess the impact of exposure-dependent differences in emergence, development time, and wing length of adult mosquitoes, both male and female, from first instar stages to adult mosquito.
- It also examined if MPs doses in a lab setting affect mosquito growth and development, as well as the length of the left wing in female mosquitoes.

Materials and Methods

- This experiment used Microplastic particles (MPPs) from microplastics polystyrene (PS) and low-density polyethylene (LDPE) in this study.
- 500 ml glass beakers (n=14) and porcelain trays were autoclaved to prevent cross-contamination.
- Two beakers with different concentrations of PS (low and high) and LDPE were used.
- The study involved beakers kept in constant conditions at NWMVCD, with constant temperature at 26.6° Celsius and 75% humidity.
- Body size was estimated using the length of the left wing from females and a dissecting microscope.



Figure 1-2: MPs and Larva Preparation

Figure 2-4: Larva Rearing

Figure 3-4: Larva Rearing

Results

Group	Survival rate	Wing Length (mm)	Male Emergence	Female Emergence	n
PS (Low)	97%	3.20	58%	42%	20
PS (High)	93%	3.25	61%	39%	20
LDPE (Low)	93%	3.22	63%	37%	20
LDPE (High)	92%	3.16	62%	38%	20
Control	93%	3.20	65%	35%	20

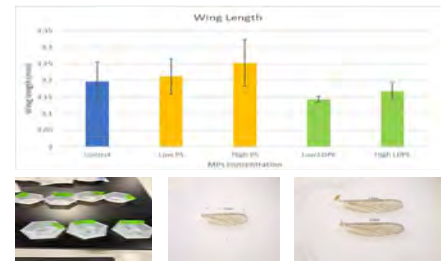
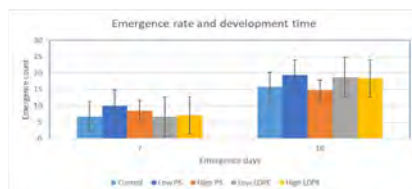


Figure 5: Adult Mosquitoes

Figure 7-8: Wing Measurement

Variable	n	F	p
PS	20	0.67	0.54
LDPE	20	0.04	0.95
Wing Length	20	0.79	0.47

Discussion

- Our findings show the features of emergence and wing length in species of *Culex* mosquitoes is unaffected by reasonably realistic dosages of 50 MPPs per milliliter or 100 MPPs per milliliter for PS and LDPE.
- We suggest that future studies recognize the complexity of MPs and create tests to better understand the effects of different MPs types, sizes, forms, and dosages on this and other mosquito species in semi-field settings rather than in a lab.

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Lookin' for Ticks (*Dermacentor*) in all the wrong places

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Introduction

On the central coast of California, it has been assumed that the locations of highest risk for encountering human-biting Ixodid tick species are natural areas such as trails, open space preserves, or other undeveloped land. This assumption has been largely supported by San Mateo County Mosquito and Vector Control District ("the District") collection data for the local Lyme disease vector tick *Ixodes pacificus*. However, the District has confirmed multiple reports of *Dermacentor* ticks in well-developed and/or landscaped suburban environments. Herein, we highlight some of the unexpected locations where the recently regionally renamed *Dermacentor similis* (previously *Dermacentor variabilis*) and/or the Pacific Coast tick (*Dermacentor occidentalis*) have been found in San Mateo County, California, by District field staff. This information may be especially pertinent in well developed, suburban settings, where residents can be caught unaware of the potential for tick exposure.

Methods/Materials

Ticks are typically collected by District staff by flagging low-lying vegetation and other surfaces with a flannel cloth, using standard collection methods as described by the CDC (2019). Ticks are identified either while alive or after being

killed with ethanol. Identification is conducted using morphological characteristics viewed through a dissecting microscope.

Tick surveys are conducted for a variety of purposes, including assessment of ticks and tick-borne pathogen presence in parks and recreation areas, inspections of residential yards as part of an ongoing District research project, and site investigations conducted in response to public complaints of tick encounters.

Results

Two species of human-biting *Dermacentor* ticks were commonly encountered in parts of San Mateo County, *D. occidentalis* and *D. similis*. Over the past few years, District staff have collected *Dermacentor* ticks from a few well-developed, suburban areas. One common factor is proximity to relatively small areas of undeveloped land. Two examples of such findings are highlighted below.

Site 1 – Apartment complex with fake rock wall (Fig. 1).

Adult *D. occidentalis* and *D. similis* were flagged from brushy vegetation at the base of a fabricated rock retention wall, which is located downhill from a small, grass-covered right-of-way. Mulch at the base of this wall was observed to be continuously wet due to drainage. The downhill



Figure 1.—Apartment complex where *Dermacentor* ticks were collected from base of rock wall. The apartment complex was in a well-developed suburban area of San Mateo County located downhill from a small undeveloped right-of-way.



Figure 2.—Undeveloped right-of-way adjacent to a fenced residential yard in which a San Mateo County resident found a *Dermacentor* tick on their dog. The dog had no recent travel history outside of the neighborhood and had not entered the right-of-way.

movement of *I. pacificus* and *D. occidentalis* has been previously documented (James et al. 2022).

Site 2 – Residential, fenced backyard next to undeveloped utility corridor (Fig. 2).

One adult *Dermacentor* tick was acquired by dog while in a solid wood-fenced backyard adjacent to a narrow strip of undeveloped land (~500 ft wide) serving as a utility corridor. This strip of undeveloped land is bordered by large suburban neighborhood on one side, and 10-lane freeway on other side. The topography of the undeveloped area is a combination of tule marsh and upland grassy area with blackberry bushes. Adult *Dermacentor* ticks were flagged from these blackberry bushes by District staff.

Discussion and Conclusion

Although multiple tick species are commonly encountered in San Mateo County, the majority of tick safety outreach and public education efforts has focused on the local vector of Lyme disease, *Ixodes pacificus*. As a result, public expectation of where ticks are present may be skewed towards environments favored by *I. pacificus*, such as oak woodlands, mixed forest, and coastal scrub environments, or areas frequented by black-tailed deer. The public and District staff alike have not anticipated encounters with other tick species, such as *D. occidentalis* and *D. similis*, occupying different environments. Although defensible from a public health perspective, tick safety

messaging solely emphasizing *I. pacificus* ecology may inadvertently reinforce misleading assumptions about where ticks can be found.

As increasing numbers of recently described tick-borne pathogens are associated with metastriate (non-*Ixodes*) tick species (Centers for Disease Control and Prevention 2020), our findings underscore both the importance of conducting field surveys in a wide variety of potential tick habitats and including these ticks and their unique ecology when crafting tick safety materials and messages.

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Lookin' for (*Dermacentor*) ticks in all the wrong places

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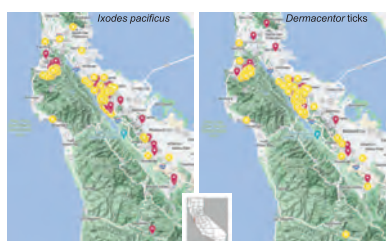
Introduction

This poster highlights some examples of "non-traditional" locations where *Dermacentor* ticks (*D. occidentalis* and *D. variabilis/similis*) have been observed in San Mateo County, California, by field staff of the San Mateo County Mosquito and Vector Control District ("District"). This information may be especially pertinent in well developed, suburban settings, where residents can be caught unaware of the potential for tick exposure.

Methods

Ticks are typically collected by District staff by flagging low-lying vegetation and other surfaces with a flannel cloth, using standard collection methods as described by the CDC¹. Ticks are identified either while alive or after being killed with ethanol. Identification is conducted using morphological characteristics viewed through a dissecting microscope.

Tick surveys are conducted for a variety of purposes, including assessment of ticks and tick-borne pathogen presence in parks and recreation areas, inspections of residential yards as part of an ongoing District research project, and site investigations conducted in response to public complaints of tick encounters.



Legend

- No ticks found
- Ticks found

The maps above show findings from residential tick inspections in San Mateo County conducted by District staff since 2022. *Dermacentor* spp. and *Ixodes pacificus* were often, but not always, found at the same sites.

Results

Two species of human-biting *Dermacentor* ticks are commonly encountered in parts of San Mateo County, *Dermacentor occidentalis* and *Dermacentor variabilis/similis*. Over the past few years, District staff have collected *Dermacentor* ticks from a handful of well-developed, suburban areas. One common factor is proximity to relatively small areas of undeveloped land. Two examples of such findings are highlighted below.

Site 1 – Apartment complex with fake rock wall



- *D. occidentalis* and *D. variabilis/similis* flagged from brushy vegetation at base of fabricated rock retention wall.
- Wall is downhill from small, grass-covered right-of-way.
- Mulch at base of wall continuously wet due to drainage issues.
- Downhill movement of *I. pacificus* and *D. occidentalis* has been previously documented².

Site 2 – Residential backyard next to undeveloped utility corridor



- *Dermacentor* tick acquired by dog while in solid wood-fenced backyard adjacent to a narrow strip of undeveloped land (~500 ft wide) serving as a utility corridor.
- Undeveloped land is bordered by large suburban neighborhood on one side, and 10-lane freeway on other side.
- Topography of undeveloped area is a combination of tule marsh and upland grassy area with blackberry bushes.
- *Dermacentor* ticks were flagged from the blackberry bushes by District staff.

Discussion

While multiple tick species are commonly encountered in San Mateo County, the majority of tick safety outreach and public education efforts has focused on the local vector of Lyme disease, *Ixodes pacificus*. As a result, public expectation of where ticks are present may be skewed towards environments favored by *I. pacificus*, such as oak woodlands, mixed forest, and coastal scrub environments, or areas frequented by black-tailed deer. The public and District staff alike have been caught off-guard by encounters with other ticks, such as *Dermacentor occidentalis* and *D. variabilis/similis*, occupying other environments. While defensible from a public health perspective, tick safety messaging solely emphasizing *Ixodes pacificus* information may inadvertently reinforce misleading assumptions about where ticks can be found.

As increasing numbers of recently described tick-borne pathogens are vectored by metastriate (non-*Ixodes*) tick species³, our findings underscore both the importance of conducting field surveys in a wide variety of potential tick habitats and including consideration of these ticks and their unique ecology when crafting tick safety materials and messages.

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2. James, D. K., et al. (2022). "Movement of *Ixodes pacificus* and *Dermacentor occidentalis* (Acari: Ixodidae) adult ticks in chaparral under natural conditions in Northern California, U.S.A." *Journal of Vector Ecology* 47(2): 188-194.
3. National Center for Emerging and Zoonotic Infectious Diseases (U.S.), Division of Vector-Borne Diseases, (2020), Guide to the surveillance of metastriate ticks (Acari: Ixodidae) and their pathogens in the United States.

Mark-release-recapture of packed and shipped *Aedes aegypti* with *Wolbachia*: implications for conducting remote Incompatible Insect Technique programs

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Abstract

Male mosquitoes containing the endosymbiont *Wolbachia* can be used as a tool to suppress wild mosquito populations through a technique termed the ‘Incompatible Insect Technique’ (IIT). IIT programs reduce wild mosquitoes via incompatible matings between released males and wild females to reduce the number of viable offspring produced in the next generation.

Successful programs rely on regular releases of incompatible males to outcompete wild males for target wild female mates. Past IIT programs have relied on local production of *Wolbachia* males to provide regular releases of incompatible males. Here, we evaluated the survival and dispersal of packed and shipped *Wolbachia Aedes aegypti* males in mark-release-recapture studies at a release site separated by over 3,600 miles from the centralized production facility. Released mosquitoes were recaptured using BG-Sentinel 2 traps collected daily for up to 7 days after release.

Wolbachia male mosquitoes packed and shipped from a centralized production facility performed similarly to males that were locally reared in the British Virgin Islands in regard to their survival, dispersal, and recapture rates. Our results support the conclusion that packing and shipping live *Wolbachia* male mosquitoes does not impact their ability to survive and disperse within release sites and suggests that IIT mosquito control programs can feasibly be conducted nearly anywhere in the world without the need for local mosquito production facilities.

A complete presentation of these data was published previously: Ohm et al. 2025. Am. J. Trop. Med. Hyg. 112: 1313–1324. <https://doi.org/10.4269/ajtmh.24-0262>

Profiling mosquito *kdr* allele L1014F in San Diego County

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Introduction

The San Diego County Vector Control Program relies on pyrethroid (synthetic pyrethrins) pesticides to suppress mosquito levels during disease threats. The increase of mosquito resistance to pyrethroid insecticides poses a threat to the control of mosquito-borne diseases (Xu et al. 2011), such as West Nile virus. It is essential to profile mosquitoes for pesticide resistance to employ effective insecticide-based mosquito control strategies.

Pyrethroid pesticides impair the insect's nervous system by binding to the voltage-gated sodium channel (VGSC) protein located on motoneurons, disrupting normal function, and causing paralysis (knock down effect) and death (Black et al. 2021). A reduction in sensitivity to these pesticides have been associated with SNPs on the *vgsc* gene (Xu et al. 2011).

The most globally prevalent pyrethroid-associated resistance genetic mutation on *vgsc*, is a single nucleotide base change from A to T, referred to as the *kdr* mutation, changing a wild-type leucine (L, Leu) to a resistant phenylalanine (F, Phe) at codon 1014 (L1014F) (Yoshimizu et al. 2020).

Previous studies have shown an average resistant allele frequency in California of 71% in *Cx. quinquefasciatus* and *Cx. pipiens* with the highest frequencies observed in the Central region and lowest in the North and South regions (Yoshimizu et al. 2020).

Methods

Mosquitoes were collected from January to December of 2023 from routine trap sites throughout San Diego County, identified and separated into single mosquito samples. Samples were then genotyped for position 1014 *kdr* SNP using the *Culex* RT*kdr* assay (Hager et al., 2022). The Applied Biosystems Design & Analysis Software 2.8.0 with Genotyping Analysis Module was used to assess the allele type. Samples which did not meet the software's automatic parameters (Fig. 1) were analyzed based on a manual call from the amplification plot. Results were mapped using ArcGIS 3.1.4.

Results

A total of 405 *Cx. quinquefasciatus* were assayed, of which 81 (20%) were homozygous for the *kdr* mutation, 189

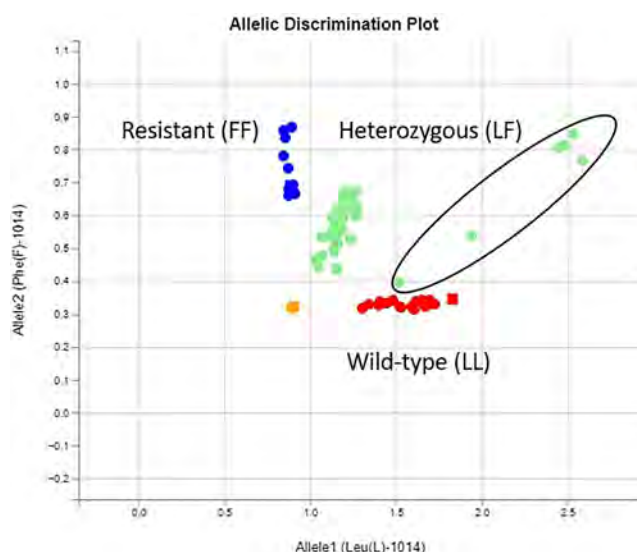


Figure 1.—An allelic discrimination plot of *Culex quinquefasciatus* samples for genotype analysis. The green samples within the black ellipse were manually determined to be heterozygous based on PCR amplification plots which showed clear presence of both alleles.

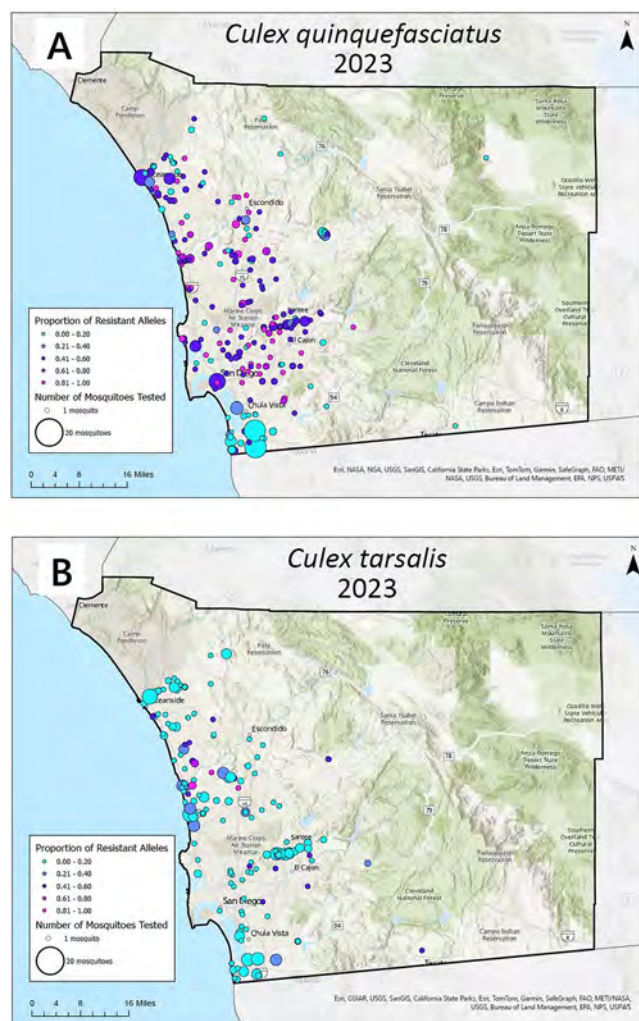
(47%) were heterozygous and 135 (33%) were wild-type. Of the 337 *Cx. tarsalis* assayed, 18 (5%) were homozygous for the *kdr* mutation, 42 (13%) were heterozygous, and 277 (82%) were wild-type. The resistant allele was present at a frequency of 0.43 and 0.12 for *Cx. quinquefasciatus* and *Cx. tarsalis*, respectively (Table 1). *Cx. quinquefasciatus* resistant alleles are widespread, except in the very southwest corner of the county (Fig. 2A), whereas *Cx. tarsalis* resistant alleles are concentrated in the central coastal areas of the county (Fig. 2B).

Conclusion

Our study found that *Cx. quinquefasciatus* resistance gene frequency was 0.43, compared to 2014 data from neighboring Orange (0.44) and Riverside (0.65) counties (Yoshimizu et al. 2020). In contrast, *Cx. tarsalis* populations had a lower overall resistant allele frequency (0.12) and have maintained their wild-type genotype possibly due to differences in pyrethroid exposure. *Culex quinquefasciatus* reside in more urban areas which may have higher encounters with pesticides from homeowner use and urban

Table 1.—Frequency of wild type and resistant *kdr* alleles in *Cx. quinquefasciatus* and *Cx. tarsalis* in San Diego County.

Species	<i>kdr</i> Genotype	TTA/TTA		TTA/TTT		TTT/TTT		Resistant Allelic Frequency
	Total Number of Samples (N)	Wild-type (LL)		Heterozygous (LF)		Resistant (FF)		
	Count	%	Count	%	Count	%		
<i>Culex quinquefasciatus</i>	405	135	33	189	47	81	20	0.43
<i>Culex tarsalis</i>	337	277	82	42	13	18	5	0.12

Figure 2.—Mapping of mosquito *kdr* allele frequencies in San Diego County. The frequency of resistant *kdr* alleles per trap site in *Cx. quinquefasciatus* (A) and *Cx. tarsalis* (B).

runoff. In contrast, *Cx. tarsalis* prefer larger freshwater sources such as lagoons, streams, and ponds where they are less of a public nuisance and therefore less likely to be exposed to urban pesticides. However, there appears to be geographic variation among the *Cx. quinquefasciatus* samples collected near the coastal border with Mexico compared to those farther north; this area represents a wildlife preserve, which may contribute to lower pyrethroid selection pressure. The Vector Control Program does not routinely use pyrethroids, therefore, selection pressures for L1014F are likely due to other sources, such as public use of pyrethroids. Future work will include additional resistance testing to gain a complete assessment of resistance profiles of San Diego County's mosquito vector populations.

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Profiling mosquito kdr allele L1014F in San Diego County

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San Diego County Vector Control Program, San Diego County Department of Environmental Health and Quality



Abstract

Adulticides are selectively used by the County of San Diego Vector Control Program (VCP) to limit the potential spread of mosquito-borne viruses. Mosquitoes of the *Culex* genus (*Culex* spp.) have been shown to exhibit resistance to pyrethrin and pyrethroid pesticides, known as knockdown resistance (kdr). The resistant phenotype has been associated with several single nucleotide polymorphisms (SNP) in the voltage-gated sodium channel (*vssc*) gene including one that results in a L1014F amino acid substitution. In this study, populations of two West Nile virus (WNV) vector species, *Culex quinquefasciatus* and *Culex tarsalis*, were genotyped for the L1014F kdr mutation using a probe-based quantitative reverse transcription polymerase chain reaction assay developed by Hager et al., 2022. A total of 405 *Cx. quinquefasciatus* and 337 *Cx. tarsalis* were assayed and showed the resistant allele present at a frequency of 0.43 and 0.12, respectively. Results indicate that *Cx. quinquefasciatus* have greater resistant genotype frequencies than *Cx. tarsalis*. Both species have lower frequency of resistance genotypes in south county than central and northern areas of the county.

Introduction

San Diego County VCP relies on pyrethroid (synthetic pyrethrins) pesticides to suppress mosquito levels during disease threats. The increase of mosquito resistance to pyrethroid insecticides poses a threat to the control of mosquito-borne diseases [2], such as West Nile virus. It is essential to profile mosquitoes for pesticide resistance to employ effective insecticide-based mosquito control strategies.

Pyrethroid pesticides impair the insect's nervous system by binding to the VGSC protein located on motoneurons, disrupting normal function, and causing paralysis (knock down effect) and death [1]. A reduction in sensitivity to these pesticides have been associated to SNPs on the *vssc* gene [2].

The most globally prevalent pyrethroid-associated resistance genetic mutation on *vssc*, is a single nucleotide base change from A to T, referred to as the kdr mutation, changing a wild-type leucine (L, Leu) to a resistant phenylalanine (F, Phe) at codon 1014 (L1014F) [3].

Previous studies have shown an average resistant allele frequency in California of 71% in *Cx. quinquefasciatus* and *Cx. pipiens* with the highest frequencies observed in the Central region and lowest in the North and South [3].

Objective

The goal of this study was to determine the frequency of kdr L1014F alleles in San Diego County's *Cx. quinquefasciatus* and *Cx. tarsalis* populations.

Methods

Mosquitoes were collected from January to December of 2023 from routine trap sites throughout San Diego County, identified and separated into single mosquito samples. Samples were then genotyped for position 1014 kdr SNP using *Culex* RT-kdr assay (Hager et al., 2020). The Applied Biosystems Design & Analysis Software 2.8.0 with Genotyping Analysis Module was used to assess the allele type. Samples which did not meet the software's automatic parameters were analyzed based on a manual call from the amplification plot. Results were mapped using ArcGIS 3.1.4.

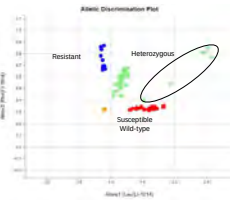


Figure 1. An allelic discrimination plot of *Culex quinquefasciatus* samples for genotype analysis. Blue data points are homozygous resistant (FF), red data points represent homozygous wild-type (LL) and green are heterozygous (LF) samples. The green samples within the black ellipse were determined to be heterozygous based on PCR amplification plots which showed clear presence of both alleles.

Results

Table 1. Frequency of wild type and resistant kdr alleles in *Cx. quinquefasciatus* and *Cx. tarsalis* in San Diego County.

Species	kdr genotype	T1A/T1A		T1A/T1T		T1T/T1T		Resistant allele frequency
		Count	%	Count	%	Count	%	
<i>Culex quinquefasciatus</i>	Total Number of Samples (N)	405						
	Wild-type (LL)	135	33	189	47	81	20	0.43
<i>Culex tarsalis</i>	Total Number of Samples (N)	337						
	Wild-type (LL)	277	82	42	13	18	5	0.12

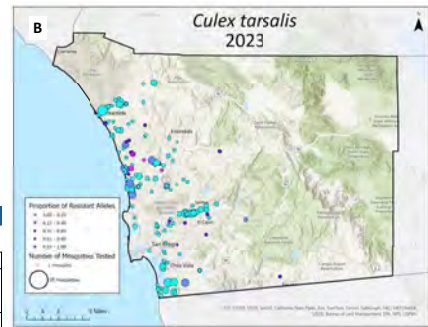
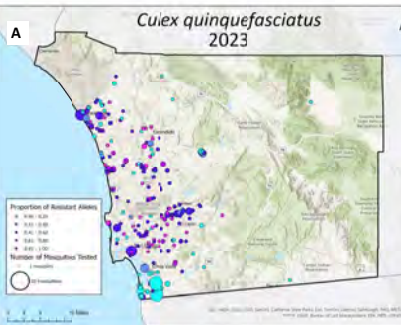


Figure 2. Mapping of mosquito kdr allele frequencies in San Diego County. The frequency of resistant kdr alleles per trap site in *Cx. quinquefasciatus* (A) and *Cx. tarsalis* (B). *Cx. quinquefasciatus* resistant alleles are widespread throughout the county, except in the very southwest corner of the county, whereas *Cx. tarsalis* resistant alleles are concentrated in the central coastal areas of the county.

Discussion/Conclusion

This study found *Cx. quinquefasciatus* resistance frequency to be 0.43, compared to 2014 data from neighboring counties, Orange County (0.44) and Riverside County (0.65) [3]. In contrast, *Cx. tarsalis* populations have lower overall resistant allele frequency (0.12) and have maintained their wild-type genotype possibly due to differences in pyrethroid exposure. *Cx. quinquefasciatus* reside in more urban areas which may have higher encounters with pesticides from homeowner use and urban runoff. In contrast, *Cx. tarsalis* prefer larger freshwater sources such as lagoons, streams and ponds where they are less of a public nuisance and therefore less likely to be exposed to urban pesticides. However, there appears to be geographic variation between the *Cx. quinquefasciatus* samples collected near the coastal border with Mexico compared to those farther north; this area represents a wildlife preserve, which may contribute to lower pyrethroid selection pressure. The Vector Control Program does not routinely use pyrethroids, therefore, selection pressures for L1014F are likely due to other sources, such as public use of pyrethroids. Future work will include additional resistance testing to gain a more complete assessment of resistance profiles in San Diego County of vector mosquitoes.

Acknowledgements

All of San Diego County VCP team!

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Date: Jan 2025

Evaluation of a multiplex qPCR assay for arbovirus detection in mosquitoes and birds: Improving consistency in results

Miguel Barretto, Ryan Clausnitzer, and Eric Haas-Stapleton*

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Abstract

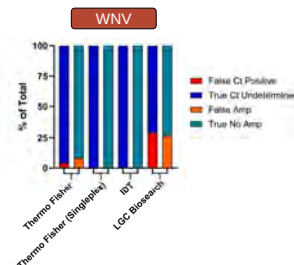
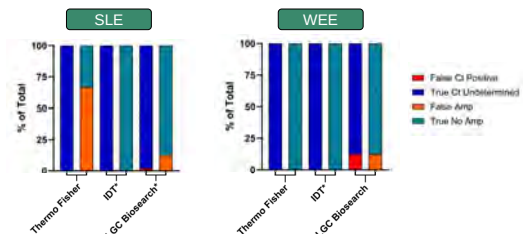
Accurate detection of arboviruses in vectors and reservoir species is critical for effective public health surveillance. To improve the reliability of a multiplex qPCR assay designed to detect West Nile (WNV), Western equine encephalitis (WEEV), and St. Louis encephalitis (SLEV) viruses in mosquitoes and birds, an evaluation was conducted after routine testing revealed infrequent but consistent false negatives. Five different assays were tested, all using the same primer and probe sequences for each virus, but varying in fluorophore types, quencher compositions and concentrations. These included a singleplex WNV and multiplex assays from Biosearch Technologies (BST; Teddington, Middlesex), Integrated DNA Technologies (IDT; Coralville, IA), and ThermoFisher Scientific (TFS; Waltham, MA). The goal of the evaluation was to identify conditions that provide more consistent results, reducing false negatives. The TFS singleplex and IDT multiplex assays showed the most consistent and accurate amplification for WNV, with no observed errors. In contrast, the TFS and BST multiplex WNV assays used the same FAM-labeled probe as the TFS singleplex and IDT multiplex, but produced false positives or negatives, indicating reduced reliability for arbovirus testing. When testing for SLEV and WEEV, the IDT multiplex assay demonstrated high specificity with no false positives detected for either cycle threshold (Ct) values or amplification status. In contrast, the TFS multiplex assay exhibited a high rate of false positive amplification for SLEV, with 66.7% of these lacking corresponding Ct values. The BST multiplex assay also showed reduced specificity, generating false positives for both Ct values and amplification status in 12.5% of cases. Overall, the IDT multiplex assay demonstrated the highest reliability across all targets, with consistent amplification and no false positives or negatives observed for WNV, WEEV, or SLEV. Based on these results, we recommend IDT multiplex assay for routine arbovirus surveillance in mosquitoes and birds, as it offers the most dependable performance for accurate detection.



Evaluating Multiplex qPCR Assays for Detecting Arboviruses: Improving Consistency in Results

Miguel Barretto, Ryan Clausnitzer, Eric Haas-Stapleton

Alameda County Mosquito Abatement District, 23187 Connecticut Street, Hayward, CA 94545

Objective	Higher WNV errors with LGC Biosearch Multiplex assay	Rationale
To improve the reliability of the multiplex qPCR assay for detecting WNV, WEEV, and SLEV, we tested four commonly used assays from different manufacturers to determine which minimized false positives.	• Thermo Fisher singleplex and IDT had the most reliable amplification and detection, with zero errors • Thermo Fisher multiplex and LGC Biosearch Multiplex had false positives or negatives even though all assays used the same fluorophore (FAM) 	Optimizing the assay will reduce the need for repeated testing, enhance operational efficiency, and provide more reliable data for public health decision-making.
Methods	No errors for SLE or WEE with IDT Multiplex	Results summary
All assays used MagMAX Elution Buffer without template nucleic acid (i.e., negative control), with identical primer probe concentrations and thermocycler conditions (n = 48 per assay). Four assays were evaluated: - Thermo Fisher multiplex - WNV-FAM, SLE-VIC, WEE-ABY - Thermo Fisher singleplex - WNV-FAM - Integrated DNA Technology (IDT) Multiplex - WNV-FAM, SLE-TET, WEE-Cy5 - QuantStudio 5 targets VIC for the TET dye - VIC: Emission-526 nm, Excitation-543 nm - TET: Emission-521 nm, Excitation-542 nm - LGC Biosearch Multiplex - WNV-FAM, SLE-Quasar 570, WEE-TAMRA - QuantStudio 5 targets NED for Quasar 570 - Quasar 570: Emission-548 nm, Excitation-567 nm - NED: Emission-548 nm, Excitation-567 nm	 • IDT had a no false positives for either Ct or Amplification Status with SLE and WEE • Thermo Fisher Multiplex had a high proportion of false positive amplification status calls for SLE with no Ct (66.7%) • The LGC Biosearch multiplex had false positive calls for both Ct and amplification status (12.5%)	• Multiplex assays from Thermo Fisher and LGC Biosearch had false positives for Ct or Amplification Status (or both) while the IDT Multiplex did not. • When the multiplex Thermo Fisher primer-probe set was used in singleplex, no false positives were found, pointing to potential cross-talk with the other dyes used in multiplex.
		Future directions
		• Evaluate different fluorophore combinations to eliminate potential cross-talk between probe dyes • Sequence false negative results to ensure no sample contamination
		Acknowledgements
		I would like to thank colleagues at Alameda County Mosquito Abatement District for their support and guidance.

Innovative 3D Printed Traps: A cost-effective solution for mosquito surveillance

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Abstract

The Marin/Sonoma Mosquito and Vector Control District sets over 1,800 conventional EVS mosquito traps annually. These traps utilize carbon dioxide and a light source to attract adult mosquitoes. Collections are used to measure adult mosquito abundance and detect the presence of arboviruses. During the 2024 adult mosquito surveillance season, staff evaluated the effectiveness of these EVS traps compared with commercially available BG Pro (Biogents, Regensburg, Germany) traps. Upon identifying differences between these traps, staff designed two 3D printed traps: one incorporating the fan from the BG Pro and another using a widely available computer fan. Measurements from a Kestrel 1000 anemometer and field studies identified that both new traps functioned better than the original EVS CO₂ traps, despite the lack of a light source. The three new traps were comparable in their functionality, but the printed computer fan trap cost significantly less than the others. The development of these innovative 3D printed traps demonstrated a cost-effective and efficient alternative to commercially available traps.



Innovative 3D Printed Traps: A Cost-Effective Solution for Mosquito Surveillance

Kelly A.L. Furey, PhD, MPH
Marin/Sonoma Mosquito and Vector Control District, Cotati, CA

ABSTRACT

The Marin/Sonoma Mosquito and Vector Control District sets over 1,800 conventional EVS mosquito traps annually. These traps utilize carbon dioxide and a light source to attract adult mosquitoes. Collections are used to measure adult mosquito abundance and identify the presence of arboviruses. During the 2024 adult mosquito surveillance season, staff evaluated the effectiveness of these traps compared with commercially available BG Pro (Biogents, Regensburg, Germany) traps. Upon identifying differences between these traps, staff designed two 3D printed traps: one incorporating the fan from the BG Pro and another using a widely available computer fan. Measurements from a Kestrel 1000 anemometer and field studies identified that all new traps functioned better than the original EVS CO₂ traps, despite the lack of a light source. The new traps were comparable in their functionality, but the printed computer fan trap cost significantly less than the others. The development of these innovative 3D printed traps demonstrates a cost-effective and efficient alternative to commercially available traps.

METHODS – TESTING TRAP EFFICACY

- Traps set ~ 20 feet from each other on same night
- Traps set in same location on consecutive nights
- Kestrel readings of fan pull

METHODS – TRAP PRINTING AND COST

Printer	Bambu Lab X1C 3D Printer with 4-filament AMS
Slicer Program	Bambu Studio
Design Programs	Tinkercad (online), Rhinoceros 8.0
Filament	Bambu Lab PETG HF - 1.75mm, dried 55C 12 hours

Furey 3000 V1		Furey 3000 V2		Furey 3000 V3 – Trap only	
Purchased Item	Cost/Trap	Purchased Item	Cost/Trap	Purchased Item	Cost/Trap
Biogents Fan	\$32.45	Trap Fan	\$11.00	Trap Fan	\$11.00
USB Adapter Cable	\$8.23	Carabiner Clip	\$0.70	Carabiner Clip	\$0.70
Carabiner Clip	\$0.70	Magnets for lid weight	\$1.90	Wheel weight for lid	\$0.34
Magnets for lid weight	\$1.90	Chain	\$0.50	Chain	\$0.50
Chain	\$0.50	Filament	\$17.41	Filament	\$21.84
Filament	\$12.61				
Total cost	\$56.39	Total Cost	\$31.51	Total Cost	\$34.38
Print time ~ 14.5 hours, 3 plates		Print time ~ 16 hours, 3 plates		Print time ~ 17 hour, 3 plates	

Cost of Biogents BG Pro ~ \$110

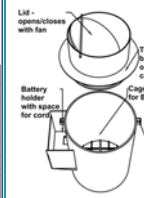
Scan QR code for files for printing the Furey 3000 V3

Trap	Catch container	Full project (for slicer)
base.stl	catch_container.stl	Furey 3000_full
top.stl	catch_container_top_ring.stl	print with settings.3mf
lid.stl	catch_container_bottom_ring.stl	
	catch_container_top.stl	

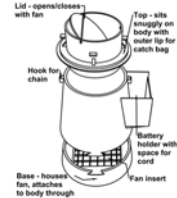


TRAP DESIGN – AN ITERATIVE PROCESS

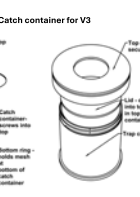
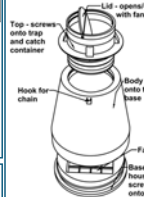
FUREY 3000 – V1



FUREY 3000 – V2



FUREY 3000 – V3



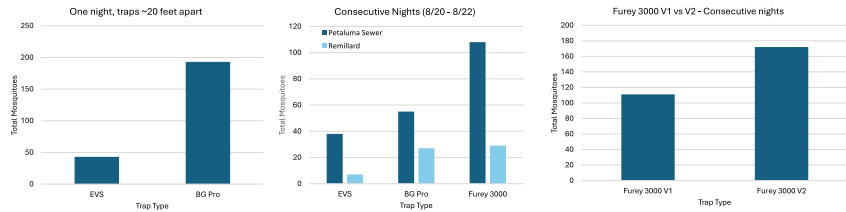
DISCUSSION

- All three of the 3D printed traps showed similar efficacy to the commercially available Biogents BG Pro trap
- The cost of the trap utilizing non-specialized parts is significantly less than the commercial trap
- Additional testing of the Furey 3000 V3 with the new catch container is needed, but initial evaluations are promising
- Furey 3000 patent pending

ACKNOWLEDGEMENTS

This product would not be what it is without the helpful discussions and field testing of many individuals, including E. Lloyd Furey, Rob McGovern, Casey Richter, Nizza Sequeira, Peter Bonkrude, Nathan Reed, Kristen Holt, Sarah Brooks, Nathan Foster, and Wilder Black.

RESULTS – TESTING TRAP EFFICACY



Trap Type	Original EVS	BG Pro	Furey 3000 V1	Furey 3000 V2	Furey 3000 V3
Kestrel Reading	0.89 m/s	2.48 m/s	1.87 m/s	1.87 m/s	2.24 m/s

Evaluating insecticide resistance of adult *Culex tarsalis* to pyrethroids and malathion in Colusa County, 2022–2024

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Abstract

Evaluating insecticide resistance is a crucial step in the control of mosquito populations that should be completed before a specific insecticide formulation is selected for vector control. Furthermore, effective control of *Culex tarsalis* is critical to public health as this species is a primary vector of West Nile virus in California. Over three consecutive years (2022–2024), bottle bioassays were performed by the California Department of Public Health Vector-Borne Disease Section, the Colusa Mosquito Abatement District, and Sutter-Yuba Mosquito and Vector Control District to determine the efficacy of three commonly used pyrethroid and organophosphate insecticides (permethrin, deltamethrin, and malathion) against adult *Cx. tarsalis* mosquitoes collected from a single site in Colusa County. Wild-caught female and male *Cx. tarsalis* were collected using EVS traps set at Colusa National Wildlife Refuge. Adults from the Kern National Wildlife Refuge *Cx. tarsalis* colony were used as the susceptible control for comparison. Assays were conducted using active ingredients according to the CDC's Bottle Bioassay Protocol outlined in the CONUS Manual for Evaluating Insecticide Resistance in Mosquitoes Using the CDC Bottle Bioassay Kit. Results indicated variable resistance of wild-caught *Cx. tarsalis* mosquitoes to permethrin (43 ug/bottle), with observed mortality ranging from 39.3% to 84.9%. *Culex tarsalis* exhibited increasing resistance to deltamethrin (0.75 ug/bottle), with mortality decreasing from 26.4% in 2022 to 12.9% in 2024. *Culex tarsalis* were initially resistant to malathion (400 ug/bottle) in 2022 with 81.1% mortality, but later demonstrated susceptibility with 100% mortality observed in 2024. Conducting additional assays to test *Cx. tarsalis* from different locations within the county, and cage trials to evaluate the efficacy of specific formulations of these pyrethroid and organophosphate insecticides under field conditions, would be helpful to better estimate insecticide resistance levels and aid management decisions to control this species within Colusa County.

Mosquito Abatement Through Empowerment (MATE) tractor farming

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Abstract

MATE (Mosquito Abatement Through Empowerment), in partnership with the Yotor Farmers Association and Rotary International, is revolutionizing malaria control in rural Ethiopia by initiating community-driven, locally funded mosquito control programs. The demonstration communities of Yejube and Addis Zemen rely primarily on subsistence farming, often using rudimentary tools and livestock. MATE's innovative model addresses both economic development and public health by introducing tractors to improve agricultural productivity and by managing standing water sources that serve as mosquito production sites.

The demonstration community surveys revealed strong local interest in mosquito control, improved farming techniques, and greater economic opportunity. Residents emphasized the burden of malaria on family health and productivity and expressed willingness to contribute to sustainable, community-led solutions. These insights directly shaped MATE's approach: improve farming yields, generate local revenue, and reinvest a portion into mosquito abatement efforts.

With two tractors funded by Rotary International and delivered to Debre Markos University and Debre Tabor University, farmers will be able to cultivate larger areas, increasing both cash and crop yields. A portion of the farming profits is earmarked to support sustainable mosquito control and improvements in local infrastructure and education—creating a self-reinforcing cycle of health and economic growth.

In February 2025, MATE and Yotor led the first tractor training session in Addis Zemen, with support from Debre Tabor University and regional agriculture leaders. Several local farmers, agriculture experts, and operators participated in hands-on sessions covering tractor safety, operations, maintenance, and field trials. Community engagement is key to the MATE approach; as trainees gain skills, they also took ownership of both farming and vector control activities. Notably, women have been empowered through this program, learning to operate and maintain agricultural machinery—an uncommon opportunity in rural Ethiopia.

As MATE moves into Phase II, the focus shifts to upscaling farming operations, tracking outcomes, and ensuring that increased agricultural revenue translates directly into mosquito abatement and broader community benefits. This holistic approach not only targets malaria reduction but fosters long-term resilience through education, infrastructure, and local ownership.



Mosquito Abatement Through Empowerment (MATE) Tractor Farming: a collaborative approach to solving public health challenges

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Objective

Malaria kills half a million people in Sub-Saharan Africa. To uplift rural communities in Ethiopia, MATE invests in tractors for mosquito control and economic empowerment, enabling them to:

- Farm larger areas to produce both cash and subsistence crops.
- Establish locally funded mosquito control programs.
- Improve infrastructure and educational opportunities.

Project Sites



Subsistence farming communities in Yejube and Addis Zemen were selected by a local non-profit and the Ethiopia Ministry of Health selected for the MATE Project.

Community Needs Survey



Two Tractors Funded by Rotary International



Two tractors purchased by Rotary in Addis Ababa and transferred to Yotor (left). The first tractor delivered to Debra Markos University near Yejube for training (right).

MATE Supporters & Advisors



Next Steps: Phase II

Develop sustainable local farming with the tractors.

Use cash crop profits to fund community needs and increase acres that are farmed.

Reserve a portion of farming profit for mosquito control & improving community infrastructure.

Measure outputs & ensure alignment with the MATE Program mission.

Acknowledgements

Completing Phase I of the MATE Project to purchase and deliver the tractors would have been impossible without the support of colleagues with Rotary International, Yotor, Alameda County MAD and the farming communities of Yejube and Addis Zemen.

William E. Walton Poster Competition

The William E. Walton Poster Competition was initiated in 2023 to recognize exceptional poster presentations in the field of mosquito and vector control research and operations. The annual competition honors the career of the late Dr. William (Bill) E. Walton, Professor of Entomology at the University of California, Riverside, who was passionate about and committed his career to the study of entomology and vector ecology. Each year the number of participants and diversity of topics in the competition has increased and serves as a showcase for innovative research and applied science in vector control and public health.

Posters have long served as an essential platform for presenting early-stage research, introducing methodological innovations, and fostering collaborative dialogue. Previous submissions reflect the remarkable diversity, creativity, and scientific rigor that define our field. Covering a wide range of topics, including operational evaluations of surveillance and control strategies, public outreach initiatives, and research on vectors and vector-borne diseases, these posters underscore the vital role of research and technology in advancing integrated vector management.

First place winners for each year

Year	Presenting Author	Poster title
2025	Alexandra Echenberg	Evaluating drone applications of larvicide to rural sites in the Coachella Valley
2024	Brittany Deegan	The impact of aromatic plants on BG Sentinel II trap efficacy
2023	Sumiko De La Vega	Evaluation of a cordless heat gun for ovicidal treatment of <i>Aedes aegypti</i> eggs

Addressing challenges in invasive *Aedes* surveillance by a Code Enforcement Program outside of a mosquito abatement district

Nader Shatara*

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Abstract

The San Francisco Department of Public Health (SFDPH) faces challenges in invasive *Aedes* surveillance because it operates without the benefit of a mosquito abatement district, its responsibilities as a health department focus on areas beyond mosquito control, and it faces information and confidentiality barriers with stakeholders and imported exotic arbovirus cases. Attempts are made to conduct surveillance at imported arbovirus locations in addition to 26 fire stations throughout the city.

In 2023 to 2024, the SFDPH received reports of 20 cases of imported exotic arbovirus cases in San Francisco. Of these 20 cases, surveillance was conducted at four locations. Seven locations lacked adequate placement points, seven others turned down our request for surveillance, and two did not provide information on their location. Of the 26 fire station surveillance sites, four fire stations had discarded their larval sources.

At the larval stage – Identifying and processing samples

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Introduction

At the Coachella Valley Mosquito and Vector Control District (District), different departments collaborate in our fight against mosquitoes and the diseases they transmit. Much of that work hinges on the surveillance of mosquitoes, not just as adults, but at the larval stage as well. With our Larval Surveillance Program, Laboratory staff identify collections to species using key characters (Meyer and Durso 2021) seen under a microscope. This information helps shape our efforts to protect the residents of the Coachella Valley.

Methods

The larval sampling process begins with Vector Control Technicians inspecting individual sites across the valley.



Figure 1.—*Cx. tarsalis* seen with 5 setal tufts in a straight row on the siphon.



Figure 2.—*Cx. quinquefasciatus* with 4 offset setal tufts on the siphon.

A number of dips are taken, specific to the habitat, and combined in a plastic sample bag, which is tied, labeled, and placed in an iced cooler for transport. The sample is processed by Laboratory staff for identification. Samples are poured into a pan and larvae are transferred into a petri dish. A stereo microscope at 75× magnification is used to identify the key characteristics of the species. Once completed, the information is entered into the Operations software.

The key characters used for identification for species found within the Coachella Valley are the number and positioning of siphon hair tufts, the number of lateral abdominal hairs, pecten spine placement on the siphon, and the shape of the siphon.

The siphon of *Culex tarsalis* (Fig. 1) has 5 tufts placed in a straight row compared to *Culex quinquefasciatus* (Fig. 2) which has 4 tufts offset from each other. In



Figure 3.—*Cx. tarsalis* with 3 lateral abdominal hairs.



Figure 5.—*Ae. aegypti* seen with a straight row of pecten spines on the siphon.

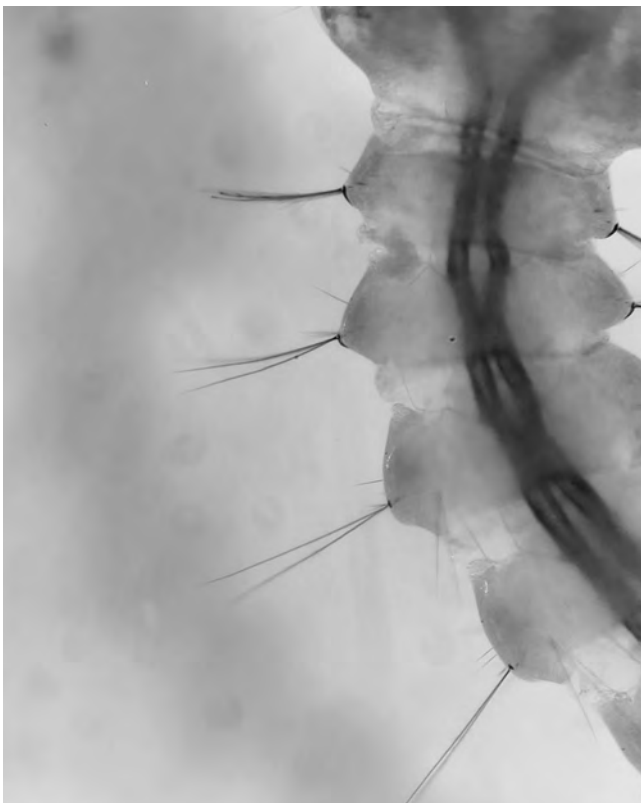


Figure 4.—*Cx. quinquefasciatus* with 2 lateral abdominal hairs.



Figure 6.—*Ae. vexans* with visible gap seen between pecten spines on the siphon.



Figure 7.—*Cx. erythrothorax* with a longer, thinner siphon.

addition, *Cx. tarsalis* (Fig. 3) has 3 lateral abdominal hairs on the 3rd abdominal segment compared to *Cx. quinquefasciatus* (Fig. 4) which has 2 lateral abdominal hairs.

In relation to pecten spines on the siphon, *Aedes aegypti* (Fig. 5) has a row of adjacent pecten spines starting at the base of the siphon as compared to *Aedes vexans* (Fig. 6) which has a similar row of pecten spines but with a noticeable gap between one or two of the distal spines.

There are also differences in the general shape of the siphon, some being shorter, fatter, or longer than others. With *Culex erythrothorax* (Fig. 7), the siphon is noticeably longer and thinner compared to *Cx. quinquefasciatus* (Fig. 8) and other *Culex* species found within the Coachella Valley such as *Cx. stigmatosoma* and *Cx. tarsalis*.

In rare cases of damaged larvae, inspecting the comb scales or hairs on the head can also help identify the species.



Figure 8.—*Cx. quinquefasciatus* with shorter and fatter siphon in comparison to *Cx. erythrothorax*.

Likewise, knowing the location and habitat of the larvae can help inform identifications.

Results and Discussion

Larval surveillance can establish the species, instar, larval habitat location, and population abundance. This information is shared throughout departments and used in a variety of ways. It creates an active and fluid map showing the distribution of vector mosquitoes throughout the Coachella Valley which allows for the prioritization of treatment zones. Larval information can help map targeted “Door-to-Door” response initiatives to combat *Ae. aegypti* as well as help plan large-scale drone, truck, and aerial applications. It aids in the investigation of human cases of vector-borne diseases, allowing the District to act promptly and effectively.

This surveillance is critical to ensure that pesticide treatments are used in a safe, efficient, and financially responsible manner.

Acknowledgements

This project would not have been possible without the insights and hard work of the following: Kim Hung, Melissa Snelling, Jacob Tarango, Alex Echenberg, Micheal Esparza, Marc Kensington, Arturo Gutierrez, Diana

Ramirez, Rick Ortiz, Gregorio Alvarado, Gerardo Avalos, Christopher Vazquez, Marco Medel, Robert Gaona, Hugo Arcos, and Marisa Kelling.

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At the Larval Stage – Identifying and Processing Samples

Eric Ortiz, Laboratory Technician, Jennifer A. Henke, M.S., BCE, and Gabriela Perezchica-Harvey, M.S.
Coachella Valley Mosquito and Vector Control District

INTRODUCTION:

At the Coachella Valley Mosquito and Vector Control District, different departments collaborate in our fight against mosquitoes and the diseases they transmit. Much of that work hinges on the surveillance of mosquitoes, not just as adults, but at the larval stage as well. With our Larval Surveillance Program, Lab staff can narrow down our findings to species, using key characteristics seen under a microscope. This information provides vital information across departments which helps shape our efforts to protect the Coachella Valley.

COLLECTING LARVAL SAMPLES:

The larval sample process begins with Vector Control Technicians who visit up to 50 sites a day. These can range from small French drains, to full 300-acre sites. A certain number of dips are performed and collected in a plastic sample bag, which is tied, labeled, and placed in an iced cooler for transport. The sample is then processed by Lab staff for identification.



Lab staff pour the sample into a pan and use a pipette to collect and place larvae into a petri dish. The petri dish is placed under a microscope to observe key characteristics in which to identify the species. Once completed, the information is entered in the Operations software.



LARVAL IDENTIFICATION: Key identifiers attributed to species:

Number and Positioning of Siphon Hair Tufts



With *Culex quinquefasciatus* (we find 4 hair tufts affixed to the siphon, positioned offset from one another. With *Culex tarsalis* we find 5 hair tufts lined up in a straight row.

Lateral Abdominal Hairs



Located at the 3rd abdominal segment, *Culex tarsalis* has 3 abdominal hairs, whereas *Culex quinquefasciatus* has 2 abdominal hairs.

Pecten Spines along the Siphon



With *Aedes aegypti*, we have the row of pecten spines starting at the base of the siphon. With *Aedes vexans*, we have a similar row of spines but with a noticeable gap in between one or two of the spines.

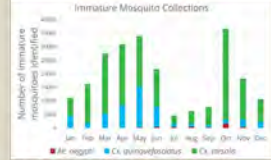
Shape of the Siphon



With *Culex erythrothorax*, the siphon is noticeably longer and thin compared to *Culex quinquefasciatus* and other *Culex* species.

In rare cases of damaged larvae, inspecting the comb scales or hairs on the head can help narrow down the species. Likewise, knowing location, habitat, and even past history can help inform what we have under our microscope. What we find throughout the year varies, and the story it tells is enlightening and incredibly valuable throughout our District.

Immature Mosquito Collections



Larval sample counts of disease vector found in the Coachella Valley in 2024.



Mosquito species sampled throughout the Coachella Valley in 2024.

CONCLUSIONS:

Larval surveillance can establish the species, instar, breeding location, and population density. This information is dispersed throughout departments and used in a variety of ways.

- Creates an active and fluid map of disease vectors throughout the Coachella Valley.
- Prioritizes treatment zones and delegates technician rollout.
- Provides information needed to run targeted "Door-to-Door" response initiatives.
- Data gathered is used in planning large-scale drone, truck, and aerial applications.
- Assists in human case vector-borne disease investigations.
- Ensures that the chemical treatments are used in a safe and efficient way, and in a financially responsible manner.



With virus-positive mosquitoes, knowing the species is imperative to keeping the public safe. As a District, our mission is to respond quickly and protect our communities. We seek to encompass all aspects of our work, down to the smallest larva, which allows us to make the largest impact.

ACKNOWLEDGEMENTS:

This project would not have been possible without the insights and hard work of the following: Kim Hung, Melissa Snelling, Jacob Tarango, Alex Echenberg, Micheal Esparza, Marc Kensington, Arturo Gutierrez, Diana Ramirez, Rick Ortiz, Gregorio Alvarado, Gerardo Avalos, Christopher Vazquez, Marco Medel, Robert Gaona, Hugo Arcos, and Marisa Kelling.

For further information, contact Eric Ortiz at the Coachella Valley Mosquito and Vector Control District at eortiz@cvmosquito.org.

Bloodfeeding patterns of *Aedes aegypti* in San Joaquin County, California

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Introduction

Aedes aegypti has high competency for arboviruses such as dengue and highly anthropophilic behavior. In tropical regions, humans make up 70-99% of *Ae. aegypti* bloodmeals (Ponlawat and Harrington 2005). In TX, HI, and FL, humans made up 31%, 53%, and 83% of bloodmeals, respectively (Tempelis et al. 1970; Tempelis 1975; Olson et al. 2020). Due to the recent introduction of *Ae. aegypti* to San Joaquin County (SJC) in 2019, determining the prevalence of human bloodmeals may determine the potential risk for arbovirus transmission among humans.

Methods

To determine which hosts mosquitoes were feeding on, the 658-bp “barcoding” cytochrome c oxidase 1 (COI) gene was extracted from *Aedes* bloodmeals, amplified, sequenced, and submitted to boldsystems.org (Ratnasingham and Herbert 2007; Thiemann et al. 2012). A nested PCR was used to limit pseudogene amplification and increase the PCR yield of the COI gene (Thiemann et al. 2012).

Results and Discussion

Aedes species, not including *Ae. aegypti*, were caught using CO₂ traps across 14 rural areas. The two bloodmeals from *Ae. nigromaculis* came from horses, whereas *Ae. vexans* fed mostly on desert cottontail (54.5%), followed by sheep (18.1%) and brush rabbit (18.1%). *Aedes melanimon* fed equally on cattle (22.7%) and desert cottontail (22.7%) followed by brush rabbit (18.2%). *Aedes aegypti* were caught using BG-Sentinel traps in 13 residential areas and fed predominantly on humans (60.5%), followed by dogs (18.4%), aligning with their well-known anthropophilic behavior.

Conclusions

In TX, Zika transmission via *Ae. aegypti* was reduced likely due to the relatively low percentage (31%) of human

bloodmeals relative to Mexico which had 98% human bloodmeals (Olson et al. 2020). In SJC, because the prevalence of human bloodmeals was between 31% and 98%, it is unclear whether dengue virus transmission would be dampened locally (Ponlawat and Harrington 2005; Tempelis et al. 1970).

Acknowledgements

We would like to thank SJCMVCD for collecting and identifying mosquitoes and for funding the project. We also thank the students from the University of the Pacific Vectorborne Disease class for helping with laboratory work.

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Bloodfeeding patterns of *Aedes aegypti* mosquitoes in San Joaquin County, California

Lemya Mohamed, Tara Thiemann, Ph.D., Shaoming Huang, Ph.D.
University of the Pacific

ABSTRACT

Aedes aegypti were first discovered in San Joaquin County, CA, in 2019. This species poses a threat to human health, as they can transmit viruses such as dengue, yellow fever, chikungunya, and Zika. Understanding the bloodfeeding patterns of *Aedes aegypti* in the county is crucial in determining risk of pathogen transmission. In this study, bloodmeals from *Aedes aegypti* and various native *Aedes* species collected in San Joaquin County were identified to host species using PCR and DNA sequencing. Feeding patterns of *Aedes* species were compared to determine specific differences in host species selection and to elucidate the portion of human bloodmeals, and thus potential risk of virus transmission, by *Aedes aegypti*.

INTRODUCTION

- *Ae. aegypti* were first detected in San Joaquin County, CA (SJC) in 2019. The other tested *Aedes* species, *Ae. vexans*, *Ae. nigromaculis*, and *Ae. melaninon*, are endemic to SJC.
- *Ae. aegypti* mosquitoes can transmit viruses like dengue, yellow fever, and chikungunya.
- Dengue virus has been detected in CA and may pose risks in SJC due to *Ae. aegypti* presence^[2].
- The majority of *Ae. aegypti* bloodmeals are human, while those of the other *Aedes* species are more variable^[3,4].
- In tropical regions, humans make up 70-99% of *Ae. aegypti* bloodmeals. In TX, HI, and FL, humans made up 31%, 53%, and 83% of bloodmeals respectively^[3,4].

Unlike WNV, which has an enzootic cycle, dengue is maintained in a mosquito-human-mosquito cycle (Figure 1).

Figure 1. Dengue transmission cycle.



OBJECTIVES

- Compare host selection patterns between *Ae. aegypti* and other *Aedes* species in San Joaquin County to illustrate the potential risk of arboviral disease transmission to humans.
- Determine the prevalence of human bloodmeals from *Ae. aegypti* in San Joaquin County.

METHODOLOGY

Mosquito Collection

- The San Joaquin County Mosquito and Vector Control District collected *Aedes* mosquitoes.
- *Ae. vexans*, *Ae. nigromaculis*, and *Ae. melaninon* were caught using a CO2 trap while *Ae. aegypti* were caught using a BG-Sentinel trap.
- All *Ae. aegypti* mosquitoes were caught in suburban residential areas while all other *Aedes* were caught in rural areas near bodies of water across SJC.

Bloodmeal Identification

- Extraction was done using the DNeasy Blood and Tissue Kit from Qiagen.
- Nested PCR was done to limit the amplification of pseudogenes and increase PCR yield.

- To determine which hosts are being fed on by the mosquitoes, a 658-bp "barcoding gene" called the cytochrome c oxidase 1 (COI) gene was amplified, sequenced, and submitted to boldsystems.org.

Blooded Mosquito

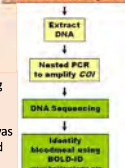


Figure 2. (Left) CO2 trap. (Right) BG-Sentinel trap.

PRELIMINARY RESULTS

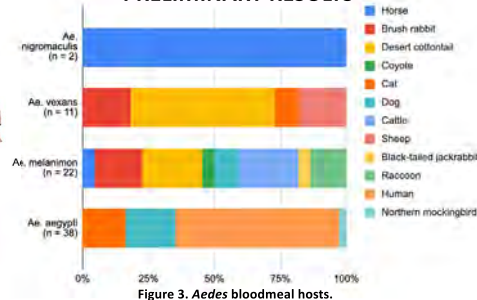


Figure 3. *Aedes* bloodmeal hosts.

- 38 *Ae. aegypti* and 36 other *Aedes* bloodmeals were identified to a host.
- The two bloodmeals from *Ae. nigromaculis* came from horses while *Ae. vexans* fed mostly on desert cottontail (54.5%) followed by sheep (18.1%) and brush rabbit (18.1%). *Ae. melaninon* fed equally on cattle (22.7%) and desert cottontail (22.7%) followed by brush rabbit (18.2%).
- *Ae. aegypti* fed predominantly on humans (60.5%) followed by dogs (18.4%).

DISCUSSION

- Non-*aegypti* mosquitoes were caught across 14 rural areas which coincided with bloodmeal hosts including cattle, sheep, and desert cottontail. The rural area also allowed for a wider range of hosts to be fed on by the other *Aedes*.
- The highest host diversity among the other *Aedes* was in *Ae. melaninon*, but it also had twice the sample size as *Ae. vexans*.
- *Ae. aegypti* mosquitoes were caught in 13 residential areas due to their behavior of dwelling alongside humans and in man-made containers like flowerpots^[5]. This contributes to the majority of bloodmeals being humans and domesticated dogs and cats.
- Having a decreased proportion of human bloodmeals from 70-99% in tropical regions to 60.5% in SJC could dampen the transmission cycle of diseases like dengue^[3]. In TX, Zika transmission via *Ae. aegypti* was reduced likely due to the relatively low percentage (31%) of human bloodmeals^[4].
- Previous work in SJC has shown that the other *Aedes* showed <10% human bloodmeals (unpublished). In this study, none of the other *Aedes* mosquitoes in this study fed on humans, only *Ae. aegypti*.
- Given the propensity to feed on humans and the traveler cases of dengue in SJC each year, the mosquitoes which are of largest risk toward human populations in SJC are *Ae. aegypti*.
- The work presented is preliminary and larger sample sizes of *Aedes* are being tested to illustrate a wider array of host bloodmeals.

ACKNOWLEDGEMENTS

We would like to thank SJCMVCD for their collection and identification of mosquitoes and for funding the project. We also thank the students from the University of the Pacific Vectorborne Disease class for helping with lab work.

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Control efforts in agriculture areas in the Coachella Valley

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Abstract

The Coachella Valley's agricultural areas provide food and support the local economy. The region's fertile soil, favorable climate, and access to water from the Colorado River allow the production of over 100 different types of crops. The area experiences scheduled flooding events for crop production and irrigation of fields and pastures. This flooding can lead to an increase in mosquito activity, particularly *Psorophora columbiae*, which is a significant pest in the region and can develop from egg to adult in just 4 days. Although this mosquito species is not known to transmit diseases to humans, it can be a nuisance to agriculture workers and residents of nearby communities. To address this issue, the Coachella Valley Mosquito and Vector Control District dedicates resources to controlling this species. The District's objectives are to maintain mosquito populations at levels that do not become a nuisance or a public health problem. This poster presents effective communication between caretakers and technicians, selecting the proper control products and application equipment, especially when navigating various types of terrain with off-road equipment due to soil and crops types.

Determinants of immature *Aedes dorsalis* distribution in a dynamic environment

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Abstract

Aedes dorsalis (summer salt marsh mosquito) inhabits dynamic tidal marshes where abiotic conditions fluctuate. This study aimed to identify the factors influencing the distribution of immature *Ae. dorsalis* in salt marsh habitats bordering the San Francisco Bay by utilizing environmental DNA (eDNA) to monitor aquatic invertebrate community composition and key abiotic parameters (water temperature and salinity).

Field surveys were conducted in tidal marsh pools during periods of known *Ae. dorsalis* larval presence. Water samples (~135 mL each) were filtered sequentially through 10 µm and 0.8 µm pore-size filters to capture eDNA, and *in situ* measurements of surface water temperature and salinity were recorded for each site. A DNA “mini-barcoding” approach targeting a 313 base-pair fragment of the mitochondrial cytochrome c oxidase I (COI) gene was used for mosquito identification. Reference DNA from adult mosquitoes (including *Ae. dorsalis* and co-occurring *Culex* spp.) was PCR-amplified, sequenced at this COI region, and validated against known GenBank sequences.

Phylogenetic analysis of the resulting COI sequences showed that the barcoded mosquito specimens formed distinct clusters by genus, confirming the utility of the 313 bp COI mini-barcode region for reliable mosquito identification. *Aedes dorsalis* larvae were observed across a range of salinities and temperatures, and statistical analysis found no significant correlation between larval distribution and water salinity ($p = 0.21$) or temperature ($p = 0.85$). These results indicated that immature *Ae. dorsalis* can tolerate broad fluctuations in salinity and temperature within marsh habitat.

As a next step, archived eDNA samples (along with ‘mock community’ controls) will be subjected to high-throughput metabarcoding. This will enable a broader ecological analysis, correlating *Ae. dorsalis* larval occurrence with marsh vegetation cover, eDNA-derived biodiversity indices, and site elevation. These efforts will help illuminate how biotic community and landscape features, in addition to abiotic factors, shape the distribution of immature *Ae. dorsalis* in dynamic salt marsh environments.



Determinants of Immature *Aedes dorsalis* Distribution in a Dynamic Environment

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Objective

Use environmental DNA (eDNA) to monitor aquatic invertebrate community structure and assess abiotic characteristics of tidal marsh habitats when immature *Aedes dorsalis* are present.

Methods

- The study was conducted in salt marsh habitats that abut the San Francisco Bay (Figure 1)
- Identifying mosquitoes using DNA 'mini barcoding'
 - Genomic DNA was extracted from adult *Aedes dorsalis*, *Culex tarsalis*, and *Culex erythrothorax* that were collected from Fremont
 - PCR was used to amplify a 313bp region of the COI gene which was sequenced and aligned to mosquito sequences from NCBI GenBank using Geneious Prime software (Figure 2 & 3)
- eDNA sample collection
 - 135 ml water samples were filtered through 10µm pore size filters to remove sediment, followed by a 0.8 µm filter to capture genetic material (eDNA)
- Aquatic environmental parameters
 - Measurements for water body surface temperature ($\pm 1^\circ\text{C}$) and salinity ($\pm 1\text{‰}$) were recorded for each sample

Study Area



Fig. 1 – Overview of marsh study areas. Heat map showing larval *Aedes dorsalis* abundance recorded in MapVision from 2020-2024. Black pins represent primary study areas with data collection taking place from August to October 2024.



Mowry Slough

Results



Fig. 2 DNA sequence alignment between complete mitochondrial reference sequences and individual mosquito barcode sequences generated using universal metazoan primers MICO1intF and JGHCO2198. (a) *Aedes dorsalis*; GenBank: PQ510210.1 (b) *Culex tarsalis*; GenBank PP960127.1

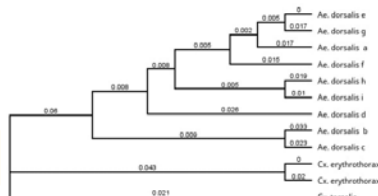


Fig. 3 Phylogenetic tree of adult mosquitoes collected in marsh habitats. Branches are labeled with nucleotide base pair substitutions per site.

Results Summary

- Barcoded mosquito specimens formed distinct clusters in the phylogenetic analysis, confirming the utility of the COI mini barcode region for identification at the genus level
- Field based measurements did not indicate a significant relationship between *Aedes dorsalis* larval distribution for salinity ($p=0.21$) or temperature ($p=0.85$)

Next Steps

- Submit archived eDNA samples and 'mock community' calibration samples for metabarcoding sequencing to assess the relationship between immature mosquito distribution, estimated biodiversity indices, vegetation cover, and elevation

Acknowledgements

I would like to thank my colleagues at Alameda County for their guidance and support.

Diel Dynamics of *Aedes aegypti* in San Joaquin County, California

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Abstract

San Joaquin County has seen an exponential growth of the *Aedes aegypti* population since its initial detection in 2019. Understanding the diel activity of *Ae. aegypti* is imperative to control their populations, minimize arbovirus outbreak risk, and reduce their biting pressure against humans. Although the diel activity in this species has been studied globally, it cannot be assumed that these observations align with all localized populations. Our study monitored the diel behaviors of *Ae. aegypti* specifically within San Joaquin County. To accomplish this, a BG-Counter unit was fitted to a BG-Sentinel trap baited with carbon dioxide and a BG-Lure. This trap setup was placed at two separate residences in Ripon, California and ran continuously for a minimum of seven days. The results indicated that *Ae. aegypti* activity peaked at sunset with a smaller peak at sunrise. In contrast, the lowest activity was during the hours before sunrise. Overall, activity was shown to continue throughout the entirety of a 24-hour period.

Effects of scales on age-grading *Culex tarsalis* using near infrared spectroscopy

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Introduction

Near infrared spectroscopy (NIRS) has been used previously to age-grade houseflies and *Aedes aegypti* based on their exterior cuticles (Joy et al. 2022; Perez-Mendoza et al. 2002). Mosquitoes are covered in chitinous scales on their thorax (Richards 1947), which may affect NIRS analysis. These scales are removed naturally over time by brushing against plants in the wild. Laboratory reared mosquitoes lack this natural removal of scales due to small cage space and lack of obstacles to brush against. Because NIRS scans the exterior cuticle hydrocarbons of the head and thorax (Escen 2023; Joy et al. 2022), it is possible that there is a discrepancy in spectra between wild-caught and laboratory reared *Culex tarsalis* used to create age-grading models. The aim of our study was to determine whether the presence or absence of scales alters the prediction of known mosquito age using NIRS.

Methods

Newly emerged female adults were separated into empty cages, maintained at room temperature, and collected at 3 ages post emergence (1 + 2, 7, and 14 days). Half of the samples for each age were skewered through the thorax and had scales removed with a paintbrush. In preparation for NIRS scanning the wings and legs on all mosquitoes were removed with fine tipped forceps. The head/thorax of each sample was then scanned using NIRS with the probe 1mm above the sample, using methods of Joy et al. (2022).

Results and Discussion

The estimated mean ages for both scaled and scaleless mosquitoes were relatively close to the known ages, except for the 7-day old group; however, the scaleless adults showed a greater variability among individual predicted ages than the scaled adults. The 7-day old adults showed atypically older age predictions in both the scaled and scaleless groups. The reason for these older predictions was unknown, but differences in the pupal cohort have been ruled out as a cause because the 2-day old adults came from

Table 1.—T-Test between scale and scaleless *Cx. tarsalis* predicted mean ages. Included in the table is the sample size for both scaled and scaleless samples as well as the mean age, T-value, and p-value.

Known Age	Sample Size (Scale)	Sample Size (Scaleless)	Mean Age (Scale)	Mean Age (Scaleless)	T-Statistic	P-value
1	15	11	2.693	1.406	−2.121	0.053
2	14	7	3.409	3.161	−0.381	0.709
7	36	13	11.156	13.949	3.862	0.001
14	28	22	9.110	10.676	2.276	0.03

P-values lesser than 0.05 are statistically significant.

the same cohort as the other age groups. There was a significant difference ($p < 0.05$) between scaled and scaleless adults for all days except for the 2-day olds (Table 1), with NIRS mean age estimates being closer to the true age for all scaleless than scaled adults, for all days except 7-day olds. These results are preliminary and require a larger sample size to determine if the trends observed here can be repeated. NIRS measurements are particularly sensitive to -CH, -NH, -SH, and -OH bonds (Joy et al. 2022), with -OH being abundant in chitin, meaning that the thin scales could be adding to the signal produce by the cuticle because scales are made of chitin (Richards 1947) as is the cuticle (Yang et al. 2021). The costs of scale removal may outweigh the benefits because NIRS has relatively fast sample processing, but scale removal increases the processing time and likelihood of damaging the sample.

Conclusions

At this time, the effect of the presence of scales on age-grading using NIRS could not be determined due to the increased variability in the scaleless adults. A greater number of samples is needed to determine if there is a difference in accuracy between scaled and scaleless females.

Acknowledgements

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contents are solely the responsibility of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention.

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Electrifying drones: Ford F-150 Lightning powers a greener mosquito control future

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Abstract

We adapted a 2024 F150 Lightning electric truck to support our drone program. Using the 96KW Pro Power accessory ports for charging the drone batteries eliminates the need for noisy gasoline engine generators. The factory provided 'Frunk' storage and an accessory organizer in the rear seat allows easy access to charging components, safety equipment, and personal/seasonal gear. After a minor 'in-house' modification of removing 1 3/8" inch from the drone height, the after-market truck bed shell, bed-slider, and trailer hitch cargo hauler provided ample storage for the drone and 18 bags of granular larvicide. Our approach resulted in a single-vehicle that was fully electric, efficient, and practical that supports our fully-electric and green drone program, which has been well received and utilized. As demand and opportunities increase, we anticipate the expansion of our program and fleet of electric vehicles.

Evaluating a drone application of VectoBac FG+ in a duck club pond

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Abstract

Coachella Valley Mosquito & Vector Control District (District) currently uses several granular larvicides with 2.8% *Bacillus thuringiensis israelensis* (Bti). These products are applied to rural water sources by drone at a rate of 11.2 to 16.8 kg/ha (10 to 15 lbs/ac). A field evaluation was performed to determine if VectoBac FG+ (6.075% Bti) was more effective at a lower application rate than other Bti granules. This lower application rate would reduce drone reloading, treatment time, and the amount of product needed. Pre- and post-treatment dip counts of field larvae and floating sentinel cages containing susceptible, laboratory-reared *Culex tarsalis* larvae were used to evaluate product effectiveness and application technique. Results showed granules were applied at the intended rate throughout the pond, and the laboratory-reared larvae in sentinel cages had high mortality rates. Unexpectedly, field larval numbers were slightly higher at most dip locations in the pond after treatment, suggesting VectoBac FG+ may not be appropriate in this type of rural mosquito habitat at this reduced application rate.

Introduction

The eastern Coachella Valley in southern California is a rural community primarily composed of agricultural land in a desert climate. It is also home to several private duck clubs which contain large manmade ponds that are flooded seasonally for duck hunting from October through February. These ponds range in area from 0.2 to 11 ha (0.5 to 28 ac) and in depth from less than 1 cm to 1 m (< 1 in. to 3 ft). Aside from the gradual inflow of water during flooding, duck club ponds are stagnant and highly organic with abundant submerged and emergent vegetation. Consequently, they become excellent habitats for rural mosquitoes, with *Culex tarsalis* being the species of greatest concern due to its potential for transmitting West Nile virus and St. Louis encephalitis virus.

Due to the large acreage of duck club ponds and their challenging muddy terrain, it is safer and more efficient to treat them with larvicides by drone. However, limitations of the drone must also be considered, including battery life and carrying capacity. When applying greater quantities of larvicide, more battery power is required, and more time is needed for refilling the drone's hopper. To address these challenges, it is beneficial to use larvicides that can be applied at a lower rate, reducing the total amount of product needed, thereby saving money and time.

The primary objective of the current study was to determine if VectoBac FG+ can be used to treat large rural larval sources in the Coachella Valley at the manufacturer's recommended rate of 5.61 kg/ha (5 lbs/ac), which is 50–67%

lower than the application rates typically used for other Bti granules.

The second objective was to develop and test a method for placing susceptible, laboratory-reared sentinel mosquito larvae into the application area to evaluate their mortality in comparison to field larvae. For this, the laboratory-reared larvae needed to be exposed to the same conditions as field larvae but contained in such a way that they may be counted 24 hours after larvicide application. Prior to this field trial, a semi-field evaluation indicated that floating larval cages inside large bins of water could be an effective way of achieving this goal.

Materials and Methods

The earthen pond used for this study was approximately 1.9 ha (4.6 ac), and located near the community of Mecca, CA. This pond receives a combination of fresh well water and overflow from a neighboring pond used for raising domestic waterfowl. The northern half of the pond was partially dry, with scattered areas of shallow, highly organic water, intermixed with emergent grass and other vegetation. The center of the pond was about 30 cm (12 in) deep with dense vegetation, and the southern half had large areas of open water from 30–60 cm (12–24 in) deep. A weather meter (Kestrel 5500, KestrelMeters.com, Boothwyn, PA) was set on a quad-pod 1 m (3 ft) above ground at the northeast corner of the pond.

Before VectoBac FG+ was applied, mosquito numbers were determined by sampling larvae at six pre-determined locations within the treatment pond and at two locations in an adjacent control pond using a 350 ml



Figure 1.—Larval sentinel cage made from a modified BioQuip emergence cage bottom, with polyester mesh liner held in place by a rubber band.

mosquito dipper (Clarke, St. Charles, IL). Larval numbers were recorded for a total of ten dips per location and then averaged.

A pair of 102-liter (27-gallon) plastic bins (Tough Tote, HDX™, The Home Depot, Atlanta, GA) which were anchored in place using a wooden stake and nylon string were positioned at 8 sites around the perimeter of the treatment pond and at 2 sites in the control pond. At each site, one bin was empty and dry, and the other bin was filled with 10 cm (4 in.) of water from the pond. Two floating sentinel cages were placed in each bin holding water. Sentinel cages were made from the bottom jars of emergence cages (BioQuip, Rancho Dominguez, CA), modified by cutting large open rectangles into their sides, covered by window screen hot-glued in place. The cages were lined with a single layer of densely woven polyester mesh (SK135 Sheer-Knit interfacing, Pellon, Clearwater, FL), held in place by a rubber band. The mesh served as a soft, water-permeable pouch that larvae could not escape, and the modified BioQuip cage served as a rigid frame to support the mesh pouch [Fig. 1].

For flotation, each cage was placed inside a foam square (approximately 19 × 20 cm in length and width and 1.9 cm thick [7.5 × 8 × 0.75 in.]) cut from larger foam boards (DIY Insulation Kit, Insulfoam®, Puyallup, WA). A hole was cut into each foam square to fit the modified BioQuip jar. The slightly wider screw-top allowed the bottom of the cage to stay submerged in water, with the open top held at the surface by the floating foam square. All cut edges of the foam were covered with strips of duct tape to prevent crumbling [Fig. 2]. Fifteen laboratory-reared, susceptible 3rd instar *Cx. tarsalis* larvae (Bakersfield strain) were placed into each floating cage.

After setup, VectoBac FG+ was applied using a drone (PrecisionVision 35X unmanned aircraft system, Central UAS Technologies, Daytona Beach, FL) with swath and



Figure 2.—102-liter (27-gallon) bin holding pond water and two floating sentinel cages, each containing 15 lab-reared *Culex tarsalis* larvae.

altitude each set at 12.2 m (40 ft). At these settings, the drone was calibrated to apply VectoBac FG+ at a rate of 5.6 kg/ha (5 lbs/ac). The total treatment area was 2.6 ha (6.5 ac), which allowed the swath to extend beyond the edges of the pond, ensuring adequate coverage in the areas where larvae were most likely to occur [Fig 3].

Immediately after treatment, the dry bins at each site were removed and the VectoBac FG+ granules collected and weighed. Using the known area of the bin's open top (0.29 m²

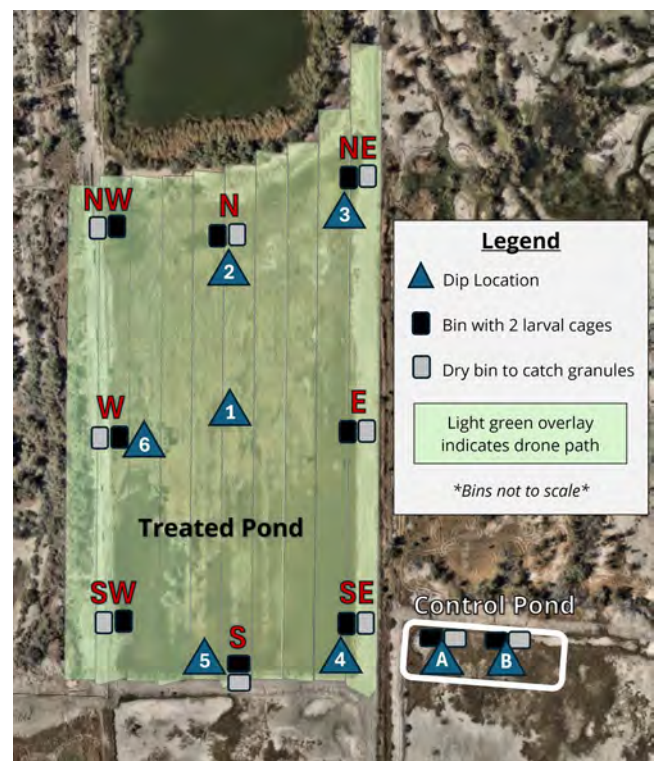


Figure 3.—Aerial view of treatment and control ponds showing bin and dip locations.



Figure 4.—Percent mortality of caged laboratory-reared larvae in bins holding pond water, and product capture rates in dry bins.

[3.14 ft²]), the rate of granule capture was calculated for each bin.

Twenty-four hours after application, the bins holding sentinel cages were collected and surviving laboratory-reared larvae from each cage were counted. The polyester pouch in each cage was removed and submerged into a pan of clear water to facilitate counting live and dead larvae. Mean post-treatment field larval numbers per dip were determined for 10 dips from each of the 6 designated dip locations.

Results

The Kestrel weather meter recorded a range of air temperatures from 22–36°C (71–97°F) throughout the 24-hour duration of this trial. The temperature was ca. 27°C (80°F) during larvicide application, and wind speeds ranged from 0–2.4 km/h (0–1.5 mph).

All dry bins placed around the perimeter of the treated pond captured VectoBac FG+ granules. The rates of

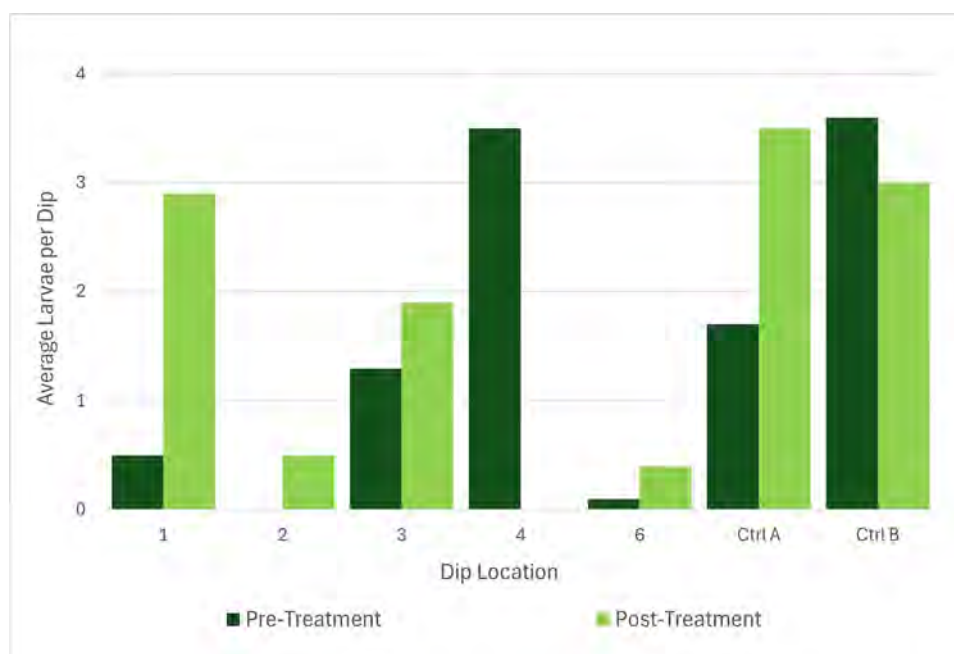


Figure 5.—Pre- and post-treatment average number of field larvae per dip at each dip location. Location 5 is excluded as it had no larvae before or after treatment.

Table 1.—Comparison of expected application time and product cost for VectoBac FG+ versus two different *Bti* granules commonly used by the District's UAS team: VectoBac GS and AQUABAC (200G).

Treatment area: 2.63 ha (6.5 ac)	VectoBac FG+ 6.075% <i>Bti</i>	VectoBac GS 2.80% <i>Bti</i>	AQUABAC (200G) 2.86% <i>Bti</i>
Application rate	5.60 kg/ha (5 lbs/ac)	14.01 kg/ha (12.5 lbs/ac)	14.01 kg/ha (12.5 lbs/ac)
Pesticide applied	14.74 kg (32.50 lbs)	36.85 kg (81.25 lbs)	36.85 kg (81.25 lbs)
Active ingredient	0.89 kg (1.97 lbs)	1.03 kg (2.28 lbs)	1.05 kg (2.32 lbs)
Hopper refills*	1	3	3
Application time**	10.11 min	14.58 min	14.55 min
Product cost for this treatment	\$142.68	\$258.38	\$149.50

* Hopper capacity = 20 lbs. Refill time = 3 min.

** Application time = flight time + hopper refill time.

application within the bins ranged from 3.6 to 10.3 kg/ha (3.2 to 9.2 lbs/ac) with the mean at 5.4 kg/ha (4.8 lbs/ac), reflecting the drone's calibrated application rate of 5.6 kg/ha (5 lbs/ac). All bins holding sentinel cages captured granules, and all but one floating cage contained granules inside with the laboratory-reared larvae. High mortality rates were observed in every bin except the NE bin, where granules did not land directly into one of the cages [Fig. 4]. Dry bins in the control pond did not capture any granules, and the laboratory-reared larvae in the control cages had an average survival rate of 95%.

Post-treatment average dip counts of field larvae increased at 4 out of 6 dip locations after applying VectoBac FG+ [Fig. 5]. Dip location 5 had no larvae before or after treatment, and dip location 4 only had larvae in an isolated pool, that was separated from the main pond. This isolated pool had the greatest number of larvae before treatment (35 per dip), and no larvae present 24 hours after treatment. The majority of larvae found before and after larvicide application were 1st-2nd instars, except in the isolated pool where most larvae appeared to be 3rd instars.

Discussion

Based on the amounts of VectoBac FG+ captured in dry bins, the drone applied this product at the desired rate of 5.6 kg/ha (5 lbs/ac). The granules also landed in all but one sentinel cage, which caused most of the laboratory-reared larvae to die within 24 hours after treatment. Placing floating sentinel cages in bins within the treatment area proved effective for evaluating larval mortality in this field trial.

The District applies *Bti* granular formulations VectoBac GS and AQUABAC (200G) at a rate of 11.2 to 16.8 kg/ha (10 to 15 lbs/ac). Using the average application rate of 14.01 kg/ha (12.5 lbs/ac), the expected application time and product cost were calculated for these two products to compare to this trial's VectoBac FG+ treatment. Table 1 shows similar amounts of active ingredient (*Bti*) would be applied for all three products, yet VectoBac FG+ would be cheaper and could be applied in less time as a result of fewer hopper refills, making it an attractive option were it to provide the expected results.

Field larval numbers did not decrease as expected after applying VectoBac FG+. However, this metric was difficult

to assess, because larvae were not present throughout the entire pond and their numbers averaged less than 4 larvae per dip before treatment. Dip locations were selected *a priori*, following the District's routine procedures. Post-treatment dips were targeted at repeat locations where larvae were found the previous day. Nevertheless, conditions seemed appropriate for larvae to ingest *Bti* from this larvicide application, and, with few mosquitoes before treatment, no larvae were anticipated to be present 24 hours after treatment. This outcome was achieved only in the isolated pool of water with no debris or vegetation.

Low mortality (6.7%) in one of two cages in the NE bin that did not receive granules indicated VectoBac FG+ must



Figure 6.—Isolated pool of water next to southeast corner of pond, where field larval dip counts were higher than anywhere in the main pond before treatment (35 larvae/dip). 100% larval mortality was achieved in this pool after applying VectoBac FG+.

land near mosquito larvae to be effective. This was further evidenced by the complete mortality of field larvae in the isolated pool of water at dip location 4. Although the water in this pool was assumed to be very similar to the adjacent pond water, it contained minimal vegetation and debris [Fig. 6]. Therefore, the rate of 5.6 kg/ha (5 lbs/ac) of VectoBac FG+ may work well in smaller sources with fewer obstructions in the water. In contrast, the main pond contained organic debris, submerged and emergent vegetation, and a dense population of water boatmen. These factors may have interfered with placing the *Bti* in close proximity to field larvae, which were scant before treatment, but present in greater numbers after treatment. This increase may have been a result of targeted sampling technique or the distribution of mosquito larvae in the pond. This trial

indicated that VectoBac FG+ may not provide effective larval control when applied at this rate in highly vegetated habitats. However, it would be worthwhile to repeat this study when field larvae are present in greater numbers before treatment.

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Evaluating pyrethroid resistance in the invasive mosquito, *Aedes aegypti*, in Madera, CA

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Abstract

The invasive mosquito, *Aedes aegypti*, was first detected in Madera, California in 2013 and has since established itself across much of the state. *Aedes aegypti* poses significant public health risks in urban environments due to its preference for blood-feeding on humans and its capacity to transmit dengue and other exotic arboviruses. Mitigating transmission primarily relies on adulticidal control. Our study evaluated the pyrethroid resistance of wild-caught adult female *Ae. aegypti* from Madera, CA compared to the susceptible Rockefeller (ROCK) laboratory strain of *Ae. aegypti*. For each pyrethroid tested, resistance was compared between groups using the CDC bottle bioassay consisting of one acetone-only control bottle and four replicate treated bottles. CDC standard concentrations of pyrethrum (15 µg/ml) and lambda-cyhalothrin (10 µg/ml) were used in each bottle. Each bottle contained approximately 25 mosquitoes, and resistance was measured using time-mortality over a period of two hours with mortality assessed at 15-minute intervals as suggested by the CDC. Two trials resulted in both wild-caught and ROCK mosquitoes reaching 100% mortality within 15 min for both pyrethroids, indicating no difference in susceptibility. Our findings indicate that the Madera population of *Ae. aegypti* remains susceptible to these pyrethroids. Our research provides valuable insights for guiding mosquito control efforts in the Central Valley where *Ae. aegypti* continues to spread.

Investigating the prevalence and ecology of a novel SFG *Rickettsia* in the San Francisco Bay Area

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Abstract

In the past decade there has been an increase in tick-borne illnesses, including those of spotted fever group rickettsiosis (SFGR) etiology. *Rickettsia* is a genus of bacteria mainly transmitted to humans through bites from arthropods, including ticks. A newly recognized human pathogen, *Rickettsia* sp. CA6269, was recently identified in California as the cause of a Rocky-Mountain Spotted Fever-Like illness. The suspected vector, *Haemaphysalis leporispalustris* (*H. lep*), commonly known as the rabbit tick, infrequently bites humans and more research is needed to understand its ecology and role in the transmission of this new pathogen. In this study, we collected and tested nymphal and adult stages of *H. lep* and *Dermacentor* tick species (the traditional vector for SFGR) to determine the vector infection, pathogen prevalence, and habitat preference of the vector in the San Francisco Bay Area. Moving forward, further research is needed to better understand this pathogen and its potential vectors to better protect public health.



Investigating the prevalence and ecology of a novel SFG *Rickettsia* in the San Francisco Bay Area

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Abstract

In the past decade there has been an increase in tick-borne illnesses, including those of spotted fever group rickettsiosis (SFGR) infections. *Rickettsia* is a genus of bacteria mainly transmitted to humans through bites from arthropods, including ticks. A newly recognized human pathogen, *Rickettsia* sp. CA6269, was recently identified in California as the cause of a Rocky-Mountain Spotted Fever-Like illness. The suspected vector, *Haemaphysalis leporispalustris* (*H. lep*), commonly known as the rabbit tick, infrequently bites humans and more research is needed to understand its ecology and role in the transmission of this new pathogen. In this study, we collected and tested nymphal and adult stages of *H. lep* and *Dermacentor* tick species (the traditional vector for SFGR) to determine the vector, pathogen prevalence, and habitat preference of the vector in the San Francisco Bay Area. Moving forward, further research is needed to better understand this pathogen and its potential vectors to better protect public health.

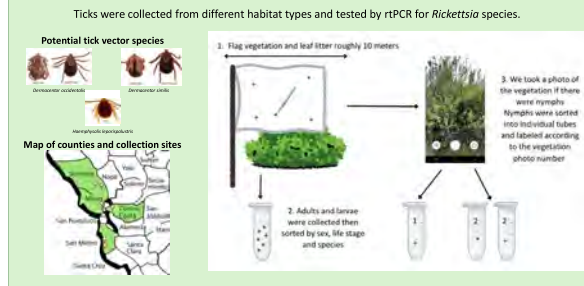
Background

- Newly recognized human pathogen, *Rickettsia* sp. CA6269, first-identified in a rabbit tick, *H. lep*. (Eremeeva et al. 2018).
- Two known cases of this pathogen causing a Rocky-Mountain Spotted Fever-Like illness were recently identified (Probert et al. 2024)
- Rocky Mountain Spotted Fever symptoms: fever, headache, rash, nausea, vomiting, myalgia, and malaise (Yoshimizu and Billeter et al. 2018)
- Case patients**
- Symptoms: fever, headache, myalgia, nausea, vomiting, acute respiratory failure, gangrene, encephalitis, and coma (Probert et al. 2024)
- Case patient 1 (July 2023): possible exposure at Alameda and Contra Costa County golf courses
- Case patient 2 (June 2004): possible exposure camping at county park and state beach in San Mateo County and rural Marin County *identified retrospectively
- The patients' samples matched the genetic sequence of *Rickettsia* sp. CA6269 found from an *H. lep* tick

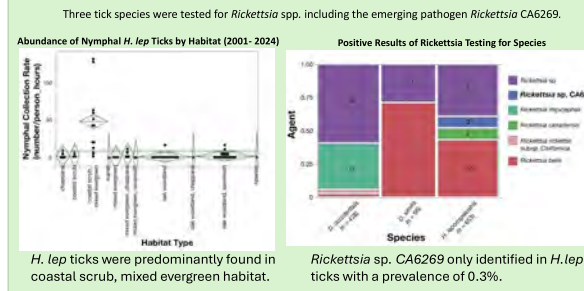
Objectives

- Explore the habitat association of the suspected vectors to understand the transmission cycle of this pathogen
- Determine the vector and infection prevalence of the emerging pathogen, *Rickettsia* sp. CA6269

Methods



Results



Results Summary

- We found a significantly higher ($p < 0.001$) abundance of *H. lep* ticks in coastal scrub/mixed evergreen habitat compared to all other habitats sampled.
- Rickettsia* sp. CA6269 was only found in *H. lep* ticks with an infection prevalence 0.3% ($n = 653$). The calculated infection prevalence for all *Rickettsia* spp. in the *H. lep* ticks was 3.5%.
- We identified six *Rickettsia* spp. in the three tick species sampled. The two ticks positive with *Rickettsia* sp. CA6269 was found in a coastal scrub, mixed evergreen habitat.

Discussion

- The mix of coastal scrub and mixed evergreen habitat type was correlated to higher abundance of nymphs collected, which we theorize to be a preferable environment for the questing ticks, likely seeking for a rabbit host.
- This *Rickettsia* sp. CA6269 was not found in the other suspected vectors, *Dermacentor occidentalis* or *Dermacentor similis*, which suggests *H. lep* ticks may be a primary vector of *Rickettsia* sp. CA6269.
- The infection prevalence of *Rickettsia* sp. CA6269 in *H. lep* ticks is low (0.3%) although is still higher than the infection prevalence of *R. rickettsii* in *Dermacentor* and *Rhipicephalus* ticks in California (0.004-0.005%) (Eremeeva et al. 2018).
- Future directions: ecological niche modeling of the habitats of *H. lep* ticks, test other tick spp. for potential vectors, and identify a reservoir host of the emerging pathogen.

Acknowledgements

Thank you to Pacific Southwest Regional Center of Excellence in Vector-Borne Diseases for funding and the California Department of Public Health for tick testing (Mary Joyce Pakigan, Erin Trent, and the Viral and Rickettsial Disease Laboratory), and San Mateo Mosquito and Vector Control District for assistance with tick collection.

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Optimizing *Culex quinquefasciatus* CQ1 Colony Supply: Quantitative analysis of key developmental parameters

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Abstract

The Shasta Mosquito and Vector Control District conducts experiments to assess the efficacy of various pesticides, which requires a reliable control mosquito population characterized by susceptibility and fitness profiles as well as an understanding of their development under insectary conditions. Our study characterized the CQ1 laboratory colony of mosquitoes over a 6 month period by examining key developmental parameters, including hatch, pupation and eclosion rates. By quantifying these factors, we were able to synchronize the development of laboratory-reared CQ1 mosquitoes with field populations, thereby ensuring a consistent supply for experimental controls. The results of our study enhanced the accuracy and reliability of pesticide efficacy assessments, ultimately contributing to more effective vector control strategies.

Optimizing urban mosquito management: A Mapping System for efficient source control in the Coachella Valley

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Introduction

Historically, urban mosquito control has been organized into zones, each managed by a technician responsible for maintaining sites mapped in a Geographic Information System (GIS). This system allowed technicians to log and review actions taken at each site, with a “History” feature displaying past entries. However, in zones with numerous mosquito sites, tracking the residual effectiveness of chemical treatments became challenging. Manually reviewing site histories was time-consuming, complicating the prioritization of follow-up efforts. To address these issues, district personnel developed a new mapping system. This system uses logic based on chemical types and expected residual durations to automatically flag sites where treatments have expired. Supervisors can toggle mapping layers to display sites by category, facilitating efficient identification of those needing attention. The system improves the assignment of daily follow-up tasks to Field Technicians, enhancing operational efficiency and ensuring timely site management across large urban zones. This poster detailed the system’s functionality, including site categorization and selection by layer, and explored the benefits and potential additional features of the system.

Area Facts

The Coachella Valley is divided into thirteen urban zones across nine cities. Technicians can be assigned to individual cities or groups of cities. There are over five thousand sites regularly inspected by technicians (Figure 1). For the purpose of this poster, swimming pools, decorative fountains, and the like were not included in the count.

Categories

Supervisors can select from twenty-four categories, either individually or in combination. Follow-up inspections for treated sites are based on 7-day, 30-day, and long-term (150-day) residual expectations. For sites with water but no larvae and treatment, the reinspection term is 30 days. For dry sites, it is 60 days. Examples of residual expectations follow:

- 30-Day Control: 0-14 days post-treatment, the site is under control.
- 30-Day Inspect: 15-29 days post-treatment, the site may be inspected to ensure product efficacy.
- 30-Day Expired: 30 days or more post-treatment, the site must be inspected and possibly retreated.

This pattern repeats for 7-Day and Long-Term products. There are also categories for neglected swimming pools and other special identifications.

Scheduling Map

Visible are sources due for follow-up inspections based on 7-day, 30-day, and long-term residuals expectations. They are all displayed as neon green dots with red rings because they received treatment upon last inspection and are now expired. Clicking on a source site reveals sub-habitat categories and inspection and treatment entry history. The counts below the map show the total number of sites in each status for all selected zones (Figure 2).

Color Coding

The system uses color coding to indicate site status. Neon green dots denote sites treated during the last visit, with rings around the dots indicating the time elapsed since treatment. Red rings indicate that the treatment has exceeded its residual period. Neglected swimming pools are identified by blue dots (Figures 3 and 4).

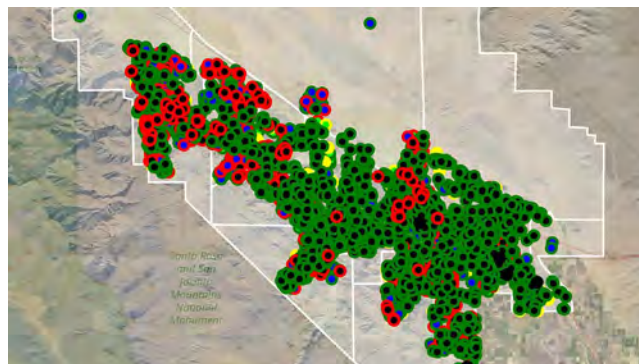


Figure 1.—Map of urban points sites in the Coachella Valley.

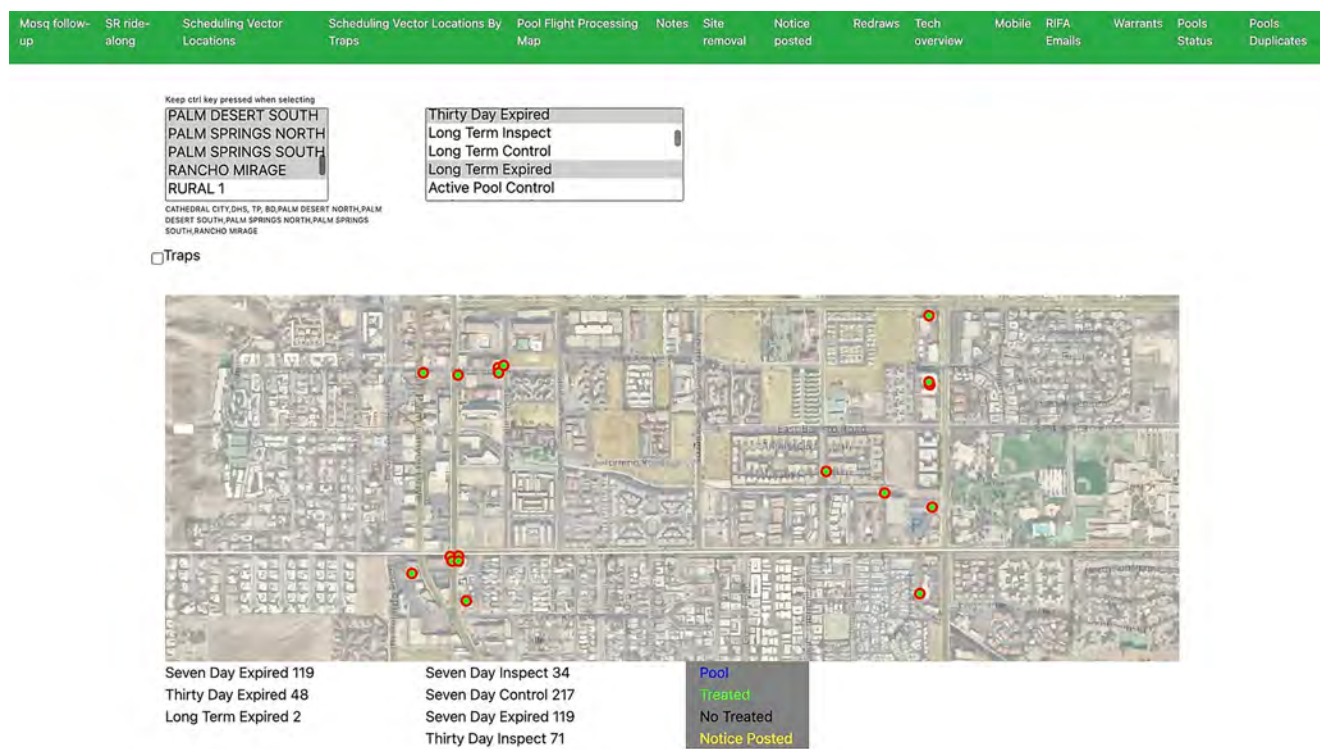


Figure 2.—Mapped sources with expired treatments.

Scheduling Sources for Inspection

Assignments are typically scheduled at the end of each prior day. Sites are assigned by choosing zones and categories. The sites are then displayed on the map and can be selected with a polygon using the mouse and ctrl key. Next, a date and a technician's name are selected from a drop-down menu (Figures 5 and 6).

The Work Assigned Map is live

The “Work Assigned Map” is live for both technicians and supervisors. After technicians inspect and/or treat sites, they submit them as “Complete.” The corresponding site

dots are instantly removed from their map, leaving only sites still in need of inspection visible. For supervisors, assigned sites remain on the map and a “C” is automatically applied next to the point to indicate a site visit was completed (Figure 7). Supervisors can visually monitor the progress of technicians individually, by supervisor group, or all at once.

Enhanced Surveillance

When there are high trap counts or virus activity, enhanced surveillance work can be scheduled by running a buffer around a specified trap. In the example, 35 sites within the buffer were not visited by technicians in the past two weeks prior to the trap collection of 4/24/2025

7-Day Residuals (Neon Green Dots)	30-Day Residuals (Neon Green Dots)	Long-term Residuals (Neon Green Dots)
7-Day Control Green Ring 1-6 Days after treatment	30-Day Control Green Ring 1-14 days after treatment	Long-term Control Green Ring 1-29 Days after treatment
7-Day Inspect Yellow Ring On the 7th day after treatment	30-Day Inspect Yellow Ring 15-29 after treatment	Long-term Inspect Yellow Ring 30-149 days after treatment
7-Day Expired Red Ring 8th day or more after treatment	30-Day Expired Red Ring 30 days or more after treatment	Long-term Expired Red Ring 150 days or more after treatment

Figure 3.—Color code legend for treated sites.

Active Pools (Blue Dots)	Mosquito Fish Pools (Blue Dots)	Dry Pools/Notice Posted (Blue Dots)
Active Pools Control Green Ring 1-89 days after inspection	Mosquito Fish Pools Control Green Ring 1-89 days after inspection	Dry Pools Compliance Green Ring 1-365 days after inspection
Active Pools Expired Red Ring 90 days after inspection	Mosquito Fish Pools Inspect Red Ring 90 days after inspection	Notice Posted Pools Yellow Ring Will remain yellow if the last inspection was "Notice Posted"
		Dry Pools Inspect Red Ring 366 days after inspection

Figure 4.—Color code legend for swimming pools.

(Figure 8). Sites assigned using this map will be displayed to technicians as they interact with the “Work Assigned Map”.

Summary

Pros

- The ability to quickly identify site status simplifies scheduling of work.
- Enhanced ability to assign and prioritize daily work.
- Efficient daily activity for technicians.

- Quick and accurate response to high mosquito trap counts or virus activity.
- Technicians gain experience in multiple zones.

Cons

- Technicians do not become as familiar with specific zones.
- Loss of rapport that can be achieved by individual technicians with residents owning permanently dry swimming pools.
- Technician instigated collaboration with city personnel.
- Limited discovery of new sites.

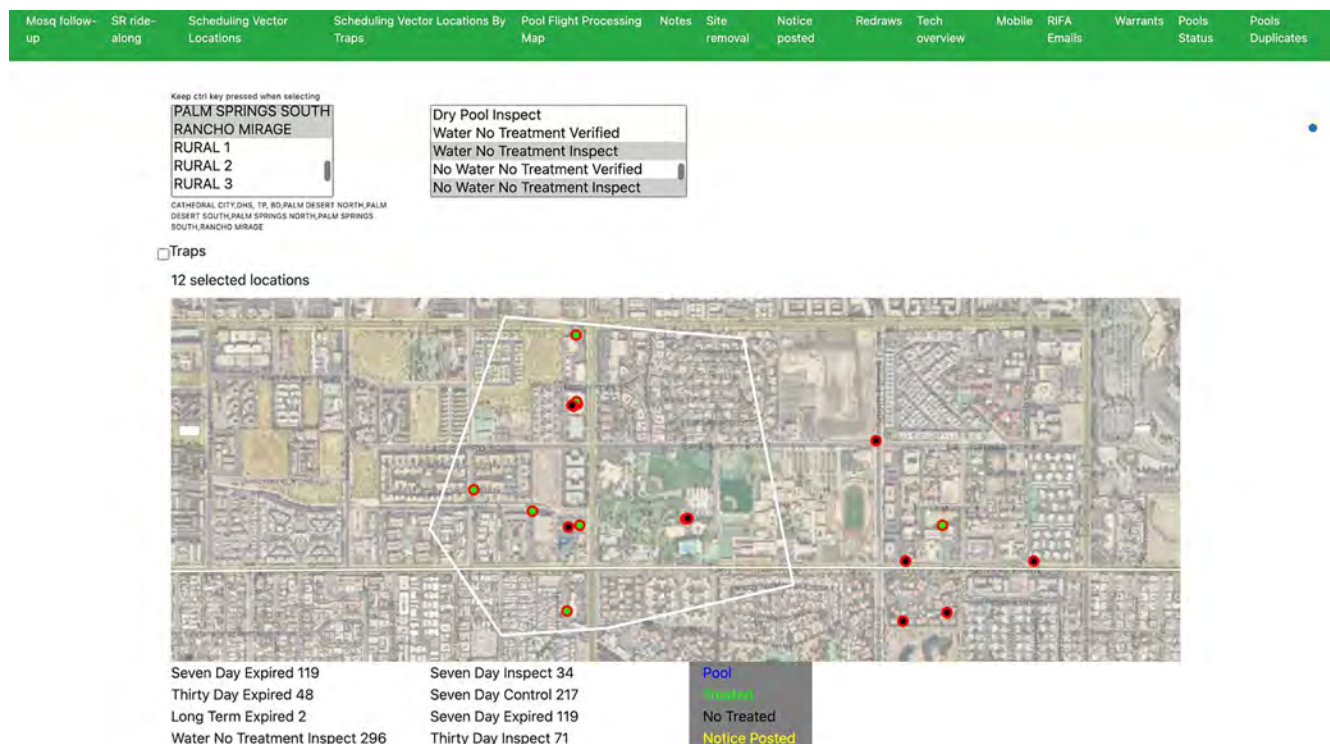


Figure 5.—Scheduling map with polygon drawn around sites to be assigned.

Keep ctrl key pressed when selecting

RANCHO MIRAGE

- RURAL 1
- RURAL 2
- RURAL 3
- RURAL 4
- RURAL 5

CATHEDRAL CITY,DHS, TP, BD,PALM DESERT NORTH,PALM
DESERT SOUTH,PALM SPRINGS NORTH,PALM SPRINGS
SOUTH,RANCHO MIRAGE

Dry Pool Inspect

Water No Treatment Verified

Water No Treatment Inspect

No Water No Treatment Verified

No Water No Treatment Inspect

☐ Traps

Scheduling Vector Locations

Scheduled Date:

mm/dd/yyyy

Technician:

Please select Technician

Submit

Cancel

12 selected locations

Figure 6.—Date and technician assignment fields.

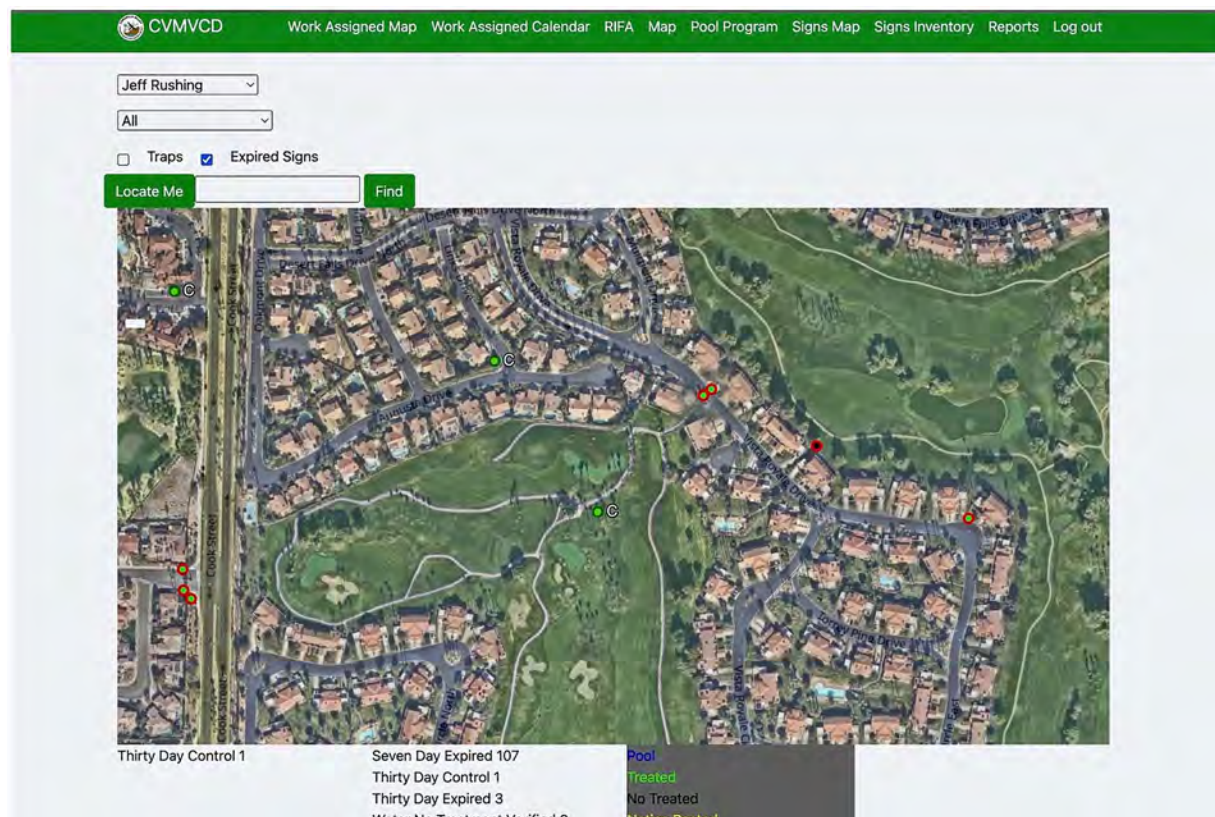


Figure 7.—Supervisor's map view of completed and not completed sites.

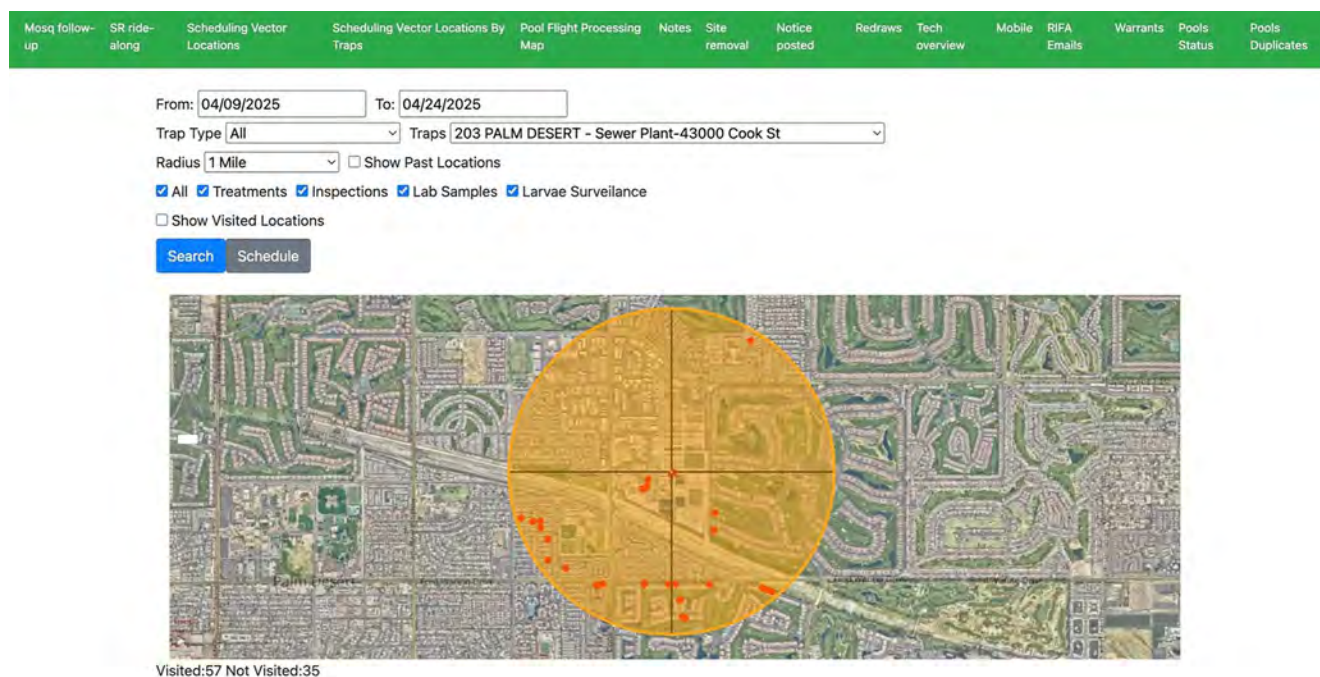


Figure 8.—Map shows sites not inspected within last 2 weeks within 1 mile radius of trap.

Conclusion

Upon comparing catch basin and drywell site data from 2022 to 2024, the District observed an increase in total inspections and treatments from 39,172 to 44,853. In 2022, inspections accounted for 61% of entries, whereas treatments made up 39%. By 2024, the proportion shifted to 50.26% inspections and 49.74% treatments. This indicated that technicians were addressing more sites requiring treatment in 2024 compared to 2022, thereby achieving better control of larval mosquitoes.

Acknowledgements

Thanks to Operations Manager Gregorio Alvarado and Field Supervisors Salvador Becerra and Antonio Molina for their vision and guidance in making the mapping system relevant and beneficial to the District's work. Special thanks to IT Department Manager Edward Prendez and IT/GIS Analyst Marko Petrovic for modeling and constructing the mapping system to meet the needs of the Operations department. Additionally, gratitude is extended to the many field technicians who embraced the new work method, thereby increasing the efficiency and relevance of site visits.

Rest in Peace, Not in Puddles: How sand is helping to solve cemetery mosquito problems

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Abstract

Cemeteries, with their numerous vases that collect water, are prime production grounds for mosquitoes, posing a public health risk. Our project aimed to reduce mosquito populations by encouraging behavior change among cemetery visitors, specifically by filling vases with sand to eliminate standing water. An inspection of over 10,000 graves identified the sections with the highest mosquito production. From this, the top 20% of graves were targeted for intervention, where sand was added to vases by our technicians. To support long-term change, signage was installed throughout the cemetery, informing the public about the benefits of using sand to prevent mosquito breeding. Additionally, sand stations were set up for public use. In addition, cemetery staff worked to improve their landscape watering strategy, further reducing sources of standing water. Monthly inspections were conducted to monitor whether gravesites retained the sand and whether visitors were refilling vases independently. Pre- and post-intervention adult mosquito trapping was carried out at 10 locations throughout the cemetery to track potential changes in the adult mosquito population. Although results on the adult mosquito population are pending, preliminary findings indicated that this intervention significantly reduced mosquito abundance, helping mitigate the potential health risks posed by vector-borne diseases in the community.



Rest in Peace, Not in Puddles: How Sand is Helping to Solve Cemetery Mosquito Problems

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Abstract

Cemeteries pose a public health risk due to their numerous water-collecting vases, which serve as prime breeding grounds for container-breeding mosquitoes such as *Aedes aegypti*. This project aimed to reduce mosquito breeding by encouraging behavior changes among cemetery patrons, specifically by filling vases with sand to eliminate standing water. An inspection of over 8,000 graves identified sections with the highest mosquito breeding activity. As a result, the top 40% of graves were targeted for intervention, where sand was added to vases by district staff. To support long-term change, concise signage was installed throughout the cemetery, informing the public about benefits of using sand in vases. Additionally, sand stations were set up for public use. Monthly inspections were conducted to monitor whether vases retained sand placement and if visitors were independently refilling them. Alongside this intervention, adult mosquito trapping was conducted at 10 locations to track population changes. Preliminary results suggest that this intervention can reduce mosquito breeding and mitigate vector-borne disease risk, but long-term success depends on sustained public engagement.

Introduction

Cemeteries are well-documented mosquito breeding sites among vector control districts.¹ Studies have highlighted that cemeteries contribute to mosquito breeding, with environmental, structural, and cultural factors all playing a significant role in mosquito populations dynamics.² However, the public often underestimates or is unaware of this issue, despite the risks posed by water-collecting vases and decorative items left at grave sites. Many cemeteries maintain lush landscapes requiring substantial irrigation, which exacerbates standing water issues. Combined with relaxed policies on grave decorations, these factors create ideal conditions for mosquito breeding. This study focuses on increasing public awareness of the problem and promoting proactive behavior by cemetery visitors. By encouraging visitors to fill vases with sand, this initiative aims to reduce mosquito breeding and subsequently lower public health risk posed by mosquito-borne diseases.

Objective

Educate and encourage cemetery visitors to fill vases with sand to prevent mosquito breeding and reduce associated public health risks.

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Fig. 1. Aerial map of Visalia Cemetery. Legend Below.



Fig. 2. Design of sign posted at three entrances and one central intersection of cemetery. Sign dimensions 36" x 48" (91cm x 121cm).



Fig. 3. Sign posted at each of the 8 sand stations throughout key areas of the cemetery. Sign dimensions 16" x 20" (40cm x 50cm).

Materials and Methods

Site Selection: Among the district's seven cemeteries, the Visalia cemetery was selected for its size, proximity to residential neighborhoods, and relaxed decoration policies. The cemetery's history, with graves dating back to the mid-1800s and 500 annual burials, makes it an active and complex site for intervention.

Adult Mosquito Trapping: Ten BG-Sentinel² traps were placed: nine along the perimeter and one in the cemetery center. Weekly trapping began during disease week 28 and tracked changes in mosquito populations.

Inspection Protocol: A preliminary walk-through identified 13 active sections (Fig. 1) along the perimeter with high visitation. These sections, totaling 8,801 graves, were inspected during disease week 31. A team of 5-6 employees recorded whether vases were dry, held water, or exhibited mosquito production. Inspections were scheduled around irrigation to avoid false positives from freshly watered sections.

Intervention Strategy: Based on inspection data, grave site rows with more than 40% holding water were prioritized. A total of 92 rows (1,407 graves) were initially filled with sand. Large, easy-to-read signs (Fig. 2) were placed at cemetery entrances to inform visitors of the intervention and encourage participation. Eight sand stations (Fig. 3) were strategically located in high-traffic areas.

Results and Discussion

Prior to the first intervention, week 37 with average temperature at 76°F (24°C), the average mosquito trap count was 28 mosquitoes per trap night (PTN). Following the intervention, counts dropped to an average of 16.5 mosquitoes PTN by week 38 (67°F, 21°C). However, populations rebounded to 33.5 mosquitoes PTN by week 39 (78°F, 26°C). In weeks 40 and 41, (71°F, 22°C), a second intervention targeted five sections with significant mosquito spikes at 36.5 mosquitoes PTN. This intervention involved sanding entire sections, adding another 43 rows (635 graves). By week 42, (67°F, 19°C), mosquito counts dropped to an average of 11.3 mosquitoes PTN, coinciding with cooler overnight temperatures. This reduction in population may be attributed to both the sand intervention and seasonal changes.

Despite these successes, challenges emerged. Graves with recently placed headstones often had sand removed, allowing water to accumulate. This highlights the need for consistent public engagement, compliance, and ongoing maintenance.

The true impact of these interventions will require years of sustained outreach targeting visitors, funeral homes, and gravestone suppliers. Early inspections before peak mosquito production and continued monitoring are critical to refining the control strategies.

Conclusion

This project demonstrates how simple, community-driven efforts can reduce mosquito breeding in cemeteries. While early results are promising, long-term success depends on active public participation and adaptable management strategies. Providing readily available sand, necessary resources, and clear signage at cemeteries can significantly enhance public compliance and the overall effectiveness of the intervention. Future efforts will focus on expanding outreach, evaluating interventions, and advocating for policy changes around cemetery practices, such as irrigation and grave decoration practices. While sand is an effective solution for reducing breeding sources, its labor and cost requirements highlight the need for shared responsibility between cemetery managers and the public. These measures, combined with continued public engagement and infrastructure support, will help ensure sustainable long-term success.

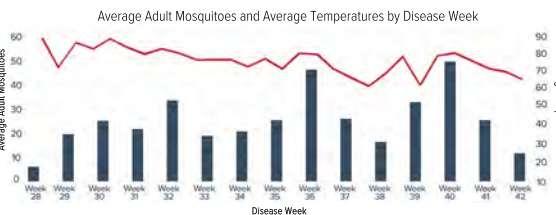


Fig. 4. Average mosquito counts of all 10 traps per disease week. Red line displays average temperature per disease week.

Testing efficacy of capturing gravid mosquitoes with CO₂ producing yeast encephalitis vector survey traps

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Introduction

Integration of BG-Pro EVS traps within routine mosquito surveillance by mosquito abatement districts has allowed for the monitoring of abundance of mosquito species and pathogen infection within affected areas. Dry ice had been the principal source of CO₂ as an attractant for host-seeking mosquitoes. While investigating the use of yeast as a possible cheaper, more sustainable, and efficient alternative, we found an increased abundance of gravid mosquitoes in our traps. Herein, we furthered our investigation into observing whether there is a greater tendency for gravid host-seeking mosquitoes to be trapped with yeast generated CO₂ compared to sublimed dry ice.

Methods

To compare dry ice and yeast as a gravid mosquito attractant, multiple traps containing Biogents-Sweetscent lures were utilized at sites within Alameda County. Sites were chosen based on mosquito abundance to obtain the greatest sample sizes to detect fluctuations in results due to weather, emergence cycles, larvicide treatments, and trap failures. The trap catches were identified to determine species, sex, and female reproductive status and the results tabulated into proportions pertaining to each site.

Results and Discussion

When comparing data within each site, test days exhibiting higher abundance (>100) resulted in CO₂ producing yeast traps having greater proportions of gravid mosquitoes than traps with dry ice. Days with relatively less

abundance (<100) were less effective in capturing gravid mosquitoes, resulting in dry ice baited traps capturing more gravid mosquitoes due to attracting more individuals overall. For *Culex erythrothorax*, there was an average 84.5% decrease in abundance when using yeast over dry ice. Although the gravid mosquito data has been unclear due to the short duration of our study, factors such as cost of materials and environmental sustainability as well as obtaining gravid mosquitoes in high abundance for virus monitoring areas may be a good reason to use yeast as a source of CO₂ for mosquito trapping.

Conclusion

The primary goal of this project was to evaluate relative efficiency of CO₂ producing yeast in BG Pro EVS traps for collecting gravid mosquitoes for arbovirus testing and surveillance. Our findings indicated that trapping with CO₂ producing yeast yielded a greater proportion of gravid mosquitoes within a trap night for *Cx. erythrothorax*, but showed a significant decrease in abundance (84.5%) compared to dry ice baited traps. These findings only applied to sites that exhibited a regular trap catch size greater than 100 individuals, and therefore these traps were not as effective in capturing gravid mosquitoes for every site. After initial costs of the Biogents-Pro EVS trap, there was a 66% decrease in cost of materials for one trap night, meaning yeast would be more cost effective than dry ice. Increasing the number of trials and lengthening the test period may provide a more precise analysis of trapping efficiency of yeast generated CO₂ for mosquito control purposes. Although dependent on abundance, yeast could be a possible lower-cost and ecologically-friendly alternative CO₂ source for capturing gravid mosquitoes for laboratory purposes.



Testing Efficacy of Capturing Gravid Mosquitoes with CO₂ Producing Yeast Encephalitis Vector Survey Traps

Eric Moyung, Ryan Clausnitzer, Eric Haas-Stapleton

Alameda County Mosquito Abatement District, 23187 Connecticut Street, Hayward, CA 94545

Objective

- Determine whether Encephalitis Vector Survey (EVS) traps with CO₂ produced by fermentative yeast would attract a greater proportion of gravid mosquitoes compared to sublimating dry ice.

Trap Sites

Figure 1. Wild-caught mosquitoes were sampled from three sites across Alameda County. Sites were chosen based on routine trapping abundance and space allotted for placement of multiple traps.



Traps Used

Dry Ice Sublimating CO₂ EVS Trap
Produces attractant CO₂ through the sublimation of dry ice (~1750L of CO₂ per 24hrs)



Reconflex Gravid Trap
Contains a water basin with detritus to provide a suitable environment for gravid mosquitoes



CO₂ Producing Yeast EVS Trap
Produces attractant CO₂ through yeast fermentative respiration (~120L of CO₂ per 24hrs)



Unbaited EVS Trap
Used as a control to test whether mosquitoes in the site would be trapped without added attractant CO₂. CO₂ traps tend to capture any mosquito attracted to a white gravid traps tend to capture egg-bearing female mosquitoes that are looking for a place to lay eggs

Results



Figure 2. Ratios of Gravid to Non-gravid Mosquitoes and Abundance at Durham Dump

- Culex erythrothorax* was the most common species collected.
- Substantial decrease in abundance on September 11.
- Before mid-September, dry ice CO₂ attracted more gravid mosquitoes; after mid-September, yeast CO₂ collected more.

Figure 3. Ratios of Gravid to Non-gravid Mosquitoes and Abundance at Del Valle Regional Park

- Lower abundance at this site with *Aedes vexans* as the dominant species.
- Gravid ratios for *Aedes vexans* were similar between CO₂ from dry ice and yeast, suggesting no preference for either CO₂ source.

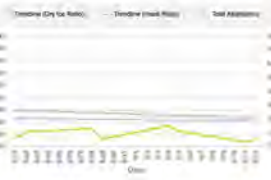
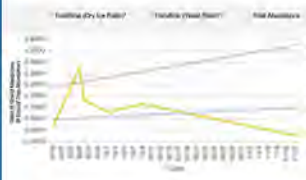


Figure 4. Ratios of Gravid to Non-gravid Mosquitoes and Abundance at Stevenson Pond

- Culex erythrothorax* was the dominant species with sustained high abundance.
- Yeast as a CO₂ source had significant and consistent gravid mosquito collection.

Figure 5. Overall Reduction in Abundance When Using Yeast CO₂ Compared to Dry Ice

- Significant loss in total abundance when trapping with fermentative yeast traps in reference to *Culex erythrothorax*.
- Exception with *Aedes vexans* with insignificant abundance lost.

Site	Abundance Lost
Durham Dump	87.38%*
Del Valle Regional Park	12.14%
Stevenson Pond	82.38%*

Cost Analysis

Figure 6. Table for Price of One Trap Night

Trap Type	Dry Ice	Yeast
Setup Cost	\$170.80	\$171.90 (Included cost of Biogenics yeast mix bag)
Refill Cost per 24hr trap	\$12.17 per 7lbs dry ice	\$4.14 per 20g Biogenics yeast and 500g sucrose

- Yeast traps cost less to refill for a single trap night but are more likely to spill and require disposal of yeast mixture.
- Dry ice is easier to transport but poses risks of frostbite and hypercapnia. Insulated ice bags require more maintenance.

Summary

- A higher proportion of gravid mosquitoes were trapped when there was greater overall abundance at the site.
- When abundance was low, dry ice increased the proportion of gravid to non-gravid mosquitoes that were trapped.
- CO₂ producing yeast in EVS traps is an effective alternative to dry ice for collecting *Culex* mosquitoes that will be tested for encephalitis viruses.
- Lower setup costs and a higher likelihood of catching blood-fed mosquitoes can support integrated vector management (IVM) efforts.
- Extending the study and expanding traps to other species and sites could yield more detailed data on yeast trap effectiveness.

Acknowledgements

I would like to thank my colleagues at Alameda County Mosquito Abatement for their knowledge and guidance.

U.A.S product characterization

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Introduction

Advances in drone technology are enhancing vector control and intervention responses to virus outbreaks. A vital aspect of Unmanned Aircraft System (UAS) treatments consists of product characterization, primarily calibration of output and swath width assessment. On average in Coachella Valley, up to 15 acres are treated/inspected per day with an All-Terrain Vehicle (ATV), whereas with drones, if weather conditions permit, up to 70 acres can be treated per day. In addition, coverage in marshes has been improved by removing constraints related to high vegetation density, making UAS technology an important feature of vector-borne disease intervention.

The primary objective of the current presentation was to demonstrate proper drone calibration procedures to ensure accurate product dispersal and thereby prevent treatment failures, pesticide resistance, and adverse environmental effects.

Methods

The first important element for calibrating granular products includes checking that the weather conditions are appropriate during the planned calibration application. Blank products without any active ingredients are used. To measure the product output rate, the drone is elevated to collect product from the spreader. The drone's motor power and door opening settings are adjusted to reach the appropriate pounds released per acre to maintain the target rate. Once the desired output rate has been attained, this calibration process is repeated two more times and an average of the output is calculated. The average output estimate then is used to determine the needed speed of the drone to ensure consistent product dispersal during a UAS mission. The equation below is used to calculate the drone's speed in miles per hour (mph) from the measured output rate.

$$\frac{\text{output rate/min} (43,560)}{\text{target rate/min} (\text{swath})} \times 60 = \text{Drone speed (mph)}$$

Once parameters have been verified and programmed into the GCS (Ground Control Station), the flight path can be determined. The flight path usually has the drone flying into the wind (Figure 1). To confirm application rates, plastic tubs (26.25" × 17.25" opening dimensions) are deployed

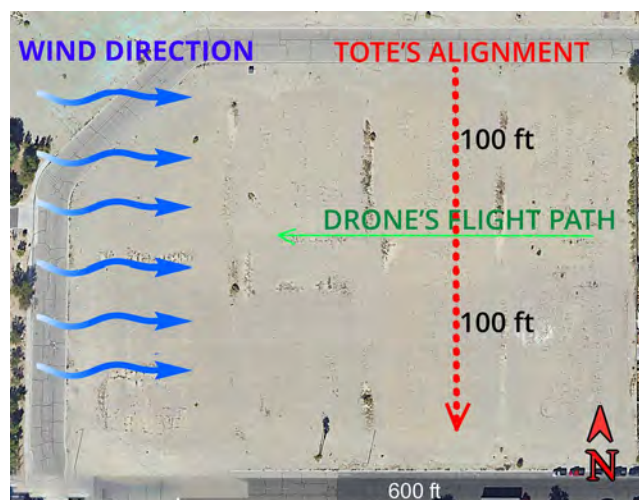


Figure 1.—Illustration showing drone flight path in relation to the wind direction. Green arrow indicates how the flight path is drawn in GCS. The red arrow are the positions of the plastic tubs. Blue arrow is the wind direction.

every 3 feet along 200 foot transects perpendicular to the drone's flight path. After a successful flight, the team transfers the contents in the tubs into small cups which are then weighed. The weight of product in each tub represents the amount of product that fell along the 200 ft distance. These values are entered into the Valent Drone Calculator (https://valentbiosciences.shinyapps.io/Swath_Analysis_Excel/) which converts these values into a graph and returns with the average pounds per acre and the predicted swath width (Figure 2).

Conclusion

These methods calculate the swath width for most granular products and the operational application rate in pounds per acre, which is essential not only to achieve the goal of mosquito suppression but to proactively mitigate the development of resistance as well as manage resource allocation.

Acknowledgements

Special thanks to Jacob Tarango for setting up the weather station. Valent Biosciences provided the drone calculator.

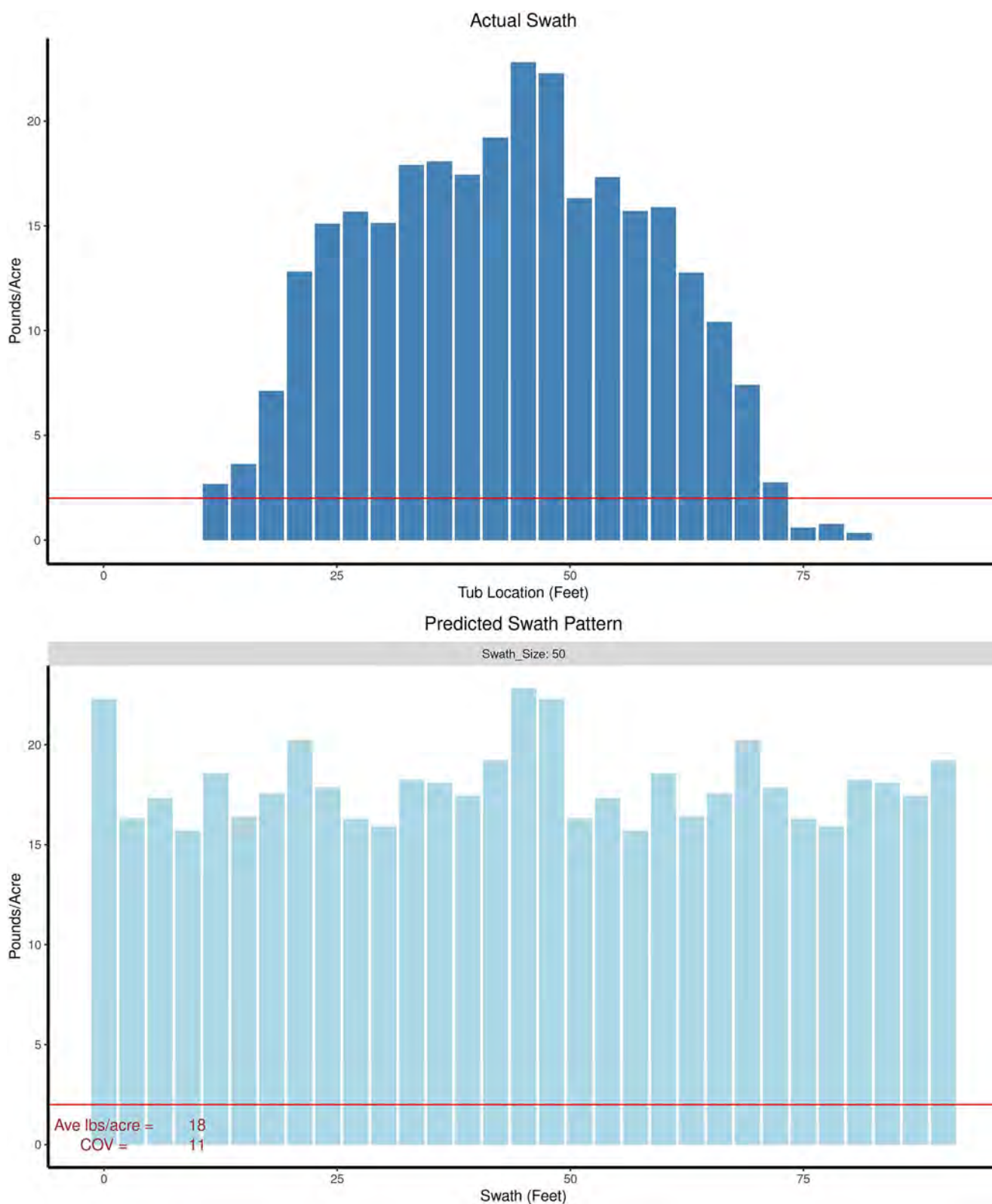


Figure 2.—Output from the Valent Drone Calculator showing the large granule (5/8 mesh) calibration results with swath width of 55 ft and application rate of 11 lb/acre. The top graph is the actual application pattern and the bottom graph is the predicted swath width.



Figure 3.—Mario Cipres, Blake Forrest, Gonzalo Arriaga, and Guillermo Rojo timing their Solo backpack output trials.

$100 \text{ linear feet} \div (\text{average time in seconds} \div 60 \text{ seconds}) = \text{feet per minute}$. The selection of a pond for this assessment is intentional, as it replicates the conditions that technicians will experience during actual treatment operations.



Figure 4.—Technician Mario Cipres running his Maruyama product formulation trials and recording his results while technician Jonathan Herrera overlooks.

Calibration Work Sheet		Solo Backpack	
Name: _____		Date: _____	
Equipment Tag Number: _____			
Travel Speed (Pond): _____ (feet/min)			
Swath Information			
Pin Swath	Coarse Fan Swath	Fan Swath	
$43560 \text{ sqft} \div \text{Swath} = \text{Linear Feet/Acre}$			
Linear Feet	Linear Feet	Linear Feet	
Output Trials & Average (Minute)			
Output Rate Pin	Output Rate Coarse Fan	Output Fan	
Trial 1.	Trial 1.	Trial 1.	
Trial 2.	Trial 2.	Trial 2.	
Trial 3.	Trial 3.	Trial 3.	
Add 3 trials & divide by 3 = Average Output Rate			
Average:	Average:	Average:	
Calibration Results			
Pin Setting Info	Coarse Fan Setting	Fan Setting	
Average Output Amount			
Floz/Min	Floz/Min	Floz/Min	
$\text{Linear Feet} \div \text{Feet/Minute} = \text{Min/Acre}$			
Min/Acre	Min/Acre	Min/Acre	
$\text{Ave. Output} \times \text{Min/Acre} = \text{Floz/Acre}$			
Floz/Acre	Floz/Acre	Floz/Acre	

Figure 5.—CVMVCD Calibration form for liquid backpacks.

To accurately calibrate liquid backpacks, VCTs fill the backpack with water and maintain consistent pressure for uniform results during output trials. Then, three trials of 1-minute intervals are timed for each spray nozzle type to determine the average output of mL/min (Figure 3). The walking speed, average output rate, and swath are used to calculate the amount of time it will take to treat an acre and the average output per acre.

To calibrate granular backpacks, technicians ensure the backpack is in good, working order prior to the event. Products are then organized by formulation and label rate. Technicians start with the smallest formulation and work up to large granules. Before starting the trials, the target rate is calculated based on the product's recommended rate and the time it takes to treat an acre. Product blanks are used to stimulate treatments with each formulation. Technicians run their equipment for one-minute intervals and collect the product into a nylon sock to weigh output (Figure 4). The lid should be closed tightly and the throttle consistent during testing for uniform results. A log of the aperture setting and bar selection should be noted for estimating the target rate. It may take several trials to find the best setting to achieve ± 0.10 lbs. the target rate. It is impossible to be 100% correct, but we strive to be as close as possible.

Results and Discussion

Calibration forms and cards were developed internally by the District (Figures 5, 6, 7, and 8). Mathematical formulas are provided directly on the calibration forms

E# _____ Solo Backpack Sprayer			
Name: _____		Date: _____ Ft/Min: _____	
Swath	Flow Rate mL	Min/Ac	Gal/Ac
Fan: _____ : _____ : _____ : _____			
C.F: _____ : _____ : _____ : _____			
Pin: _____ : _____ : _____ : _____			

Figure 6.—CVMVCD Calibration card for liquid backpacks.

or within the CVMVCD Technician Manual, offering staff the necessary guidance during the calculation process. Upon completion of the calibration procedure, technicians document their output information on the

<u>Calibration Work Sheet</u>		<u>Manzanita</u>	
Name: _____		Date: _____	
Equipment Tag Number: _____		Swath: _____	
Travel Speed (Fpd): _____ (ft/min)			
<u>Collected Product Output</u>			
Application Rate	Product	Formulation	
Aperture Selection	Target Rate	Bar Selection	
1-2-3-4-5-6-7-8-9		Left-Center-Right	
Trial 1		LBS	
Trial 2		LBS	
Trial 3		LBS	
Average Collected Output		LBS/MIN	
Minutes/Acre	Pounds/Minute	Pounds/Acre	
<u>Collected Product Output</u>			
Application Rate	Product	Formulation	
Aperture Selection	Target Rate	Bar Selection	
1-2-3-4-5-6-7-8-9		Left-Center-Right	
Trial 1		LBS	
Trial 2		LBS	
Trial 3		LBS	
Average Collected Output		LBS/MIN	

Figure 7.—CVMVCD Calibration form for granular backpacks.

Name: _____		Date: _____		Equip# _____	
Walking Speed: _____					
Product	Bar	Aperture	Output	Min./Acre:	
Sand				lbs. / Acre.	
Metalarv				lbs. / Acre.	
Small				lbs. / Acre.	
Large				lbs. / Acre.	
Oct/2024					

Figure 8.—CVMVCD Calibration card for granular backpacks.

designated forms. This information is retained at the District and by each technician for future reference. The finalized calibration cards are secured within wallets and affixed to application equipment, ensuring immediate accessibility during field operations.

Conclusion

Calibration is critical to vector control operations by ensuring the correct amount of pesticide is applied to the target area. Technicians at CVMVCD achieve consistent and accurate application rates and minimize uncertainty during pesticide treatments by calculating swath measurements, walking speed, and output amounts. The hands-on calibration events in the fall highlight the importance of team coordination and collaboration. CVMVCD's commitment to precision and teamwork ultimately enhances our mission to reduce the transmission of vector-borne diseases in the Coachella Valley.

Acknowledgements

I thank my fellow Lead Vector Control Technicians, Gonzalo Valadez and Vincent Valenzuela, for their valuable calibration training and information. I also thank the MVCAC, our General Manager Jeremy Wittie, Operations Manager Gregorio Alvarado, and my supervisor Jonathan DeSantis for the opportunity to present this poster. I appreciate the feedback from our Vector Ecologist Kim Hung and Biologist Alex Echenberg. Additionally, thank our Outreach Department, Fernando Gutierrez, Hugo Arcos, and Robert Gaona, for documenting our calibration events. Lastly, I am grateful to the Operations team and all the District staff for their unwavering support and commitment to protecting public health.



WHY CALIBRATE? ENSURING ACCURACY IN MOSQUITO CONTROL

Marisa Kelling, Lead Vector Control Technician
Coachella Valley Mosquito and Vector Control District, Indio, CA

ABSTRACT: The Coachella Valley Mosquito and Vector Control District's (CVMVCD) mission is to reduce the transmission of vector-borne diseases. The Coachella Valley provides various habitats for mosquitoes, including agricultural runoff, catch basins, duck clubs, drywells, and swimming pools. Our calibration practices allow us to protect humans, livestock, pets, wildlife, crops, and the environment by ensuring safe and accurate applications of pesticides. Application equipment calibration is a legal requirement and is done biannually. It is crucial for the success or failure of a pesticide treatment. Therefore, every time a vector control technician applies a pesticide, steps must be taken to ensure the correct amount of pesticide is applied. At the District, we calibrate urban equipment in the spring and equipment used for large, rural applications in the fall. The Operations department organizes a team event where all technicians use duck club ponds to establish accurate walking speeds during fall calibration. In this poster, I will explain why we calibrate, the principles of calibration, and the coordination involved in calibrating operations equipment.

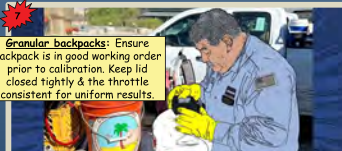


Planning: Supervisors work with the stakeholders of duck club ponds to organize the date and location for our team calibration event.

Technicians are grouped into smaller teams to promote collaboration. Lead technicians assign equipment to each technician, and it is up to the individual to ensure their backpacks are ready for the mission.



Liquid backpacks: Fill backpack with water and consistently keep it pressurized for uniform results. Time 3 trials of 1-minute intervals for each spray nozzle type to determine the average output of mL/min.



Granular backpacks: Ensure backpack is in good working order prior to calibration. Keep lid closed tightly & the throttle consistent for uniform results.

Organize products, formulations, & rates. Start with the smallest product & work up to large granules.



1. PPE & specialized gear 2. Measuring devices (liquids) 3. Nylon sock (granules) 4. Timer 5. Scale (granules) 6. Equipment 7. Product Blanks 8. Paperwork & forms



Use the walking speed, average output rate, and swath to calculate the amount of time it will take to treat an acre and the average output amount per acre.

$43560 \text{ sq ft/acre} \div \text{swath} = \text{linear feet/area of an acre}$
 $\text{Linear feet} \div \text{feet/minute} = \text{minutes/acre}$
 $\text{Average output} \times \text{minutes/acre} = \text{Fl. Oz./acre}$
 $\text{Fl. Oz./acre} \div 128 \text{ fl. Oz./gals} = \text{gallons/acre}$



Calculate the target rate based off the product's recommended rate & the time it takes to treat an acre.

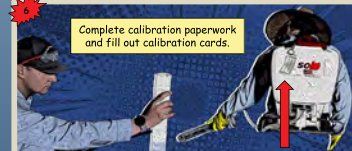
$\text{Product rate} \div \text{min/acre} = \text{target rate lb/min}$



Walking speed: CVMVCD coordinates an annual calibration event for technicians to familiarize themselves with walking in difficult terrain.

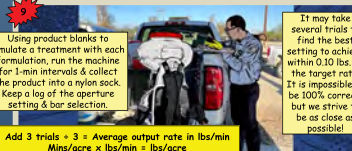
Calculate: $100 \text{ ft} \div (\text{avg. sec} \div 60 \text{ sec}) = \text{feet/minute}$

100 linear feet is visibly marked in chosen duck pond. Applicators walk the 100 linear ft with their equipment and are timed for three trials to determine an average amount of time in seconds.



Complete calibration paperwork and fill out calibration cards.

Calibration and chemical cards should be attached to equipment for every use!



Using product blanks to simulate a treatment with each formulation, run the machine for 1-min intervals & collect the product into a nylon sock. Keep a log of the aperture setting & bar selection.

Add 3 trials $\div 3 = \text{Average output rate in lbs/min}$
 $\text{Mins/acre} \times \text{lbs/min} = \text{lbs/acre}$

It may take several trials to find the best setting to achieve within 0.10 lbs. of the target rate. It is impossible to be 100% correct, but we strive to be as close as possible!

Complete calibration paperwork and fill out calibration cards.

ACKNOWLEDGEMENTS:

Thank you to my fellow Lead Vector Control Technicians, Gonzalo Valdez & Vincent Valenzuela for their calibration training and information. Thank you to the M&VCA, our District Manager, Jeremy Wilkie, Operations Manager, Gregorio Alvarado, and my supervisor Jonathan DeSantis for the opportunity to show this poster. Thank you to our Vector Ecologist, Kim Hung and Biologist, Alex Echeverria, for their feedback. Thank you to our Outreach Department, Fernando Delacruz, Hugo Arred, and Robert Green for documenting our calibration events. Thank you to the Operations Team and the rest of the District staff for their support and their commitment to protecting public health.



PHOTO CAPTIONS:

1. Eric Ortiz wading through a duck pond in Mecca, CA. Supervisors Antonio Molina and Jeffrey Kuehling helping coordinate this year's calibration event. 3. Blake Forrest wading through a duck pond in Mecca, CA for a timed walking speed trial. 4. Marisa Cipres, Blake Forrest, Gonzalo Arriaga, & Guillermo Rojas timing their Solo backpack output trials. 5. Marisa Kelling holding up a measuring beaker after recording output results. 6. Blake Forrest measuring and Mayra Espinoza draining liquid backpacks. 7. Ramon Gonzalez ensuring his Maruyama backpack is free of debris before starting the calibrating process. 8. Marisa Cipres running his Maruyama product formulation trials and recording his results while Jonathan Hernandez oversees. 9. Jonathan Leung recording his finished Maruyama results. 10. OPS department group photo in Mecca, CA.

For further information, contact Marisa Kelling at the Coachella Valley Mosquito and Vector Control District at mkelling@cvmosquito.org

Yeast vs dry ice baited EVS trap efficacy comparison

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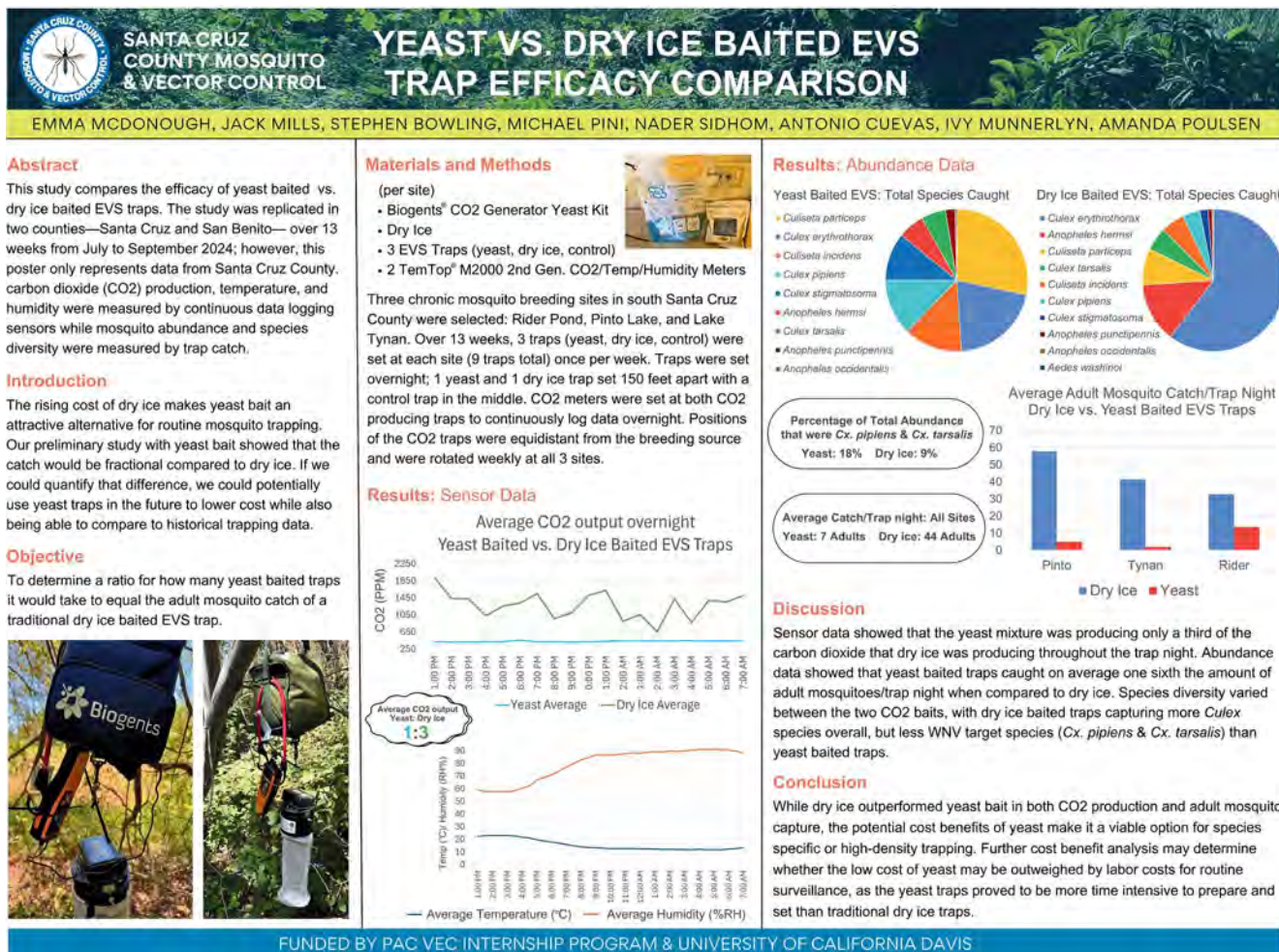
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Abstract

Our study compared the efficacy of yeast vs dry ice baited EVS traps in Santa Cruz County. Three traps were set in each of three sites each week from Jul - Sep, including: 1 yeast baited EVS trap, 1 dry ice baited EVS trap, and 1 unbaited control EVS trap. At each site the 2 CO₂-baited traps were positioned 150 feet apart and the unbaited control trap was centrally located, equidistant from the mosquito source. Carbon dioxide (CO₂) production, temperature, and humidity were measured by continuous data logging sensors, whereas mosquito abundance and species diversity were measured by counts of each adult mosquito species per trap night.

Sensor data showed that the yeast mixture produced only a third of the CO₂ that dry ice produced throughout the trap night. Abundance data showed that yeast baited traps caught on average one sixth the number of adult mosquitoes/trap night when compared to dry ice. Species diversity varied between the two CO₂ baits, with dry ice baited traps capturing more *Culex* species overall, but less WNV vector species (*Cx. pipiens* and *Cx. tarsalis*) than yeast baited traps.



Buzzing into action: Navigating dengue with dynamic outreach and rapid response

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Abstract

In 2024, the San Gabriel Valley Mosquito and Vector Control District (SGVMVCD) faced an unprecedented rise in locally acquired dengue cases in Baldwin Park and El Monte. By Mid-October, the Los Angeles County Department of Public Health (LACDPH) had identified eight cases across the county and escalated the level of activity in Baldwin Park to that of a localized outbreak.

This presentation explores SGVMVCD communications and outreach response to the detection and subsequent small outbreak of locally acquired dengue cases within its jurisdiction. It examined how the district swiftly adjusted and pivoted its outreach strategy to ensure timely and effective messaging that empowered residents to protect themselves from mosquito-borne diseases. As the situation evolved, SGVMVCD escalated its communications efforts, leveraging multiple platforms and tailored strategies to reach diverse audiences. The presentation highlighted the critical role of internal communications in maintaining a coordinated response and outlined best practices for engaging with partner agencies during crisis communications. Key insights were shared on how the district maintained flexibility, amplified its messaging, and collaborated effectively across different agencies to manage this public health risk.

Changing behavior at cemeteries to reduce the risk of mosquitoes through messaging

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Introduction

The risk of mosquito production at cemeteries is great due to the items visitors leave at gravesites that can hold water, including cans, bottles, candles, vases, toys and plants with saucers. There are also maintenance issues that cemetery workers can control to reduce the risk of mosquitoes by fixing leaky irrigation valves, properly disposing of tire piles, and properly storing grave and vault liners left outdoors. However, change at cemeteries can be slow and limited. In response, the Contra Costa Mosquito and Vector Control District (District) developed a collaborative initiative to educate and equip cemetery managers and workers with effective tools to limit mosquito production and thereby protect public health.

Methods

Previous efforts to elicit a positive response from cemetery managers to reduce the risk of mosquito production yielded little to no compliance, therefore the District developed a multi-part plan to decrease mosquito production at cemeteries. The plan featured a dual approach to develop tools for both District employees and cemetery managers and workers. The tools for both cemetery inspections and communication with cemetery managers were standardized through the use of a Mosquito Inspection Protocol and an Inspection Report. For cemetery managers and workers, the District shared educational messaging and tools during a Cemetery Workshop.

Results

The Cemetery Workshop was held during California Mosquito Awareness Week in April 2024. During the workshop, District management shared messaging on the importance of working together to protect public health by using effective mosquito prevention measures at cemeteries and reviewed the importance of complying with California Code of Regulations Title 16, § 2333, which requires cemeteries to maintain maintenance standards that control vermin and insect problems.

The workshop began with viewing the video, “One Mosquito Bite Can Change a Life Forever,” which discusses the danger that one infective mosquito can pose to the lives

of people who can become sick or die, and the sorrow for those who love them. Then, there were presentations from the District General Manager, who talked about the importance of mosquito prevention efforts at cemeteries and the legal considerations involved if no efforts were made. The District’s Scientific Programs Manager spoke about the frequency of mosquito production at cemeteries and the risk that invasive *Aedes aegypti* could pose if produced in cemetery vases. The Vector Control Planner covered common mosquito habitats within the cemetery, methods for eliminating standing water, and the application of sand in vases. The Public Affairs Director shared messaging and ways cemetery managers and workers could encourage visitors to change their behavior by stop using water in vases or leaving other items on gravesites that can produce mosquitoes.

During the first two weeks after the Workshop, District employees conducted initial inspections of Contra Costa County’s cemeteries. If they found areas of mosquito production in cemetery vases and other items on gravesites, they pointed them out to cemetery managers and workers and pointed out the other areas on cemetery grounds that could produce mosquitoes. They provided Inspection Reports so that cemetery workers could follow up on items that were noted for potential or current mosquito production. During these inspections, District employees found a broken pipe, leaking valves, and clogged drains at one cemetery. To assist the cemetery manager and the cemetery’s maintenance supervisor in resolving the issues to reduce mosquito production, a District supervisor created a map that provided specific locations of the water-related issues.

Discussion

During the subsequent weeks after the Cemetery Workshop, District employees continued to inspect Contra Costa County cemeteries using the Mosquito Inspection Protocol and issued each cemetery manager an Inspection Report. So far, the District has witnessed some positive results. Many of the cemeteries that have received inspections appear to be taking the issue of mosquitoes at cemeteries seriously. Some are posting signs inside their offices and outside with messaging on reducing the risk of mosquitoes at gravesites. One cemetery now includes discussions about mosquitoes and preventative measures

with families when they come to make burial arrangements for their loved ones. Some cemeteries have hired additional workers to remove standing water and fill vases with sand, others provide sand to visitors to fill cemetery vases on their loved one's graves, while other cemeteries have begun to drill holes in cemetery vases to prevent standing water. Coincidentally, as some cemeteries began taking positive steps toward reducing mosquito production, the District detected *Ae. aegypti* at one Contra Costa County cemetery in Aug 2024. In response, the District worked rapidly to control the mosquitoes and used this detection as a reminder of the danger of not taking steps to reduce the risk of mosquitoes at cemeteries.

Conclusions

As we take the next steps in the District's effort to work with cemeteries to protect public health, the District's Public Affairs team began working on cemetery signs and printed materials to help cemeteries disseminate information to visitors and nearby communities on their efforts to reduce the risk of mosquitoes. The effort to prompt cemetery management to comply continues to be important as District employees have found that there are still cemeteries where water remains in vases, and there is not enough progress made in filling the vases with sand. One issue the District has learned is visitors who dislike having sand in their vases will dump it out, because they maintain that they have spent thousands of dollars on a cemetery plot and believe they

should not be dictated what to do by the cemetery management. During the 2025 mosquito season, the District will continue to inspect and work with cemeteries that are open to change and move forward with abatement proceedings with those cemeteries that refuse to make the changes necessary to protect public health for their workers, visitors, and the nearby communities.

Acknowledgements

Paula Macedo, DVM, Ph.D., General Manager; Steve Schutz, Ph.D., Scientific Programs Manager; David Wexler, Operations Manager; Nola Woods, Public Affairs Director; Tim Mann, Operations Supervisor; Terry Davis, Operations Supervisor; Jeremy Shannon, Operations Supervisor; Andrew Pierce, Public Information & Technology Officer; Wayne Shieh, IT Systems Administrator; Contra Costa Mosquito and Vector Control Technicians and Inspectors: Olivia Zaragoza, Joe Cleope, Steve Fisher, Lawrence Brown, Patrick Vicencio, Brandon French, Christopher Doll, Josefa Cabada, John Boyd, Charles Baek, Miguel Munoz, Godwin Baladad, Osvaldo Mendoza, Miaja McCauley, Shaun Redman, David Obrochta, and Cameron Oliver.

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Amplifying hosts: developing community leaders to spread prevention information

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Abstract

Vector control staff often work with limited time and resources. EcoHealth Vector Education (EcoHealth) is a program of the San Gabriel Valley Mosquito and Vector Control District (District) that partners with schools and teachers to amplify the District's message. EcoHealth has implemented community education strategies, including a professional development program to train middle school teachers to facilitate Operation Mosquito G.R.I.D., a middle school community science program. The train-the-trainer program curriculum covers the importance of vector control, mosquito basics, and best practices for program facilitation. EcoHealth provides teachers with pacing guides, hands-on materials, and facilitation support to successfully implement the program. Since 2022, seven teachers have been trained by District staff, yielding 2,081 students to-date that have received mosquito education facilitated by these trained teachers and additional teachers they personally recruited and trained on their campuses. Results indicate that teachers feel properly trained to teach the content and find that the program is a valuable tool to teach scientific skills and stewardship. Community training programs, such as the teacher training save the District significant time, money, and resources while empowering community leaders to amplify source reduction and disease prevention messages.

Introduction

The San Gabriel Valley Mosquito and Vector Control District (District) is located in Los Angeles County. The District provides mosquito control services to more than 1.5 million residents in the San Gabriel Valley, and some unincorporated portions of Los Angeles County. There are more than 550 schools and 24 school districts within the District's jurisdiction. The District has two, full time education specialists, both with active California teaching credentials, who provide K – 12 educational services, including the train-the-trainer style teacher professional development program.

The Operation Mosquito G.R.I.D. (Growth Reduction, Increase Detection) program was created in 2021 to provide community science opportunities during the pandemic. Through the middle school community science program, students learn about mosquitoes, collect stagnant water samples around their homes, eliminate mosquito sources, set oviposition cups, and analyze and submit their data to the District. Participating teachers are provided with all required materials and conduct the program from their classrooms and students' homes.

In 2022, the teacher training program for Operation Mosquito G.R.I.D. was developed as a tool to amplify the educational reach of the program, without multiplying the amount of work, resources, and time spent at local schools. Train-the-trainer programs have been used to teach content knowledge and programs (Harper et al., 2024) in many fields including public health, education, and healthcare (Yarber et al., 2015). Some of the main draws for this

training are its cost effectiveness, reaching large audiences, and direct access to communities that trainees serve (Yarber et al., 2015; Harper et al., 2024). This strategy has become pivotal to the District's EcoHealth Education Program as local transmission of dengue fever and the devastating Eaton Fire have called for increased flexibility of staff workflows and limited time for educational outreach.

Methods

In the Summer of 2022 and 2024, four and three middle school teachers (trainees), respectively, signed up for the Operation Mosquito G.R.I.D. train-the-trainer program. These seven middle school teachers were offered three Continuing Education Units (CEUs) via Cal Poly Pomona. To receive CEUs, trainees were required to attend a virtual half day of training, a full day of training in the laboratory, implement the middle school community science program in their middle school classroom, and submit backyard water sample data and oviposition paper data to the District.

The training program was promoted via email to local teachers and administrators, social media posts, and the District's quarterly newsletter sent to educators.

The goals of the two training days were to build trainees' knowledge base and confidence as topic experts in their classrooms. During the first day of the summer training, trainees learned about vector control, mosquito biology, mosquito-borne diseases, and the collaborative efforts of the local, county, and state public health agencies that work to prevent the spread of disease. Guest speakers included

vector control staff as well as local and state public health experts. All presenters used PowerPoint via Zoom to deliver the content.

On the second day, class was held in-person at a laboratory. Trainees learned to identify mosquitoes at every stage and experienced each step of the Operation Mosquito G.R.I.D. program. They practiced all of the hands-on activities that would be required by middle school students, such as collecting water samples from backyard sources, analyzing water samples, setting oviposition traps, analyzing oviposition papers, and reporting data using Padlet.com — an online education platform used by EcoHealth to collect students' surveillance data. Once the trainees experienced the steps as "students," the District's education specialists provided best practices for teaching the material to middle schoolers. The program provided trainees with a Google Drive folder complete with parent permission slips, pacing guides, insect keys, worksheets, and facilitation support to successfully implement the program. Time was set aside to review the library during the training to familiarize trainees with the resources available to them. To increase participation by the trainees, the education specialists emphasized the potential impact of middle school students' actions on their private properties, such as keeping properties free of stagnant water and taking proactive measures to prevent mosquito bites.

At the end of the in-person training, trainees completed a training evaluation survey via SurveyMonkey. Participating middle school students submitted pre-assessments and post-assessments via SurveyMonkey, as well as one-, three-, and six-month post-assessments. Students additionally submitted photos of their water sample and oviposition data to Padlet.com, allowing for an assessment of the trainee's instruction and completion of the program. Trainees also completed a reflection survey at the end of the program after students had completed the program in class.

Results and Discussion

In 2022, four teachers were trained during the first train-the-trainer program. Those initial teachers personally trained three additional teachers at their schools. In 2024, three additional teachers were trained by District staff, for a total of 10 teachers prepared to teach the material without education specialists physically present on campus. To date 2,081 middle school students have been impacted by the trainee-led Operation Mosquito G.R.I.D. program or by a colleague on campus who they personally trained. This number of impacted students will naturally increase as trained teachers continue to teach new cohorts of students in their science classes in the coming years.

The cost for the 2024 in-person training was \$765.10: Meta and Google Ads (\$186), lunch and snacks (\$188.38), CEUs from Cal Poly Pomona (\$360), and Clip-on microscope adaptors (\$30.72). This cost excluded the cost of communications department and laboratory materials, and the staff time spent planning, preparing, and facilitating the program.

During the program, trainee success and the success of the training program itself was measured in multiple forms. First, trainee surveys were disseminated at the end of the in-person training during the summer and in fall once the program had been implemented with middle school students. The surveys demonstrated the program enhanced the trainee's science curriculum, offering real world science, that was highly interactive and promoted mosquito awareness and science skills such as using hands-on investigations, data collection and communicating results based on scientific findings. After implementing the program, one trainee stated, "I learned that many of the students and families were unaware of the [effects] of mosquitoes" (8th Grade teacher survey response, 11/1/2022). Another trainee emphasized the enhancement of the curriculum and connection to science standards: "GRID project is well organized, public health science investigation to eradicate mosquitoes. It not only aligns with NGSS, it is also highly interactive and educational for our students" (8th Grade teacher survey response, 11/13/2024).

Trainees learned enough information to feel confident teaching the subject matter in their classrooms to facilitate the program. One trainee stated, "I got to learn a lot about mosquitoes and how to lead students through real world experience regarding mosquito populations in the community" (7th Grade teacher survey response, 11/20/2024). Another trainee added, "I liked being able to actually go through the steps of GRID that our students would be going through. I also thoroughly enjoyed and learned a lot from the speakers and understanding the philosophical and scientific reasonings behind every step of [GRID]" (Anonymous teacher survey response, 7/21/22). Walking through the steps and providing trainees the justification for each step helped with buy-in and boosted their understanding of the program's components.

Via the surveys, trainees also provided program improvement suggestions such as less trapping, increased time frames for assignments, live progress reports, and using Google Forms instead of SurveyMonkey. Survey-suggested improvements have helped address the ever-changing school landscape and meet the current needs of teachers, keeping the program relevant and manageable for participating teachers.

Middle school student surveys were also conducted to determine knowledge gain and self-efficacy differences prior to and six weeks after the trainee-led Operation Mosquito G.R.I.D. program. Using a SurveyMonkey questionnaire, an increase in knowledge was found. Knowledge gain was measured by two questions. First, "Water trapped for longer than one week can grow mosquitoes", 53% of the middle school students answered "Yes" in the pre-assessment, and 90% responded "Yes" in the post-assessment (Figure 1). For the question, "Removing items that can trap and hold water is a good way to keep mosquitoes from growing," 69% responded "Yes" in the pre-assessment, with an increase to 93% in the post-assessment (Figure 2). This emphasizes the successful implementation of trainees' educational content and facilitation.

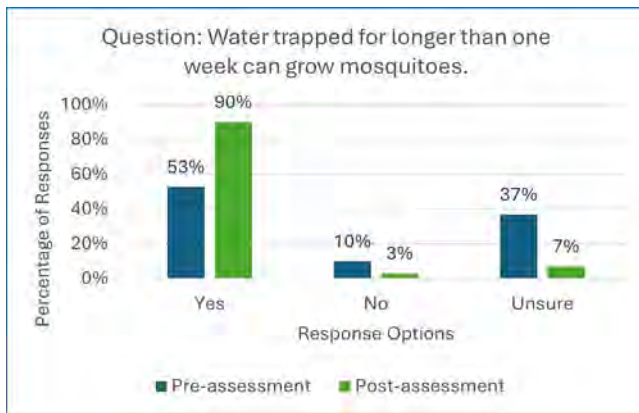


Figure 1.—Comparison of middle school student pre-assessment to post-assessment data 6 weeks later. Students were presented with the statement “Water trapped for longer than one week can grow mosquitoes”, 53% of the middle school students answered “yes” in the pre-assessment, and 90% responded “yes” in the post-assessment.

Trainees successfully trained middle school students to submit water samples and oviposition data via Padlet.com. By training the trainees to use Padlet during the laboratory day, they successfully facilitated student data submissions using photos or videos of their samples, eliminating the need for District staff to be present and verify results. Not only did the analysis of their own data increase the impact of the program for students, but it also allowed the District’s education specialists to receive data virtually from multiple schools in one day, rather than spacing out physical school visit dates for data analysis.

Although the program ran smoothly at most schools over the three-year period, trainees faced barriers as they facilitated the program. Some examples included unmaintained school microscopes, school District approval of Padlet.com on student devices, language barriers, low audio on student training videos, and limited cell phone reception during data analysis submission. Due to the detection of locally acquired dengue near one of the schools and growing parental concerns, the program was paused, and the outcomes of these students were not included in this report as the program’s timeline and process diverged greatly from the training procedure. Districts running similar programs should constantly gather feedback from trainees to address issues swiftly and remain flexible to find creative solutions to unforeseen obstacles.

Conclusion

Utilizing the train-the-trainer model to educate local middle school teachers increased the reach of the education program and expanded the program to new grade levels, while reducing time and resources spent. The District successfully incentivized local teachers with CEUs, science curriculum enhancements, and further development of students’ science skills, making the program beneficial for all involved.

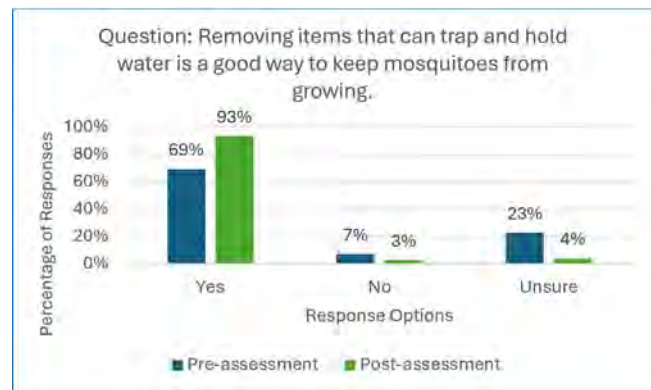


Figure 2.—Comparison of middle school student pre-assessment to post-assessment data 6 weeks later. Students were provided the statement “Removing items that can trap and hold water is a good way to keep mosquitoes from growing.” 69% responded “yes” in the pre-assessment, with an increase to 93% in the post-assessment.

To successfully train trainees, it is imperative to develop and facilitate activities during the training that mirror activities trainees will be expected to facilitate, allowing for practice, processing, and immediate feedback. Additionally, program leaders should have easy-to-implement resources readily available for trainees and that may be adjusted according to their needs. Effort should be made to assess the trainees and their students to adapt and improve the program over time.

As trainees continue to teach the material with new cohorts of students and to other colleagues, the one-time training reaps further rewards. Agencies should consider train-the-trainer programs with partners or other interested parties to expand their reach. As vectors and their diseases continue to increase in the state, vector control’s messages need to spread wider and faster. Empowering community leaders to spread mosquito education helps amplify the District’s message and makes disease prevention a shared responsibility.

Acknowledgements

Thank you to the SGVMVCD communications team and the participating teachers who have contributed an immense amount of time and energy to make this program possible.

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Not just another tool in the social media toolbox: How Nextdoor can target your communication

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Introduction

During a public health emergency, the ability to communicate with impacted residents is critically important. The Contra Costa Mosquito and Vector Control District (District) detected invasive *Aedes aegypti* in the city of Antioch in the fall of 2024. As District staff worked to provide important public health information to Antioch residents, we quickly learned how the social media vehicle Nextdoor.com differed from other social media channels. It can be used to disseminate specific, timely, and geographically targeted messaging that could increase the likelihood that residents would know why the District was conducting surveillance and control in their neighborhood. By increasing awareness through educational social media posts, the District experienced decreasing opposition to surveillance activities.

Nextdoor as a media option

Nextdoor, unlike other social media options, prioritizes community-oriented discussions about subjects including local news, information about lost pets, and safety updates. Nextdoor also stands apart from other social media platforms by focusing exclusively on hyper-local connections with neighborhoods within cities. Users of Nextdoor are required to verify their home address to ensure that they only see content relevant to their immediate community and that interactions among users remain within the same neighborhood. Nextdoor also limits who can see user posts to their immediate neighborhoods, thereby reducing the potential for broader social media exposure.

Although private businesses that have created a Nextdoor account usually pay a monthly fee to conduct advertising directed at a wider customer reach, public or government agencies can create a Nextdoor account and have access to the residents who have Nextdoor accounts in every neighborhood within a specific jurisdiction. That allows the government agency to provide targeted messaging to any and all account holders at no cost. The District has the ability to post content to all 1,008 neighborhoods throughout the 19 cities in Contra Costa County. Nextdoor also has a direct messaging app, that allows residents to send comments or questions directly to the account holder, and the account holder can, in turn, respond to the resident. In addition, Nextdoor offers the option to allow residents to

comment on an agency's post or the agency can opt to keep the discussion closed.

Use of Nextdoor in response to *Aedes aegypti*

When the District detected invasive *Ae. aegypti* in September 2024 in the city of Antioch, the District's initial response was to mail letters to the affected neighborhood informing residents of the mosquito issue and that staff would be conducting door-to-door inspections. As trapping efforts expanded, so did the discovery of additional *Ae. aegypti*. In addition to sending out several news releases and posting information countywide to District social media channels regarding the growing public health concerns in specific neighborhoods of the city, District staff decided to target the information being disseminated via Nextdoor to neighborhoods where these mosquitoes were being found in real time. Neighborhoods in Antioch that were targeted for information and updates including why the mosquitoes are dangerous, what District staff were doing to control the mosquitoes, and why residents needed to contact the District upon receiving a letter in the mail.

As surveillance and trapping for *Ae. aegypti* continued, the number of neighborhoods included in subsequent Nextdoor posts expanded and questions that arose from residents regarding door-to-door inspections were addressed. Specifically, residents wanted to know that District staff would be identifiable, wearing a uniform and having an ID badge. Residents wanted to know exactly what the inspection consisted of, and if there were any fees associated with the inspection. The District answered those questions by posting messages and photos of staff in uniform in a post that targeted 13 neighborhoods in Antioch where door-to-door activities were taking place (Fig. 1). The District also held a community meeting at an Antioch park and invited residents in the affected areas via a neighborhood-specific post to meet District staff in-person and ask questions about upcoming larval mosquito control inspections and operations.

Conclusion

Targeting social media posts to specific neighborhoods within a city or county may increase community engagement, and be a vehicle for addressing the needs and concerns of residents within that specific area. Also, by making sure posts are relevant to the current situation

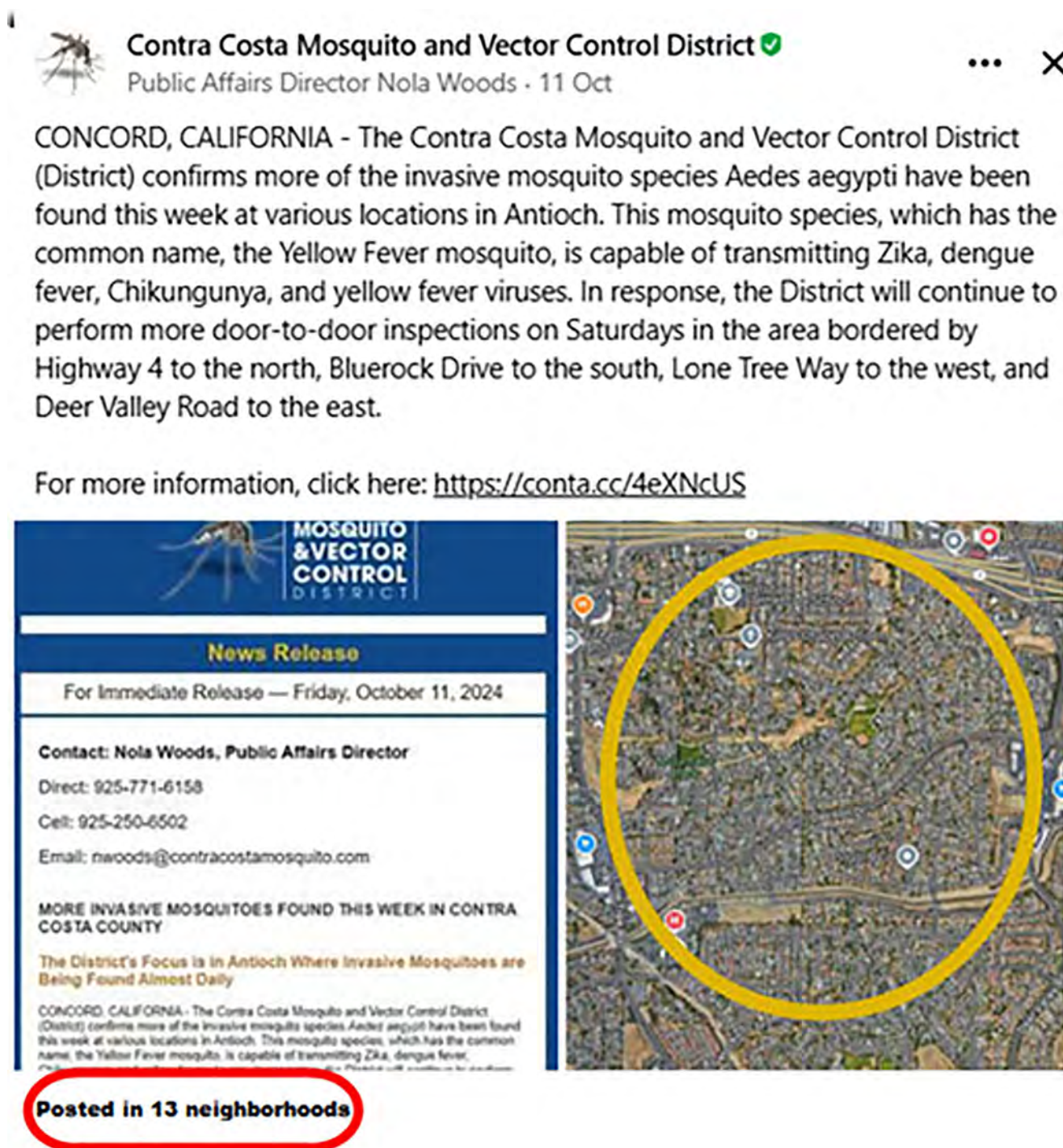


Figure 1.—Screenshot of a Nextdoor social media post that was posted in 13 specific neighborhoods in the city of Antioch informing residents of the expanding discovery of invasive *Aedes aegypti* mosquitoes.

creates a more meaningful experience for Nextdoor app users within that neighborhood. Lastly, and quite possibly most important, targeted posts can encourage connectivity among residents while building a stronger sense of community and a partnership with public health agencies and mosquito control districts.

Acknowledgements

The author would like to express his appreciation to staff for their dedication to protecting public health during residential door-to-door inspections in neighborhoods where *Aedes aegypti* mosquitoes were discovered.

Where shall we start? How to start building a classroom-based Mosquito and Vector Control curriculum

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Abstract

With the expansion of invasive species throughout California, mosquito and vector control agencies need to engage diverse communities in different forums. One avenue for engagement is through in-classroom presentations to students. Working directly with schools enables a quick route to new audiences and the ability to distribute targeted messages directly to geographic specific communities. To achieve these benefits, educators need to first develop and then implement their classroom presentations. Not all presentations are equal, and not all presentations suit all educators.

In 2022 we started the Mosquito and Vector Control Educators Group (MVCED) specifically to learn from educators throughout the US about their programs. As the group has expanded to nine states and over 40 educators, we have noticed similar patterns and challenges, including how to build a classroom program. We encourage mosquito and vector control educators to think about these main components when designing or redesigning their education programs. This presentation is the beginning of a toolkit for new and continuing mosquito and vector control educators.

Guiding questions for developing a program:

- *Which grades to target?* Certain ages, mainly younger children are more receptive to messaging about intervening in the mosquito life cycle than older ages.
- *Which standards will align with the lesson?* Our group recommends targeting standards in life science and Next Generation Science Standards; however, many lessons also infuse math and health science standards. Many teachers feel a lot of pressure to align every moment in their classroom to a state standard. Mosquito and Vector Control Educators can justify their presence in the classroom if they meet state standards for the specific grade.
- *What format will work best for district staff?* Educators should think about the amount of energy and content they plan to put into their presentations. Some educators choose to hold the attention of entire classes for hours at a time, whereas others have only fifteen minutes in a classroom and will choose to frontload content with teachers before going into the classroom. Determine what will work best for the educator and then modify the presentation based on which types of presentations are easiest to book with schools.

The following questions can help guide which messages to highlight during a classroom-based presentation:

Are there specific topics or messages the district wants in schools? Depending on the mosquito issues the specific district contends with, some may choose to concentrate about West Nile virus while others highlight dengue. Certain districts may have many private and unmaintained pools in their area while others only have well maintained municipal pools. Educators should know about their district needs and develop messages based on what would resonate best.

Are there certain populations or locations the district wants to work with more? Class education extends beyond the classroom to families and school administrators. If there are families that have limited English skills, or there is an area where the district doesn't conduct many service requests, classroom presentations are a great way to start learning more about the area and to assess the challenges residents may have in accessing services.

What staff time (including prep) and budget is available? Many Mosquito and Vector Control Educators hold multiple roles in their districts, and therefore when building a program educators must consider the amount of money and staff time to devote to classroom presentations.

Three things to keep in mind when preparing a school-based program:

1. Look at the district mission and prioritize messages that resonate within a classroom. Children may not remember the names of mosquito borne diseases, but they have all experienced a mosquito bite. Discuss ways mosquitoes make being outside unpleasant and how annoying mosquitoes can be.
2. In which ways can children intervene with a mosquito issue? Many of the messages districts promote require action from an adult- cleaning an unmaintained pool, treating ponds with bacteria, using repellent and so on. Children need to know what they can do on their own. Highlight things they may be responsible for such as picking up toys in the

yard or clearing out a dog's water bowl daily is an opportunity for them to build the habit of eliminating mosquitoes.

3. What are their strengths in the classroom? Think about which grades or ages to work with. Consider how the educator wants to present in the classroom—are they silly, are they scientifically focused, do they like to sing a lot? Different educators can bring different styles and it helps to design a program that works well for the educator. Honestly assess classroom management skills and ensure the program goals are manageable. Educators typically become more comfortable in classrooms over time, and can enhance their goals when they feel stronger

in the classroom. Educators should also consider which size of groups they wish to present to. Some educators will split classes in half in order to present to smaller groups, while some prefer multiple classes at once. It depends on what will work for both the educator and the school. We encourage mosquito and vector educators to use our guiding questions and resources as a support to build a strong in classroom program for students at the district.

We hope these questions are helpful and we look forward to compiling more information into a toolkit for new and continuing mosquito and vector educators in the future.

Neighbors love free stuff: Using Nextdoor as an outreach platform

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Introduction

Public education – including sharing information about basic vector biology and personal protective measures – is a core component of an integrated vector management strategy. However, the impact of public education programs is typically difficult and resource-intensive to quantitatively measure. The current study aimed to: 1) raise awareness of the San Mateo County Mosquito and Vector Control District (District) among District residents, 2) leverage the social media platform Nextdoor to advertise a District program, and 3) assess knowledge/attitudes/practices of residents regarding ticks and Lyme disease in the District.

Methods

The free kits included a variety of tick-bite prevention education materials and tick removal tools. The kits were advertised in several posts on Nextdoor as well as using other social media platforms and through collaborations with other organizations and agencies. Residents were able to self-select into a knowledge/attitudes/practices study – they took an online pre-test, a post-test shortly after receiving their tick-bite prevention kit, and a post-test at least one month after receiving their kit. The study materials were reviewed and approved by the Institutional Review Board of the University of California, Santa Barbara (IRB 2-24-00321).

Results and Discussion

Over 1,300 residents signed up for free tick-bite prevention kits, more than half (51%) of whom found

out about the program via the District's posts on Nextdoor. The program resulted in multiple outreach presentations to groups of dog owners, staff at a local park, and others at risk of encountering ticks in their daily activities. This resulted in an increase of more than 800 subscribers to the District's monthly newsletter. Preliminary results of the knowledge/attitudes/practices study indicated that participants increased their scores from pre-test to post-test and maintained the increase in score in the second post-test as well.

Conclusions

Residents were receptive to vector education efforts via less-traditional routes (advertising on social media, written and video materials for in-home review) and such education can be measured by measuring residents' knowledge, confidence, and likelihood of using known personal protective measures to reduce encounters with ticks and risk of Lyme disease transmission.

Acknowledgements

Authors thank all the residents who signed up for kits, especially those who enrolled in the knowledge/attitudes/practices study, as well as the San Mateo County Mosquito and Vector Control District staff who helped deliver the kits. CDPH staff were helpful in the study design process. The Pacific Southwest Center of Excellence in Vector-Borne Diseases funded (through grant to Kacie Ring) a portion of this study.

Would you like I.C.E. with that? An all-encompassing guide to living with the new mandates of EV compliance

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Abstract

Each Mosquito Abatement or Vector Control District within California is nearing the requirement to comply with the California Air Resources Board (CARB) Advanced Clean Fleets Program (ACF). Depending on where your District is on the Technology Acceptance Model (TAM), you are likely to fall somewhere between hopefully being able to retire before it becomes required or already having to replace your entire Internal Combustion Engine (ICE) fleet for newer electric vehicles (EVs). Our presentation covered CARB requirements, small off-road engines (SORE), and TAM modeling to aid in implementation, as well as Contra Costa Mosquito and Vector Control District's pathway to EV adoption.

California CLASS: A Local Government Investment Pool option for mosquito abatement districts

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Abstract

In the face of increasing fiscal challenges, especially from the spread of invasive *Aedes*, mosquito abatement districts must optimize their investment strategies to ensure financial stability and growth. This presentation explores the significant benefits of utilizing the California CLASS Local Government Investment Pool (LGIP) as a strategic tool for local government investment. California CLASS offers a professionally managed, high-quality, and diversified portfolio, specifically designed to meet the unique liquidity and return needs of local public agencies. By pooling resources, participants benefit from economies of scale, reduced administrative costs, and enhanced investment opportunities that might be inaccessible to individual entities. Moreover, California CLASS emphasizes transparency, with regular reporting and adherence to stringent regulatory standards, ensuring the security and integrity of public funds. Through case studies and comparative analysis, this talk demonstrates how leveraging California CLASS can lead to improved cash flow management, increased yield potential, and strengthened fiscal health for mosquito control districts. Attendees will gain insights into the operational mechanics, risk mitigation strategies, and performance metrics that underscore the value of California CLASS, empowering them to make informed decisions that align with their financial objectives. This presentation highlights the strategic advantage of adopting California CLASS in fostering sustainable financial practices and achieving long-term economic resilience for local communities.

Cybersecurity readiness: Safeguarding your network infrastructure

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Abstract

This presentation focuses on the significance of cybersecurity readiness in today's digital landscape. The rapid digital transformation and increased reliance on cloud computing, the Internet of Things (IoT), and remote work setups have led to a sharp increase in cyber threats. Organizations and individuals face significant financial losses, reputational damage, and operational disruptions due to cyberattacks. This discussion explores the critical factors contributing to the growing number of attacks, including the rise of Cybercrime-as-a-Service (CaaS) and credential theft.

Through real-world cases, we illustrated the consequences of insufficient cybersecurity measures. The 2024 Change Healthcare cyberattack, which stemmed from missing multi-factor authentication (MFA), resulted in a \$22 million ransomware payment without data recovery, affecting over 100 million users. Similarly, Lincoln College's closure in 2022 underscores the importance of robust data backup strategies.

To mitigate risks, individuals must adopt proactive measures, such as using strong passwords, enabling MFA, staying alert to phishing attempts, and keeping software updated. Leaders, on the other hand, should focus on cybersecurity education, enforcing strict authentication policies, establishing backup and recovery plans, and continuously refining cybersecurity strategies. This overview highlights practical steps organizations can take to fortify their defenses and offers free resources, such as those provided by the National Cybersecurity Alliance and cybersecurity service vendors, to promote ongoing awareness and preparedness. By integrating these strategies, businesses and individuals can better safeguard their networks against evolving cyber threats.

Empowering improvement: Building growth through supportive field evaluations

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Abstract

Performance assessments are crucial for evaluating the effectiveness of vector control programs, but inconsistent evaluation processes can create ambiguity and hinder communication among staff. Without clear expectations, it becomes difficult to align individual tasks with organizational objectives, ultimately affecting program success and the prevention of vector-borne diseases. Additionally, the lack of standardized procedures may lead to unaddressed obstacles in the workflow, resulting in inefficiencies and increased errors.

In this presentation, we discussed how implementing field evaluations of staff helps create a consistent framework that aligns employees with common goals, reduces ambiguity, and fosters collaboration. These assessments allow us to evaluate both individual effectiveness and existing procedures, providing valuable feedback for refining operations. By continuously assessing staff performance and operational processes, we enhance team functionality and strengthen our overall efforts to prevent vector-borne diseases. Additionally, recognizing outstanding contributions boosts morale and encourages continued high performance.

Indoor Fish Rearing: A sustainable shift from traditional outdoor storage

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Alameda County Mosquito Abatement District (District) began its mosquitofish program by gathering fish from natural sources and maintaining them in outdoor tanks (Fig. 1). Over the past 35 years, the District has been purchasing mosquitofish from increasingly scarce fish farms that are neither local or dependable. In the Fall of 2022, the District began planning for a revised fish production program, and in November 2023, the Board of Trustees approved funding for facility construction. In July of 2024, a new 1,115 sq/ft Aquaculture Facility was completed to establish a sustainable and productive indoor mosquitofish rearing program (Fig. 2).

Every district takes a unique approach to its mosquitofish program, but all share a common goal: producing mosquitofish as a clean, biological method for larval mosquito control. As natural sources of fish become scarcer and collecting them become increasingly labor-intensive, we recognized the need to shift from fish storage to active rearing.

Transitioning from simple outdoor storage to an indoor fish rearing program takes significant planning. Research and development were the first steps, and as with any local government project, financial planning and Board approval are essential. Once funding was secured, Requests for Proposals (RFPs) and Requests for Qualifications (RFQs) from architects and general contractors were solicited,



Figure 1.—Outdated outdoor fish storage structure that was demolished to make space for an indoor fish rearing facility.



Figure 2.—Exterior of the new, 1,115 sq/ft indoor Aquaculture Facility for rearing fish year-round.

reviewed, and selected. A project manager oversaw every stage of construction, ensuring each component was carefully reviewed and completed according to specifications. This process seemed just as time-consuming as gathering fish from the field—but it was necessary.

As construction was completed, new and existing equipment was installed in the new facility (Fig. 3) and a new approach to fish production began. We look forward to assessing this initiative and presenting data on the success of our sustainable indoor fish-rearing program at future conferences.



Figure 3.—Indoor fish-rearing tanks with water conditioners, climate control, and automatic fish feeders.

Delta MVCD Mosquitofish Program: Current and future directions

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Mosquitofish (*Gambusia affinis*) serve as an effective biological control method for reducing mosquito populations, primarily *Culex* larvae and pupae. In response to the increasing need for effective mosquito management, the Delta Mosquito and Vector Control District (District) developed an indoor mosquitofish rearing facility in 2020 (Fig. 1). The mosquitofish program consists of breeding, growing, acclimating, holding, and distributing mosquitofish to various mosquito larval sources. Although the program has been operational for four years, new challenges continue to emerge such as: managing water mold (*Saprolegnia spp.*),



Figure 1.—Indoor rearing facility for breeding and growing mosquitofish.



Figure 2.—Outdoor facility for acclimating and holding mosquitofish ready for distribution.

treating bacterial disease outbreaks, optimizing brood tanks for fry production, and regulating water parameters from city municipal sources. Future projects will assess the efficacy of mosquitofish in reducing mosquito larval densities in different field conditions, exploring methods to increase fry production, and refining the acclimation process to reduce transfer stress (Fig. 2). This program is an important part of the District's integrated vector management strategy and aims to provide a reliable, year-round supply of mosquitofish to support control operations and meet public demand.

Origin and evolution of the Lake County Mosquitofish Program over time

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Abstract

The Lake County Vector Control District (LCVCD) has owned and operated a 2.74-acre satellite pond property for the last 62 years. Although it was originally purchased to study different pesticide and biocontrol strategies to control the Clear Lake gnat, the objectives of the LCVCD have changed over the decades to the management of mosquitoes. Because of this new direction and the money generated by a benefit assessment passed in 2009, the LCVCD constructed an entirely new mosquitofish-rearing facility to accommodate the community's existing and future biological control needs. Since the construction of this new facility, we have learned a lot about the dynamics of building, operating, and maintaining a large-scale mosquitofish program. Many successful strategies have been implemented at our pond property throughout the years that have greatly enhanced our fish production and are worth sharing with others who have the same goals.

The Clear Lake gnat Problem

The current presentation describes the origin of the LCVCD and the evolution of our programs to eventually include the current mosquitofish-rearing facility. Lake County is unique in that it is centered around Clear Lake (Figure 1). Clear Lake is the largest natural lake entirely within the borders of California and is one of the oldest lakes in North America at an estimated 2.5 million years old (Lake County 2025). It is eutrophic which means it is shallow, warm, nutrient-rich, and abundant with fish and wildlife. These eutrophic conditions created unique problems. In the mid-1900's, the Clear Lake gnat (*Chaoborus astictopus*), a non-biting phantom midge, emerged from the lake by the billions in huge mating swarms causing a nuisance for residents and tourists alike (Figure 2). Historical data from Lindquist and Deonier 1942 indicated that one light trap set for 2 hours caught 88 pounds of gnats! County residents at the time claimed it was difficult to breathe during the gnat hatches. They were so abundant and irritating that the public demanded a solution to this problem. Therefore in 1948, the county voted to establish the Lake County Mosquito Abatement District (LCMAD) (later changed in 1995 to Lake County Vector Control District) to combat these massive swarms of irritating gnats while also controlling mosquitoes.

Within the first couple of years, gnat research and lake treatments were well underway (Figure 3). Different oils and chemicals were studied and applied on Clear Lake and local ponds by use of boats, barges, and airplanes with great success (Lindquist and Deonier 1942). Neighboring Mendocino, Butte, Placer, and as far south as Santa Cruz, Monterey, and San Luis Obispo counties, noticed the

progress LCMAD was having with gnat problems and requested assistance. As the years went by, the LCMAD budget and assets grew. This resulted in the purchase of our lakefront property, construction of a pier to house boats and barges, and eventually a laboratory and shop.

Historically, the pesticides used were not target specific. Eight years after the first lake treatments began for the Clear Lake gnat, high levels of DDD (dichlorodiphenyldichloroethane), an organochloride similar to DDT and widely heralded at the time as the "miracle insecticide", were found in the food chains around the lake. At this time our Board emphasized the dire necessity for more intensified entomological research into other control methods.

The 1960's ushered in a wave of new ideas using biological control methods including the use of bacterial and protozoan diseases as well as fish to eliminate gnats and mosquitoes (Figure 4). Because the local fish had shown little to no sign of consuming gnat larvae, emphasis was placed on finding and importing a new fish species into California that would readily consume gnats in Clear Lake. In collaboration with Fish and Game, the LCMAD considered shad, inland silversides, and emerald shiners; but because a biological introduction into Clear Lake would be a permanent one, a place was needed to evaluate species for possible introductions.

In 1962, LCMAD purchased a 2.74-acre satellite property about a mile from our District headquarters, constructed 11 ponds, and dug a 300 ft. on-site well (Figure 5). For many years, these experimental ponds housed several imported species of fish that were carefully monitored, studied, and successfully bred and acclimated to California weather. These new fish showed encouraging progress for gnat removal. After the introduction of inland silversides,



Figure 1.—Clear Lake in Lake County, California is the largest natural lake entirely within the borders of California. The lake encompasses roughly 68 square miles.

Menidia beryllina, into Clear Lake and other lakes and ponds throughout California, the Clear Lake gnat rapidly declined in abundance. These gnats still exist in Clear Lake and throughout California, but at very diminished and manageable numbers.

After the introduction of the new fish species into the lake and the subsequent reduction in gnat abundance, gnat control was discontinued, and the satellite experimental pond property fell into disrepair. As the decades passed by,



Figure 2.—The Clear Lake gnat (*Chaoborus astictopus*) plagued Lake County in the early 1900's.



Figure 3.—The Lake County Mosquito Abatement District owned and operated several barges to administer chemical treatments to Clear Lake and hired airplanes to spray floating gnat egg drifts. In some instances, gnat egg accumulations were reported to be 1 to 20 feet wide and extend for miles along the shoreline.

the property became overgrown, the old ponds no longer held water for long periods of time, and the plumbing was broken. Antiquated barges were stored there and the property was used for various District training exercises.

Most mosquitofish (*Gambusia affinis*) were collected off-site during this time. We utilized local golf course ponds, private ponds and pools throughout the county, and would purchase fish from our neighbor in Glenn County, an hour



Figure 4.—The biological control research efforts of the 1960's included various species of parasitic *Pythium*, spore-forming bacteria, and protozoans to eliminate gnats.



Figure 5.—Mosquito Abatement Experimental Pond property purchased in October 1962.

and a half away. The process of retrieving fish great distances from LCVCD headquarters and bringing them back for public distribution and release into rice fields was very time consuming and a great expense for our District.

New Fish Production Facility

In 2009, LCVCD passed a benefit assessment that allowed the construction of a new mosquitofish-rearing facility and rejuvenation of the old satellite property. The old ponds and buildings were demolished, all the trees removed, and construction started in 2015. The basic layout of the renovated property consisted of a large shop and pesticide storage shed near the entrance, two $\frac{1}{4}$ acre ponds in the middle, and a round, deep fifth-acre pond in the rear of the property (Figure 6). The two $\frac{1}{4}$ acre ponds are identical and have been used for many comparison studies.

The new pond design mimicked that created by Woody Schon from the Sacramento-Yolo Mosquito & Vector Control District, and is designed for maximum mosquitofish production. The finer details of its construction were presented in 2017 (Courcier 2017). Herein, the key functions of this pond design and some of our newest and most successful additions were presented.

The ponds were lined and the bottoms contoured to be shallower around the perimeter to assist in seining fish towards a concrete ramp at the end (Figure 7). They are 5 feet deep at their deepest point and have rip rap rocks

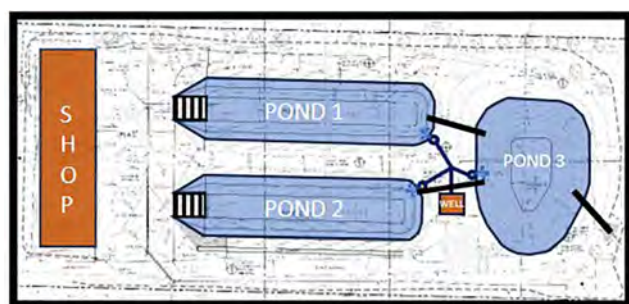


Figure 6.—The basic layout of the newly renovated Lake County Vector Control District satellite pond property.



Figure 7.—One of the $\frac{1}{4}$ -acre ponds partially filled to demonstrate the contoured bottom. Other features include a concrete ramp at the end and rip rap rocks around the perimeter.

around the perimeter to promote the production of newly released fry and provide them a place to hide.

Successful management of pond systems requires maintaining water quality, weed management, oxygenation, food and water distribution, predation control, and waste removal. However, one of the biggest challenges when working with shallow pond systems is weed management. Since the inception of these ponds 9 years ago, one pond has been treated with AquaShade and the other with a fertilizer (Figure 8). One method dyes the water blue and the other promotes an algae bloom, but both of these methods implement the same basic goal of prohibiting sunlight from reaching the bottom of the ponds promoting weed growth.

Identifying and enumerating phyto- and zooplankton within the ponds has allowed an analysis of how fertilizing and dyeing methods affect plankton production and therefore fish nutrition. It is my belief that the more natural planktonic food produced, the healthier the fish will be.

The introduction of oxygen is very important for the fish to withstand nightly decreases in dissolved oxygen (DO) caused by respiration in plants, and for the microorganisms in the sediment at the bottom of the ponds that are providing waste removal and sediment reduction. To pump fresh oxygen into the ponds, we purchased solar aerators from Keeton Industries/ProLake (Figure 9 A, B). These 300w



Figure 8.—A side-by-side comparison of the two $\frac{1}{4}$ -acre ponds. One is treated with a fertilizer and the other with the solar wave-length inhibitor, AquaShade.



Figure 9.—(A, B) Solar aeration units pump fresh oxygen into our pond systems from the bottom. (C) PVC elbows have been added to our water mains to eject and aerate all new water entering the ponds. Both of these systems provide ample oxygen into our pond systems to maintain our fish and muck-eating microorganisms.

solar panels charge for four hours during the sun's highest peak of the day and provide 20 hours of bubble production. These units cost \$7k-10k depending on the type of battery selected, with lithium-ion batteries being the most expensive. Each unit includes the solar panel, batteries, compressor, timer, and a cabinet, and can run 2 aeration heads at a time. These aeration units require very little maintenance and provide outstanding water quality and oxygen at all levels within the pond systems.

In addition, oxygenation is provided at the water source. The water supply at the ponds comes from a well so it is completely devoid of oxygen. We have created PVC elbows that fit onto the water mains to eject water into an arcing pattern that falls and oxygenates new water entering the ponds (Figure 9 C). Union coupling joints were also added to allow elbow adjustments to the left or right and move water around the ponds and prevent stagnation.

Pond systems are complex and may take many years to optimize. Last year was the first year there was a reduction in aquatic pond weeds and an increase in water quality, and it is largely due to all of these successful additions. Currently, the ponds are healthy, relatively weed-free, and abundant with mosquitofish, and we hope to be able to maintain these optimum conditions for many years to come.

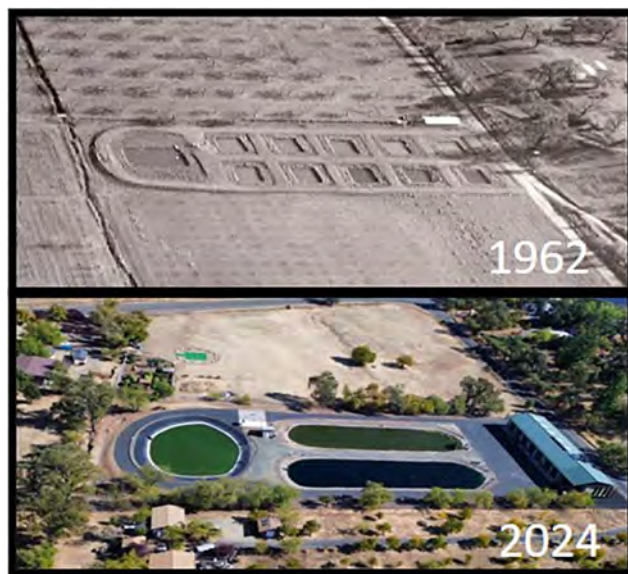


Figure 10.—Aerial photos of the 2.74-acre satellite property circa 1962 and 2024 (drone photo credit to Seth Burgoyne from BrickBody Drones).

This presentation summarized the origin and evolution of the Lake County Vector Control mosquitofish program (Figure 10). We went from a small District with a \$500 budget that borrowed the local high school boat, to the current large District we are today with very successful programs such as the mosquitofish program. It is my hope that through programs like ours, we can forge the way to a healthier more hospitable world for the future.

Acknowledgements

I thank my coworkers in the field for their support in helping make this program successful and our Board of Trustees that have always encouraged and promoted our growth. It is a pleasure to be serving Lake County.

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Maintaining tanks and earthen and lined ponds for fish culture

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Abstract

The Sutter-Yuba Mosquito & Vector Control District has a fisheries program as part of its Integrated Pest Management (IPM) strategy for controlling mosquito larvae in a variety of environments. The program focuses on three fish rearing facilities, including intensive aquaculture tanks, earthen ponds, and lined ponds. Constant challenges include water quality maintenance, filamentous algae and sludge removal, re-grading of ponds, and predator exclusion barriers and eradication. Maintaining fish rearing sites are dependent on season and weather. Ideal weather is conducive for drying time, regrading soil, and sanitizing tanks and lined ponds. Each site is serviced according to its specific needs. Maintaining fish rearing environments and employing best management practices contributes to the success of raising healthy fish and producing high yields. The goal is to improve growth and reproductive rates of the Western Mosquitofish (*Gambusia affinis*) by providing optimal living conditions through maintaining facility rearing facilities.

Sacramento-Yolo Mosquito & Vector Control District program overview for culturing *Gambusia affinis*

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Abstract

The Sacramento-Yolo Mosquito and Vector Control District (District) operates a robust fisheries program dedicated to the production and distribution of mosquitofish (*Gambusia affinis*) as part of a broader integrated pest management (IPM) strategy. Covering Sacramento and Yolo counties - and serving nearly 1.8 million residents - the District manages a unique landscape where dense urban neighborhoods are situated beside rice fields, wetlands, and rich bird habitats along the Pacific Flyway. Each year, the District produces approximately 3,500 pounds of mosquitofish from twenty-one outdoor earthen ponds located on its 27-acre facility in Elk Grove. These fish are deployed into rice fields, wetlands, and smaller mosquito breeding sources such as neglected swimming pools and livestock ponds to help control mosquito larvae and reduce reliance on pesticides.

Over the years, several improvements have been made to increase production and streamline operations. The program shifted to solar-powered aeration systems to stabilize nocturnal dissolved oxygen levels and retrofitted the ponds with bird exclusion fencing and netting to protect fish from predation. Program technicians also built an on-site fish trailer to increase efficiency during the short stocking season, allowing fish load preparation in advance of application. District fish are raised using a sustainable yield approach, and water chemistry is monitored closely with a custom-built Fisheries mobile application that centralizes data from ponds and tanks. Experimentation with low-cost filtration and holding systems to expand summer capacity will continue. Although challenges remain—from short stocking windows to persistent weed control—the program continues to adapt and improve, always working toward the ultimate goal: safer communities through intelligent, sustainable mosquito control.

Standard Operating Procedure essentials for mosquitofish program success

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Abstract

In July 2024, the Orange County Mosquito and Vector Control District (District) completed an update and enhancement of its Mosquitofish Program Standard Operating Procedure (SOP). The current presentation focused on the following: (1) The purpose and benefits of SOPs, (2) the SOP preparation process, and (3) discussion of the essential content of the District Mosquitofish Program SOP. Mosquitofish program SOPs are of critical importance in successful management and operation and may be modified and adjusted to serve the needs of any statewide mosquitofish program, regardless of their size and scope.

The purpose of an SOP is to create a document that contains centralized and easily accessible information, resources, and protocols that support the main purpose and function of a program. There are numerous benefits associated with having established SOPs, such as improving program quality, maintaining uniformity, minimizing errors, providing clarity, improving training, ensuring accountability, improving safety, and increasing efficiency. A well-organized and carefully written SOP prescribes effective actions and resources needed to achieve program objectives.

Mosquitofish programs that plan to prepare an SOP can expect to invest several months to a year organizing the information and materials necessary for this document, depending on the size and needs of the operation. It is recommended that one program staff member commit to the preparation of the SOP with dedicated blocks of time set aside each week and/or month for this project. Additional staff may assist with the collection of information and/or the review of the document for initial and final edits. Once completed, appropriate personnel should review and familiarize themselves with the details of this document and amend the SOP, as needed, to address any modifications to the program.

The District Mosquitofish Program SOP includes six essential sections of information and resources, including a Table of Contents, Program Introduction and Layout, Program Protocols and Maintenance, Program Vendors and Consultants, Literature Cited, and an Appendix. A Table of Contents provides a framework and roadmap for simple navigation of all document content. The Program Introduction and Layout serves the purpose of clearly defining and describing the program's background, purpose, goals, structure, and function. The core of this document, Program Protocols and Maintenance, includes all obligatory protocols and pertinent maintenance duties needed for optimal program function. A Program Vendors and Consultants component is included in the SOP, because the program relies heavily on a variety of aquaculture components and other resources, various equipment, and additional technical support and resources, all of which are carefully listed and described in this section. Finally, any supporting publications reviewed or referenced in the SOP may be listed in Literature Cited, while manuals and related documents that are supplemental to sections of the SOP can be included in the Appendix. The organization and content of this SOP have proven indispensable to District mosquitofish program.

The objective of the current presentation was to share and make available information and resources to MVCAC membership for the preparation and implementation of a functional mosquitofish program SOP. Mosquitofish programs interested in obtaining a copy of this District's SOP may do so by request from the presenter. Any constructive comments or suggestions regarding this SOP layout and content are welcomed and appreciated.

Controlling mosquitoes in large complex sewage-contaminated flood area

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Abstract

As a result of the winter 2024 storms and widespread flooding, a storm runoff channel berm broke at the beginning of February in the Tijuana River Valley (TJRV) that previously constrained water flows to join the Tijuana River. Over 300 acres of land became flooded with raw sewage-contaminated water, including roads, trails, a campground, wildlife reserves, and State, Federal and private properties. Contaminated flows continued at >100 M gallons per day throughout the spring and summer and resulted in standing water that supported produced large numbers of vector mosquitoes. Recognizing the potential for the flood waters to exponentially increase disease-transmitting mosquitoes, including *Culex tarsalis*, the Vector Control Program (VCP) increased surveillance in TJRV to monitor mosquito populations and assess potential risks to public health. In April 2024, coinciding with the start of the mosquito season in the region, mosquito activity began increasing, reaching almost 140 times greater than the historical 5-year average for *Cx. tarsalis* mosquitoes in the TJRV. In response, the VCP undertook extensive efforts to coordinate with multiple agencies and suppress mosquito populations via land and air insecticide applications under challenging and changing conditions. Fortunately, these efforts reduced mosquito levels to near pre-flood levels and no pathogens were detected. This presentation described the strategies and techniques the VCP used to achieve these results.

An evolving approach to neglected swimming pools

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Abstract

Neglected swimming pools within Contra Costa County continue to be a significant source of mosquito production. Beginning in 2019 the District implemented changes to its approach to addressing neglected swimming pools. This presentation discussed the evolution of the District's program, its failures, and its successes.

Evaluation of RT-qPCR reagents for mosquito pool and dead bird testing

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Introduction

In 2005, the Sacramento-Yolo Mosquito & Vector Control District (SYMVCD) established diagnostic capacity to test field-collected mosquitoes for West Nile virus (WNV), St. Louis encephalitis virus (SLEV) and western equine encephalitis virus (WEEV) RNA and augmented this capacity in 2010 to include dead bird testing to enhance surveillance for arbovirus activity within SYMVCD borders. Monitoring both mosquito populations and dead birds for arboviruses is an integral part of the integrated vector management program at SYMVCD. It is critical to continually evaluate new testing protocols and to ensure assay methodologies provide optimized testing specificity and sensitivity. To test whether SYMVCD's current testing methodology maintained the highest specificity and sensitivity standards, a comprehensive evaluation of reverse transcription quantitative polymerase chain reaction (RT-qPCR) reagents was performed. Overall, two RNA extraction kits, two RT-qPCR master mixes, and two primer/probe mixtures (PPM) were evaluated.

Materials and Methods

Sample types and collection methods

The analysis was done on a subset of WNV-positive and WNV-negative field collected mosquito and dead bird samples from the 2024 testing season. The mosquito species utilized in the study included pools of *Culex tarsalis* and *Culex pipiens* that were collected and tested for routine WNV surveillance. The dead bird samples included multiple bird species and sample types including: oral swabs stored in viral transport media (only collected from American Crows, *Corvus brachyrhynchos*), brain aspirates, and maggots. A range of sample types was included from both mosquitoes and birds to ensure the different methodologies provided consistent results for all potential sample types tested during the surveillance season.

Sample processing and evaluations

First, the RNA extraction was examined. The extraction process began by homogenizing all sample types. Mosquito pools were homogenized in viral transport media (VTM) consisting of Dulbecco's modified eagle medium (ThermoFisher; Waltham, MA), 500 U/mL penicillin

(source), 0.5 mg/mL streptomycin (GeminiBio, West Sacramento, CA), 250 µL/mL amphotericin B (GeminiBio, West Sacramento, CA), and 20% fetal bovine serum (ThermoFisher; Waltham, MA). All dead bird sample types were homogenized in 5× viral lysis buffer (ThermoFisher; Waltham, MA). The samples were homogenized using two 5 mm glass beads (ThermoFisher; Waltham, MA) shaken for three minutes in a Sample Prep 8000D (Spex; Metuchen, NJ). Two different extraction kits were assessed, the MagMAX™ CORE Nucleic Acid Purification Kit (hereafter Core kit; ThermoFisher; Waltham, MA) and the currently utilized MagMAX™-96 Viral RNA Isolation Kit (hereafter Viral RNA kit; ThermoFisher; Waltham, MA). The kits were tested with matched sample sets consisting of both WNV-negative and WNV-positive mosquito and dead bird samples. All samples were RNA-extracted using the KingFisher™ Flex Purification System magnetic particle processor (ThermoFisher; Waltham, MA). The extraction plates include both positive and negative controls.

All RNA extracted samples were tested in parallel using the VetMAX™ Fast Multiplex Master Mix (hereafter VetMAX; ThermoFisher; Waltham, MA) and the currently utilized TaqMan™ Fast Virus 1-Step Master Mix (hereafter Fast Virus; ThermoFisher; Waltham, MA). The master mix supplied all necessary enzymes and buffers to run the PCR reaction to completion, except for the primer and probe mixture (PPM). To increase throughput, reactions were conducted in 96-well plates, each containing positive and negative extraction controls as well as standard RT-qPCR positive controls and a 'no-template' water control. All samples were analyzed by RT-qPCR using a QuantStudio 5 Real-time PCR system (ThermoFisher, Waltham, MA). Following 40 cycles of PCR, the QuantStudio 5 software was used to assign a value for each sample in the form of a cycle threshold value (Ct). The Ct value measures the amount of RNA present and is the number of cycles required for a sample to cross a fluorescence threshold, indicating the presence of a nucleic acid target. These Ct values were the metric used to compare all test parameters.

The last assessment compared an in-house prepared primer and probe mixture (hereafter in-house PPM) for WNV, WEEV, and SLEV and a set of primers and probes designed by Life Technologies specific for the same viruses that came pre-mixed in a 20× solution (hereafter Life Tech PPM; ThermoFisher, Waltham, MA). The in-house PPM

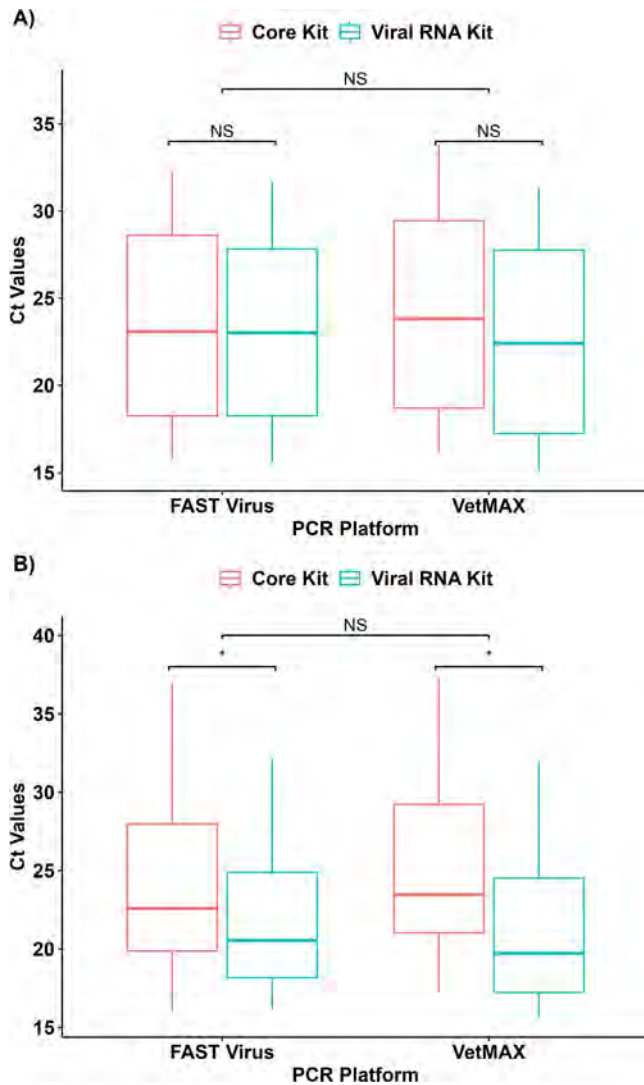


Figure 1.—Box plots comparing the distribution of Ct values across two extraction kits [Viral RNA (elution volume = 50 μ l) and Core extraction kits (elution volume = 100 μ l)]. Followed by RT-qPCR with two different master mixes (VetMAX and FAST Virus). Notches represent the median and 95% confidence intervals. A) dead bird samples; B) mosquito samples.

was routinely used for dead bird and mosquito sample testing and utilizes previously published primer and probe sequences (Brault et al., 2015). The sequences used for the Life Tech PPM were proprietary. The PPM were tested against RNA standards for WNV, SLEV, and WEEV, and no-template water controls and tested with the Fast Virus master mix.

Statistics

A two-way analysis of variance (ANOVA) compared Ct values across different extraction kits and PCR platforms for two sample types: mosquito pools and dead bird swabs. The analysis was followed by Tukey's post-hoc test for pairwise comparisons. All analyses were done in R statistical software version 3.6.0 (R Core Team, 2023).

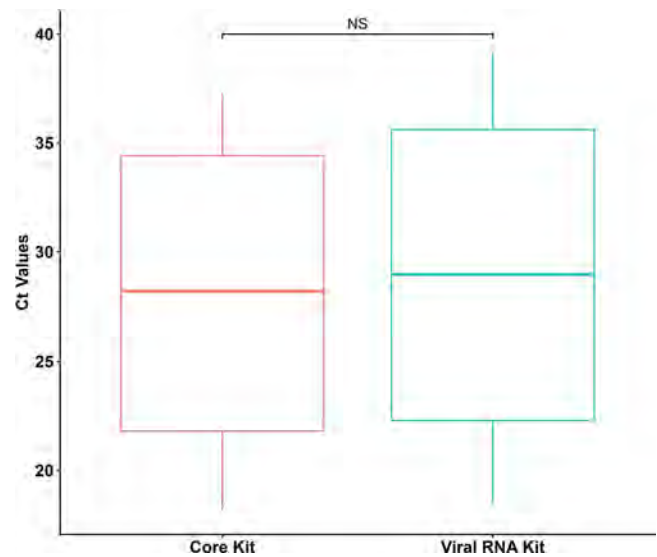


Figure 2.—Box plots comparing the distribution of Ct values of mosquito samples extracted with the Viral RNA (elution volume = 50 μ l) and Core (elution volume = 50 μ l) kits followed by RT-qPCR using the Fast Virus master mix.

Results and Discussion

The dead bird sample results showed no significant difference (Fig. 1A) in the performance of the VetMAX and Fast Virus [$F(1, 116) = 0.04, p = 0.844$] master mixes and extraction kits [$F(1, 116) = 0.46, p = 0.500$]. However, the mosquito samples had significantly lower Ct values when extracted with the Viral RNA kit compared to matched samples extracted by the CORE kit [$F(1, 116) = 6.35, p = 0.013$] (Fig. 1B), regardless of the PCR platform [$F(1, 236) = 0.10, p = 0.753$]. This difference was consistent across both master mix kits and was attributed to the extraction process. A lower Ct value signifies a higher viral load, indicating that the Viral RNA kit had greater sensitivity than the Core Kit. However, when the protocols were reviewed, there was a discrepancy, where the manufacturer protocol called for a higher elution volume in the Core kit (100 μ l) compared to the Viral RNA kit (50 μ l). The elution buffer is what the isolated RNA is suspended in, so differences in elution volume directly impact the concentration of the extracted RNA, potentially leading to changes in sensitivity. To make the two kits comparable, the elution volume was adjusted for the Core kit to match the volume used in the Viral RNA kit. Following this adjustment, there was no significant difference in Ct value between the two extraction kits [Fig. 2; $F(1, 62) = 0.11, p = 0.743$]. It was not clear why the elution volume had an impact on the Ct values of the mosquito samples, but not the dead bird samples, but these data illustrate the importance of testing reagents against all sample types.

When the primer/probe mixtures were compared, there was no significant difference in the resultant Ct values (Fig. 3) [$F(1, 102) = 0.07, p = 0.794$], between the current primer/probe (mean $\pm$ SE = 25 ± 5.08) and the commercially available primer/probe (24 ± 5.08). Although

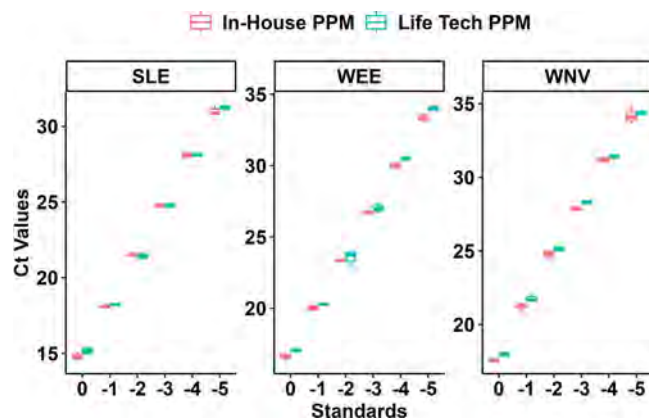


Figure 3.—Box plots comparing Ct values of RT-qPCR reactions conducted with the Viral RNA extraction kit, Fast Virus master mix and two different primer/probe mixtures (in-house PPM and Life Tech PPM). Each primer/probe mixture was tested against a 10-fold dilution series of nucleic acid target of the triplex (WNV, SLEV, and WEEV).

the two mixtures produced similar results, a further consideration is the effort required to prepare primer/probe mixtures. Preparing primer and probe solutions for a triplex RT-qPCR assay requires six primers and three probes. These reagents must be ordered individually, properly diluted, and then combined into a primer/probe mixture so that all the primers and probes can be added to the RT-qPCR reaction in a single pipetting action. This speeds processing and reduces errors compared to adding each primer and probe individually. However, preparing the primer/probe mixture is a time-consuming process, but it is worthwhile when testing a high volume of samples. The commercial option tested comes at an increased cost compared to prepared primer/

probe mixtures, but may be a good solution for agencies that have lower sample volumes.

Conclusion

There are a wide range of reagents and methods available for arbovirus testing. Our study evaluated alternative reagents for RNA extraction, PCR master mix, and primers and probes. When used according to the manufacturer protocol the Core kit yield slightly higher Ct values compared to the Viral RNA kit, indicating greater sensitivity; however, when the Core kit protocol was adjusted so that both kits had the same elution volume, the performance of the two kits was comparable. Overall, the PCR master mixes and primer/probe mixtures had similar performance and either would be a good choice for testing dead birds and mosquito pools. When selecting reagents and protocols, there are multiple important considerations such as testing volume, ease of use, and cost. Each lab must balance these factors against the sensitivity and specificity of reagents and protocols. Our results demonstrated that other reagents are available that provide the same level of sensitivity and specificity currently achieved, thus providing future options and flexibility for our testing program.

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Exploring resistance in *Culex tarsalis*: linking bottle bioassays to sentinel cages to field trials

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Introduction

One of the research priorities for the CDC Centers of Excellence was to evaluate the impact of insecticide resistance on operational mosquito control. To approach this priority, the Thiemann Laboratory at the University of the Pacific and the Sacramento-Yolo Mosquito and Vector Control District collaborated on a project to make connections among laboratory resistance assays, semi-field sentinel cage trials, and changes in the abundance in target populations that result from insecticide applications.

Methods

Designed as a multi-year project, the first insecticide tested was a natural pyrethrin. *Culex tarsalis* females from Natomas, CA were used for all aspects of the study. Standard CDC bottle bioassays were conducted, with and without piperonyl butoxide (PBO). Additionally, mosquitoes were placed in bottles coated with the formulated product, Pyronyl 525 Oil Concentrate [active ingredients: 5% pyrethrins, 25% (PBO)]. In the field, mosquitoes were placed in tambourine cages and treated with an aerial application of the same formulated product. Thirty carbon dioxide-baited traps were set the nights before and after the insecticide application to measure changes in abundance. Field trials were repeated weekly for three weeks in July/August 2023.

Results and Discussion

In bottle bioassays, results for standard CDC assays with pyrethrins + PBO were similar to those for formulated

Pyronyl 525. Mortality at 1 hour in the bottles with PBO synergist (~65%) was in the range of 1-hour mortality for females deployed in sentinel cages (54-85%). At 2 hours in the bottle with formulated product, mortality was >95%; this was also true at 12 hours after exposure in the sentinel cages. Although there was week-to-week variation likely due to environmental factors, there was a decline in *Cx. tarsalis* abundance following the aerial Pyronyl 525 application.

Conclusions

In this study, bottle bioassays with pyrethrin + PBO and with formulated product were good predictors of results from sentinel cage trials. Although field trial results varied, consistent *Cx. tarsalis* mortality was associated with the Pyronyl 525 applications. Further comparisons of various mosquito populations and different insecticides will be required to determine how bottle bioassay or sentinel cage data can be used as a predictor of the results of operational mosquito control.

Acknowledgements

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Resisting resistance: Identifying biochemical biomarkers for pyrethroid resistance in *Aedes aegypti* mosquitoes

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Abstract

Over the past decade, invasive *Aedes aegypti* have increasingly colonized California, complicating vector control efforts due to its resistance to pyrethroids, the primary insecticides used for adult control. This study elucidated and evaluated potential biochemical biomarkers associated with metabolic mechanisms conferring pyrethroid resistance. By dissecting these mechanisms, our research seeks to facilitate the development of targeted assays for novel resistance biomarkers to improve monitoring and control capabilities.

West Nile virus and Mosquito Control at East Side Mosquito Abatement District, Modesto, California

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Abstract

Historically, human cases of West Nile virus (WNV) at East Side Mosquito Abatement District (East Side MAD) jurisdiction have overwhelmingly been occurring within the City of Modesto. Challenges caused by large underground and above ground larval sources, and the expansive tree canopy within the city seems to have presupposed this status of WNV endemicity. We recently mapped all underground sources into our data collection software. In 2024 with full crew of ‘curb riggers’, small zone sizes for ‘above ground’ control technicians, and an aerial assault by contracted aircraft, we saw a significant reduction in mosquito abundance, WNV-positive mosquito samples, and WNV human cases.

Restricting mosquito use of underground storm drain systems in the Coachella Valley by application of botanical repellents

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Introduction

Mosquito-borne diseases continue to pose a persistent threat to global health, annually claiming over a million lives and debilitating millions more through illnesses such as malaria, dengue, Zika, and West Nile. These diseases exact not only a devastating toll on human health but also impose considerable socioeconomic burdens, particularly in tropical and subtropical regions (Achee et al., 2012). Rapid urbanization has compounded this challenge, as human-engineered environments inadvertently create ideal breeding grounds for mosquitoes. Among these, Underground Storm Drain Systems (USDS) have emerged as significant yet underappreciated habitats for mosquito proliferation. These subterranean networks, characterized by dark, humid, wind-protected conditions and often containing stagnant water, are particularly conducive to the survival and reproduction of *Culex quinquefasciatus*, a principal vector of West Nile virus and lymphatic filariasis (Strickman & Lang, 1986; Reisen et al., 1990; Suman et al., 2022).

The intricate vulnerability of urban landscapes is vividly illustrated by the role of USDS in sustaining mosquito populations. Designed to manage stormwater and runoff in urban areas such as southern California, these systems often hold stagnant pools of runoff water, providing optimal developmental and resting sites for mosquitoes. This dynamic is particularly pronounced for *Cx. quinquefasciatus*, whose remarkable adaptability to polluted environments allows it to exploit these urban refuges (Wangai et al., 2020). As an opportunistic feeder on both humans and animals, *Cx. quinquefasciatus* functions as an efficient bridge vector for zoonotic pathogens, posing an challenge to public health (Sutthanont et al., 2022). Consequently, mosquito production within USDS exacerbates nuisance issues and significantly amplifies the risk of arbovirus transmission (Strickman & Lang, 1986; Reisen et al., 1990; Su et al., 2003).

Managing mosquito populations within USDS presents unique and multifaceted challenges. These systems often deliver untreated water directly to natural water bodies, limiting the use of conventional insecticides. Additionally, the dynamic hydrology of USDS frequently flushes away larvicides, while high organic content compromises the efficacy of microbial agents (Des Lauriers et al., 2006; Mulla et al., 1986). Although physical barriers and insecticides such as Bti and pyriproxyfen have been

explored, their success has been inconsistent, often constrained by water replacement rates and organic interference (Mian et al., 2020; Mian et al., 2017). Furthermore, the reliance on chemical insecticides for mosquito control has become increasingly untenable, as excessive use has led to widespread resistance in mosquito populations, eroding the efficacy of organophosphates and pyrethroids (Bisset et al., 1990; Low et al., 2013; Peiris & Hemingway, 1993; Suman et al., 2022). This escalating resistance underscores the imperative for the development of innovative and sustainable alternatives in vector control.

Spatial repellents (SRs) offer a transformative approach to mosquito management by altering mosquito behavior rather than relying on direct lethality. These SR compounds emit volatile chemicals that disrupt host-seeking, resting, and/or oviposition behaviors, thereby reducing human-vector interactions (Achee et al., 2012; Fongnikin et al., 2024; Swai et al., 2023). SRs have demonstrated significant efficacy against *Cx. quinquefasciatus*, with notable reductions in feeding and resting activities (Sathantriphop et al., 2015). Although synthetic SRs such as transfluthrin and metofluthrin have shown promise, the growing interest in botanical repellents reflects an increasing emphasis on environmental sustainability (Peach et al., 2019).

Botanical essential oils (EOs) stand out as compelling alternatives to synthetic compounds due to their rich composition of bioactive compounds such as terpenoids, phenols, and aldehydes (Regnault-Roger, 1997; Gupta et al., 2023). These compounds offer robust mosquito repellency while minimizing environmental impact. For instance, citronella oil has achieved repellency rates exceeding 90% against *Cx. quinquefasciatus*, and peppermint oil demonstrates both larvicidal and repellent properties (Ansari et al., 2000; Nerio et al., 2010; Ramar et al., 2017). Recent studies also have highlighted the synergistic potential of EO combinations, such as lemongrass and cinnamon bark oils, which effectively reduce human-vector contact in field trials (Peach et al., 2019). Despite these advances, the application of spatial repellents to manage mosquitoes in complex urban systems like USDS remains largely uncharted. The volatile organic compounds (VOCs) emitted from decomposing organic matter in USDS may interact with repellent chemicals, potentially modulating their efficacy (Suman et al., 2022). Investigating these interactions is essential to optimizing repellent performance in such challenging environments.

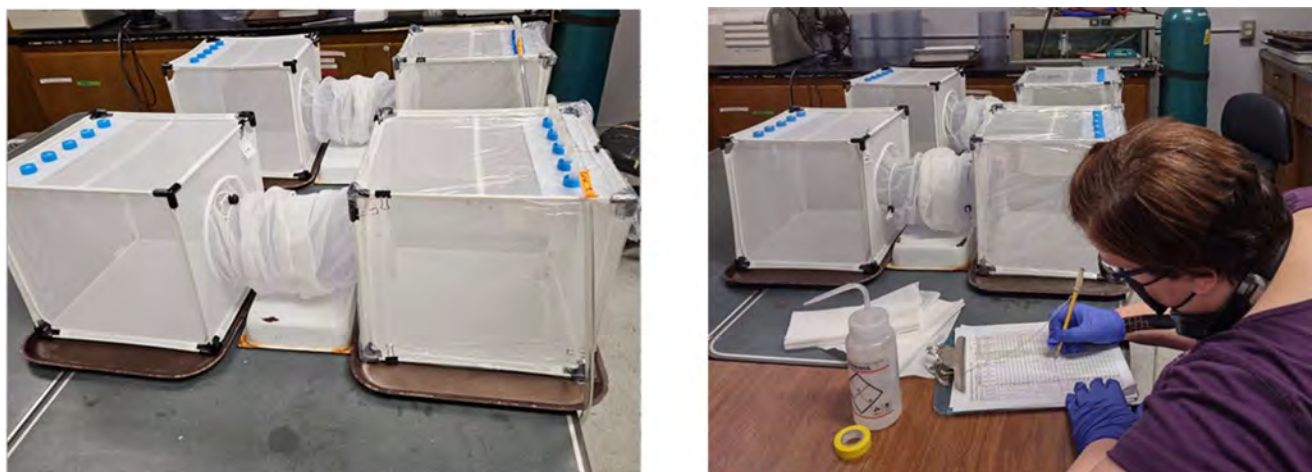


Figure 1.—Assay chamber for repellent testing: Left image - Adult mosquitoes moved freely between repellent (right) and escape (left) cages via a connected PVC pipe. Right image - A spatial repellency trial being conducted in the laboratory.

Our study systematically evaluates the efficacy of spatial repellents in simulated USDS conditions through three primary objectives. First, it quantifies the spatial repellency of botanical and synthetic repellents using controlled assays to assess behavioral effects on mosquitoes. Second, it examines synergistic interactions between repellents to determine whether combinations enhance efficacy. Third, it explores the influence of VOCs emanating from USDS water on repellent performance, addressing critical environmental factors.

By integrating robust experimental methodologies with an environmentally conscious framework, this study aims to redefine mosquito control strategies in urban settings. The findings are intended to inform evidence-based public health policies, mitigate mosquito-borne disease transmission, and promote ecological sustainability. By focusing on the potential of botanical spatial repellents, this research addresses the pressing need for effective and sustainable mosquito control solutions tailored to urban environments.

Materials and Methods

Mosquito rearing

The mosquitoes used in this study (*Culex quinquefasciatus*) were laboratory-reared from a strain collected from the Coachella Valley. Colonies were maintained under controlled laboratory conditions, including a temperature of $26 \pm 1^\circ\text{C}$, relative humidity of $70 \pm 10\%$, and a 12:12 light-dark cycle. Adult females aged 5–9 days, nulliparous, non-blood-fed, and sugar-fed, were selected for experiments to standardize responses. Mosquitoes were deprived of sugar but provided water for 12 hours prior to assays to enhance their behavioral responsiveness to repellents. Mosquitoes were chilled before being transferred to the assay chambers to facilitate handling.

Repellent compounds

The study evaluated a range of repellents, including botanical oils (citronellol, geraniol, eugenol, and

cinnamaldehyde), essential oil mixtures (peppermint and geranium oil) and positive controls (0.25% pyrethrum extract with $> 50\%$ pyrethrins and DEET). Botanical oils were selected based on prior published evidence of their efficacy against mosquitoes and were acquired commercially. To ensure consistent chemical properties, all repellents were confirmed for purity through gas chromatography-mass spectrometry (GC-MS). Mineral oil was used as a negative control. Combinations of individual repellents were also tested to identify potential synergistic interactions that might enhance their efficacy.

Excito-repellency assay

Repellent efficacy was assessed using a modified excito-repellency chamber based on Roberts et al. (1997). The assay chamber included two compartments: a repellent cage and an escape cage, with the two cages connected by a 6-inch diameter PVC pipe (Figure 1). The top of the repellent cage contained five slots to hold 15 ml plastic tubes that were perforated along the upper half of the tube to allow release of volatiles from compounds within each tube. The tubes either remained empty (dummy tube) or contained a cotton wick saturated with approximately 6 mL of a single repellent compound (volatile release tube). Airflow from a vinyl tubing circuit connected to each tube with a mean flow rate of 0.7 m/s measured over 40 seconds per connector ensured consistent volatile distribution into the repellent cage. The repellent cage was covered with plastic wrap to concentrate volatiles, while the escape cage remained uncovered. For additional details of this assay design, please refer to the Mid-year Report (submitted in June 2024).

For each assay, 30 female mosquitoes were introduced into the repellent cage and acclimated for 5 minutes. The repellent treatment was initiated by replacing dummy tubes with 1-5 volatile release tubes. Mosquito behavior and location were observed at 1, 5, 10, 20, 30, and 40 minutes. Observations included mosquito position across six areas (four quadrants within the repellent cage, within the escape port, and within the escape cage), level of excitatory activity

(e.g., flying or walking), and evidence of knockdown effect. At the end of the assay, mosquitoes were transferred to clean containers and monitored for mortality after 24 hours.

To evaluate synergistic effects, combinations of the most promising individual repellents were tested. Individual repellents were placed into separate volatile release tubes to prevent interactions in the liquid phase. Assays monitored mosquito escape rates to determine additive or synergistic effects. Different combinations of repellents were tested across multiple replicates to ensure statistical robustness.

Data Analysis

Escape rates in excito-repellency testing were evaluated using survival analysis with Cox proportional hazards models to account for time-dependent variations in mosquito behavior. Synergistic effects were assessed using generalized linear models (GLMs) incorporating interaction terms to detect additive or multiplicative effects. Post hoc pairwise comparisons were conducted using Tukey's test with p values adjusted for multiple comparisons. All analyses were conducted using R (version 4.2.2), with statistical significance set at $p < 0.05$ to ensure rigorous evaluation of results.

Results

The percentage of mosquitoes escaping from the release chamber varied significantly across treatments and concentration levels (Fig. 2A, top panel). The strongest spatial repellency was observed at the highest concentration (5 tube) for both eugenol and geraniol, which elicited significantly greater escape rates than the mineral oil control. Peppermint oil and citronella oil also showed significant repellency at the 5 tube concentration, although their effects were less pronounced. Notably, eugenol remained highly effective even at the intermediate concentration (3 tube), exhibiting repellency comparable to its 5 tube level. In contrast, cinnamaldehyde, geranium oil, and DEET did not differ significantly from the control at any of the concentrations tested. At the lowest concentration (1 tube), none of the compounds induced escape behavior above baseline levels.

Walking behavior increased significantly in mosquitoes exposed to several treatments, particularly peppermint oil at the highest concentration (5 tubes), which elicited the strongest response. Geranium oil (5 tubes), cinnamaldehyde (5 tubes), and peppermint oil at the intermediate concentration (3 tubes) also led to significantly elevated walking activity compared to the mineral oil control (Fig. 2B). Additionally, combinations of peppermint and eugenol (each at 1 tube) produced a robust walking responses, suggesting additive or synergistic effects. In contrast, the control treatment with mineral oil exhibited minimal walking behavior, indicating that the absence of repellents led to lower mosquito activity, with mosquitoes predominantly resting in the release cage (Fig. 2B).

The percentage of mosquitoes exhibiting flying behavior varied notably across treatments (Fig. 2C). The most pronounced increase in flight activity was observed with peppermint oil at the intermediate concentration (3 tubes), which led to significantly greater flying percentages than the control. Geranium oil (5 tubes) and combinations of peppermint and eugenol also resulted in elevated flying behavior. These treatments appeared to stimulate mosquito activity within the release cage without necessarily prompting escape, suggesting a behavioral agitation effect rather than strong spatial repellency. In contrast, flying activity in the mineral oil control group remained low, indicating limited mosquito activity in the absence of repellents.

Walking and flying behaviors offer important insights into how spatial repellents modulate mosquito activity within a treated environment, even when they do not result in high escape rates. Elevated walking or flying, particularly in the absence of substantial escape, suggests that mosquitoes are behaviorally disturbed or agitated by the volatile compounds but are not receiving a sufficiently strong directional cue to exit the chamber. This pattern is evident in the response to peppermint oil at the highest concentration, which triggered considerable walking and flying but only moderate escape, in contrast to eugenol at an intermediate concentration, which elicited high escape rates with comparatively low locomotor activity. These contrasting outcomes underscore that increased mosquito activity does not necessarily correlate with strong spatial repellency.

Instead, heightened walking and flying may reflect deterrence of resting behavior. Such a response may indicate that mosquitoes are unable or unwilling to settle in the treated area, which could displace them from potential resting or oviposition sites without necessarily prompting directed escape. For instance, combinations of peppermint and eugenol altered mosquito behavior—stimulating walking and flying—without inducing consistent escape responses. This may reflect behavioral interference or sensory overstimulation, in which the agitation caused by one compound disrupts the escape-inducing signal of another, resulting in increased undirected movement rather than effective repellency.

These behavioral profiles highlight the importance of evaluating spatial repellents using multiple metrics. High levels of walking or flying in the absence of escape may signal subthreshold concentrations that disrupt behavior but do not achieve true repellency. Conversely, treatments such as eugenol at an intermediate concentration, which prompt escape with minimal agitation, are more indicative of effective spatial repellency. Ultimately, integrating escape, walking, and flying responses provides a more comprehensive understanding of repellent action—distinguishing between mere behavioral disruption and true spatial deterrence. This distinction is especially important in operational contexts, where deterrence from resting and oviposition sites may be as critical as inducing escape, particularly in applications such as treated USDS chambers.

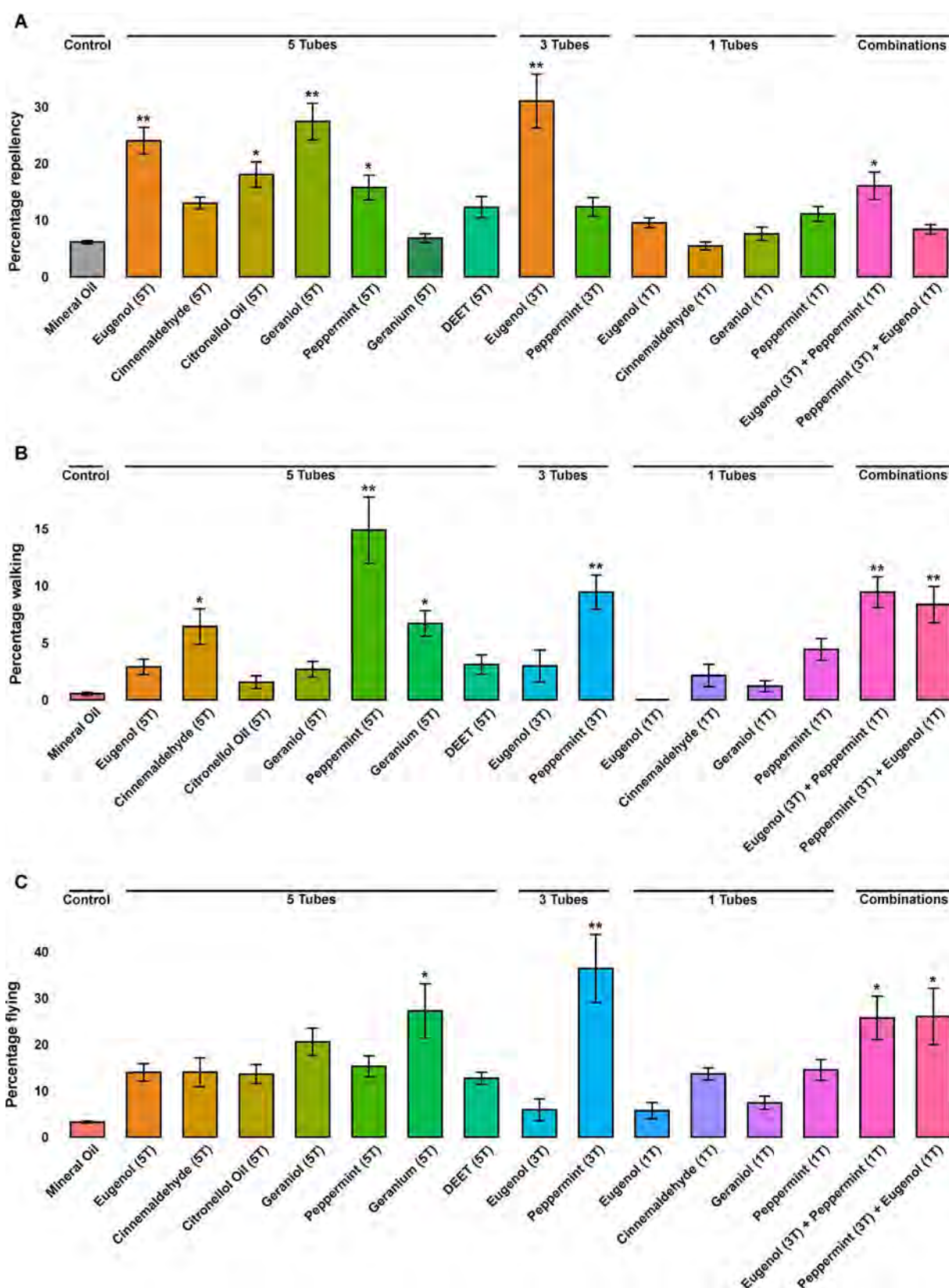


Figure 2.—Spatial repellent effects of botanical oils on mosquito behavior. A) Percentage of mosquitoes escaping from the release chamber across treatments, indicating spatial repellency. B) Percentage of mosquitoes exhibiting walking behavior, reflecting partial repellency or agitation. C) Percentage of mosquitoes exhibiting flying behavior, suggesting agitation rather than escape. Error bars represent the standard error of the mean (SEM), and asterisks indicate statistically significant differences compared to Mineral oil (control) ($p < 0.05$).

Discussion

This study evaluated the effectiveness of several botanical oils as spatial repellents for potential mosquito control applications in underground storm drain systems (USDS) in the Coachella Valley. Laboratory assays revealed that multiple botanical oils elicited behavioral responses in *Cx. quinquefasciatus* when compared to the negative control (mineral oil). Notably, eugenol and geraniol were highly effective at inducing escape behavior, a clear indicator of spatial repellency. In contrast, peppermint oil and combinations of peppermint and eugenol primarily elevated mosquito activity—manifested as increased walking and flying—rather than consistently promoting escape.

A concentration-dependent response was evident across most treatments, with higher volatile concentrations resulting in more pronounced behavioral effects. The greatest escape rates were observed with eugenol and geraniol at moderate to high concentrations, underscoring their promise as potent spatial repellents for enclosed settings like USDS chambers. Conversely, peppermint oil and cinnamaldehyde predominantly stimulated agitation without directing mosquitoes to leave the treated space. This excitatory effect was most pronounced at higher concentrations of peppermint oil, which increased activity within the chamber but did not lead to substantial escape. Interestingly, the combination of eugenol and peppermint at a 3:1 ratio amplified excitatory behaviors relative to either oil alone, while reversing the ratio to 1:3 diminished this effect, resembling the activity profile of peppermint oil alone.

These findings highlight the importance of integrating both escape and excitatory behaviors—walking and flying—when assessing spatial repellent efficacy. Although escape reflects a direct repellency effect, increased locomotor activity also may deter resting behavior and reduce the likelihood of mosquitoes using treated areas as shelters or oviposition sites. In the context of USDS chambers, where mosquitoes seek refuge from harsh outdoor environments, the ability of botanical oils to prevent resting or oviposition—even without inducing escape—may still confer vector control benefits. This behavioral disruption could have physiological consequences by increasing energy expenditure, potentially lowering mosquito survival and limiting the likelihood of pathogen transmission, especially for viruses requiring an extended extrinsic incubation period.

Ongoing studies are examining the interaction between botanical oils and volatile compounds naturally present in water collected from USDS sites. These assays will compare mosquito behavior in the presence of botanical oils alone, USDS water samples alone, and combinations of both, to determine whether any synergistic or antagonistic interactions influence escape, walking, or flying behavior. These results will inform whether environmental volatiles modulate repellent efficacy, thereby refining strategies for field deployment in complex chemical environments.

In conclusion, our study demonstrates that botanical oils—particularly eugenol and geraniol—are promising

candidates for environmentally sustainable spatial repellents. Oils that stimulate behavioral excitation, such as peppermint and cinnamaldehyde, may complement repellent oils by deterring mosquito resting even without promoting escape. Combining these behavioral effects may enhance control outcomes by simultaneously reducing mosquito occupancy and disrupting key behaviors required for survival and reproduction in USDS environments. Further field studies are warranted to assess the impact of these compounds under natural conditions. Formulations that combine repellency with behavioral interference may provide the greatest reduction in mosquito use of USDS and help mitigate the risk of mosquito-borne disease transmission in urban settings.

Acknowledgments

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Suppression of *Aedes aegypti* using Incompatible Insect Technique in the British Virgin Islands

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Abstract

BugOut *Wolbachia* is a community-driven campaign to suppress *Aedes aegypti* from Virgin Gorda in the British Virgin Islands (BVI). The project is a partnership between Green VI, a non-profit NGO based in the BVI, and Verily's Debug Team, a global team experienced in using *Wolbachia* male releases for suppression of *Aedes aegypti*. The program combines traditional source reduction efforts with incompatible male releases to suppress *Ae. aegypti* on Virgin Gorda. *Wolbachia* male releases began in January 2023 and have resulted in up to 94% suppression of the wild *Ae. aegypti* population in select treatment areas.

Fine-scale modeling of West Nile virus vector dynamics to inform vector control trial design

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Introduction

Optimal design of field trials for West Nile virus (WNV) vector control strategies benefits from fine-scale spatio-temporal prediction of WNV transmission risk to guide trial placement toward locations and times where vector and virus activity is expected to be highest and to facilitate comparison of intervention effects between areas with similar baseline vector metrics. This approach maximizes statistical power to detect intervention effects. Building on preliminary analyses that characterized historical patterns of WNV activity in the city of Davis, we conducted mosquito sampling along transects intended to capture expected gradients in vector abundance and WNV infection prevalence throughout the 2023 summer season. The results informed models that revealed spatio-temporal associations between risk factors – such as proximity to larval habitats and urban edges – and vector abundance and prevalence. These insights into spatial and seasonal variation of vector dynamics across Davis have been used in the design of an ongoing two-year trial evaluating a novel WNV vector control strategy.

Methods

During the 2023 summer season, mosquitoes were collected every other week for 12 weeks using CO₂-baited traps placed along five transects throughout Davis, California. Transects began at the urban-agricultural edge of the city and extended to the city center, and were placed in the Mace, El Macero, Wildhorse, northwest, and west regions of Davis, respectively. Mosquitoes were sorted by species and *Culex tarsalis* females were submitted in pools of ≤ 50 for WNV testing by PCR.

Spatial variables of interest included trap distance from urban edge, area of rice contained within an 8-km radius of each trap as a spatial proxy for the extent of *Cx. tarsalis* larval habitat (Smith et al., 2004), land cover composition within a 300-m radius of each trap, and average surface imperviousness within a 300-m radius of each trap. Land cover and imperviousness data were sourced from the National Land Cover Database (USGS, 2021) with a resolution of 30 m², whereas rice field spatial polygons were provided by the Sacramento-Yolo Mosquito and Vector Control District. Associations between each spatial variable

and *Cx. tarsalis* abundance and WNV prevalence were evaluated using generalized additive models developed using R Statistical Software (v4.3.3; R Core Team 2024) using the mgcv package (Wood 2017).

Results

Of the four spatial features of interest, only distance from urban edge, area of rice within an 8-km radius, and proportion of cultivated crop land within a 300-m radius yielded statistically significant associations with vector abundance and WNV prevalence when controlling for other variables. Cultivated crop land was dropped as a primary variable of interest due to insufficient sample size but was controlled for as a potential confounder. All confounders were selected based on a combination of a directed acyclic graph and variable-by-variable analysis, and included the presence of specific land cover types (cultivated crops, developed open space, developed low intensity, and developed high intensity), the application of ultra-low volume (ULV) insecticide prior to trap placement, and either distance from urban edge or area of rice, respectively. Time, represented in weekly units, was included as a smoothed interaction term with the primary spatial variable based on initial analyses indicating significant fluctuation in distance to urban edge and area of rice effect depending on time of the season.

Both distance from urban edge and area of rice exhibited generally monotonic associations with *Cx. tarsalis* abundance. Increasing trap distance from urban edge was associated with lower abundance, whereas increasing the area of rice within an 8-km radius of a trap was associated with higher abundance. By contrast, the relationship between both spatial variables and WNV prevalence in *Cx. tarsalis* was more complex. In the early season, greater distance from urban edge was associated with decreased prevalence. However, the effect reversed in late season, with a sharply positive slope reflecting higher WNV prevalence at greater distances from the urban edge. The relationship between area of rice and WNV prevalence was more consistent over time but nonlinear, displaying a bimodal peak in prevalence at low and high values of total rice area.

Information gleaned from these models was used to estimate spatial blocks within Davis for which vector abundance and WNV prevalence would be comparable.

Each block was then assigned two control (untreated) and two vector intervention (treated) stations for an ongoing vector management trial. The trial is a two-year study that is scheduled to end in late summer 2025, after which the effects of the ivermectin intervention will be evaluated within the spatial blocks.

Conclusions

The models developed in this study demonstrated detectable and statistically significant associations between specific spatial variables and vector measures of risk, and further highlighted meaningful interactions between time and these effects. The results indicated that fine-scale spatio-temporal resolution of WNV risk is achievable, though relevant spatial features will vary by landscape and primary WNV vector species. Importantly, spatial predictions of WNV activity and the distribution of local vector populations can be used to support the evaluation of novel vector control trials by informing trial placement: improving spatio-temporal comparability of vector metrics maximizes the trial's power to detect the true effect of intervention.

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First year of targeted sterile insect releases to control *Aedes aegypti* in the West Valley region of southern California

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Introduction

Mosquito-borne viruses such as dengue, Zika, and chikungunya are emerging public health threats in parts of the United States, driven in part by the spread of *Aedes aegypti*. Conventional control methods relying on insecticides face increasing challenges, including insecticide resistance and community hesitancy. As a result, innovative, non-chemical strategies such as the Sterile Insect Technique (SIT) and biological control are gaining attention. Our study evaluated the effectiveness of deploying sterilized male *Ae. aegypti* alone and in combination with In2Care[®] Mosquito Stations in the West Valley region of Southern California. The goal was to assess the population suppression potential of SIT and its synergy with biocontrol tools in a real-world, urban setting.

Methods

The study was conducted from March 2024 to November 2024 across 25 sites in the West Valley area of San Bernardino County, California. X-ray-sterilized *Ae. aegypti* males were released biweekly into nine selected “SIT-only” sites with sixteen additional sites receiving both SIT and In2Care[®] Mosquito Stations. Mosquito populations were monitored using BG-Sentinel traps. Additionally, the egg hatching rate was assessed by collecting eggs from females that mated with irradiated males to evaluate the impact of SIT on reproductive suppression. Public service request logs were also analyzed to evaluate the trend of mosquito-

related service requests before and during SIT interventions.

Results

In SIT-only neighborhoods, female *Ae. aegypti* abundance declined by 44% compared to the previous year. Sites treated with both SIT and In2Care[®] showed a greater reduction of 65%, indicating a synergistic effect between the two interventions. Evaluation of the hatch rate revealed that more than 99.6% of eggs collected from females that mated with irradiated males failed to hatch, confirming successful sterilization of irradiated males. Furthermore, service requests related to mosquito nuisance dropped by 45% in treated neighborhoods, indicating a noticeable reduction in biting pressure.

Conclusion

This study demonstrated that SIT, especially when integrated with other Integrated Vector Management (IVM) strategies such as In2Care[®] stations, is an effective, environmentally friendly tool for reducing *Ae. aegypti* populations in urban environments. The high rate of induced sterility and significant reductions in mosquito abundance support the potential of these interventions as components of IVM strategy. As vector-borne disease threats grow in the U.S., these findings offer a scalable, sustainable model for controlling *Ae. aegypti* by integrating additional innovative tools such as SIT into the existing IVM toolbox.

Facilitating California District-led Sterile- and Incompatible-insect programs (SIT & IIT) against *Aedes aegypti* and *Aedes albopictus*

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Abstract

Districts are seeking additional tools to control invasive and medically-important mosquitoes. However, some Districts lack the internal resources for near term implementation of autocidal methods. This presentation discussed ongoing examples of collaborative California partnerships intended to accelerate progress and provide Districts with an ability to ‘try out’ and explore whether these technologies meet expectations and align with District needs.

From the sewers to the streets: Investigating Norway rat activity above and below ground in the City of Oakland

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Abstract

Norway rats (*Rattus norvegicus*) are commensal synanthropes whose primary habitat is the densely populated urban environment. They are commonly associated with sewers, subway systems, and human dwellings. These rodents can transmit a host of zoonotic diseases, and they have long been recognized as a public health threat.

Norway rat populations flourish in urban sanitary sewers, which provide a predator-free, climate-controlled environment for proliferation. They can then use cracks in the sanitary sewer system to disperse above ground, infesting human domiciles and potentially causing disease. For decades, the main method of controlling Norway rat populations in sewers has been sewer baiting, the practice of baiting sanitary sewers with rodenticide.

Sewer baiting is a particularly attractive intervention for vector control districts, because it requires minimal resources and does not require public compliance or the assistance of other civic agencies. However, literature on the effectiveness of sewer baiting is sparse. Further, questions about sewer baiting's effectiveness imply that sewer populations of Norway rats are a driver of human contact with Norway rats above ground. This is an assumption that is not consistently supported by literature.

The Alameda County Vector Control Services District (ACVCSD) was established in 1984 by the Alameda County Board of Supervisors to provide comprehensive vector control services to residents of the county. Vector Control Biologists (VCBs) at ACVCSD respond to requests for service (RFS) made by residents of Alameda County by providing inspections and recommendations for reported vector-related issues. In 1987, the City of Oakland recognized that it had severe rodent issues as a result of its sewer infrastructure and voted to provide additional funding to control rodents in its sewers. The resulting contract between ACVCSD and the City of Oakland involves the inspection of 8,000 manholes per year and treatment of manholes that have Norway rat activity. ACVCSD also receives requests for service (RFS) from residents of Alameda County for various vectors, including urban rodents.

ACVCSD uses both passive surveillance of human-rodent interactions above ground via RFS's and active surveillance of Norway rat activity in sewers via the District's sewer baiting activities in Oakland. From these two surveillance methods, we spatially and temporally analyzed data generated over a five-year period to determine whether sewer activity of Norway rats correlated with above-ground contact with Norway rats in the City of Oakland. This information may help fill in gaps in our knowledge of rodent control on a public health scale and inform future IPM control strategies.

Improvements to the Contra Costa Mosquito and Vector Control District Rodent inspection and monitoring program

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Introduction

The primary goal of our Mouse and Rat program is to provide inspections and advice to the public to help prevent rodent infestations. A re-evaluation of the program was performed to identify areas for improvement. By updating the literature, evaluating inspection tools, increasing the training of technicians, and changing control material product selection, the Contra Costa Mosquito and Vector Control District (District) streamlined service response and made monitoring more efficient and manageable. The result is better communication and education and setting clear expectations with the public.

One of the services that the District provides are rat and mice inspections to residents of Contra Costa County. The program consists of an exterior rodent inspection and advice. During the Covid epidemic, inspections were limited to the exterior of the structure. Currently the District continues to only inspect the exterior of the structure and interior of the garage identifying entry points, focusing on proper exclusion and eliminating food and harborage areas, aspects critical to successful rodent control. Exclusion offers the best long-term rodent control solution.

Problems and Changes

In the past all rodent inspections were performed by three rodent technicians. As we evaluated workloads and found that service requests were increasing, it became necessary to cross-train all 14 zone technicians to be able to provide all the services offered by the District within each geographic zone area.

In 2024 1,283 rodent inspections were performed, a 25% increase over the 1,026 inspections performed in 2023. A number of concerns by District staff indicated a need to review and make changes to the rodent program. At one point the District had deployed over 240 poison bait stations which became difficult to manage and maintain. Although rodenticide labels require that all stations maintain a constant supply of fresh bait for at least 10-15 days or until rodent activity ceases, many District stations were unmaintained, damaged or missing.

Some stations were observed with muddy paw prints of skunks and racoons indicating that wildlife routinely visited these rodent stations. Because consistent gnawing damage

created risk for non-target bait exposure, a different station was necessary. The plastic Protecta LP stations were removed from the field and replaced with heavier vandal resistant steel Ultrabait VR stations. Protecta LP stations cost approx. \$16.58 ea. whereas Ultrabait VR cost approx. \$27.00 ea.

Technicians are responsible for identifying rodent hot spots within their zone. Each technician utilizes 10 or more stations to monitor for activity. Placement locations are identified by triangulating rodent service requests and identifying secluded harborage sites. In keeping with rodenticide label requirements, bait station locations are within 50/100 feet of a structure and are not located on private property. These stations can be moved as needed by the technician within their zone. Each station starts baited with Non-toxic Detex monitoring blocks. Based on activity Detex may be replaced with a rodenticide that is selected based on the environmental conditions and target species. This provides a more focused and secure monitoring/control program.

Additional tools and training are provided for technicians. All technicians are equipped with a flashlight, small mirror, small truck mirror mounted on a curved 4' pole and the Frye Inspection tool (F.I.T.) developed by Dr. Matt Frye, Cornell University. New York State Integrated Pest Management Program. Mirrors are an important inspection tool, because they allow inspectors to direct sunlight into dark areas and out-perform flashlights (on a sunny day). Having a long-handled mirror allows for easy inspection in hard-to-reach areas as well as the underside of stucco or siding where it meets the foundation.

Residents often request inspections after having received multiple inspections from private pest management companies. Technicians are trained to navigate these situations by sticking closely to the information contained in our brochure and by only commenting on the findings they observe. The District does not offer referrals and technicians do not comment on the quality of previous inspections. When possible points of entry are identified and recommendations are provided for exclusion. Sometimes seeing is believing. I have seen the use of the F.I.T. tool which matches the dimensions of a mouse and rat skull convince residents by visually showing them the capabilities of rodents to enter small openings. Recently a resident shared that they had two companies inspect their home, one company said that an opening was too small for a rodent to

enter, whereas the other stated it needed to be sealed. The resident said “I don’t know who to believe”. The District technician used F.I.T. tool and confirmed to the resident that the opening needed to be sealed. This tool builds trust and credibility with the public and costs around \$5.00.

Conclusions

The changes described have provided a more secure baiting program and improved our efficiency to respond to service request.

Surveillance of *Rickettsia* species in Alameda County associated with wildlife and commensal rodents

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Abstract

In 2018, Alameda County Vector Control Services District began a county-wide surveillance program to ascertain what *Rickettsia* species are present in fleas removed from wildlife and commensal rodents. The same animal and insect vectors that are known from documented disease transmission cycles for Murine typhus, *R. typhi*, and flea-borne typhus, *Rickettsia felis*, also occur in Alameda County. Our study has focused primarily on commensal rodents that are associated with homeless encampments in the County and wildlife from urban areas. To date we have found several *Rickettsia* species in Alameda County associated with the fleas removed from wildlife and commensal rodents.

Investigating the effects of prescribed fire on tick abundance in Santa Clara County grasslands

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Introduction

Despite the significant impact of tick-borne disease on public health, tick control methods are limited to acaricide applications and host reduction or exclusion which are not feasible at a large scale (Fritz and Kjemtrup 2003). In recent years, tick control studies have focused on prescribed fire due to its potential to cause tick mortality or unfavorably alter tick habitat and it is already in use by public land managers to restore native landscapes and reduce wildfire intensity (Gleim et al. 2019; Guo and Agosto 2022; Gallagher et al 2022; Francos and Úbeda 2021). In Santa Clara County, Santa Clara County Parks (SCCP) employees anecdotally reported less tick activity in grasslands managed with fire, but to date, there have been no studies documenting the effects of prescribed fire on tick abundance in these habitats. In the current study, Santa Clara County Vector Control District (SCCVCD) and SCCP monitored tick abundance in three grasslands with varying levels of fire management.

Methods

Three 10-acre grassland plots within Joseph D. Grant County Park were selected for the study: a control plot with no record of fire management in the last decade, a single burn plot, and a multi burn plot burned four times between 2019–2024 (Figure 1). During the study, a burn was conducted in July 2022 at both the single and multi-burn plots and a second burn was completed in October of 2023 at the multi burn plot. The habitat was primarily composed of invasive grasses, i.e. wild oat (*Avena* sp.), with sparse clusters of coyote brush (*Baccharis pilularis*) and a few valley oak trees (*Quercus lobata*).

Ticks were sampled each month at ten 50-m transects per plot using a tick drag and three CO₂ baited traps operated for 22–24 hours (Newman et al. 2019). The overall effect of prescribed fire on tick count was analyzed using a negative binomial model with the multi-burn and single-burn sites categorized as replicate treatments. Then, a one-way repeated measure analysis of variance (ANOVA) was used to test the effect of burn treatment type on total tick count per site per month. Six collection dates where no ticks were collected from any

plots were omitted from analysis. A Tukey HSD test was then used to determine whether both treatment types significantly differed from the control.

Annual Daubenmire vegetation surveys were conducted to document changes in plant composition and restoration of native species (Bonham et al. 2004). Climate loggers were used to record hourly temperature and relative humidity. The temperature and humidity data from the three climate loggers at each plot were averaged to produce a representative climate dataset for each burn type. A one-way ANOVA was used to compare climate among plot types during the typical tick questing period (09–1500h). The same analysis was used to determine difference in native and invasive stem counts.

Results

From June 2022–June 2024, a total of 164 adult ticks were collected: 160 *Dermacentor occidentalis*, 3 *Dermacentor similis*, and 1 *Ixodes pacificus* (Table 1). The negative binomial results indicated that overall, the burn treatment sites had significantly lower tick counts than the control ($p = 0.0009$). The ANOVA used to analyze separate treatment types was significant ($p = 0.002$) indicating a difference in tick counts among treatments (Figure 2). The Tukey HSD determined the single burn plot had significantly less ticks when compared to control ($p = 0.0088$), and the multi-burn plot tended to have lower tick counts, but results were not statistically significant ($p = 0.1053$).

No significant difference in temperature was found among the three plots, but humidity did differ. The multi burn plot had significantly higher humidity levels than the single burn and control plots ($p = 0.0002$). Native and invasive stem counts didn't differ significantly among plots, but the multi burn plot trended toward a significantly higher native plant population ($p = 0.08$) (Figure 3).

Discussion

Overall, tick abundance was lower in grassland plots managed with fire as compared to the control. However, only the plot treated with a single burn had significantly lower tick counts than the control. The plot treated with repeat fire had less ticks than the control, but was not

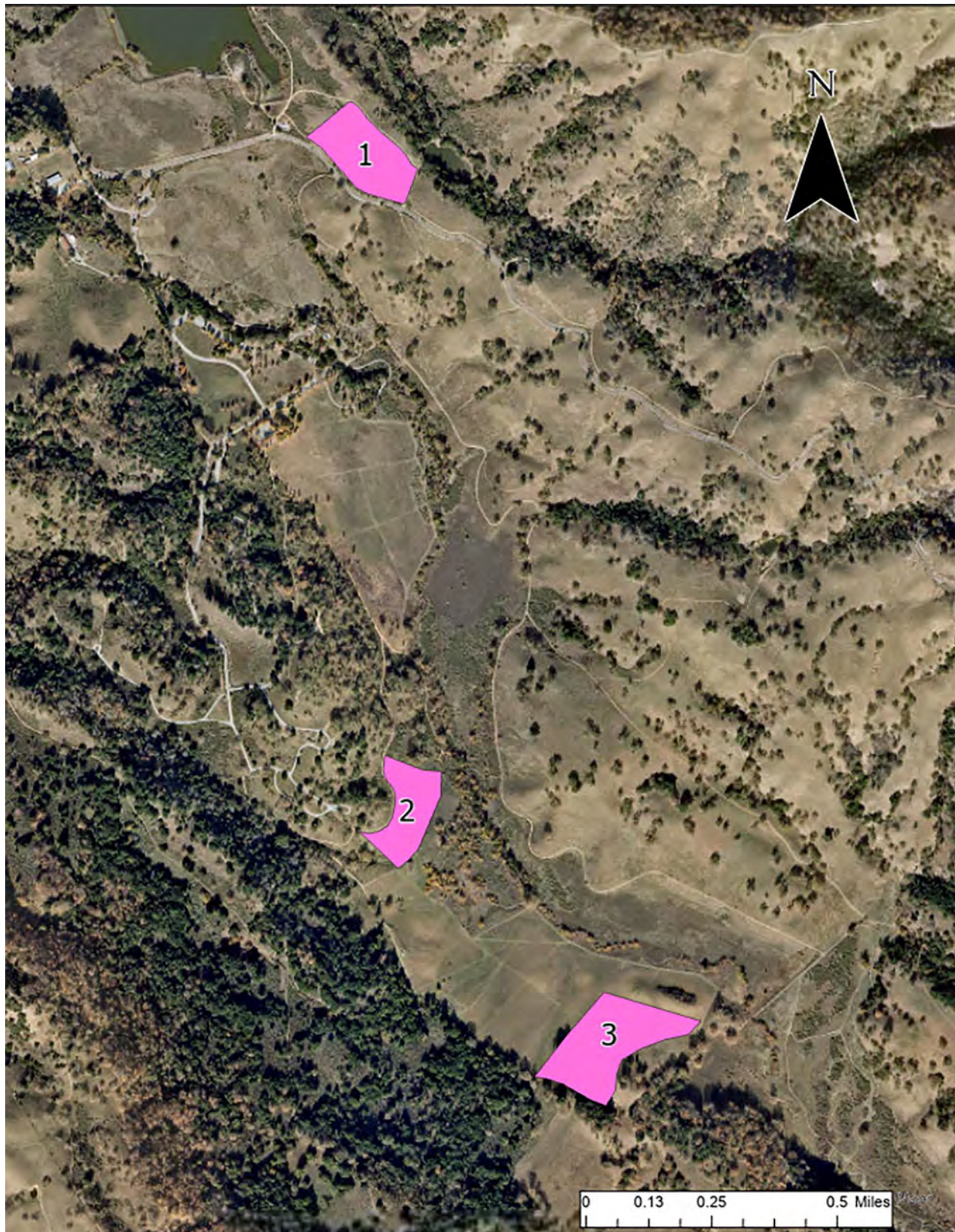


Figure 1.—Map of study plots at Joseph D. Grant County Park: 1) Control 2) Single Burn and 3) Multi-burn.

Table 1.—Number of ticks collected by month by plot type.

Month	Multi Burn	Single Burn	Control
Jun-22	2	1	1
Jul-22	0	0	2
Oct-22	15	0	9
Nov-22	8	14	10
Jan-23	4	3	9
Apr-23	2	2	9
May-23	4	1	8
Jun-23	0	0	2
Oct-23	8	0	10
Nov-23	4	1	1
Feb-24	0	0	4
Mar-24	0	0	10
Apr-24	0	2	13
May-24	0	0	4
Jun-24	0	0	1
Total	47	24	93

statistically significant. This result contrasts with studies conducted in forested habitats which found that repeated burns were necessary for tick population reduction (Gleim et al. 2019). As fires in grasslands are low intensity and vegetation regeneration occurs quickly, the long-term change in plant composition that result from repeat burns may be more important than the immediate reduction in ground cover.

As ticks extract water from the surrounding air, humidity levels regulate their ability to ascend stems and quest for hosts (Lane et al. 1995). Native grasses retain more moisture than invasive grasses, resulting in higher water retention in the soil and superior forage quality for host animals (Potts et al. 2012; Elstein 2004). By the last vegetation survey in spring of 2024, 39% of the multi-burn plot was composed of native plants, whereas only 21% of the single burn plot was composed of native plants (Figure 3). The multi-burn plot also had significantly higher humidity levels than the control and single burn plots during the typical tick questing period (09–1500h).

Although we were unable to survey the abundance of potential host animals, mule deer (*Odocoileus hemionus*), wild boar (*Sus scrofa*), coyotes (*Canis latrans*), California deer mice (*Peromyscus californicus*), and California ground squirrels (*Otospermophilus beecheyi*) were observed within the plots. Mule deer, a probable host for adult ticks, prefer foraging in areas managed with prescribed fire due to an increase in forage quality, especially 2–3 years post burn (Roerick et al. 2019). Larval and nymphal ticks typically rely on smaller animals to acquire a blood meal. Studies have shown that grassland prescribed fire can result in an immediate increase in deer mouse populations and long-term increase in rodent species diversity (Kirchner et al. 2011), indicating that the restoration of native vegetation in grasslands may create more favorable habitat conditions for

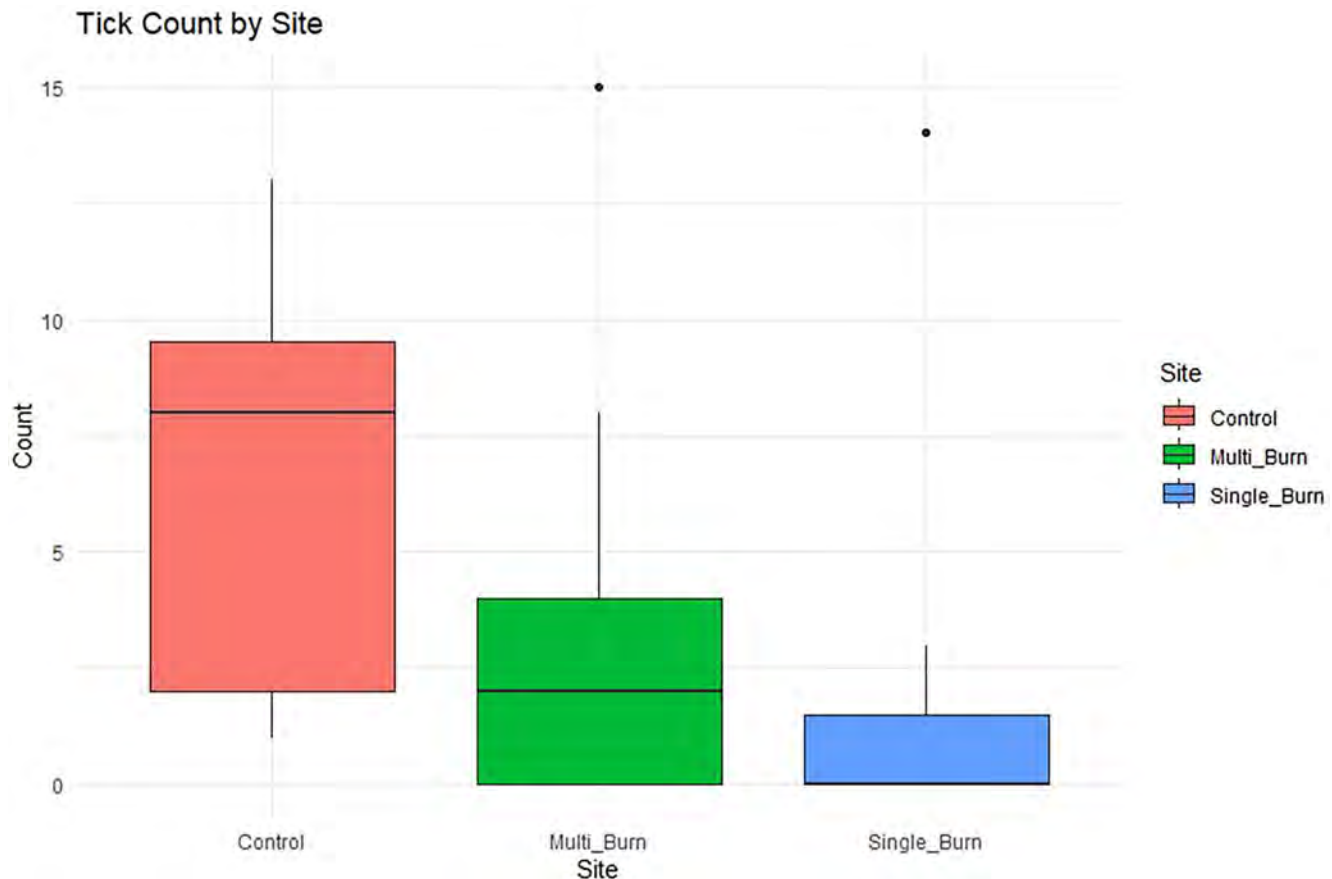


Figure 2.—Overall tick count by site type.

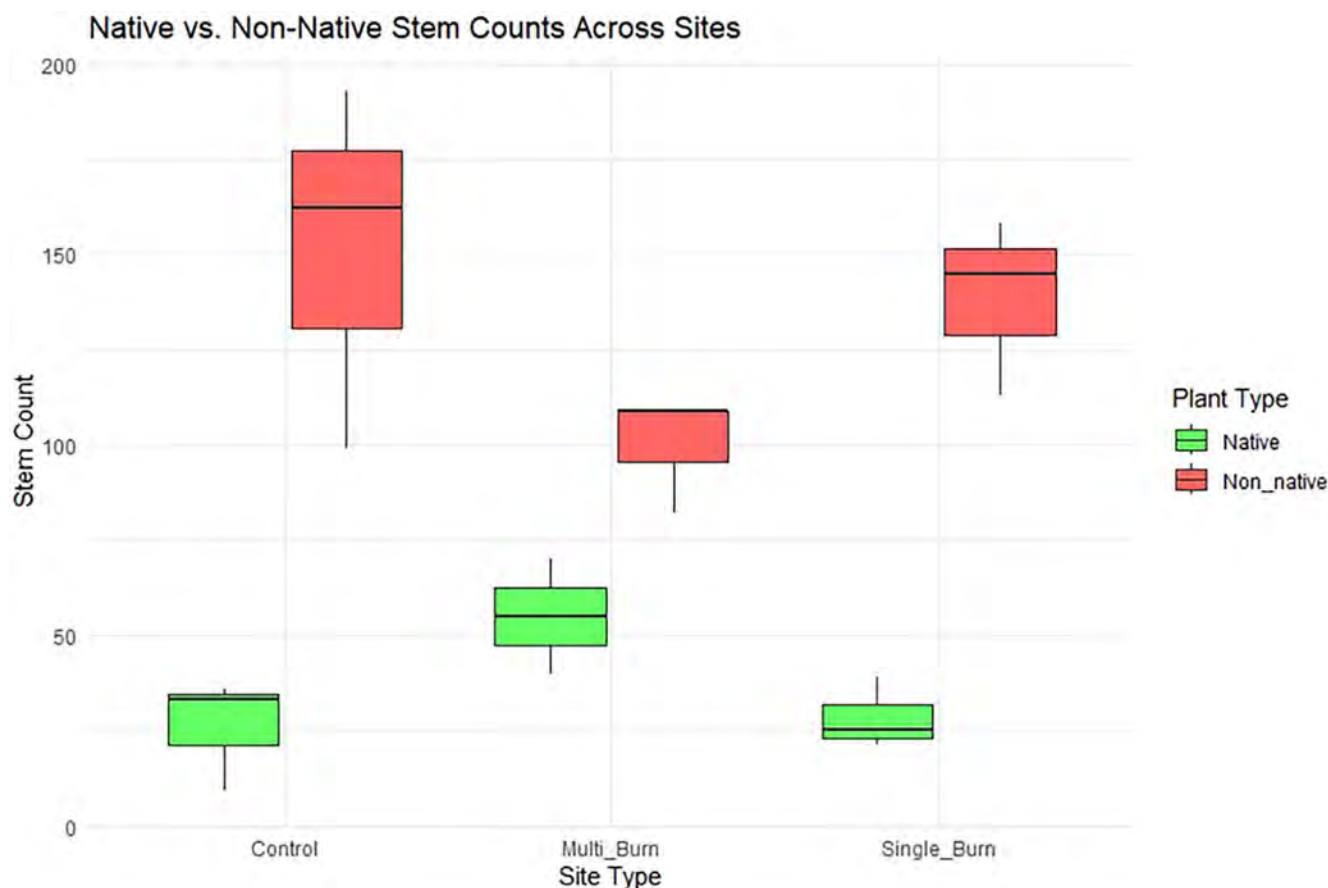


Figure 3.—Native and Non-native plant stem counts by site type.

ticks and bloodmeal sources when compared to a single burn treatment.

Conclusions

Further investigation into the relationship between native grasses and host attraction to the landscape is needed for a deeper understanding of how tick populations are influenced by prescribed fire in grasslands. Public land managers should remain vigilant regarding the potential rise in tick activity that may accompany the restoration of native grasslands.

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What's new with VectorSurv

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Introduction

The VectorSurv team develops the VectorSurv family of software tools to help mosquito and vector control agencies throughout California and the rest of the United States manage their data, make data-informed decisions, and understand the intensity of vector borne disease transmission (VectorSurv Development Team, 2025). Thanks to annual support from the State of California, the VectorSurv team continues to develop and improve new and existing functionality based on the needs of these agencies. Here, we review recent additions to the VectorSurv Gateway interface, new tick surveillance functionality, the VectorSurv Maps, and discuss the evolution of the VectorSurv application programming interface (API). This review also will highlight development priorities for the coming year.

Methods

The VectorSurv team collaborates with the CalSurv Steering Committee, the member agencies of the Mosquito and Vector Control Association of California (MVCAC), and other state and local public health agencies that use VectorSurv to identify needs and define developmental projects. Recently, we enhanced our tick surveillance capabilities in collaboration with the MVCAC Vector and Vectorborne Disease Committee, made improvements to the VectorSurv Maps, and developed tools for data integration using the VectorSurv API. Current and future development will provide agencies with the ability to document and view their service visits, gain additional benefits from various calculators, and better understand insecticide resistance using improved bottle bioassay data functionality.

Results and Discussion

In 2024, VectorSurv developed a new version of the mosquito collection and pool forms using the new VectorSurv interface. The forms were made available to VectorSurv users for beta testing during the 2024 season. After responding to feedback, the VectorSurv team has officially released the new forms, providing a modern, easy-to-use, and mobile-friendly interface for managing surveillance data.

The number of dengue infections in California has increased in recent years, including local dengue transmission in southern California during 2023 and 2024 (California Department of Public Health, 2025). To assist public health agencies in understanding the risk of dengue transmission, VectorSurv has updated the dengue risk map with a new method for estimating transmission risk. The dengue risk map uses environmental data along with *Aedes aegypti* surveillance data to estimate the risk of local dengue transmission upon introduction of an imported infected individual.

Working with the MVCAC Vector and Vectorborne Disease Committee, we have designed a map displaying tick surveillance data. The map allows VectorSurv users to view tick data from the perspective of either abundance or pathogen infections. The abundance view depicts tick relative abundance spatially as a 'heat map'. The pathogen view shows the percentage of pools that test positive for tick-borne pathogens. Both views can be filtered to include specific data for species, date of collection, collection method, sex, and life stage.

Another project that resulted from our collaboration with the Vector and Vectorborne Disease Committee was the tick abundance anomaly calculator. The tick abundance anomaly calculator provides a view of tick abundance and density for a designated place for a configured time frame, along with the previous five years of data for the same time frame for comparison. The data to be included in the calculator can be filtered based on species, sex and life stage, spatial region, collection method, and collection conditions. This tool allows users of VectorSurv to understand the abundance of ticks throughout the season and identify changes and trends. The tick abundance anomaly calculator is the first of the VectorSurv calculators to use our new calculator interface and includes the ability to save, load, and share calculation settings.

One of the recent focus areas of VectorSurv is data interoperability. The VectorSurv team continues to evolve the VectorSurv API to improve the ability to access VectorSurv data. The VectorSurv API is a RESTful interface that allows VectorSurv users to manage or retrieve data using programs they create or using other popular software applications. Anyone with a VectorSurv account can connect business intelligence software tools such as Power BI or Tableau to create visualizations, vector surveillance data

management software to manage their data, or create their own tools using Python, R, or any other programming language they are familiar with.

This year, we introduced *vectorsurvR*, an easy-to-use package for R, that abstracts the technical details of accessing the VectorSurv API to allow users with limited programming experience to retrieve their VectorSurv data using R in a format that is easy to understand and use for analysis or report generation. The *vectorsurvR* package currently provides functions to retrieve mosquito and tick datasets, and perform calculations similar to the abundance anomaly, infection rate, and vector index calculators found within VectorSurv. *VectorsurvR* will empower VectorSurv users to analyze their data and create reports using real-time VectorSurv data.

Development that is in progress or planned includes service visit functionality, an improved interface for calculators, and an enhanced experience for managing and using bottle bioassay data. Agencies that use VectorSurv will soon have a way to view and manage their service visits using the upcoming service visit module. Service visit functionality is currently being tested and is expected to be released soon. This functionality will allow local agencies to record information about their work including location, work done, inspection results, and insecticide applications made during the visit. The existing calculators will be updated to use the same interface as the tick abundance anomaly calculator, including the ability to save calculator settings and easily download chart images. The VectorSurv

team will be collaborating with the MVCAC Integrated Vector Management Committee to update bottle bioassay data management, calculator, and resistance map in VectorSurv to make it easier for agencies to manage and use bottle bioassay data to understand the intensity and distribution of insecticide resistance.

Acknowledgements

We thank our partners, especially the Mosquito and Vector Control Association of California and the California Department of Public Health, for their many essential and long-standing contributions to CalSurv's mission to strengthen the evidence basis for vector-borne disease control. CalSurv is made possible by funding support from the State of California and Epidemiology and Laboratory Capacity for Infectious Diseases Cooperative Agreement number NU50CK000539 from the US Centers for Disease Control and Prevention.

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VectorSurv tools for tick and tick-borne pathogen surveillance

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Introduction

VectorSurv provides a range of data management and analysis tools used by vector control agencies for the surveillance and control of vectors and vector-borne pathogens (VectorSurv Development Team, 2024). As tick and tick-borne pathogen surveillance has increased in importance within California, VectorSurv has developed tools to assist vector control agencies with the management and analysis of their tick related data. Here, we provide an overview of these tools, including VectorSurv's tick collection and pool forms, application programming interface (API) connectivity, and tick-focused maps and calculators that support decision-making.

Methods

The VectorSurv team identifies development projects by working with a variety of stakeholders including users of the VectorSurv system in state and local public health agencies, the CalSurv Steering Committee, and the member agencies of the Mosquito and Vector Control Association of California (MVCAC). Projects are prioritized based on the value they provide to the vector-control community, development effort required, and scheduling constraints. Recent and current tick and tick-borne pathogen development projects include the improvement of the tick collection and tick pool forms within VectorSurv's interface, the addition of the tick abundance anomaly calculator and tick maps as analysis tools for VectorSurv users, and the development of tick-specific connections to the VectorSurv API to allow for agencies to easily access their tick data within VectorSurv using computer code to develop customized reports and data visualizations.

Results and Discussion

To better align the VectorSurv tick functionality with the needs of vector control agencies within California, the VectorSurv team worked with the MVCAC Vector and Vector-Borne Disease Committee to identify ways to enhance tick surveillance capability in VectorSurv. To

address these needs, VectorSurv improved the tick collection and tick pool forms within the VectorSurv interface to allow for simplified data entry and to increase variable fields important to VectorSurv users. These include vertebrate host options and new fields to allow agencies to record additional collection conditions including moisture, exposure to sunlight, and vegetation.

VectorSurv provides calculators to analyze surveillance data to make data-based decisions. In 2024, the VectorSurv team released the Tick Abundance Anomaly Calculator to enhance VectorSurv users' ability to analyze their tick collection data. The user can select time frames, time intervals, and spatial regions, then analyze the density of ticks, the total number of ticks collected, or the total number of ticks per collection. The user can also filter their tick data for the calculations by trap type, species of interest, life stage, attachment and bloodfed status, or environmental conditions during tick collections.

VectorSurv maps are useful tools to visualize surveillance data. In 2024, the VectorSurv team introduced a tick map, which allows users to view tick data using either abundance or pathogen views. Both views allow the user to filter the data by surveillance year, disease week, trap type, species, life stage, and either county or agency spatial boundaries. The abundance view makes it easy for users to see the total number of ticks collected within the specified spatial boundaries and provides graphical breakdowns of the total ticks collected by species and the disease week of their collection. The pathogen view displays either the percentage of tick pools that have tested positive or the minimum infection rate of tick pools tested within the selected spatial boundaries. This view also provides a summary of the percentage of ticks that have tested positive for the pathogens that the user has selected.

The VectorSurv team strives to provide VectorSurv users with access to their data in a modern, standardized interface so they can view or manage their data using whatever tools best fit their workflows. The VectorSurv API is a RESTful API that allows for the integration of VectorSurv data into other software applications using a framework already used by many software developers. Using the VectorSurv API, VectorSurv users and software developers can easily manage

or view VectorSurv data, including tick collection and tick pathogen testing data. Additionally, the vectorsurvR R package provides users with a user-friendly way to access their tick related data independently of their mosquito data using the VectorSurv API.

Acknowledgements

We thank our partners, especially the Mosquito and Vector Control Association of California and the California Department of Public Health, for their many essential and long-standing contributions to VectorSurv's mission to strengthen the evidence basis

for vector-borne disease control. VectorSurv is made possible by funding support from the State of California and Epidemiology and Laboratory Capacity for Infectious Diseases Cooperative Agreement number NU50CK000539 from the US Centers for Disease Control and Prevention.

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Beyond the Gateway: Making decisions with your VectorSurv data

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Introduction

VectorSurv enables users to make real-time, data-driven control decisions using built-in tools in the VectorSurv Gateway and through code-based access to other popular software tools such as Tableau, Power BI, Python, and R using the VectorSurv API (<https://vectorsurv.org/docs/vector-surv-api/>). The online VectorSurv calculators and maps allow users to quickly determine when action thresholds have been met, yet users may want to use their VectorSurv data differently, such as to create customized visualizations, automated reports, or interactive dashboards.

Results

The vectorsurvR package allows for a more streamlined connection and workflow in R (De Cesaris 2024). To complement the package, VectorSurv recently released a YouTube playlist, “R video series: Decision making and reporting” (<https://youtube.com/playlist?list=PLbSUxtSzuqQFhsquussQDNJdwt2-k1bh&si=L53LNKgUSfBKO2vM>). The series was designed to be useful to beginners and seasoned R experts alike. The series starts with an introduction to R, including what users need to get started, and then walks through how to use the vectorsurvR package

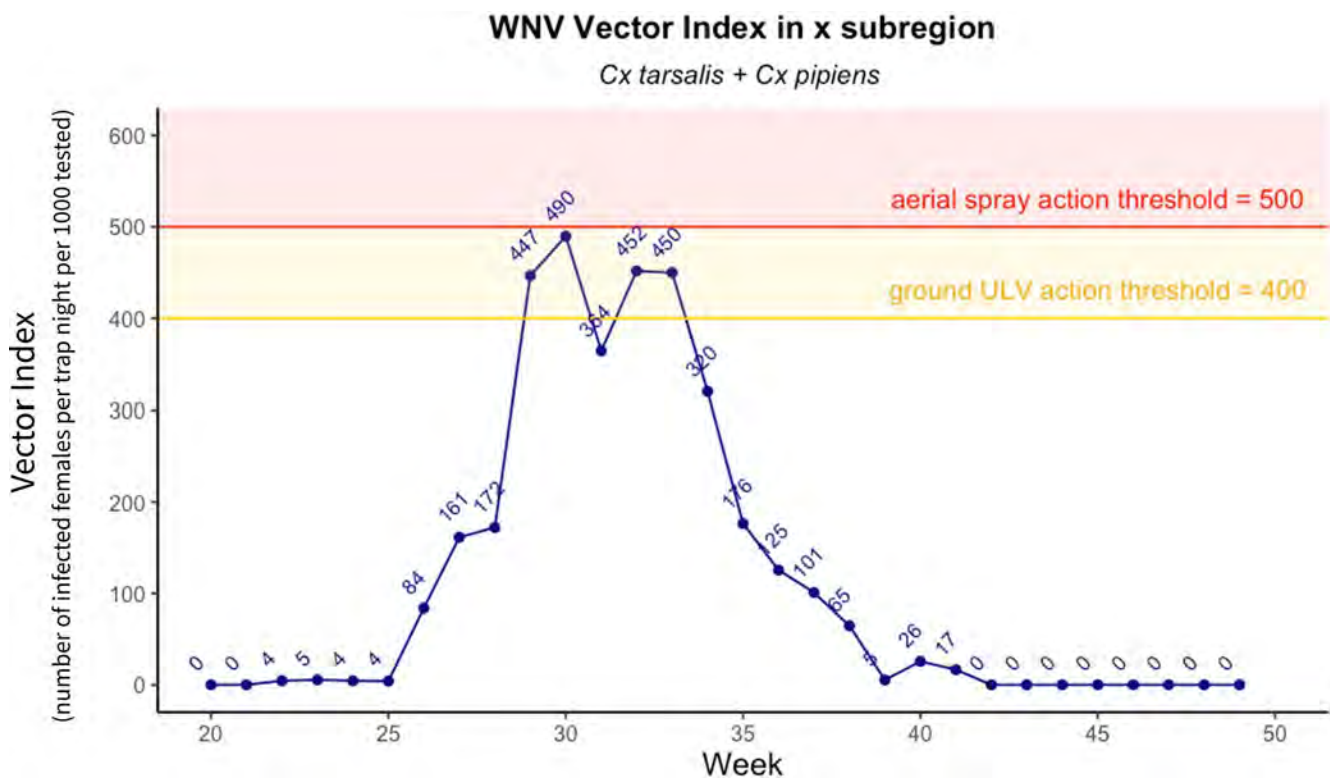


Figure 1.—Vector index visualization developed in R using the vectorsurvR package. R allows for customizable visualization of VectorSurv data, including the ability to add action threshold markers for quick decision-making and intervention. Users can also change colors, fonts, text size, and axis labels.

to access data through the VectorSurv application programming interface (API), filter and sort data, and create visualizations.

The current presentation provided an example of a customized vector index visualization in R, demonstrating the ability to visually add action threshold markers and customize font, colors, and data shown (Figure 1, R Core Team 2024). Additionally, the presentation included a demonstration of how to create a report into HTML and Word. Following this presentation, Kim Hung of the Coachella Valley Mosquito and Vector Control District, and Jung Ma of the San Gabriel Valley Mosquito and Vector Control District, demonstrated how they use Tableau and PowerBI with the VectorSurv API, respectively, to make data-based vector control decisions for their districts to limit the spread of vector-borne diseases. Users can learn more about how to use the API to create tools in other software like PowerBI and Tableau in the VectorSurv documentation (<https://vectorsurv.org/docs/vectorsurv-api/>).

Conclusions

VectorSurv continues to enhance user ability to make real-time data-driven decisions to control the spread of vector-borne diseases in California through the built-in tools and API connections, including the vectorsurvR package. The VectorSurv team will continue to enhance the capabilities of these tools and aims to make additional data types and analytical tools available in the future.

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Real-Time Dengue risk mapping Using VectorSurv

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Abstract

This study introduces the updated dengue risk index map provided by VectorSurv. The dengue risk index is based on adult female *Aedes Aegypti* counts from CO₂-baited and BG Sentinel traps as recorded in VectorSurv by local and state vector surveillance and control programs, combined with weekly temperature data, to gauge the potential risk of dengue spread. The relative risk calculations incorporate key parameters such as mosquito density, biting rate, survival, and the incubation period of dengue virus in the vector to provide real-time, dynamic estimates of dengue transmission risk at the census county subdivision level. This allows users to more effectively monitor and understand spatial and temporal variations in transmission potential, providing valuable insights for proactive vector control and public health interventions.

Generating Tableau reports with VectorSurv data

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Abstract

Tableau, a visual analytics platform by Salesforce, is being used by the Coachella Valley Mosquito and Vector Control District to understand trends from mosquito trap collections and arbovirus test results submitted to California's VectorSurv. This presentation shared several reports created using Tableau, including the "Mosquito Traps Dashboard," designed to support technicians by providing detailed abundance data. We examined a graph comparing weekly BG-Sentinel trap collections inside and outside treatment areas, helping us evaluate the impact of area-wide larvicide applications on adult *Aedes aegypti* abundance. The PESP table was created in Tableau to simplify preparation of annual reports. Finally, we are in the process of creating graphs for arbovirus risk assessment to streamline the process of generating reports. An overview showed how we developed calculations behind the visuals, the challenges encountered, and the benefits of using Tableau.

Creating Reports in Microsoft Power BI using the VectorSurv Gateway

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Abstract

Vector control agencies rely on data reports to make informed decisions across multiple departments. Compiling data and generating reports on a routine basis can be a lengthy and repetitive process with possibilities of inaccuracies due to human error. At the San Gabriel Valley Mosquito and Vector Control District a semi-automated method of generating surveillance reports has been developed using Microsoft's Power BI and connecting it to the VectorSurv Gateway, a database where mosquito data is entered and stored. This process involves melding and organizing data sources in Power BI through data manipulation and using the compiled data to set up calculations and visualizations such as tables, maps and graphs in a predetermined layout. After the report file is set up, the displayed calculations and visualizations semi-automatically update as the VectorSurv database is updated with new data entries. Making new reports for isolated events also becomes a simplified process when the relevant data is already compiled and organized. This method of generating reports has reduced the amount of time and effort needed to produce complex deliverables and has improved accuracy by eliminating many opportunities for human error.

Using R to design Mosquito Trapping Routes, Visualize Data, and Run Statistical Analysis

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Abstract

R is a free software used widely in life sciences for statistical analysis and data visualization. At Santa Clara Vector Control we utilize R in a variety of projects to increase replicability and save time. In 2022 we implemented a R script designed by an undergraduate intern to create mosquito sampling routes around dead birds that test positive for West Nile Virus. Throughout 2023 and 2024 we have utilized R to automate graph generation for mosquito reports using Calsurv data. R has also been an invaluable statistical analysis tool as we have implemented more complex study designs to evaluate the efficacy of new products and tools in our attempts to control *Aedes aegypti* in our region. In this presentation we describe our use cases for this program, provide example data inputs and graphical outputs and share our troubleshooting strategies.

Evaluating Vectech IDX™: AI-driven identification for enhanced vector management

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Abstract

Assessing and advancing cutting-edge technologies that are designed to optimize mosquito surveillance strategies are crucial given the complex challenges presented by our rapidly changing environments. Vectech's Identification-X (IDX) machine offers an innovative way to identify and count adult mosquitoes and train artificial intelligence (AI) software. In collaboration with Vectech, staff at Placer Mosquito and Vector Control District (District) have identified and imaged ~5,100 adult mosquitoes since 2021. Using the most recent software update, we aimed to evaluate accuracy improvements by incorporating a diverse range of mosquito species and genera. To achieve this, 100 female specimens of ten wild-caught mosquito species were imaged. The species included *Anopheles freeborni*, *Anopheles franciscanus*, *Culex tarsalis*, *Culex pipiens*, *Culex stigmatosoma*, *Culex erythrothorax*, *Aedes vexans*, *Aedes melanimon*, *Aedes aegypti*, and *Aedes sierrensis*. Of the ten species analyzed, six had an identification accuracy of 96-100%. Given the software's current accuracy for *Culex tarsalis*, follow-up examinations were conducted to determine at what point consistent specimen degradation impacted the machines' ability to correctly identify mosquito samples of this particular species. Finally, we compared the identification accuracy and speed of individual vector control technicians (VCTs) with the imaging accuracy and speed of the IDX machine to determine operational efficiency of this device within a mosquito surveillance program. Results indicated that the IDX machine is as accurate and efficient as a VCT but is prone to misidentification of morphologically similar mosquito species when specimens are degraded.

Introduction

Placer Mosquito and Vector Control District (District) is dedicated to assessing and advancing cutting-edge technologies to optimize mosquito surveillance strategies. This commitment is driven by the need to address the complex challenges presented by rapidly changing environments, including urban development (Duval et al., 2023; Fletcher et al., 2023) and agricultural practices (Wang et al., 2024). As urbanization and agricultural intensification reshape landscapes and ecosystems, traditional surveillance methods must evolve to accurately track mosquito populations and control strategies (Wheeler et al., 2022). Therefore, it is essential to explore and integrate innovative monitoring tools that can adapt to these shifting conditions, improve data collection, and enhance our ability to respond to mosquito population trends.

Vectech (Baltimore, MD) is a public benefit corporation that provides AI technology-based solutions. They have been developing the Identification-X machine (IDX) (Figure 1) with AI-based software that utilizes supervised machine learning to identify adult mosquitoes from images (Brey et al., 2022; Gupta et al., 2024). Although the AI requires significant training to develop accuracy, there are a number of disease-transmitting species, and therefore practical applications for this technology. In 2021, the

District began a collaboration with Vectech to provide training material and testing for the IDX machine. Since that time, over 5,100 adult mosquitoes were identified and imaged, representing 24 distinct species from 5 genera.

Our study evaluated the current species identification accuracy of the IDX system for a group of local mosquito species. We also compared potential discrepancies in identification between the first and second image perspectives. Two additional investigations were conducted: the first assessed the impact of progressive specimen degradation on the system's ability to identify *Cx. tarsalis* females, whereas the second compared the identification accuracy and efficiency of individual vector control technicians to that of the IDX system. This work aimed to demonstrate that advanced AI-driven software has the potential to have practical applications for improving vector surveillance within mosquito control programs.

Methods

To train the IDX machine, mosquito specimens were first identified by a trained vector control technician. The specimens then were carefully placed into wells within a specially designed IDX imaging tray, allowing for 12

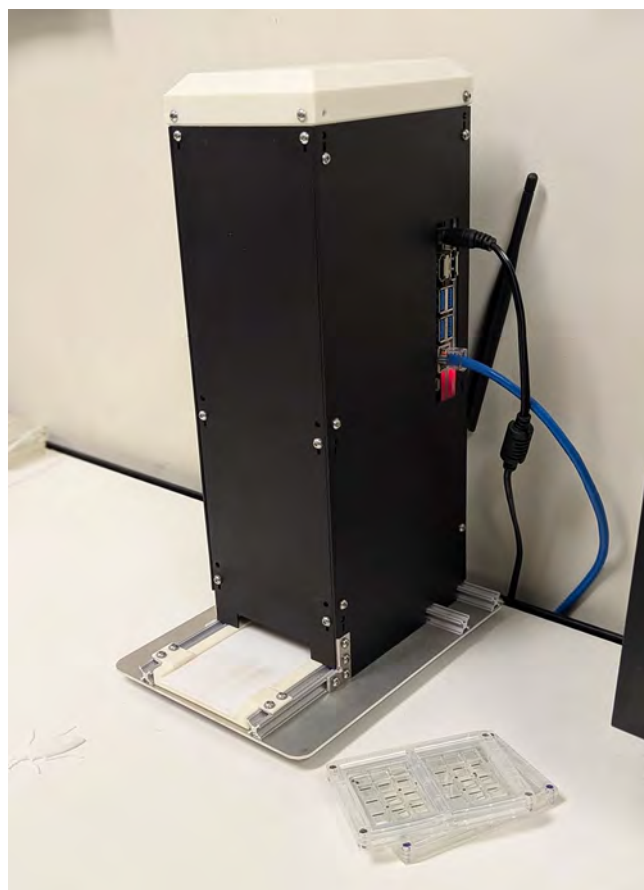


Figure 1.—Vectech's Identification-X (IDX) machine.

specimens to be imaged at a time. After loading, the tray was placed into the machine, where images of the entire tray were captured. The machine analyzed these images to separate the specimens by insect type, genus, species and sex. At this stage, the user enhances the dataset by assigning manual identifications (genus, species, and sex) to each specimen. The tray was then removed, inverted, and reinserted to acquire additional images from a different orientation. As more specimens are imaged and annotated, the overlap between user and machine identifications improves, thereby building a robust dataset to enhance the IDX machine's identification accuracy.

The primary objective of the current study was to evaluate the current accuracy of the IDX machine. This was assessed by imaging ten wild-caught mosquito species, with at least 100 specimens per species, focusing predominantly on female mosquitoes. The species included *Anopheles freeborni*, *An. franciscanus*, *Culex tarsalis*, *Cx. pipiens*, *Cx. stigmatosoma*, *Cx. erythrothorax*, *Aedes vexans*, *Ae. melanimon*, *Ae. aegypti*, and *Ae. sierrensis*. In the larger 2021–2024 dataset, images were excluded from the analysis if a paired first and second image were not available. The analysis included both yearly species accuracy and the agreement between the first and second images. For

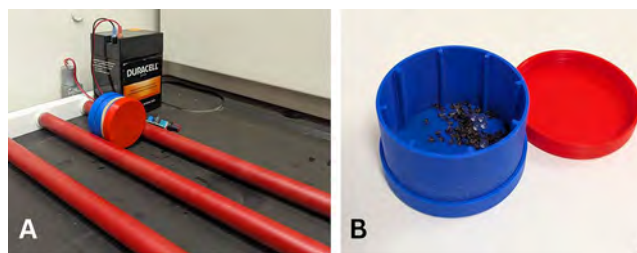


Figure 2.—District (A) bottle roller and (B) tumbler with beads and mosquitoes.

each species and year, the percentage of specimens correctly identified in both images was calculated.

To determine at what point specimen degradation impacted the machines' ability to correctly identify *Cx. tarsalis*, 100 laboratory-reared KNWR (Kern National Wildlife Refuge 1900) adult females were frozen in a –80 °C freezer for several days. Specimens were then thawed and imaged with the IDX machine before being placed in a 3D-printed tumbler (Figure 2), with 5, 4 mm borosilica beads (Avantor Inc.). The tumbler was then placed on a 3D printed bottle roller rotated at 370 RPMs. The tumbler was removed from the bottle roller and specimen images were taken after 2, 4, 8, 12, 20, and 28 min. Percent of specimens identified accurately was calculated at each time point. The trial was discontinued when the IDX machine failed to correctly image > 20% of the specimens. The latest IDX software update, V5.0.4, assesses both images to make a final identification determination referred to as 'fused'. Fused identifications were used in this and the following analyses.

To compare the identification accuracy and speed of VCTs with the imaging accuracy and speed of the IDX machine, 4 technicians identified mosquitoes from 3 mixed batches of species each containing 50 specimens (Table 1). Mosquitoes used in the mixed batches were part of a pre-identified reference collection, and identifications

Table 1.—Mosquito species diversity of batches 1–3.

Batch 1		Batch 2		Batch 3	
Species list	# of specimens	Species list	# of specimens	Species list	# of specimens
<i>Aedes aegypti</i>	3	<i>Aedes melanimon</i>	3	<i>Aedes aegypti</i>	9
<i>Aedes nigromaculis</i>	2	<i>Aedes vexans</i>	4	<i>Aedes melanimon</i>	11
<i>Aedes sierrensis</i>	7	<i>Culex erythrothorax</i>	9	<i>Aedes nigromaculis</i>	6
<i>Aedes vexans</i>	1	<i>Culex pipiens</i>	13	<i>Aedes sierrensis</i>	8
<i>Anopheles freeborni</i>	10	<i>Culex stigmatosoma</i>	10	<i>Aedes vexans</i>	8
<i>Culex pipiens</i>	10	<i>Culex tarsalis</i>	11	<i>Anopheles punctipennis</i>	3
<i>Culex stigmatosoma</i>	8			<i>Culex erythrothorax</i>	5
<i>Culex tarsalis</i>	9				
TOTAL	50	TOTAL	50	TOTAL	50

Table 2.—2024 sample size and identification accuracy for 6 local mosquito species.

Genus	Species	Sample size 2024	Identification Accuracy 2024
<i>Culex</i>	<i>tarsalis</i>	105	100.0%
<i>Aedes</i>	<i>vexans</i>	108	100.0%
<i>Aedes</i>	<i>melanimon</i>	101	99.0%
<i>Aedes</i>	<i>sierrensis</i>	166	98.7%
<i>Culex</i>	<i>pipiens</i>	100	98.0%
<i>Anopheles</i>	<i>freeborni</i>	112	96.4%
<i>Aedes</i>	<i>aegypti</i>	110	95.4%
<i>Culex</i>	<i>erythrothorax</i>	133	60.3%
<i>Anopheles</i>	<i>franciscanus</i>	103	*0%
<i>Culex</i>	<i>stigmatosoma</i>	143	*0%

* Listed species are still in the training phase of early data acquisition.

were confirmed prior to the experiment. The identification time and the percent accuracy were recorded for each VCT. Each batch utilized different species mixtures to evaluate consistency across varying species compositions. Specimens were replaced with the same species, if damaged during the identification process. Batch accuracy percentages, mean identification time, and mean accuracy were calculated for each participant in the trial.

Results

Data was exported from the IDX machine and assessed in Tableau, an analytics platform geared towards data organization and visualization. Of the ten species analyzed, six had well-established image training datasets from previous years, with at least 200 existing images per species. These species had an accuracy of 96% or higher, with identification of *Cx. tarsalis* and *Ae. vexans* reaching 100% accuracy (Table 2). In contrast, four other species had fewer than 50 historical training images from Placer MVCD, but two (*Ae. aegypti* and *Cx. erythrothorax*) likely contained enough training images from offsite locations to have a moderate accuracy of 95.4% and 60.3%, respectively. At the time of testing both *An. franciscanus* and *Cx. stigmatosoma* were not yet included in the deployed identification algorithm for our region and were thus not appropriately scored for accuracy by IDX.

Identification accuracy for most mosquito species improved between 2021 and 2024 (Figure 3). This was due, in part, to the increased number of mosquito images captured by the District over the years, as well as system updates and the inclusion of image datasets from other districts and institutions. The District provided a large number of *Cx. tarsalis* images for IDX training from 2021 to 2024, with identification accuracy showing steady but substantial increases over time. In 2022 and 2023 the District provided images for *Ae. sierrensis*, but the overall accuracy remained at 0% until after a software update resulted in increased identification accuracy for this species.

When comparing the identification accuracy of the first and second images for 2024, there was a 100% accuracy across all species. In contrast, specimens imaged in 2021 had many mismatched identifications, resulting in an agreement between

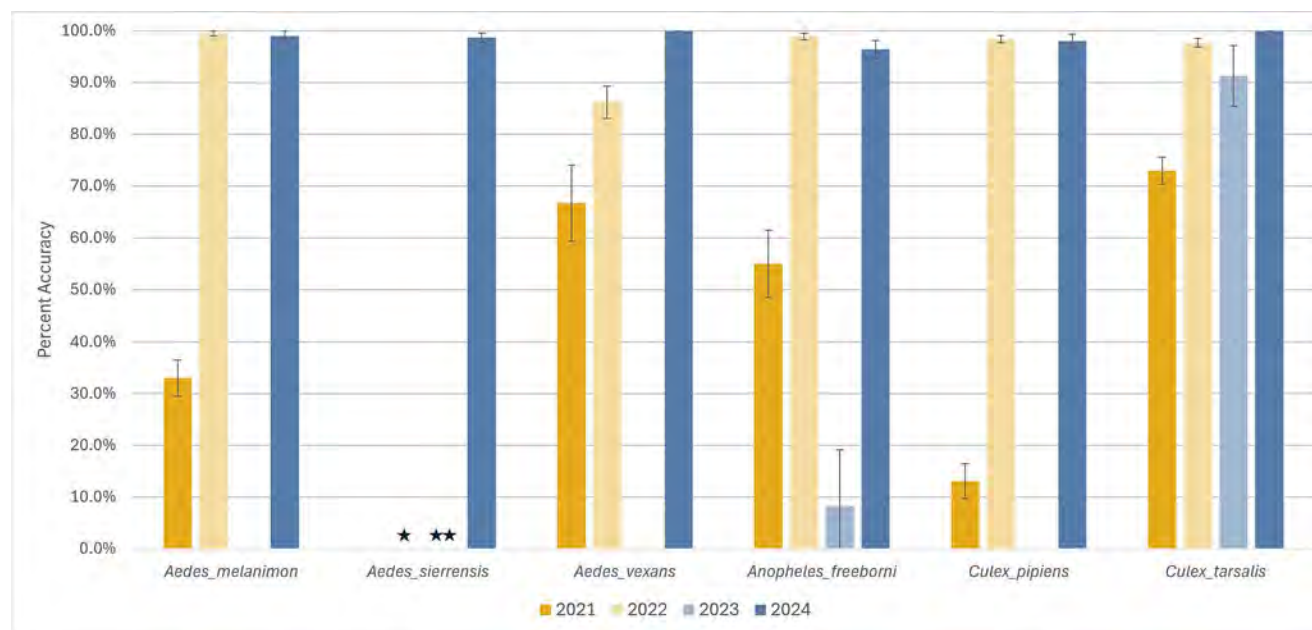


Figure 3.—Vectech IDX machine identification accuracy (2021–2024) for species with ≥ 200 specimens examined. Percent accuracy $\pm$ SE of the percentage. *Ae. sierrensis* collected and imaged with a 0% accuracy, *2022 (n = 32), **2023 (n = 499).

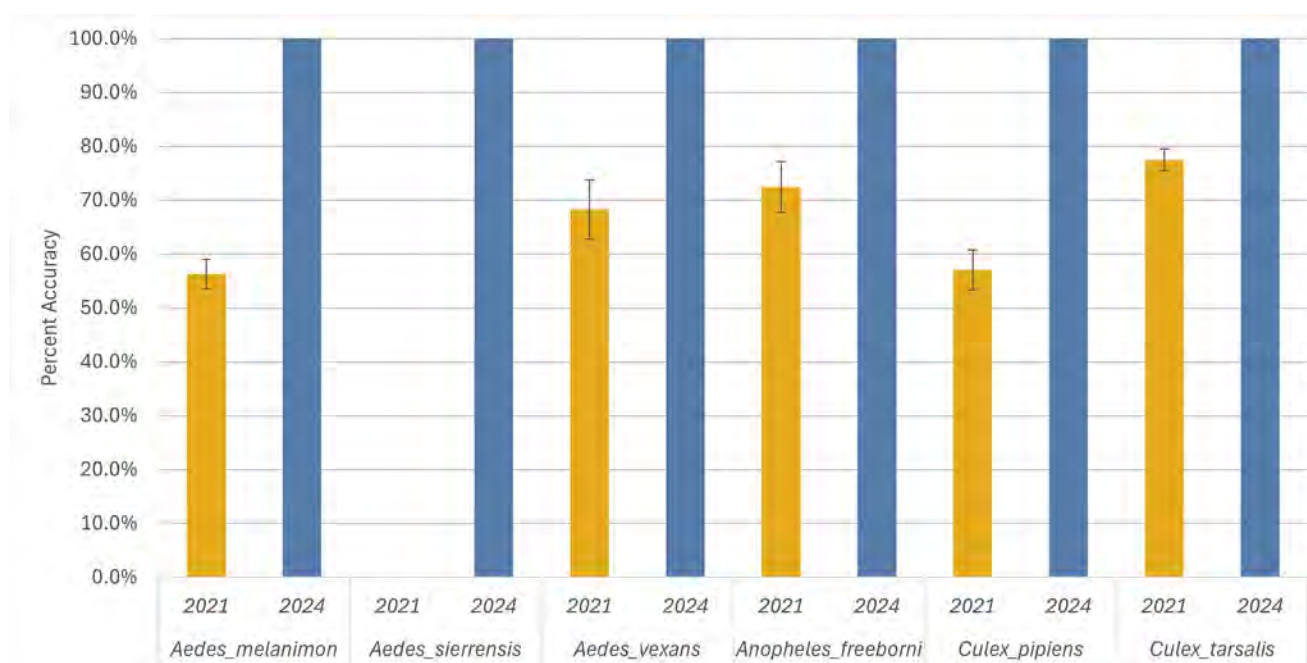


Figure 4.—Vectech IDX machine identification accuracy agreement between first and second image, comparing 2021 and 2024. Percent accuracy $\pm$ SE of the percentage.

56.3% - 77.5% (Figure 4). This indicated that software updates and increasing images resulted in improved agreement between images of the same specimens.

Results indicate that as specimens experienced the progressive loss of scales, legs, and other morphological features within the tumbler (Figure 5), the accuracy of the IDX machine in identifying specimens declined after 4 min (Figure 6). By 28 min, the identification accuracy for *Cx. tarsalis* was reduced to 12% (Figure 6). As correct species identification decreased, the IDX system increasingly assigned alternative identifications, often misclassifying specimens as morphologically similar species such as *Cx. pipiens* or *Cx. tritaeniorhynchus*. In many cases, specimens were categorized as “unknown”.

Results of the comparisons between 4 VCTs and the IDX machine regarding identification speed and accuracy indicated that the IDX machine may be as accurate and as

efficient as a vector control technician with 22 months of experience, but was only half as fast as a senior experienced technician (Table 3).

Discussion

The IDX system uses a convolutional neural network (CNN) for computer vision tasks to reliably identify certain species of mosquitoes. The data presented here demonstrated that providing progressively more training specimens enhances the accuracy of the IDX machine. However, the results also indicated that updates to the system’s algorithm were a crucial factor in improving overall identification accuracy. Due to the subtlety of certain morphological traits, the learning model may require larger sets of training images to accurately differentiate between certain species, such as *Cx. erythrothorax* and *Cx. pipiens*. *Anopheles franciscanus* and *Cx. stigmatosoma* were two species in this examination that had fewer than the recommended number of training images and required further algorithmic updates to improve performance, leading to low identification percentages. During the initial phase of data capture, algorithm accuracy percentages were not immediately established. Instead, captured images undergo a preliminary evaluation to assess both image quality and identification accuracy prior to the deployment in the IDX software, and the collection of additional specimen images. At this early stage of species-specific data acquisition, laboratory-reared specimens are often utilized, as they are more likely to exhibit complete scale patterns and well-defined identifying characteristics, which facilitate more reliable model training.

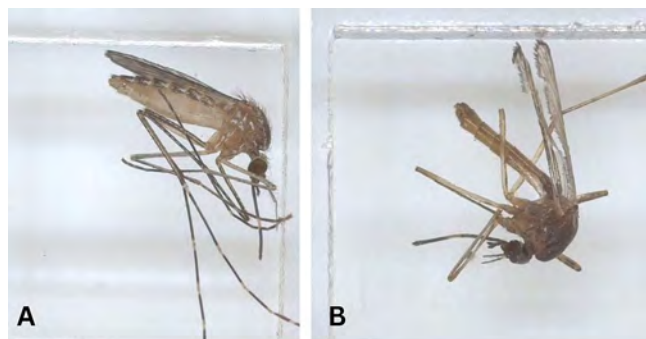


Figure 5.—Colony-reared *Culex tarsalis*, after 0 (A) and 28 (B) minutes within the tumbler.

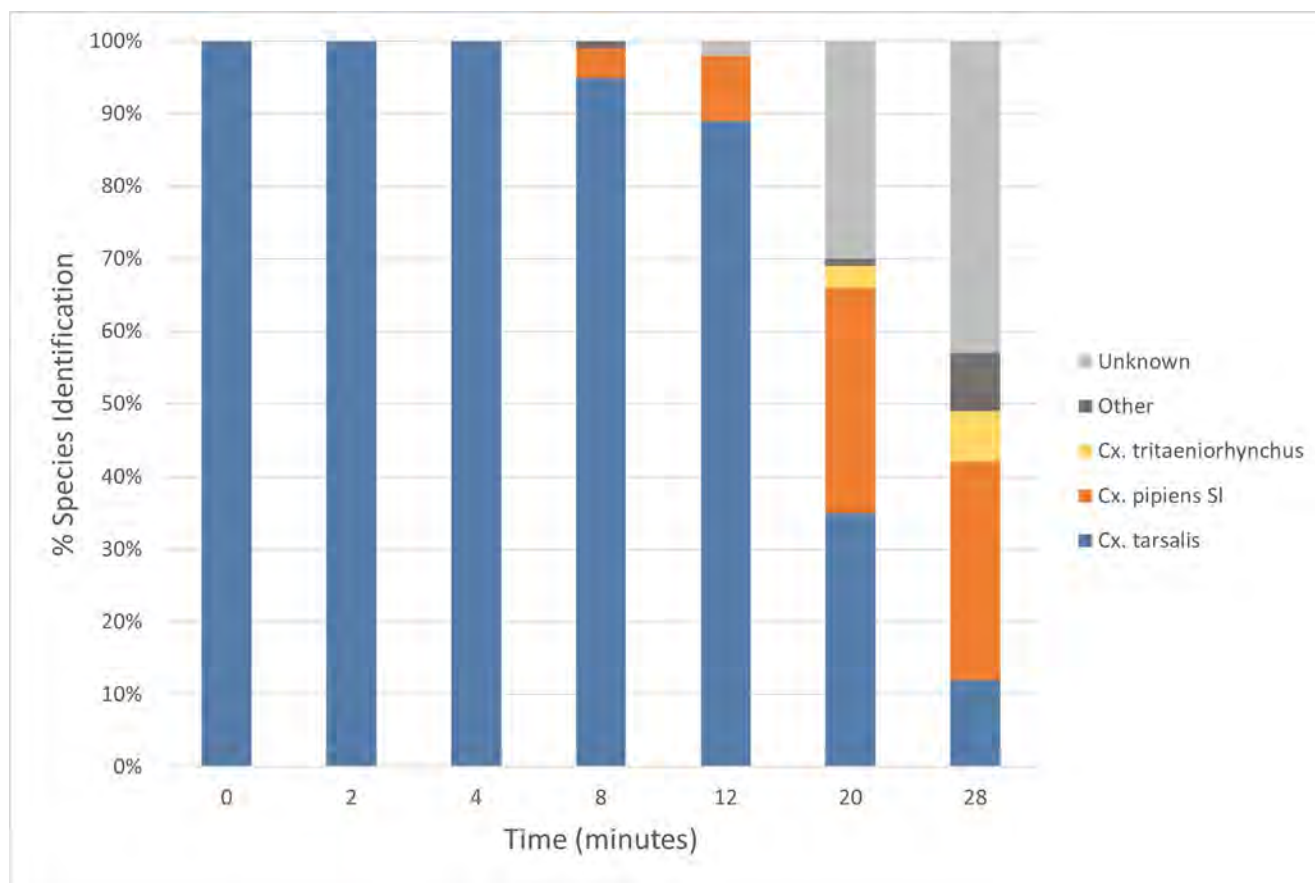


Figure 6.—Fused IDX machine identification accuracy of colony-reared KNWR *Cx. tarsalis* specimens through timed degradation within a tumbler.

To continue to increase current accuracy and to build larger species lists, additional specimens are required, not only locally, but nationwide to capture the diversity within and among species. Although it is important to provide quality specimens for training purposes, all wild-caught specimens will experience some level of degradation due to aging processes in the field, or through mechanical degradation in collection traps. Rarely do vector management staff deal with pristine specimens. The amount of degradation can heavily impact the identification accuracy by both vector control

technicians and the IDX machine. For this reason, it is also valuable to provide the IDX with damaged mosquito specimens so that it can train on these more difficult specimens. Our study provided preliminary data to demonstrate what the identification outcome was for poor quality specimens, but further work is needed to clarify the level of accuracy sufficient for operational purposes. The stakes in misidentification are high, with broad impacts to operations and control efforts for species that transmit important pathogens, and the ability of the IDX to designate specimens as “unknown species” may increase user confidence.

Our research has shown that as the system currently stands, the Vectech IDX may be a helpful device for districts with limited staffing resources that may only be able to hire seasonals with limited experience or training in adult mosquito identification. The IDX machine has gone through many software updates thus far and is slated to continue to do so, streamlining and refining for accuracy and efficiency, and developing larger species datasets that encompass wider geographical areas.

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Table 3.—Identification time and accuracy for 4 vector control technicians (VCT) with between 7 and 144 months of experience and the IDX machine.

VCT Experience (months)	Batch 1		Batch 2		Batch 3		Avg. Accuracy	Avg. Time
	Accuracy	Time (min)	Accuracy	Time (min)	Accuracy	Time (min)		
144 (VCT II)	100.0%	9:26	96.0%	9:38	98.0%	7:17	98.0%	8:47
32 (VCT II)	100.0%	8:46	94.0%	12:43	98.0%	9:28	97.3%	9:35
22 (VCT I)	98.0%	10:28	98.0%	20:13	86.0%	20:06	94.0%	16:55
7 (VCT I)	98.0%	22:25	86.0%	13:54	92.0%	28:25	92.0%	21:34
IDX	97.5%	18:04	96.9%	20:22	89.7%	14:33	94.6%	16:31

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A GIS protocol for high-resolution mapping of mosquito abundance using mosquito trapping data and land use variables

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Introduction

High resolution mapping of mosquito populations is critical for vector control districts to conduct their surveillance and control operations. Mosquito trap density can vary widely across space, and there is a need for mapping methods that can both acknowledge sampling effort and predict mosquito abundance beyond trap locations. We present a new protocol that is generalizable to any agency in the U.S.A. using ArcGIS for mapping short-term predicted mosquito abundance at fine spatial scales by incorporating vector surveillance data and publicly available data sets.

Kriging is a family of geostatistical techniques used to estimate spatial relationships and predict values where observations do not exist using data from observed locations. It has several limitations, including that it requires intensive manual adjustment by analysts to achieve accurate models and that it assumes a single spatial relationship model across the study area. Among the kriging approaches, empirical Bayesian kriging (EBK) improves upon other methods by using local spatial relationship models with subsets of sampling data, making it ideal for highly variable processes like mosquito abundance (Krivoruchko and Gribov 2019). Empirical Bayesian kriging regression takes the process one step further by combining EBK with other spatial covariates to produce more accurate predictions than either EBK or regression alone (Krivoruchko and Gribov 2019).

In the current paper, we describe a protocol for mapping short-term predicted mosquito abundance using empirical Bayesian kriging regression with mosquito surveillance trapping data and spatial covariates to enhance spatial prediction in unsampled areas. To demonstrate the use for real-time vector control operation planning, we apply the protocol to one week of *Aedes aegypti* trapping data in Miami-Dade County, Florida during July 2-8, 2023.

Methods

The protocol is presented using an example of *Ae. aegypti* trapping data in Miami-Dade County, Florida during July 2-8, 2023. All analyses were conducted in ArcGIS Pro 3.3 (ESRI, Redlands, CA, USA). First, the counts of *Ae. aegypti* females were transformed to a normal distribution using

$\ln(y+1)$. *Aedes aegypti* have been previously shown to prefer highly urbanized and densely populated areas (Hopperstad et al. 2021). Therefore, land cover data from the National Land Cover Dataset 2021 and census tract population data from the American Community Survey were retrieved from the ArcGIS Living Atlas (U.S. Geological Survey, 2023; U.S. Census Bureau, 2023). Non-urban land cover types were aggregated into a single “non-urban” category to simplify the prediction model. Population density for each census tract was calculated using the reported total population and area in square miles from the American Community Survey. The EBK Regression Prediction tool then was used with urban environment and population density as explanatory rasters to produce an interpolated map of predicted *Ae. aegypti* female abundance and prediction standard errors for July 2-8, 2023 across Miami-Dade County.

Results and Discussion

The output of this protocol is a map of predicted *Ae. aegypti* female abundance and an accompanying map of standard errors of the predictions (Fig 1). This high-resolution map can be used to identify areas of high abundance at an appropriate spatial and temporal scale for targeting vector control operations. The map of standard errors can be used to measure uncertainty in abundance predictions, locating areas where the counts from traps were highly variable or too sparsely sampled to support operational decision-making. Critically, this protocol is also adaptable to regional needs. Our example used a short timescale (one week) to demonstrate the use for real-time operation planning, but the same protocol could be applied using other trapping timescales over which the counts of *Ae. aegypti* can be assumed to be relatively stable (e.g., monthly during a peak period in summer). In addition, a variety of other spatial covariates specific to regional needs and particular mosquito species could be incorporated as explanatory rasters in the EBK regression analysis.

Conclusions

We presented a novel protocol for producing high resolution maps of *Ae. aegypti* abundance for use in targeting

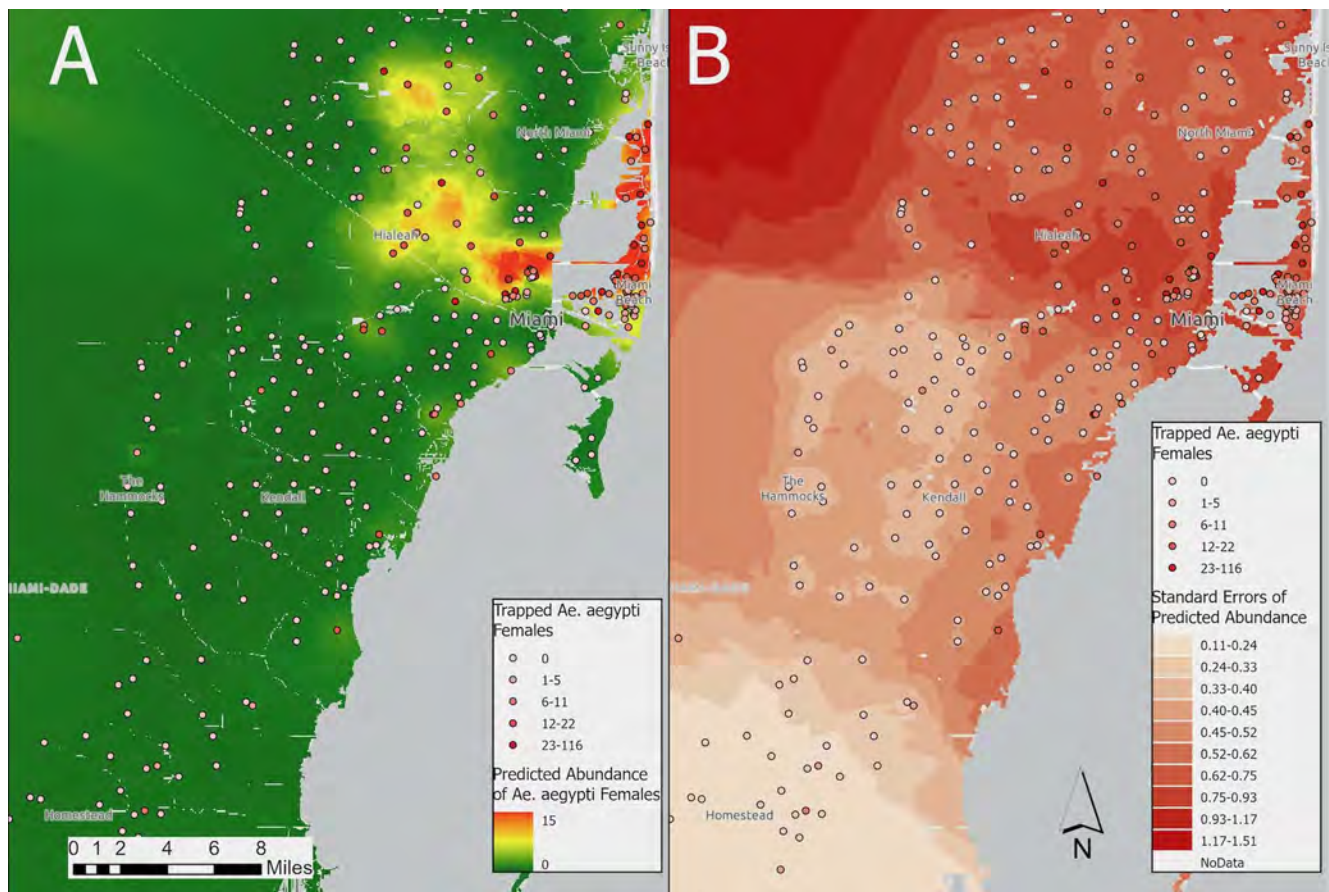


Figure 1.—Maps of predicted *Aedes aegypti* female abundance and associated standard errors during July 2-8, 2023 in Miami-Dade County, Florida, USA. Points represent trapping locations and are colored according to the number of *Ae. aegypti* females trapped. **(A)** Predicted abundance using empirical Bayesian kriging with urban land cover and population density as spatial covariates. **(B)** Standard errors of *Ae. aegypti* female abundance predictions from empirical Bayesian kriging model.

vector control operations. This protocol uses EBK Regression Prediction, a tool in ArcGIS Pro that combines the benefits of both EBK and regression analysis to produce better predictions than either alone. Critically, this protocol is adaptable, and it can be conducted using a variety of trapping timescales and can incorporate a variety of predictors specific to regional spatial features and target species.

Acknowledgements

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Tracking Heat Illness

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Introduction

Outdoor workers in mosquito and vector control districts face a heightened risk of heat illness due to the nature of their work, which is often performed in remote environments with fluctuating temperatures. Heat-related illnesses can range from mild discomfort to life-threatening conditions, impacting worker health, productivity, and the ability of the district to fulfill its public health mission. Cal/OSHA regulations 8 CCR § 3395 (<https://www.dir.ca.gov/title8/3395.html>) mandate specific measures to prevent heat illness, including monitoring, hydration, and rest protocols. A 2024 Cal/OSHA inspection of the Contra Costa Mosquito and Vector Control District (District) revealed specific areas where the existing heat illness prevention program was inadequate, prompting a complete revision to ensure full compliance and enhance worker safety. This document details the development and implementation of a comprehensive, automated heat illness prevention program aimed at meeting Cal/OSHA requirements, improving worker safety, and minimizing the occurrence of heat-related illnesses among District employees.

Methods

District management, in collaboration with staff, revised the heat illness policy and procedures using Cal/OSHA's template. To address the challenges of monitoring variable temperatures across Contra Costa County, the District developed automated programs that utilize real-time weather data. This system continuously tracks temperatures spatially across the county utilizing government owned weather stations, updating every 10 minutes to provide current conditions. In addition, the automated programs include a 5-day average heat wave calculation, which compares the current day's high temperature to the prior 5-day average high temperature for each location and provides an automated morning email notification of any heat waves in the county so that staffing can be adjusted to comply with regulations. When temperatures reach or exceed 95°F, supervisors initiate high-heat procedures. When temperatures indicate a heat wave (the current day's high is 10°F or more above the 5-day average high temperature and over 80°F), heat wave procedures are initiated.

Key components of the District's revised heat illness procedures include:

- Pre-shift meetings: Conducted by supervisors, these meetings review the day's heat illness risk, emphasize hydration and rest protocols, and reinforce workers' rights to take cool-down breaks.
- Automated 5-day average heat wave calculation: This program compares the current day's high temperature to the 5-day average high temperature for each location. If the difference is 10°F or greater, and the daily high is over 80°F, a heat wave is declared, triggering additional precautions.
- Buddy system: During heat waves, field employees are required to work in two-person teams when outdoors for more than 15 minutes, ensuring mutual monitoring and support.
- 30-minute call schedule: On high heat days (95°F or above), supervisors implement a 30-minute call schedule to check in with field employees. These calls verify water intake, encourage extra breaks and shade use, and assess overall well-being.

Results and Discussion

The automated system has proven highly effective in tracking temperatures and providing timely alerts, enabling supervisors to proactively adjust staffing and implement preventive measures. The 30-minute call schedule has significantly improved communication and monitoring of employees working in high-heat conditions, fostering a safety-conscious work environment. The 5-day average heat wave calculation ensures accurate identification of heat waves as defined by Cal/OSHA, triggering the buddy system and other protective measures. Furthermore, the automated system has streamlined the monitoring process, freeing up supervisor time and reducing reliance on manual calculations.

Conclusions

Implementing a Cal/OSHA-compliant heat illness prevention program requires a multifaceted approach that combines policy revision, automated monitoring, and enhanced communication strategies. The automated system developed and implemented by the District effectively addresses the challenges of monitoring variable temperatures and ensuring worker safety in a demanding field environment. This program demonstrates the feasibility and benefits of adopting a proactive, technology-driven approach to heat illness prevention.

Epidemiological trends of dengue in California

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Abstract

From 2016 through 2023, 1,204 dengue cases were reported to the California Department of Public Health (CDPH), with an annual mean of 151. All but 2 cases reported in 2023 were associated with international travel. As of 1 Apr 2025, there were 711 cases of dengue with illness onset in 2024 reported to CDPH, more than 2.5x the previous high of 267 cases in 2019. Most of these cases were travel associated, with 83% reporting recent travel to Latin America or the Caribbean. In addition to the travel associated cases, there 18 locally acquired cases: 14 from Los Angeles County, 3 from San Diego County, and 1 from San Bernardino County. Local transmission was likely driven by record high numbers of returning travelers with dengue infections, which in turn was driven by record high transmission of dengue in the Americas, where almost three times as many cases were reported than during the previous record high in 2023.

Out for Blood: Standardizing local dengue case monitoring

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Abstract

In 2024, human dengue infections saw a substantial increase across endemic regions in the Americas, leading to the declaration of a public health emergency in Puerto Rico and prompting the CDC to issue a health advisory to the United States, citing an elevated risk of dengue virus infections among travelers. This rise was reflected in the number of cases reported in California, whereby early October 2024, the total number of cases had already surpassed the year-end total for 2023. Dengue cases within the San Gabriel Valley Mosquito and Vector Control District's service area mirrored this statewide increase. To respond consistently for each case, a standardized surveillance schedule was implemented and executed. This schedule combined the Enhanced Surveillance model regularly utilized by the San Gabriel Valley surveillance department and the California Department of Public Health's recommended 45-day monitoring window for local transmission of Aedes-borne diseases. This surveillance model informed control teams responding to several local dengue transmissions within the District's service area, offering insight for when additional treatments might be necessary. By standardizing its response to local dengue infections, the San Gabriel Valley Mosquito and Vector Control District was able to quickly mount a multifaceted communicative, surveillance, and control operation for multiple dengue infections in a short time frame.

MVCAC Managers vs. Biologists: who can predict West Nile virus disease?

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Abstract

A series of national forecasting challenges have been conducted in recent years to assess the ability of modelers to predict cases of human disease caused by West Nile virus (WNV). Previously, all forecasts have been the result of statistical or mathematical models fitted to previous data. In 2024, CDPH organized a California-focused forecasting challenge that was limited to 13 counties that have had histories of the highest incidence of WNV disease in the state. The focus on a smaller number of counties, each of which is served by at least one vector control district, provided an opportunity to assess the ability of people to predict human disease cases as a more realistic contrast to the performance of computational methods. This presentation described a collaboration between DART and personnel from 13 MVCAC member districts to obtain and evaluate monthly predictions of WNV disease for all of the counties within the 2024 challenge.

2024 West Nile Virus California Forecasting Challenge

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Abstract

In April 2024 the California Department of Public Health (CDPH) launched the pilot West Nile Virus (WNV) California Forecasting Challenge, in which modeling teams from multiple institutions were tasked with submitting county-level predictions of binned WNV case incidence for each remaining month of the year. This effort was inspired by the 2020, 2022, and 2023 CDC National WNV Forecasting Challenges but targets California's counties to make the forecasting results more relevant to local health jurisdictions and mosquito control entities. Four different institutions submitted a total of 20 unique models with diverse methodologies, broadly grouped as: statistical (5), mechanistic (1), machine learning (7), and human judgement (7). As the WNV season has progressed, ensemble forecasts were shared internally at CDPH and preliminary analyses on different modeling approaches were performed to determine which models have been most accurate. With this pilot challenge, we aim to set up a framework to support county-level forecasting and vector-borne disease control efforts in California in coming seasons and to compare our results with findings from the CDC WNV Challenges, in which simple models trained on historical WNV case counts were found to outperform more complicated machine learning and statistical approaches.

Low risk for locally acquired Chagas disease in California: A review of human cases and triatomine submissions, 2013–2023

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Abstract

Chagas disease is caused by infection with the protozoan parasite *Trypanosoma cruzi*, which is carried in the guts of triatomine insects (also known as kissing bugs). Transmission typically occurs when the infective stage of the parasite in triatomine feces encounters mucous membranes or bite wounds. Infection is most common in rural and peri-urban parts of continental Latin America where triatomines inhabit human dwellings. Triatomine bugs infected with *T. cruzi* are also present across the southern United States, yet relatively few locally acquired infections have been documented. Rather, most reported cases have plausible exposure in Latin America. In California, the widespread distribution of *T. cruzi*-infected triatomines suggests a potential risk of local transmission. Here, we summarize triatomine submissions and human case reports made to the California Department of Public Health between 2013 and 2023. Nearly one-third of 226 adult triatomine submissions were positive for *T. cruzi* via PCR. None were linked to any of the 40 human *T. cruzi* cases reported in the same period. Human cases were assessed for likelihood of local transmission. Country of birth, travel history, and location of primary residence suggested non-local transmission for approximately 80% of the cases. Information on country of birth and travel history were missing from the remaining case reports; local transmission could not be ruled out for these cases, as the patients resided within 400 meters of potential triatomine habitat. Despite the presence of triatomines, *T. cruzi*, and human cases in California, statewide data indicates the risk for locally acquired Chagas disease is very low.

Evaluation of different approaches to detect the flea borne disease, *Rickettsia typhi*

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Introduction

Flea-borne diseases, such as *Rickettsia typhi*, pose health risks to humans who frequent areas with flea-infested animals, including parks with ground squirrels or unhoused encampments with rat infestations (Yomogida et al. 2023, Nelson et al. 2018). To address this, the Santa Clara County Vector Control District (District) is researching optimal flea collection methods to effectively sample these sites and test the fleas for *Rickettsia typhi* and, potentially, other pathogens. Three distinct collection techniques were analyzed to determine the best approach.

Methods

The three flea collection methods analyzed included collecting fleas from rats captured at unhoused camps, collecting fleas from ground squirrel burrows in public parks, and collaborating with the Peninsula Humane Society & Society for the Prevention of Cruelty to Animals (Wildlife Center) to provide ectoparasites removed from submitted animals. Norway rats were sampled at unhoused camp sites in San Jose, California using Tomahawk Collapsible Squirrel Traps (model 201) and were euthanized using a Euthanex induction chamber (Zp-700) before combing and brushing for ectoparasites. Flea sampling from ground squirrel burrows utilized a swabbing technique based on a swatch of terrycloth attached to a 5 ft length of plumber's snake that was inserted into underground squirrel burrows and withdrawn into a Ziploc bag. The Wildlife Center was provided with sample vials to collect ectoparasites from arriving animals. These three methods were evaluated based on safety, the number of flea species identified, workload

calculations (material preparation, field work, cleaning and lab work), and general challenges.

Results

Flea collection at unhoused camps posed significant safety concerns, including exposure to Sharps containers, unhygienic conditions, and unpredictable interactions with people and dogs. Public Park collections had manageable safety risks with personal wearing protective equipment. The collaboration with the Wildlife Center had no safety concerns for Vector Control staff.

Flea collection in unhoused camps often resulted in work equipment loss due to theft, whereas public park collections were subject to permit restrictions. The success of the Wildlife Center collaboration was dependent on the continuation of the partnership.

As shown in Table 1, the unhoused camp method was the most labor-intensive, yielding five different flea species. The public park burrow sampling required significantly less effort than the unhoused camp method but yielded only four species. The most time-efficient approach, which also resulted in the highest flea species diversity (six), was collaboration with the Wildlife Center.

Conclusions

There was no single best method for flea collection, the choice depended on District priorities and resources. The least efficient approach, collecting fleas at unhoused camps, remains important because it targets the most vulnerable part of the population. Additionally, maintaining strong collaboration with nearby wildlife centers, such as the

Table 1.—Flea species, total number of each species, and the time required to collect a flea (preparation time + fieldwork/total number of fleas collected) across the three different collection methods.

	Collection at unhoused camps	Collection at public parks	Collaboration with the Wildlife Center
Flea species found	115 <i>Nosopsyllus fasciatus</i> , 15 <i>Ctenocephalides felis</i> , 8 <i>Leptopsylla segnis</i> , 7 <i>Hoplopsyllus affinis</i> , 5 <i>Ctenocephalides canis</i>	177 <i>Hoplopsyllus anomalus</i> , 70 <i>Oropsylla montana</i> , 10 <i>Nosopsyllus fasciatus</i> , 1 <i>Echidnophaga gallinacea</i>	326 <i>Ctenocephalides felis</i> , 33 <i>Pulex irritans</i> , 26 <i>Echidnophaga gallinacea</i> , 24 <i>Orchopeas howardii</i> , 23 <i>Nosopsyllus fasciatus</i> , 9 <i>Oropsylla montana</i>
Time invested to collect a single flea	6 h	19 min	0.75 sec

Peninsula Humane Society & SPCA, was productive for obtaining a large number of fleas with minimal workload. As for *Rickettsia typhi* testing, the Wildlife Center collected the most *Ctenocephalides felis* which commonly transmits Murine typhus (U.S. Centers for Disease Control and Prevention 2025) and therefore should be considered the most efficient trapping method. None of the methods collected Oriental rat fleas (*Xenopsylla cheopsis*), also an important vector of *R. typhi*.

Acknowledgements

We sincerely thank the Peninsula Humane Society & SPCA for their invaluable collaboration, which provides us with flea specimens. We also appreciate the support of San Jose Parks, Recreation, and Neighborhood Services for granting us the

necessary permits. Lastly, we extend our gratitude to everyone involved in collecting the flea samples.

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Recent plague activity in California: A winter domestic cat plague case and other surveillance oddities

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Abstract

Yersinia pestis, the flea-borne pathogen that causes plague, is maintained by various rodent species and their fleas in endemic regions of California. Plague occurrence is cyclical and periods of increased (i.e., epizootic) activity are associated with heightened disease risks for humans and pets. The last human case of plague in California was in 2020, and subsequent plague activity in rodents and carnivores has been low in most areas where surveillance was conducted. However, in December 2023, the first confirmed case of feline plague since 2011 was reported in a domestic cat from Sierra County. Surveillance conducted by the California Department of Public Health, Vector-Borne Disease Section (VBDS) from late January to early May 2024 found only three deer mice (*Peromyscus maniculatus*) positive for antibodies to *Y. pestis* (i.e., seropositive) adjacent to the residence where the cat hunted. Seropositive deer mice are typically indicative of spillover transmission during times of increased plague transmission among reservoir rodents and deer mice are not typically involved in maintaining *Y. pestis* during enzootic periods. In the central Sierra Nevada region, VBDS detected elevated seroprevalence of *Y. pestis* in carnivore samples from Yosemite National Park and adjacent areas. Forty-one percent (14/34) of carnivore samples collected from late 2023 to early 2024 in this area were seropositive, suggesting that elevated plague activity had recently occurred in local rodent populations. Plague was not detected by VBDS rodent surveillance at nearby Yosemite locations in 2023, but rodent trap success was low relative to prior years and undetected plague activity was suggested as a possible cause for the apparent decreased rodent abundance. Surveillance by VBDS in 2024 found limited evidence of *Y. pestis* activity in Yosemite rodents; just one seropositive deer mouse. Plague activity in rodents and carnivores elsewhere in the state during this period was low with no obvious epizootics. The plague detections in Sierra County and Yosemite illustrate the focal, transient, and sometimes cryptic nature of plague in California and the value of maintaining multiple surveillance components.

A Treehouse Tale of Soft Tick Relapsing Fever

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Abstract

Soft tick relapsing fever (STRF), a tick-borne disease caused by the bacterium *Borrelia hermsii*, is maintained in California in multiple rodent reservoir species and transmitted by *Ornithodoros* soft ticks. The primary vector, *O. hermsi*, is mainly found in forested foothill and mountainous regions above 4,000 feet. These ticks inhabit bird and rodent nests and present a public health risk when the nests are found in or adjacent to inhabited buildings. Soft ticks typically feed for only a few minutes at night, painlessly attaching to a host, so people are often unaware that they have been bitten. Here, we present an investigation of an unusual STRF case where the patient slept in a residence without evidence of rodent activity and was likely exposed during the daytime in a rodent-infested treehouse. Furthermore, this patient presented with unusually severe symptoms. This case highlights the potential severity of this disease and the importance of conducting an environmental investigation, allowing us to identify unusual exposure circumstances and provide control recommendations to help prevent additional cases.

Investigation of a novel spotted fever group rickettsiosis in California

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Abstract

There has been a dramatic increase in spotted fever group rickettsioses over the last decade in the United States. In 2023, a newly recognized rickettsial pathogen known as *Rickettsia* sp. CA6269 was identified as the cause of a severe case of spotted fever like illness in the San Francisco Bay Area. This presentation describes the ensuing environmental investigation and identifies future research objectives for understanding this newly identified human pathogen.

Enhancing *Dermacentor* tick surveillance

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Introduction

The County of San Diego Vector Control Program (VCP) monitors over 4,300 square miles of service area with hundreds of hiking and walking trails that can harbor a variety of tick species, including *Ixodes pacificus*, *Dermacentor occidentalis*, and *Dermacentor similis*.

With the VCP expanding diagnostic testing and the periodic re-emergence of tick-borne pathogens such as *Rickettsia rickettsii* and *Rickettsia rickettsii* subspecies *californica* (also known as *Rickettsia* 364D and *Rickettsia philipii*) in routine sampling, an updated approach for the surveillance of tick populations and disease response was developed. This new approach focused on providing a detailed tick flagging documentation plan using mapping applications to create 400 meter (quarter mile) transects. Our goals were to improve the understanding of local tick ecology, test our new methodologies, and increase the efficiency and precision of surveillance efforts which help identify areas in the San Diego County at increased risk for tick bites and tick-borne pathogens.

Methods

Adult ticks were collected within 400 meter transects from four trails in late winter and again in late spring/summer between 0800 and 1200h, using the flagging method and were sorted by species and sex (Figure 1). The AllTrails® application was used to establish the transects and to create visual maps of where ticks have been detected positive for pathogens (Figure 2). Environmental conditions were documented including temperature, humidity, trail orientation/directional face of slope, and general vegetation/habitat. Ticks were speciated, bisected, and then sorted into pools of up to 10 ticks per pool, with the second half of the tick frozen at -80°C for further testing. Pooled samples were tested for rickettsial pathogens via PCR using primers that detected multiple spotted fever group rickettsia (PanR) and specific primers for *R. rickettsii* and *Rickettsia rickettsii* subspecies *californica* (Karpathy et. 2019, Kato et al. 2013, Stenos et al. 2005). Tick halves from positive pools then were tested individually.

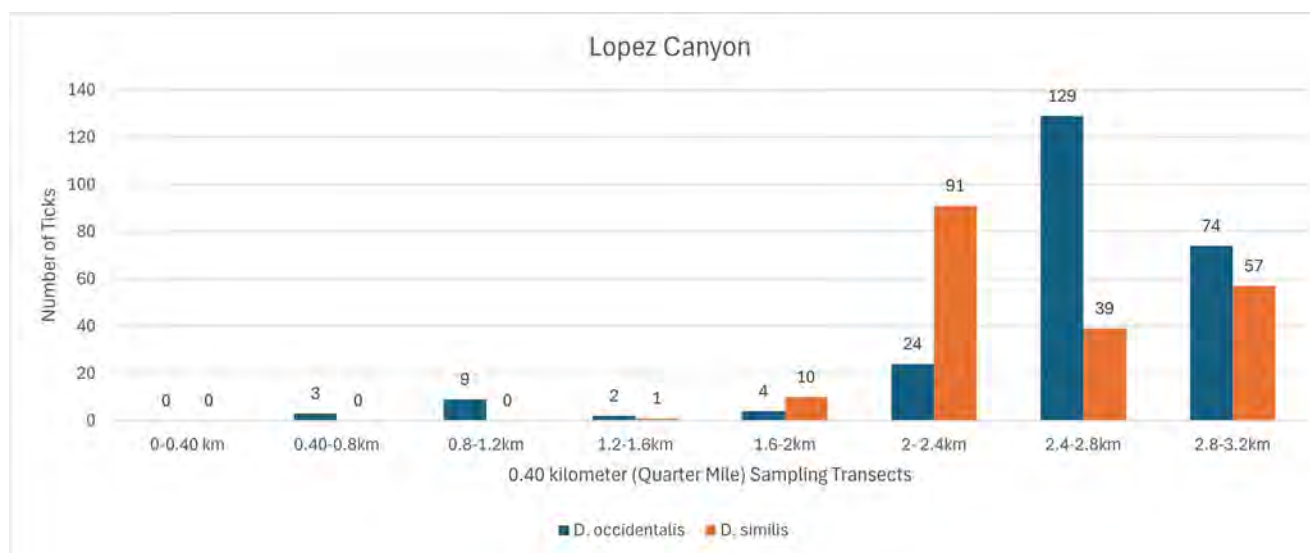


Figure 1.—*Dermacentor occidentalis* and *Dermacentor similis* captures along 400 meter transects of the Lopez Canyon trail. Distances are measured from trailhead out and back where one side of the trail was sampled going out and the other side of the trail was sampled on the return.

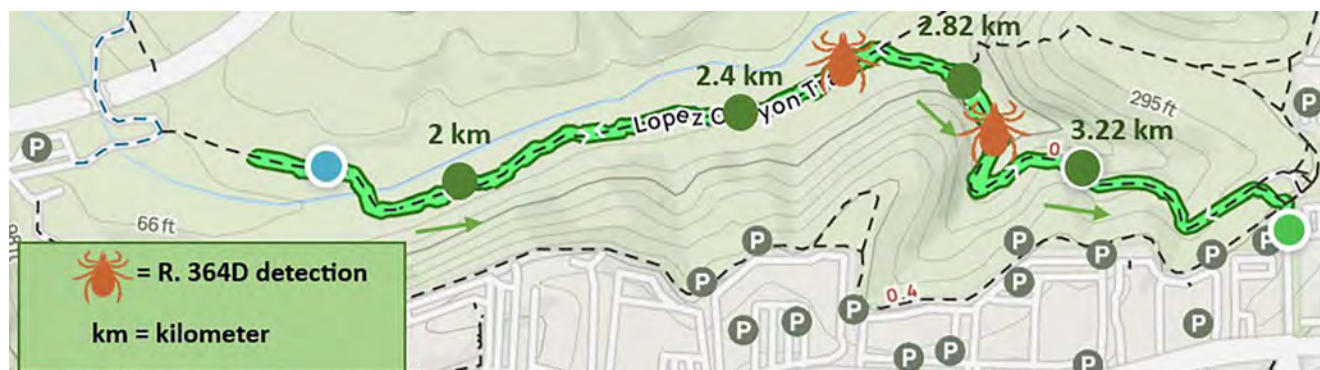


Figure 2.—AllTrails® transect map of Lopez Canyon trail showing locations of *Rickettsia* 364D – pathogen positive tick detections. Map shows the 1.6 to 3.2 km tick sampling section of the inclined northern face of Lopez Canyon trail. Green dots delineate 400m transects; green arrows indicate sampling direction; R. 364D = *Rickettsia rickettsii* subspecies *californica*.

Results

Between February and March (collection 1), *Dermacentor* captures were predominantly *D. occidentalis* (99.3%), with *D. similis* accounting for only 0.7%. From May through July (collection 2), *D. occidentalis* declined to 36.5%, while *D. similis* increased to 63.5%. Temperature and humidity averaged 61 °F (STD 4.2) and 68% humidity (STD 11.8) during collection 1 and 76 °F (STD 7.9 °F) and 53% humidity (STD 6%) during collection 2. This shift in species composition from spring to summer is characteristic in San Diego County (Vo and McDivitt, unpublished data, 2015–2024). *Rickettsia* 364D was detected predominantly in collection 1, with 25 of the 28 total detections (89%) made at this time (Table 1). In contrast, only 3 detections (10.7%) were recorded during collection 2. All detections were from *D. occidentalis*.

North-facing slopes had the highest tick captures (713) and the most detections of Pan-*Rickettsia* (57) and *Rickettsia* 364D. Thirteen out of the 31 (41.9%) 364D detections were collected from a north-facing slope. In contrast, south-facing slopes had the lowest tick captures (3.7%) and pathogen (3.2%) detections. East and west-facing slopes showed moderate tick captures and 364D detection (22.6%), whereas flat zones had moderate tick

captures (11.3%) and low pathogen detection (9.7%) (Table 2).

Terrain inclining from the trail, such as banked hillsides and brushy backslope areas, exhibited the highest tick captures (972 ticks), with 28 out of the 31 (90.3%) ticks testing positive for the pathogen *Rickettsia* 364D. Flat zones had moderate tick captures (120 ticks), with only 3 ticks testing positive for the pathogen *Rickettsia* 364D. Terrain declining from the trail had the lowest tick captures (31 ticks) and no detections of *Rickettsia* 364D (Table 3).

Discussion and Conclusion

Understanding spatial patterns of tick infestation is critical for developing targeted vector surveillance in hilly or brush-dominated regions of California and highlights the role of environmental and seasonal factors in determining tick and pathogen dynamics, particularly in regions with varied topography. Our project aimed to improve understanding of local tick ecology, pilot new field methodologies, and enhance the efficiency and precision of surveillance efforts to identify areas in San Diego County at elevated risk for ticks and tick-borne pathogens. Segmenting trails into 400-meter (quarter mile) sections proved effective for detecting tick hotspots on trails and identifying geographical features

Table 1.—Tick and *Rickettsia* detections.

Trail	Date	Total Ticks	<i>D. occidentalis</i>	<i>D. similis</i>	Pan R	<i>R. rickettsii</i>	<i>R. 364D</i>
1 Feb – March							
Rueda Canyon	2/1/24	72	72	0	6	0	5
Mother Miguel	2/12/24	237	237	0	21	0	11
Lopez Canyon	2/14/24	191	186	5	23	0	2
Mule Hill	3/5/24	215	215	0	22	0	10
		715	710	5	72	0	28
2 May – July							
Lopez Canyon	5/30/24	254	59	195	12	0	0
Mother Miguel	6/13/24	59	42	17	7	0	1
Rueda Canyon	6/14/24	57	48	9	5	0	2
Mule Hill	7/31/24	38	0	38	2	0	0
		408	149	259	26	0	3

Collection 1 is from Feb – March 2024 and Collection 2 from May – July 2024. Ticks were collected from previously *Rickettsia* positive trails. Pan R = Pan-*Rickettsia*; 364D = *Rickettsia rickettsii* subspecies *californica*.

Table 2.—Tick captures and pathogen detection by directional face of the sampled terrain.

Directional Face	Total Ticks	Pan R Positive	Rickettsia 364D Positive
North	713	57	13
South	41	2	1
East	88	10	7
West	154	17	7
Flat	127	12	3
Total	1123	98	31

Rickettsia 364D = *Rickettsia rickettsii* subspecies *californica*.

that may play a role in tick abundance and pathogen presence. Preliminary findings indicated that environmental factors—such as solar exposure, terrain, and seasonality—play a key role in tick species distributions and the potential occurrence of *Rickettsia* pathogens. Tick captures were highest on inclined north-facing slopes, possibly due to denser underbrush, frequent host pathways, and moisture-retaining microhabitats. Interestingly, the fewest ticks were collected on terrain that sloped away from trails. The cause for this distribution is unknown but might relate to differences in sampling technique, as the flagging angle is more obtuse on ground that slopes away from the flagger or, perhaps, to tick preference for upward sloping terrain from trails which could facilitate questing for hosts. Although current data provide early insights into seasonal and spatial trends, additional sampling is essential to validate these patterns and monitor long-term changes in tick populations

Table 3.—Tick captures and pathogen detection by slope.

Slope	Total Ticks	Pan R Positive	Rickettsia 364D Positive
Incline	972	83	28
Decline	31	2	0
Flat	120	13	3
Total	1123	98	31

Rickettsia 364D = *Rickettsia rickettsii* subspecies *californica*.

and pathogen dynamics. These initial results also highlighted the potential need for updated public outreach and educational materials to raise awareness on *Rickettsial* pathogens that may cause human disease.

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Rhipicephalus sanguineus infestation in South Sacramento - A case study

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Abstract

The brown dog tick, *Rhipicephalus sanguineus*, is an important vector of *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever (RMSF) in North America. Because of the vector's widespread distribution in California, RMSF remains an important public health threat in the state. During the 2024 season, the Sacramento - Yolo Mosquito and Vector Control District detected a brown dog tick infestation in South Sacramento that encompassed four different adjoining properties. In partnership with the University of California Davis, we established a surveillance and abatement program for the localized infestation which included tick surveillance, residual barrier spraying and providing dog tick and flea collars as well as information on RMSF and brown dog ticks in both English and Spanish.

Surveillance for mosquito-borne virus activity in California, 2024

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Abstract

In 2024, 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 32 of 58 counties in California, and St. Louis encephalitis virus (SLEV) activity was reported from 5 counties. A total of 131 human WNV cases were reported, and non-human WNV activity was detected among horses, dead birds, mosquitoes, and sentinel chickens. No cases of SLEV disease were identified, although enzootic SLEV activity was detected in mosquitoes from five counties. In addition, a total of 18 locally acquired cases of dengue were identified from 3 counties.

Introduction

The California Arbovirus Surveillance program is a cooperative effort among 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 2024, 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 32 (55%) of 58 counties in California (Table 1), while SLEV activity was reported from 5 (9%) 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 antibodies using an IgM enzyme immunoassay (EIA) and/or an IgM immunofluorescence assay (IFA). Specimens from case-patients with early season onset or from counties with enzootic SLEV activity also received testing using an SLEV IgM MAC-ELISA and plaque reduction neutralization tests (PRNT) at VRDL. Additional WNV infections were identified through screening for RNA performed by blood centers.

In 2024, a total of 131 symptomatic and 19 asymptomatic infections in blood donors with WNV were reported (Tables 1 and 3). Of the 131 symptomatic cases, 103 (79%) were classified as West Nile neuroinvasive disease (WNND) (e.g., encephalitis, meningitis, acute flaccid paralysis, or other neurologic dysfunction) and 28 (21%) were classified as non-neuroinvasive disease; 12 (9%) cases were fatal. Cases were residents of 24 counties, and 84 (64%) were male. In 2024, WNV disease incidence in California was 0.33 cases per 100,000 persons. Incidence was highest (5.34

Table 1.—West Nile virus activity in California by county, 2024. Humans include asymptomatic infections detected through blood bank and organ donor screening.

County	Humans	Horses	Dead birds	Mosquito pools	Sentinel chickens
Alameda	0	0	53	19	2
Alpine	0	0	NT	NT	NT
Amador	0	0	NT	NT	NT
Butte	12	0	4	70	28
Calaveras	1	0	NT	NT	0
Colusa	1	0	1	0	2
Contra Costa	5	0	6	10	8
Del Norte	0	0	NT	NT	NT
El Dorado	0	0	0	NT	NT
Fresno	20	0	2	208	NT
Glenn	2	0	NT	0	NT
Humboldt	0	0	0	NT	NT
Imperial	0	0	NT	3	NT
Inyo	0	0	NT	0	NT
Kern	12	0	0	48	NT
Kings	7	0	NT	87	NT
Lake	0	0	3	13	7
Lassen	0	0	NT	NT	NT
Los Angeles	29	1	17	206	42
Madera	0	0	1	110	NT
Marin	0	0	0	0	NT
Mariposa	0	0	NT	NT	NT
Mendocino	0	0	NT	NT	NT
Merced	3	0	1	44	12
Modoc	0	0	NT	NT	NT
Mono	0	0	NT	0	NT
Monterey	0	0	NT	NT	NT
Napa	0	0	0	0	NT
Nevada	0	0	0	NT	1
Orange	1	0	1	53	NT
Placer	0	1	4	93	NT
Plumas	0	0	NT	NT	NT
Riverside	6	0	4	217	NT
Sacramento	7	1	66	53	1
San Benito	0	0	NT	0	0
San Bernardino	7	0	10	76	NT
San Diego	2	1	8	0	NT
San Francisco	0	0	0	0	NT
San Joaquin	2	1	6	188	NT
San Luis Obispo	0	0	0	0	NT
San Mateo	1	0	37	0	0
Santa Barbara	0	0	0	0	NT
Santa Clara	6	0	301	34	NT
Santa Cruz	0	0	0	0	NT
Shasta	1	0	0	37	2
Sierra	0	0	NT	NT	NT
Siskiyou	0	0	NT	NT	NT
Solano	0	0	2	13	8
Sonoma	0	0	0	0	NT
Stanislaus	6	0	0	56	NT
Sutter	1	0	0	32	20
Tehama	1	1	NT	NT	6
Trinity	0	0	NT	NT	NT
Tulare	4	0	1	243	10
Tuolumne	0	0	NT	NT	NT
Ventura	0	0	0	0	0
Yolo	9	0	6	66	3
Yuba	4	1	2	32	7
State Totals	150	7	536	2,011	159

NT = None tested.

Table 2.—St. Louis encephalitis virus activity in California by county, 2024.

County	Humans	Mosquito pools ¹	Sentinel chickens
Fresno	0	14	NT
Imperial	0	1	NT
Kings	0	3	NT
Madera	0	4	NT
Tulare	0	12	0
State Totals	0	34	0

¹ Positive mosquito pools included *Culex pipiens* (1), *Cx. quinquefasciatus* (25), and *Cx. tarsalis* (8).
NT = None tested.

cases per 100,000 persons) in Butte County, whereas Los Angeles County reported the most cases (29, 22% of total) (Figure 1, Table 3).

The median age of those with WNND was 63 years (range, 7 to 91 years), and among cases with non-neuroinvasive disease the median age was 56 years (range, 34 to 82 years). The median age of the 12 WNV-associated fatalities was 72 years (range, 21 to 86 years). Dates of symptom onset ranged from May 28 to October 24, with the peak occurring during epidemiological week 33 (August 11–August 17), when 16 (12%) symptomatic infections occurred.

In addition to human cases of WNV, 18 locally acquired cases of dengue virus were identified in 3 counties: Los Angeles (14), San Bernadino (1), and San Diego (3). This is the second consecutive year that locally acquired cases of dengue, a virus transmitted by invasive *Aedes* mosquitoes, have been identified in California.

Mosquito Surveillance

In 2024, mosquito testing was performed at DART and 13 local mosquito and vector control agencies. A total of 1,257,701 mosquitoes (49,167 pools) collected in 40 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 triplex RT-qPCR.

West Nile virus was detected in 2,011 mosquito pools from 25 counties (Tables 1 and 4), and the statewide annual minimum infection rate (MIR-defined as the minimum number of infected female mosquitoes per 1,000 tested) of WNV in all mosquitoes tested was 1.6. During California's peak transmission period (July – September) the statewide MIR in *Culex* mosquitoes was 2.8 and 4 counties reported MIRs greater than 5.0, the epidemic threshold value defined in the statewide response plan (Figures 2 and 5) (California Department of Public Health, 2024). West Nile virus was detected from six *Culex* species (*Cx. erythrorhax*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. stigmatosoma*, *Cx. tarsalis*, and *Cx. thriambus*) (Table 5). WNV positive pools were collected from March 8 to December 12, with the peak

occurring during epidemiological week 33 (August 11–August 17).

SLEV was detected in 34 mosquito pools from 5 counties (Table 2). Statewide, the SLEV MIR in all mosquitoes tested was 0.03; the MIR was highest (0.3) in Kings County. St. Louis encephalitis virus was identified from three *Culex* species (*Cx. pipiens*, *Cx. quinquefasciatus*, and *Cx. tarsalis*) collected from May 14 to October 8.

In addition, a total of 13,399 *Ae. aegypti* were tested for chikungunya, dengue, and/or Zika viruses; all were negative.

Chicken Serosurveillance

In 2024, 24 local mosquito and vector control agencies in 20 counties maintained 75 sentinel chicken flocks (Table 4). Blood samples were collected from chickens every two weeks and tested for antibodies to WNV, SLEV, and WEEV by an EIA at the CDPH Vector-Borne Disease Section and one local agency. Presumptive positive samples were confirmed by IFA or western blot. Of 4,889 chicken blood samples tested from 495 chickens, 159 (32%) seroconversions to WNV were detected among 45 flocks in 16 counties (Tables 1 and 4). Seroconversions to WNV occurred between July 15 and November 19, with the peak occurring during epidemiological week 33 (August 11–August 17). No SLEV seroconversions were detected.

Dead Bird Surveillance

In 2024, the WNV and Dead Bird Call Center and website received a total of 6,702 dead bird reports from 54 counties (Table 6). Tissue samples from dead bird carcasses or oral swabs transferred to RNA preservation cards (Fortius Bio, San Diego, CA) were tested by RT-qPCR by the DART laboratory and 13 local agencies, or by IHC at the California Animal Health and Food Safety Laboratory. Of the 1,931 bird carcasses that were deemed suitable for testing, WNV was detected in 536 (28%) carcasses from 37 counties (Tables 1 and 6). Thirty-four different bird species tested positive for WNV: 72% were American crows, 5% were California scrub-jays, 8% were other corvids, and 15% were non-corvid or unknown species. Positive birds were detected from January 11 to December 11, with the peak occurring during epidemiological week 35 (August 25–August 31).

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 2024, WNV infection was confirmed in 7 horses from 7 counties (Table 1). A single horse (14%) was euthanized because of their infection.

Discussion

In 2024, 32 (55%) of 58 counties reported WNV activity. A total of 131 symptomatic human cases were reported from

Table 3.—Reported West Nile virus human cases by county of residence and year, California, 2014–2024.

County	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2024 incidence per 100,000 person-years	Ten-year incidence per 100,000 person-years
Alameda	0	0	1	0	1	0	0	1	1	0	0.00	0.02
Alpine	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Amador	0	1	0	1	1	0	0	0	0	0	0.00	0.76
Butte	53	21	4	12	5	4	13	3	18	11	5.31	6.99
Calaveras	0	0	0	0	0	0	0	0	1	1	2.23	0.45
Colusa	1	2	0	0	1	0	0	1	1	1	4.60	3.22
Contra Costa	1	4	4	4	1	4	2	1	10	4	0.35	0.31
Del Norte	0	0	0	0	0	0	0	0	0	0	0.00	0.00
El Dorado	0	1	0	0	0	1	1	0	3	0	0.00	0.32
Fresno	8	14	13	14	51	10	14	30	23	17	1.67	1.91
Glenn	19	6	0	2	0	1	2	1	4	1	3.48	12.53
Humboldt	0	0	0	1	0	0	0	0	0	0	0.00	0.08
Imperial	1	0	3	0	3	1	0	0	0	0	0.00	0.44
Inyo	0	0	4	0	0	0	0	0	0	0	0.00	2.12
Kern	11	17	30	13	28	8	8	22	16	12	1.32	1.81
Kings	0	8	5	0	3	2	8	7	9	6	3.93	3.14
Lake	2	1	0	1	0	2	0	0	6	0	0.00	1.79
Lassen	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Los Angeles	286	151	277	43	31	90	16	61	62	23	0.25	1.06
Madera	4	6	2	4	3	6	3	3	9	0	0.00	2.51
Marin	1	0	0	0	0	0	0	0	0	0	0.00	0.04
Mariposa	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Mendocino	2	0	0	0	0	0	0	0	0	0	0.00	0.22
Merced	1	0	10	2	10	12	6	7	8	3	1.04	2.05
Modoc	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Mono	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Monterey	0	1	0	1	0	0	0	0	2	0	0.00	0.08
Napa	0	0	0	1	0	0	0	0	0	0	0.00	0.07
Nevada	2	0	0	1	0	0	0	0	1	0	0.00	0.40
Orange	92	32	33	9	5	17	3	9	6	1	0.03	0.66
Placer	0	7	0	9	1	2	2	2	6	0	0.00	0.70
Plumas	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Riverside	127	11	32	15	12	10	3	0	20	4	0.25	0.96
Sacramento	4	25	6	15	4	7	6	5	53	7	0.44	0.84
San Benito	0	0	0	0	0	0	0	0	0	0	0.00	0.00
San Bernardino	54	8	57	9	7	3	1	4	28	5	0.23	0.81
San Diego	42	20	2	2	3	1	3	3	0	2	0.06	0.24
San Francisco	0	0	1	0	0	0	1	0	0	0	0.00	0.02
San Joaquin	2	13	14	14	7	2	7	4	15	2	0.25	1.01
San Luis Obispo	0	0	0	0	2	0	2	0	2	0	0.00	0.22
San Mateo	0	0	0	0	0	0	1	1	3	0	0.00	0.07
Santa Barbara	0	0	0	0	0	0	2	0	0	0	0.00	0.05
Santa Clara	8	1	0	1	1	0	3	1	3	4	0.21	0.12
Santa Cruz	0	0	0	0	0	0	1	0	0	0	0.00	0.04
Shasta	3	1	1	1	0	2	3	1	6	1	0.56	1.06
Sierra	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Siskiyou	1	0	0	0	0	0	0	0	0	0	0.00	0.23
Solano	1	4	1	0	1	1	2	2	6	0	0.00	0.40
Sonoma	0	0	0	0	0	0	0	0	1	0	0.00	0.02
Stanislaus	13	26	28	15	16	35	5	15	33	5	0.91	3.48
Sutter	2	12	3	1	1	1	0	1	7	1	1.00	2.90
Tehama	5	5	2	2	0	2	0	3	0	1	1.56	3.11
Trinity	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Tulare	13	10	12	8	24	7	8	15	25	4	0.84	2.63
Tuolumne	0	0	0	1	0	0	0	0	0	0	0.00	0.18
Ventura	6	7	1	2	2	0	0	0	1	0	0.00	0.23
Yolo	8	16	6	11	1	4	3	3	39	9	4.06	4.51
Yuba	10	11	1	2	0	0	0	1	5	4	4.78	4.06
Total WNV Cases	783	442	553	217	225	235	129	207	433	131	0.33	0.86
Asymptomatic Infections	77	41	47	26	18	28	19	14	40	19		
Total WNV infections	860	483	600	243	243	263	148	221	473	150		

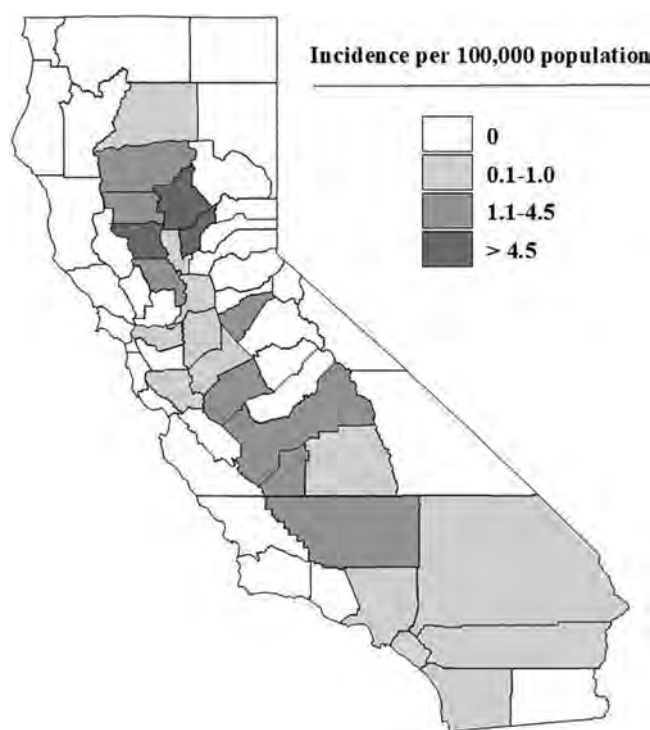


Figure 1.—Incidence of human cases of West Nile virus by county in California, 2024.

24 counties, which was the lowest number of cases reported in California since 2021, lower than during 2009–2011 (Figure 3), and lower than the 5-year average. Los Angeles County reported the most cases ($n = 23$), but the incidence was highest in Butte County (Table 3). Non-human WNV activity was reported from 32 counties and all environmental indicators were lower than the previous year. The number of WNV positive dead birds was lower than reported in 2023, but higher than the five-year average. In contrast, the number of WNV positive mosquito pools was lower than both what was reported in 2023 and the five-year average. The percentage of WNV positive sentinel chicken flocks was also lower compared to previous years (Figures 4, 5 and 6; California Department of Public Health). Notably, the number of WNV equine cases was the lowest observed since WNV emerged in CA in 2003. 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 for these two closely related flaviviruses. 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. However, in 2024, no human cases of SLEV were identified, marking the first time since its reemergence in 2015 that no cases were reported. A total of 34 SLEV positive mosquito pools were reported from 5 counties (Table 2), which is the lowest number of SLEV positive mosquito pools reported in

California since it reemerged. No SLEV chicken seroconversions were identified (Table 2). All environmental indicators of SLEV were low, supporting the absence of any identified human cases. The lowest number of reported SLEV positive counties, mosquito pools, and human cases since the reemergence of SLEV contrasts with the highest reported numbers of these same indicators in 2023 and highlights the dynamic nature of enzootic arboviral transmission.

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. Despite the identification of 18 locally acquired cases of dengue and the concurrent absence of dengue virus positive mosquito pools, 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 introduction of these exotic arboviruses into California.

Conclusions

Environmental activity of WNV and SLEV was lower in 2024 compared to recent years. WNV remains the greatest vector-borne disease threat in California, with over 8,000 cases and more than 300 fatalities reported since its initial detection in 2003. Human cases of WNV were the lowest since 2021, no human cases of SLEV were reported. Local transmission of dengue virus was observed for the second year, highlighting the importance for ongoing surveillance and awareness of potential human disease risk stemming from imported cases. Environmental detections of WNV often preceded the detection of human cases, supporting the value of environmental surveillance to direct mosquito control efforts and decrease the risk arboviral disease transmission in California.

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Table 4.—Results of mosquito and sentinel chicken testing for West Nile virus by county, California, 2024.

County	No. mosquitoes tested	No. mosquito pools tested	WNV + pools	No. flocks	No. chickens	No. WNV positive flocks	WNV + sera
Alameda	37,804	1,510	19	3	21	1	2
Butte	18,131	404	70	7	43	7	28
Calaveras				1	10	0	0
Colusa	450	9	0	1	10	1	2
Contra Costa	17,899	484	10	4	24	2	8
Fresno	56,498	1,698	208				
Glenn	400	8	0				
Imperial	4,774	243	3				
Inyo	338	10	0				
Kern	19,929	563	48				
Kings	9,887	262	87				
Lake	9,877	409	13	2	14	2	7
Los Angeles	160,176	3,944	206	19	105	13	42
Madera	31,298	726	110				
Marin	2,702	145	0				
Merced	34,281	997	44	4	24	3	12
Mono	53	2	0				
Napa	3,740	219	0				
Nevada				6	35	1	1
Orange	90,144	3,830	53				
Placer	24,434	1,883	93				
Riverside	226,073	7,001	217				
Sacramento	36,959	3,601	53	3	16	1	1
San Benito	222	134	0	1	8	0	0
San Bernardino	69,851	2,530	76				
San Diego	23,139	2,567	0				
San Francisco	5	1	0				
San Joaquin	55,307	1,740	188				
San Luis Obispo	753	18	0				
San Mateo	6,115	546	0	2	13	0	0
Santa Barbara	3,478	168	0				
Santa Clara	10,665	2712	34				
Santa Cruz	1,002	96	0				
Shasta	24,550	827	37	3	18	2	2
Solano	22,227	613	13	3	21	2	8
Sonoma	12,426	448	0				
Stanislaus	27,786	998	56				
Sutter	10,120	286	32	5	35	5	20
Tehama				3	30	1	6
Tulare	156,781	5,310	243	1	10	1	10
Ventura	373	8	0	3	29	0	0
Yolo	41,535	2,038	66	2	15	2	3
Yuba	5,519	179	32	2	14	1	7
Total	1,257,701	49,167	2,011	75	495	45	159

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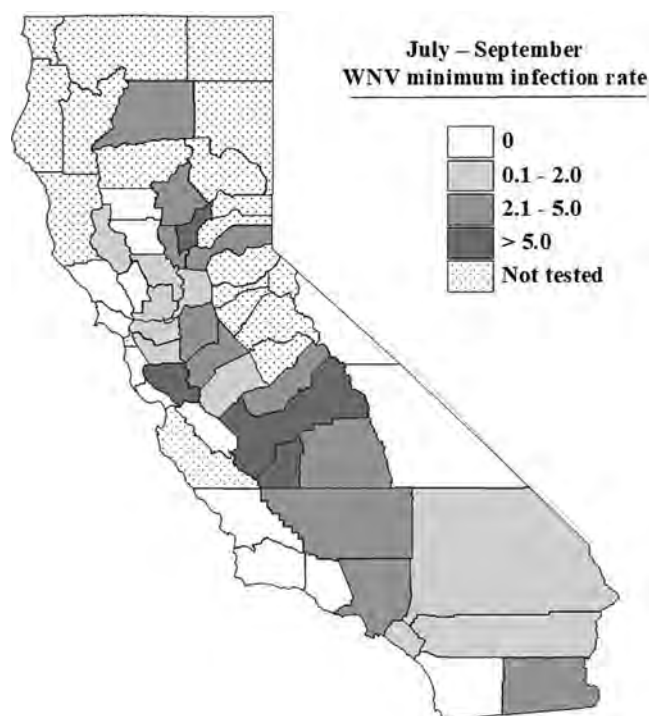


Figure 2.—West Nile virus minimum infection rate in *Culex* mosquitoes, by county, California, July–September, 2024. Minimum infection rate is defined as the minimum number of infected female mosquitoes per 1,000 tested.

Table 5.—Mosquito species tested for West Nile virus, California, 2024.

<i>Culex</i> species	No. pools	No. mosquitoes	WNV +	MIR
<i>Cx. erythrothorax</i>	2,345	87,180	7	0.1
<i>Cx. pipiens</i>	8,973	131,435	204	1.6
<i>Cx. quinquefasciatus</i>	19,535	558,690	818	1.5
<i>Cx. restuans</i>	7	7	0	0.0
<i>Cx. stigmatosoma</i>	1,007	12,623	37	2.9
<i>Cx. tarsalis</i>	16,337	457,512	943	2.1
<i>Cx. thriambus</i>	89	239	2	8.3
<i>Culex</i> species	2	2	0	0.0
All <i>Culex</i>	48,295	1,247,688	2,011	1.6
<i>Anopheles</i> species	No. pools	No. mosquitoes	WNV+	MIR
<i>An. franciscanus</i>	2	22	0	0.0
<i>An. hermsi</i>	2	30	0	0.0
All <i>Anopheles</i>	4	52	0	0.0
<i>Aedes</i> species	No. pools	No. mosquitoes	WNV+	MIR
<i>Ae. aegypti</i>	504	3,146	0	0.0
<i>Ae. melanimon</i>	31	956	0	0.0
<i>Ae. notoscriptus</i>	1	1	0	0.0
All <i>Aedes</i>	536	4,103	0	0.0
Other species	No. pools	No. mosquitoes	WNV+	MIR
<i>Arthropoda</i>	1	1	0	0.0
<i>Culiseta incidens</i>	302	5,171	0	0.0
<i>Culiseta inornata</i>	22	585	0	0.0
<i>Culiseta particeps</i>	6	51	0	0.0
Unknown	1	50	0	0.0
All other	332	5,858	0	0.0

Table 6.—Dead birds reported, tested, and positive for West Nile virus, California, 2024.

County	Reported	Tested	Positive	Percent
Alameda	515	187	53	28
Alpine	0			
Amador	7			
Butte	56	19	4	21
Calaveras	7			
Colusa	3	1	1	100
Contra Costa	439	67	6	9
Del Norte	2			
El Dorado	54	6		
Fresno	91	6	2	33
Glenn	4			
Humboldt	7	2		
Imperial	1			
Inyo	2			
Kern	22	1		
Kings	9			
Lake	28	14	3	21
Lassen	3			
Los Angeles	800	126	17	13
Madera	14	1	1	100
Marin	53	1		
Mariposa	4			
Mendocino	9			
Merced	40	6	1	17
Modoc	0			
Mono	2			
Monterey	30			
Napa	22	5		
Nevada	23	4		
Orange	276	62	1	2
Placer	208	91	4	4
Plumas	7			
Riverside	145	45	4	9
Sacramento	737	325	66	20
San Benito	4			
San Bernardino	133	37	10	27
San Diego	246	108	8	7
San Francisco	80	8		
San Joaquin	137	31	6	19
San Luis Obispo	29	5		
San Mateo	550	160	37	23
Santa Barbara	29	5		
Santa Clara	1,077	436	301	69
Santa Cruz	70	9		
Shasta	20	2		
Sierra	0			
Siskiyou	2			
Solano	113	38	2	5
Sonoma	117	10		
Stanislaus	102	10		
Sutter	42	6		
Tehama	8			
Trinity	0			
Tulare	38	13	1	8
Tuolumne	2			
Ventura	81	15		
Yolo	179	66	6	9
Yuba	23	3	2	67
Totals	6,702	1,931	536	28

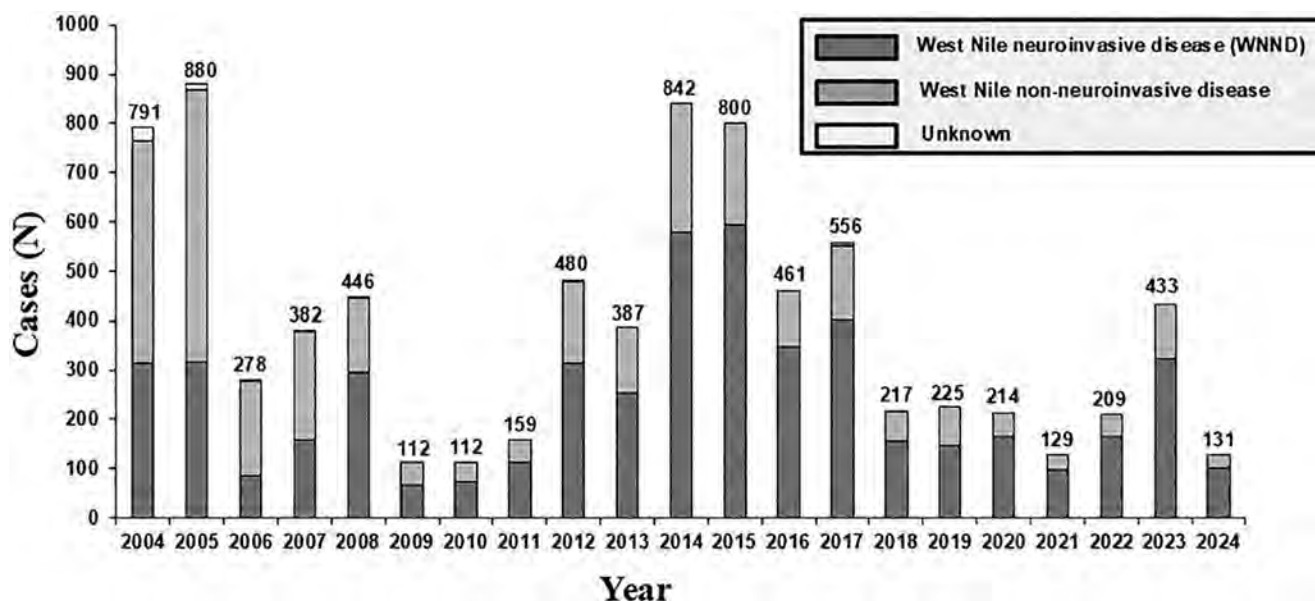


Figure 3.—Human cases of West Nile virus in California, by year, 2004–2024.

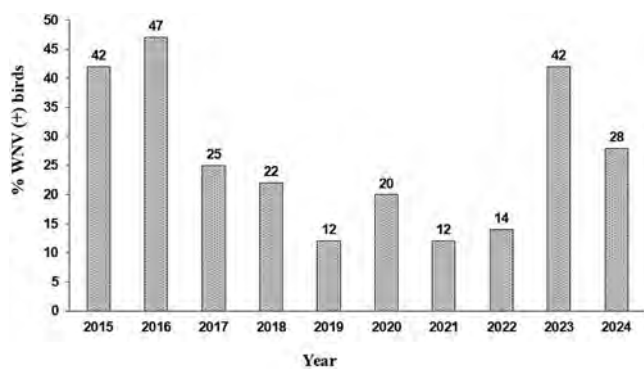


Figure 4.—Percentage of dead birds positive for West Nile virus in California, 2014–2024.

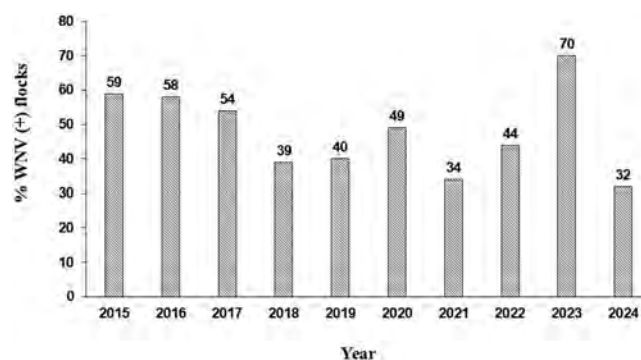


Figure 6.—Percentage of sentinel chicken flocks in California with one or more birds positive for antibodies to West Nile virus, 2014–2024.

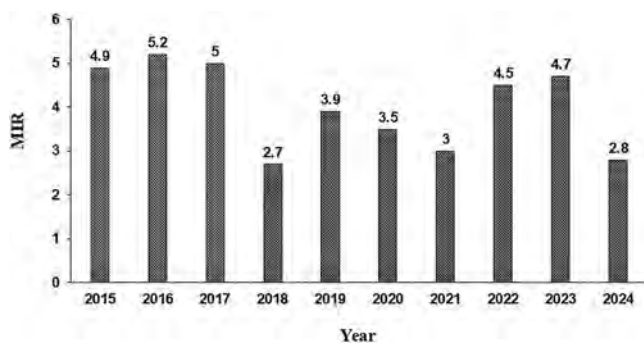


Figure 5.—Minimum infection rate (MIR) in *Culex* females per 1,000 tested for West Nile virus in California, July–September, 2014–2024.

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A decade of truck mounted larviciding

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Abstract

In September 2011, *Aedes albopictus* re-emerged in the city of South El Monte. Despite the district's valiant door-to-door efforts to clear each property, we faced significant challenges, including limited property access and labor-intensive house inspections. Recognizing the need for a long-term control strategy, we collaborated with Peter Dechant, formerly of Valent BioSciences, to utilize truck-mounted larviciding. We experienced mixed results with the Curtis Dyna-Fog LV8 and the Buffalo Turbine. Ultimately, we purchased the A1 Super Duty Sprayer, which also could be used for marsh treatments. This presentation discussed the spray machines trials and the application of the A1 orchard sprayer for larviciding mosquitoes in residential areas and marshes.

Mist blower rotary atomizer adjustments and droplet data collection

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Introduction

Mist blower sprayers were originally developed for agricultural applications, but have been adapted for mosquito control to include low-volume (LV) spraying. The current presentation outlines the adjustments, operational considerations, and droplet data collection strategies used to optimize the performance of the A1 Super Duty fan air driven mist sprayers outfitted with rotary atomizers (Fig. 1A & B).

System Modifications

To increase efficiency and control in urban mosquito control operations, the A1 SuperDuty sprayer was upgraded by the Sacramento-Yolo Mosquito & Vector Control District (District) with several key features in addition to the factory added rotary atomizer in place of the more commonly found Tee-Jet spray tips:

- A 200-gallon tank for extended spray duration.
- A high-flow electric pump (5.3–14 GPM) to ensure consistent flow replaced the standard belt driven roller pump.

- RPM sensors, cameras, and quick-disconnect fittings were added for flexible field trials using a small 5-gallon tank.

These upgrades improved coverage, facilitated real-time data collection, and maintained consistent product delivery under varied conditions in product efficacy trials by removing as many variables as possible.

Rotary Atomizer Design and Operation

The rotary atomizer, borrowing from aircraft spray technologies, can produce mono-dispersed droplets in the 50–400µm range by rotating at 2,000–10,000 RPM. Blade pitch rotation adjustments control the RPM and consequently droplet size. Unlike conventional sprayers, atomizer rotation is powered by airflow while the pesticide is sprayed internally and expelled through perforations that generate fine droplets as the rotating tube with holes cuts the droplets into fine particles. (Fig. 2A, B & C).

Operational Challenges and Considerations

The variability of individual sprayers and environmental factors (e.g., wind, humidity, obstacles) can markedly affect



A



B

Figure 1.—A) A1 SuperDuty A1 fan air driven mist sprayer – Manufacturer Photo. B) A1 SuperDuty Mist Blower modified and mounted on District Truck.



Figure 2.—A) Split detail close-up drawing of Micronair Rotary Atomizer - Manufacturer photo. B) Close up of Rotary Atomizer propeller base. C) Rotary Atomizers mounted on airplane wing – Micronair Manufacturer photo.

droplet dispersal and efficacy. We noticed early in our testing that there were three areas of variability affecting swath width as determined by sentinel mosquito mortality. These included:

- **Sprayer variability** – Each unit may produce a different droplet spectra due to engine RPM, fan speed, atomizer RPM and pump flow rates.
- **Drift and droplet management** – Balancing droplet size to limit over-drift and achieve target coverage, while not spotting vehicles or other residential items near the application site.
- **Product-specific requirements** – Different products require specific droplet sizes for maximum efficacy, such as preventing droplet drying in the drift layer. This requires adjusting the atomizer RPM due to product viscosity which may slow the RPM thus creating too large of a droplet; for example, with the engine on high, the blower air speed spins the atomizer at 10,000 RPMs, but with spray on it slows to 9,4500 RPMs.

Calibration and Field Testing

Calibration of each sprayer and individual atomizer involves ensuring accurate flow rates per acre and RPM settings based on product viscosity to obtain optimal droplet size for each product. Atomizer rotation speed may be measured using handheld tachometers; however, to read the RPM with pesticide passing through the system, the tachometer must be mounted on the inside of the tube to avoid pesticide spray from blocking the tachometer eye (Fig. 3).

Liquid larvicide products were first evaluated using a “+” shaped transect with dry cups, spray cards, and spinners positioned from 0 to 500 feet at 50-foot intervals along the + transect lines, conducted during early morning hours when spray operations would normally occur. These trials were timed to replicate application conditions and were validated with dyed droplet cards and sentinel mosquito mortality assessments. We drove the two outside edges (sides A, B, C and D) of the transect sides depending on wind direction. Each station was set 50-ft apart with dry cups and spray cards at



Figure 3.—RPM meter positioned in front of spinning Micronair Rotary Atomizer.

each station to help measure true swath width mortality as defined by sentinel mosquito mortality data (Fig. 4).

Applications in Residential Settings

After repeated field trials produced satisfactory swath widths, droplet spectrums and cup mortalities, we tested applications in residential areas. Before each residential application, staff placed dry cups in front and back yards to verify accurate droplet dispersal. Post-application the cups were collected, filled with water and larvae placed into them to evaluate larval mortality which then was related to wind data at application. The urban applications verified the field trial data (Fig 5 & 6).

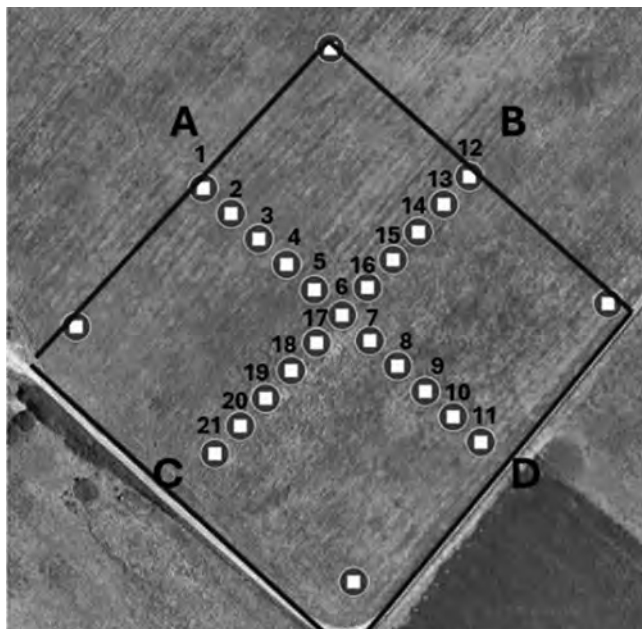


Figure 4.—Photo of field trial showing intersecting transect grid setup.



Figure 5.—Open dry cup placement under bush in urban setting.

Conclusion and Future Considerations

The adapted mist blower spray systems enhanced effectiveness in mosquito control operations with the added flexibility to change droplet sizing while maintaining the same desired flow rates. Future directions include exploring the use of adjuvants and identifying the lowest effective application rates and atomizer RPMs for large scale urban applications.



Figure 6.—Larvae placed in hydrated cups to bioassay post application mortality.

Which Way to WALs? A new method for setting up WALs transect trials

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Introduction

The spread and establishment of *Aedes aegypti* and *Ae. albopictus* throughout California has driven the need for innovative new strategies for larval mosquito control. One such technique is the use of Wide Area Larvicide Spraying (WALS) that utilizes a mist blower to deliver VectoBac WDG to cryptic larval sources throughout the urban landscape. The Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) routinely uses this approach to control *Ae. aegypti* in urban areas, but many questions remain such as: what are the best application protocols, how do other larvicides compare to VectoBac WDG, and what are the best methods for determining whether the application “worked.”

Transect trials are ideal for evaluating equipment setup, droplet spectrum, and effective swath width. These trials are typically conducted in an open field at the same time as planned WALs missions. Monitoring stations are set up with both bioassay cups and droplet deposition cards at increasing distances downwind from the spray path. Bioassay cups are returned to the lab where water and larvae are introduced to determine whether an effective larvicide dose reached the cup. Droplet cards are read for droplet size and density.

Previously, SYMVCD set transect arrays the morning of a trial after determining the wind direction. Because trials took place during pre-dawn hours, a laser pointer was used to set the transect orientation and then transect distances were measured using an open reel tape measure or a walking wheel starting at the drive path. However, early morning wind patterns can be variable, especially as dawn approaches. Shifting winds can lead to a situation where previously set transects are no longer aligned with the prevailing wind direction. Off angle wind drift can lead to longer drift paths than expected, or the applied product to completely bypass sampling cups and cards. Wind shifts of up to 30 degrees can be compensated for by extending the drive path by at least 200 ft before and beyond the transect, but off angle winds lead to longer than expected drift distances. When winds are more than 30 degrees out of alignment with the transect, reorientation is necessary prior to continuing the trial, requiring extra time, and possibly compromising the study overall. To avoid the necessity of transect shifting in response to changing wind conditions SYMVCD has begun using a new method for setting up

WALS trials. This transect set up uses a cross pattern that can accommodate all wind directions.

Material and Methods

Transect points were geocoded prior to field deployment. The day before the planned trial, the geocoordinates were used to set out small orange cones with reflective tape at each transect point, eliminating the need to measure transects the morning of the trial. Instead, immediately before the start of a trial, a tripod that supported a cup and droplet card was deployed to each transect location (Fig. 1).

Once all the tripods were set out, the wind direction was determined. Wind speed and orientation were measured with



Figure 1.—Transect marking cone and tripod used to deploy droplet cups and cards.

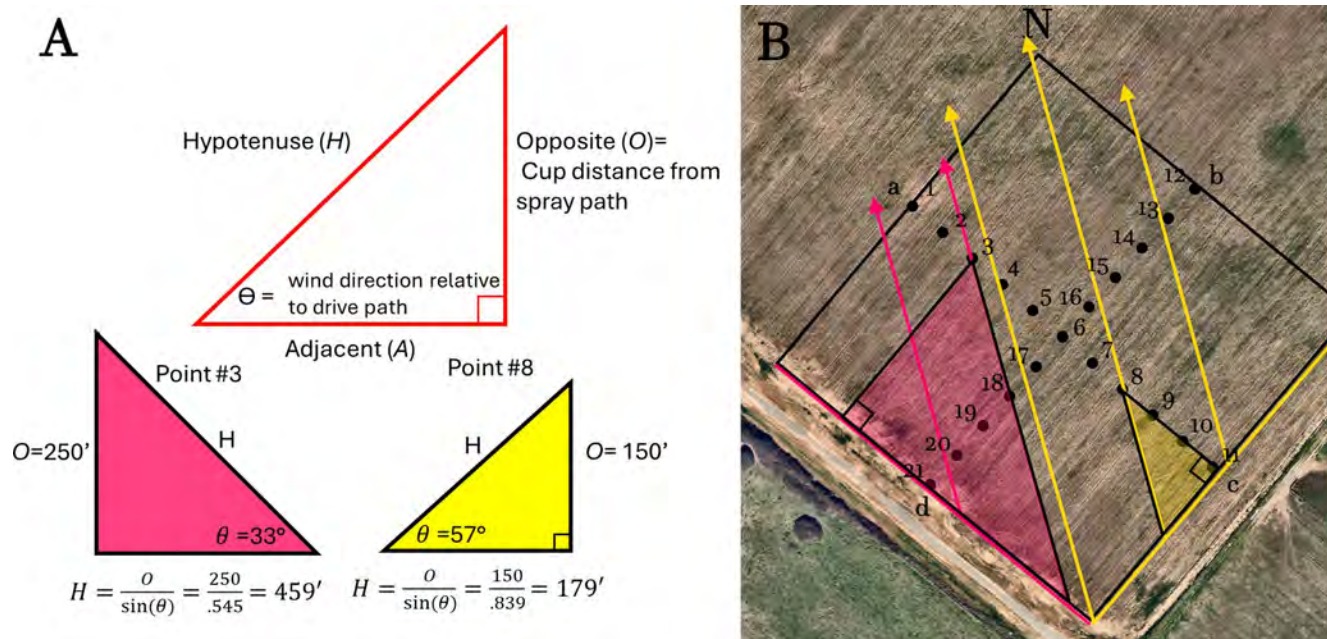


Figure 2.—A) Examples of right triangles, known distances (based on the study transect where points were spaced every 50 ft), and the formula used to solve drift distances are provided. B) Transect set up with overlaid triangles described in 2A, the black box is the drive path, the arrows represent the 168° wind direction and the arrows are colored coded to show which drive path represented in deposition to the transect locations. The west to south drive path (pink) hit cups 1, 2, 18, 18, 19, 20, and 21. The south to east drive path (yellow) hit all remaining cups.

a Kestrel 5500 weather meter (Kestrel Instruments; Boothwyn, PA) set at 5 and 30 ft above the ground. Additional wind direction readings were taken using wind detector dust (Dead Down Wind; Covington, GA) and a compass. Puffs of dust were released, and a compass used to determine wind direction based on the direction the dust was carried. This method was especially useful when winds were below the activation threshold for the Kestrel anemometer and wind vane (< 1 mph).

The advantage of having a transect perfectly perpendicular to the wind direction is that drift distances are equivalent to the transect spacing. When this is not the case, trigonometric ratios are useful for determining the distance the larvicide traveled from the release point to the cup and droplet card. The drift distance is essentially the hypotenuse of a right triangle. The opposite side of the triangle is the distance of the transect point from the drive path, and the angle is calculated based on a 360 degree azimuth scale where true N is $0/360$ degrees (Fig. 2A). The transect used in this study had crossed perpendicular lines that ran from $0 - 180^\circ$ and $90 - 270^\circ$. The transect spacing started at the drive path and transect locations were placed every 50 ft. The entire transect area was 500 ft x 500 ft, to accommodate the targeted 300 ft swath width of a typical WALS application (Fig. 2B). The potential drive paths for the transect were along the main cardinal directions. To solve the drift distance the transect orientation and the direction of the wind were required.

Wind directions are described based on the angle of origin. If winds were moving across the study transect from 168° , that direction could be compared to the angle of the

drive path (for our transect 225°), to solve one acute angle of the right triangle (for this example 57°) (Fig. 3). This angle and the distance of the transect point from the road are all that are required to solve for the drift distance (hypotenuse) using the formula:

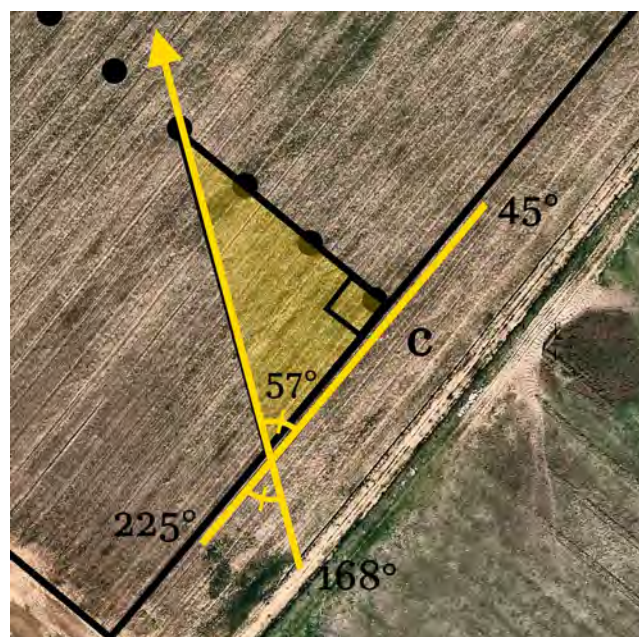


Figure 3.—Example calculation for solving the drift angle relative to the drive path. Orientation of the drive path must be known; in this example the drive path ran SW (225°) to NE (45°).

$$H = \frac{O}{\sin(\theta)}$$

H = drift distance

O = distance of the transect point from the drive path

θ = angle between drive path and drift distance
(hypotenuse)

When solving the drift distances, it is critical to know which drive path resulted in deposition at transect points. On the described transect, typically two sides of the transect were driven. For instance, when there was a southerly wind, the east to south and south to west drive paths were used to make sure all transect points were ‘hit’ with product. However, some of the cups were hit by the east to south route and the rest were hit by the south to west. Understanding which drive path resulted in deposition is needed because it changes the angle between the road and the drift distance. Once one drive path triangle is solved, the other drive path is the same triangle (assuming the wind direction did not change during the application). However, the triangle is a mirror image so that the acute angles are switched, but the formula is otherwise the same.

To determine which drive path hit each transect point, a to-scale digital map was created. Using a digital compass, a line representing the wind direction was drawn on the map. By dragging the line across the map drift patterns were visualized to determine which drive path resulted in deposition at each location, drift distances were then solved accordingly.

Results and Discussion

This new method of setting up transects for WALs trials has multiple advantages including faster set up time, less disruption from changing winds, and many transect points that can be used for evaluating application parameters. As described, this transect had 21 data collection points of increasing distance from the spray path. Depending on the

wind direction, each of these points may have a unique distance from the spray path or there were up to 11 repeat distances. This occurred when the wind was perfectly aligned with one of the transects, and therefore all the transect points in the crossed transect were equidistant from the spray path.

Although solving the drift distance with this new method is not as straightforward as a single transect, perfect alignment can be difficult to achieve and off angle wind drifts across the single transect can lead to spurious and incorrect interpretation of the results. Accurate wind measurements are paramount, and wind detector dust and a compass are simple, inexpensive tools that have great utility for wind direction monitoring. When using this cross pattern design, prevailing wind directions, drive paths, and cardinal alignment are key considerations when designing a transect and must be known to solve drive distances. WALs applications require wind to drive larvicide across urban landscapes and into cryptic sources. A stable repeatable method for evaluating WALs applications allows for optimization of spray protocols and the opportunity to adapt new products to the WALs method. That has special importance for the control of invasive *Ae. aegypti* and *Ae. albopictus*, two species that are extremely difficult to control in the urban landscape. We plan to improve application methods to control these mosquitoes across our urban landscapes.

Acknowledgements

We would like to thank our industry partners from Central Life Sciences (Samer Elkashef, Loren Cunningham, and Dan Killingsworth) and Clarke (Nancy Voorhees, Andrew Rivera, and Ryan Arkoudas) for getting up early with us to figure out how to implement this new strategy. Also, we thank Mark Palmer for helping us remember how to do trigonometry.

Trending techniques for analyzing aerial and ground-based mosquito control applications

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Abstract

Mosquito application technologies and techniques are constantly advancing in response to the need for efficacious control efforts. At the same time, vector control professionals are very much aware of the need to reduce reliance on pesticides that continue to increase resistance in vector populations. With innovations such as drones and enhanced area-wide liquid larvicide sprayers becoming part of routine application equipment, the need to define and determine Standard Operating Practices (SOPS) for these applications becomes greater. Based on field experiences and applied research recommendations, this presentation offered suggestions for enhancements to common techniques that should be considered when developing an SOP for an efficacious mosquito control program.

Optimizing Unmanned Aircraft System operations at Placer Mosquito and Vector Control District with AirData

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Abstract

The Placer Mosquito and Vector Control District (PMVCD) has successfully implemented the AirData™ data management system to enhance the operation and oversight of its unmanned aircraft systems (UAS) program. AirData is a robust, comprehensive platform specifically designed to streamline UAS operations, allowing PMVCD to effectively manage a variety of operational processes, including flight tracking, pilot management, mission planning, regulatory compliance, and maintenance. By integrating all aspects of UAS management into one centralized system, PMVCD significantly improved both operational efficiency and regulatory oversight.

AirData's core functionality includes the ability to upload, store, and review detailed flight logs and telemetry data. These logs provide vital flight information such as altitude, speed, battery life, and geographic location of flight paths, allowing PMVCD to monitor the performance of its UAS fleet in real-time. These data can be accessed through the platform's intuitive interface, ensuring that both vector control technicians and administrative staff can retrieve comprehensive information regarding flight operations.

In addition to flight tracking, AirData plays a critical role in managing UAS mission planning. Remote pilots can submit detailed mission plans through the platform, including specifying flight locations, points of interest, and mission timings. Administrative staff can then access these mission plans, providing them with a clear overview of both past and upcoming operations. This streamlined planning process allows for better coordination, ensures that resources are allocated efficiently, and helps staff know the whereabouts of ongoing operations.

AirData's mobile application further enhances the functionality of the system by allowing remote pilots to complete essential pre-flight tasks, such as risk assessments and pre-flight aircraft checklists, directly from their smartphones. The app also facilitates the automated generation of flight notifications, which are sent to relevant administrative staff when a risk assessment is completed. This feature ensures that all stakeholders are promptly informed and notified of any UAS operations taking place that day.

Another key advantage of AirData is its ability to support the maintenance and regulatory compliance needs of PMVCD. The system enables tracking and management of UAS maintenance schedules and battery usage, helping to ensure the longevity and performance of the aircraft fleet. Additionally, AirData generates essential regulatory reports, such as Public Certificates of Authorization (COA) and FAA ADX Reports, which are crucial for maintaining compliance with federal regulations. These reports are automatically generated monthly, reducing the administrative workload and ensuring that PMVCD stays current with its regulatory obligations.

By integrating flight data, mission planning, pilot management, maintenance tracking, and regulatory reporting, AirData provides a unified platform that supports the effective management of PMVCD's UAS program. This integration not only streamlines operational processes but also enhances the district's ability to make informed decisions, ensure compliance, and maintain a high level of operational efficiency. Ultimately, AirData contributes to the success of PMVCD's vector control efforts by providing the district with the tools necessary to integrate this technology into our daily operations while maintaining safe and efficient UAS operations.

AMCA Drone Program Update 2025

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Abstract

The American Mosquito Control Association (AMCA) Drone Program aims to decrease the barriers for drone use and encourage the mosquito control community to embrace this technology to better protect public health (For more information visit <https://www.mosquito.org/uas/>). The AMCA has been working on developing a program to support AMCA members who are planning to use or are using unmanned aircraft system (UAS or drones) in their mosquito control programs. This project was initiated at the 2024 AMCA meeting in Dallas, however, we are still waiting for the Federal Aviation Administration (FAA) to approve regulatory exemptions. This presentation provides updates on the development of the AMCA drone program, progress on regulatory exemptions, and discusses the state of the drone industry as it relates to mosquito control agencies.

Spectroscopic age grading of *Aedes aegypti*: Techniques and Confounding factors

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Introduction

Age grading of mosquitoes is important because older mosquitoes may have taken multiple blood meals and persisted for the duration of a pathogen's extrinsic incubation period, which is a prerequisite for transmission (Ernst et al, 2017). Spectroscopic methods for age grading mosquitoes may offer significantly faster, cheaper, and more accurate results in *Aedes aegypti* when compared to traditional age-grading methods, which makes them a promising technology for use as surveillance tools and in studying the effects of mosquito control on the age structure of wild mosquito populations. In this presentation, we discuss the current progress of our project studying the effects of potential confounding factors such as wild/insectary rearing status, geographic origin, and number of blood meals taken to determine the versatility of both near-infrared spectroscopy (NIRS) (Joy et al. 2022) and Raman spectroscopy (Gao et al. 2023) in the age grading of *Aedes aegypti*.

Methods

Female *Ae. aegypti* collected across the Los Angeles Basin area and genetically similar control mosquitoes reared in the Davis Arbovirus Research and Training (DART) insectary were frozen at -70C and shipped to partner laboratories for NIRS and Raman spectroscopy. Mosquitoes that underwent NIRS had their legs and wings removed, and 50 near-infrared spectra (800-2500 nm) were recorded from each of their thoraxes, whereas mosquitoes that underwent Raman spectroscopy were liquified, treated with silver nanoparticles, and 20 spectral samples were taken from the resulting solution for each mosquito. In both cases, 80% of mosquito spectra were used to train a machine learning model to predict the age of *Ae. aegypti* specimens, while the remaining 20% were used to validate the models.

Results and Discussion

Mosquitoes varying in geographic origin and blood meal status have been processed, but the majority of collected

wild mosquitoes have not yet finished processing. Raman spectroscopy maintained good predictive value both within and across the two Los Angeles regions of origin for DART colony-reared mosquitoes (Lakewood and Tujunga), although blood feeding reduced accuracy. For NIRS spectroscopy, region of origin also was not a significant confounder. However, mosquitoes taking their first blood meal showed a significant increase in predicted age across both colonies, which will be investigated further as both a potential confounder for NIRS and a potential means to determine the number of blood meals a mosquito has taken.

Conclusions

Geographic origin of laboratory-reared unfed *Ae. aegypti* appears to have no significant effect for both NIRS and Raman spectroscopic age grading protocols. Blood feeding may moderately affect Raman spectroscopic age grading, and significantly skewed our current NIRS age grading models upwards (older). Moving forwards, in addition to completing additional analysis of wild-caught *Ae. aegypti*, we will train NIRS age grading models on both unfed and blood fed mosquitoes to attempt to remove the confounding effects of blood feeding.

Acknowledgements

Thanks to GLACVCD for collecting the mosquito samples for this experiment, and to our partners at University of Massachusetts Amherst and University of Arizona in Lili He and Michael Riehle's laboratories.

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Integrating eDNA and qPCR for early detection and surveillance of mosquito larval habitats

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Introduction

Traditional morphological keys for distinguishing *Aedes aegypti* from *Ae. sierrensis* require expert entomologists and often fail on damaged or atypical specimens, whereas advanced eggshell imaging is not practical for routine use (Faull et al. 2016; Matsuo et al. 1974; de Moraes et al. 2019). To address these challenges, we designed a multiplex qPCR assay that discriminates between these two species via mitochondrial COI SNPs. Beyond direct specimen testing, we extended the assay to eDNA captured from larval habitat water, such as phytotelma and artificial containers, to detect mosquito presence even after larvae have emerged or have been vacated. This combined approach reduces field effort, enables earlier interventions, and uncovers unoccupied larval habitats that might otherwise have been overlooked.

Methods

Frozen water samples (100-150 mL) from known larval habitats were thawed and pre-filtered through 10 µm nylon mesh to remove debris. Triplicate 25 mL aliquots were vacuum-filtered onto 0.22 µm PES membranes; all apparatus components were decontaminated between samples with 10% bleach, Alconox detergent, deionized water rinses, and ethanol drying. Filters were aseptically cut into ~3 mm pieces and beaten with acid-washed glass beads in autoclaved tubes. eDNA was extracted using a modified Qiagen DNeasy Blood & Tissue protocol, eluted in 50 µL buffer, and 5 µL was used per 20 µL multiplex qPCR reaction targeting *Ae. aegypti* and *Ae. sierrensis* COI probes.

Results

Triplicate qPCR of 25 mL eDNA from a flowerpot container (> 2 liters) with 14 *Ae. aegypti* larvae yielded a mean Ct of 25.153 ± 0.165 , with no false positives. Treehole samples with water that contained *Ae. sierrensis* produced a mean Ct of 29.788 ± 0.014 , also without false positives. The ~4.6-cycle difference indicates a higher eDNA concentration in the artificial container. These data

confirm the assay's high sensitivity, specificity, and quantitative discrimination of *Ae. aegypti* across distinct larval habitats.

Conclusion

The multiplex qPCR assay can differentiate *Ae. aegypti* from *Ae. sierrensis* in both specimen and eDNA samples, with zero false positives in flowerpot and treehole tests. Its rapid, non-invasive detection capability for eDNA samples can surpass traditional morphological methods and supports early, informed vector control. Future work should assess performance in mixed-species eDNA samples and expand primer-probe sets to other container and treehole *Aedes* species as well as other container-breeding genera, further enhancing comprehensive mosquito surveillance.

Acknowledgements

We thank the Sacramento-Yolo Mosquito and Vector Control District, Placer County Mosquito and Vector Control District, Orange County Mosquito and Vector Control District, Coachella Valley Mosquito and Vector Control District, San Joaquin County Mosquito and Vector Control District, East Side Mosquito Abatement District, Butte County Mosquito and Vector Control District, and Santa Cruz County Mosquito and Vector Control District for providing water samples containing *Ae. aegypti* and *Ae. sierrensis* larvae. Preparation of this Extended Abstract was AI assisted.

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Battle of the BG's: A comparative analysis of Biogents sentinel and BG Pro traps for *Aedes aegypti* surveillance in Sacramento, California

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Abstract

The Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) is dedicated to enhancing its surveillance methodologies for invasive mosquito species. This includes *Aedes aegypti* populations that are expanding into residential areas of the Sacramento and Yolo Counties. Previous studies conducted by the SYMVCD indicated that the BG Sentinel traps were the 'gold standard' for invasive mosquito surveillance. The current study compared the effectiveness of the BG Sentinel and the smaller, more inconspicuous BG Pro traps. This trial was conducted for eight weeks in a residential neighborhood in Sacramento CA. Study sites were selected based on historical data of *Ae. aegypti* abundance and mosquito control application history. Each trap type was deployed for one night at each site for eight weeks. Four sites were randomly assigned to start with BG Sentinel and the other four with BG Pro traps. After each collection, the traps were switched, repeating this cycle throughout the study period. *Aedes aegypti* catch numbers were recorded for each trap; these data were analyzed alongside environmental factors, including temperature, humidity, and wind speed. The BG Sentinel trap captured significantly more female *Ae. aegypti* than the BG Pro; however, there was no statistically significant difference in male *Ae. aegypti* capture between the trap types.

Introduction

Controlling *Aedes aegypti* presents many challenges to vector control districts and public health agencies across the globe, because the species is highly adapted to urban environments and a competent vector for medically important arboviruses, including dengue (DENV), Zika (ZIKV), yellow fever (YFV), and chikungunya (CHIKV) (Matthews 2019). As a result of unique physiological and behavioral adaptations such as desiccation-resistant eggs and human host preference, *Ae. aegypti* geographic range has rapidly expanded into the western region of the United States (Facchinelli et al. 2023; Pless et al. 2022). Therefore, when *Ae. aegypti* was first detected in Southern California in 2013, public health agencies and vector control districts utilized all facets of established Integrated Vector Management (IVM) programs, and emphasized community outreach campaigns to encourage residents to report day-biting mosquito activity and reduce standing water sources around their homes (Pless et al. 2017; Gloria-Soria et al. 2014). However, despite all efforts to contain the mosquito, *Ae. aegypti* rapidly acclimatized to the Mediterranean climate of the Golden State and has since been detected in over 40% of California counties (CDPH 2025).

In addition to the rapid expansion across California counties, this invasive mosquito species has increased its abundance in certain areas, leading to a stable expansion of their invasive range. Increased abundance has led to an increase in human vector contact. In 2023 the first two cases

of locally acquired DENV were reported in Los Angeles County (Birhanie et al. 2025; CDPH 2025; Feaster et al. 2024). Subsequently in 2024, there were 15 additional locally acquired DENV cases (Birhanie et al. 2025).

Although the majority of these cases were confined to the Los Angeles area, locally acquired DENV cases were also detected in San Bernardino and San Diego counties, leaving many California residents and public health agencies concerned about possible endemic disease transmission (CDPH 2025).

Although locally acquired DENV cases have not been reported from Sacramento or Yolo counties, the SYMVCD has observed an increase in *Ae. aegypti* range and abundance. One example is the residential community of South Natomas, Sacramento County. *Aedes aegypti* was first detected in this neighborhood in 2022 and has continued to expand its range and catch rates over the past two years. During 2023 the area required 637 chemical control treatments in response to high trap counts, larval detections, and service requests, emphasizing the importance of surveillance in providing data to guide control efforts.

Presently, SYMVCD utilizes Biogents BG Sentinel (BG-S; Biogents USA; Cary, NC, USA) traps for *Ae. aegypti* surveillance across both counties. This host-seeking trap exploits mosquito feeding biology. Female *Ae. aegypti* have olfactory receptors designed to detect carbon dioxide (CO₂) and odors produced by their hosts. BG-S traps work with a combination of visual and sensory cues such as releasing CO₂ and octanol (oct-1-en-3-ol) to simulate a

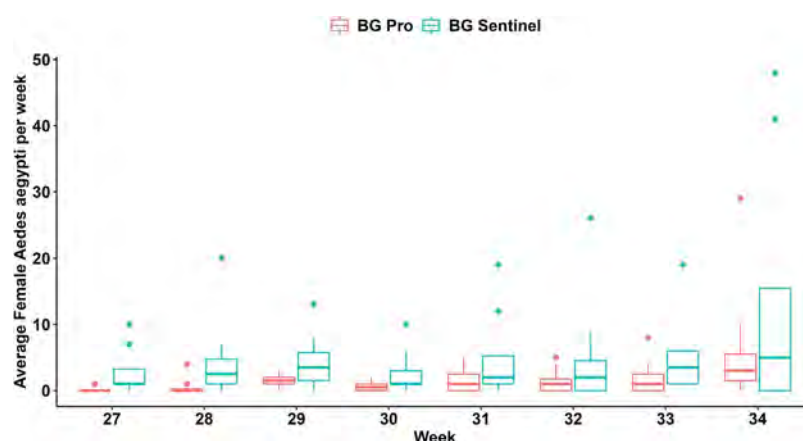


Figure 1.—Average female *Ae. aegypti* capture per trap per night between trap type. Points represent outliers and correspond to trap type.

human host (Le Goff et al. 2016). The BG-S has become the industry standard for *Aedes* collection (Degener et al. 2021; Staunton et al. 2019; Williams et al. 2006). However, despite the efficacy of the BG-S trap, the SYMVCD is interested in incorporating smaller and more portable traps for urban trapping efforts. In 2023, SYMVCD conducted a comparative analysis between an encephalitis vector surveillance (EVS) trap supplemented with a BG-Lure and a BG-S trap in South Natomas. This study found the BG-S trap to be the most effective at collecting both male and female *Ae. aegypti* (Clack et al. 2024). In continuation during 2024 the BG-S trap was compared to the Biogents Pro trap (BG-P; Biogents USA, Cary, NC, USA).

Methods

The BG-S trap was compared to the smaller and more portable BG-P over an eight week study period from 1 Jul to 21 Aug 2024 in South Natomas. Eight homes were selected for the trial based on historical adult or larval *Ae. aegypti* detections. Before the start of the study mosquito control interventions were performed in response to *Ae. aegypti* detections in the area. Study sites were balanced with respect to 2024 treatments prior to the start of the study to include four sites previously treated with larvicides and four sites previously treated with adulticides. Larvicide applications included: Sumilarv (MGK, Minneapolis, MN, USA), Natular DT Clarke, Roselle, IL, USA)info), and/or Natular XRT (Clarke; Roselle, IL, USA). Adult mosquito control products included the residual barrier sprays OneGuard (MGK; Minneapolis, MN, USA) or Lambda CS (Central Life Sciences; Schaumburg, IL, USA), and ultra-low volume (ULV) applications with Deltagard (Bayer; Cary, NC, USA).

To ensure the implementation of a balanced study design and limit the introduction of potential location and treatment biases, a randomization generator in excel was used to construct two groups, labeled A and B, each with two sites that were previously treated with either a larvicide or adulticide. Throughout the study, WALS applications and localized treatments were made as necessary. To minimize

potential bias due to WALS applications, all trap sites were included on local WALS routes so that they received the same larvicide treatments. There were two trap nights per week at each site. On the first trap night Group A sites were set with a BG-P and Group B set with a BG-S, on the second night the trap type was rotated so that equal numbers of trap types were set on each study night. Both trap types were baited with dry ice pellets and BG-Lure. BG-Lure is a blend of volatile compounds including lactic-acid, ammonia, and hexanoic acid, which mimic human odor (Khan et al. 2024). Although the lure and ice can both be contained within the BG-S, due to the small, portable nature of the BG-P, the BG-Lure was placed inside the trap unit, next to the fan, and the manufacturer ice bag was suspended just above the trap entry point. The containers for both trap types hold approximately 1.5 kg of dry ice. Traps were set in the same location regardless of trap type. The BG-S traps were powered with a 12V sealed lead acid (SLA) battery, and BG-P traps were powered with a 6V SLA battery.

Statistical analyses were done in R v4.2.1 (R Core Team 2022). Kruskal-Wallis rank sum tests were used to examine variances in male and female *Ae. aegypti* capture rates across trap types and trapping block/location.

Results

There was a significant difference in female *Ae. aegypti* capture rates across trap types, $H(1) = 14.38, p < 0.001$, with the BG-S catching an average of 5.4 ± 2.9 females compared to the BG-P catching an average of 1.7 ± 1.9 females per trap night (Fig. 1). However, there was no difference in male *Ae. aegypti* catch rates between traps $H(1) = 2.9, p = 0.08$, with the BG-S catching an average of 2.6 ± 2.4 males compared to the BG-P with an average of 1.5 ± 2.0 males (Fig. 2).

Discussion

Overall, the BG-S collected significantly more female *Ae. aegypti* per trap per night compared to BG-P (Fig. 1). Trap set up may have played a role in the capture disparity.

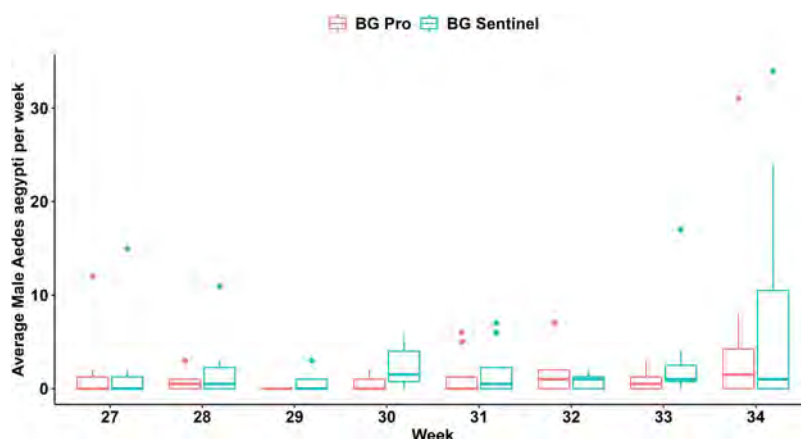


Figure 2.—Average male *Ae. aegypti* capture per trap per night between trap type. Points represent outliers and correspond to trap type.

According to the Biogents BG-P manual, the trap can be deployed in three different ways: 1) EVS-Style (suspended), 2) CDC-Style (suspended), and 3) Sentinel-Style (grounded) (Biogents 2024). For our study, we opted for the sentinel-style set up but used dry ice pellets as the CO₂ source rather than a CO₂ cylinder. Because the BG-P was smaller and more portable, the manufacturer ice bag was suspended just above the mouth of the trap and the BG lure was placed within the body of the trap near the fan unit to promote circulation of BG-lure attractant. This was in contrast to the BG-S, a much larger trap unit, which contained both the dry ice container and the lure within the unit, possibly leading to a better dispersal of the olfactory attractants (Biogents 2017).

Another factor that may have impacted BG-P trap performance was the dry ice deployment method. As stated above, the BG-P traps were deployed with the manufacturer ice bag whereas the BG-S traps were set with a modified insulated beverage container with a hole drilled in it for CO₂ release. Although both dry ice containers were insulated, the dry ice bag appeared sublime faster. During the first few weeks of the study, following multiple observations of the ice bag being completely empty at trap pick up, a low temperature thermometer was used to monitor how long it took the dry ice to sublime in the ice bags. Dry ice typically was completely sublimed in the dry ice bags by 0200-0300h. Once the dry ice was completely sublimed one of the traps most powerful attractants was no longer present. Previous studies have shown the combination of dry ice and BG-Lures yields a significant increase in overall mosquito capture rates in comparison to baiting with a single host-seeking attractant (Gillies 1980; Ventura et al. 2024). The early loss of one host-seeking attractant could have reduced *Ae. aegypti* capture rates during the first diel activity peak which typically occurs between 0700-1100h (Le Goff et al. 2017; Ventura et al. 2024).

Conclusion

Overall, the goal of the study was to conduct a comparative analysis between two Biogents trap models: the BG-S and BG-P. This study once again demonstrated why

the BG-S is the industry gold standard for *Ae. aegypti* surveillance. The BG-S consistently collected more female *Ae. aegypti* than the BG-P. However, the two trap types performed similarly with male *Ae. aegypti* collection. Additionally, although the goal of the study was primarily focused on *Ae. aegypti* capture, both trap types captured *Culex pipiens* and *Culex tarsalis*, further emphasizing the functionality of these traps in not only collecting invasive mosquitoes but also WNV vectors in urban and suburban communities.

Furthermore, as we continue to adapt to the increased geographic range and localized expansion of *Ae. aegypti* in Sacramento and Yolo counties, it is imperative to evaluate surveillance practices, ensuring the implementation of surveillance tools and strategies that will enhance our understanding of population dynamics, assist in our ability to assess potential arboviral transmission risk by invasive and endemic vectors, and guide our urban IVM response efforts to minimize any potential public health risks. Additionally, in areas where increasing *Ae. aegypti* abundance is observed, SYMVCD should continue to evaluate the efficacy of current and future smaller and portable surveillance tools, that will enable more comprehensive trap efficacy evaluations. Future studies should focus on evaluating catch rate differences between front yard and backyard trapping. Providing insight into where residents are most likely to come into contact with this invasive species and where IVM response efforts should be primarily targeted. Lastly, similar studies should be replicated in other known areas of infestation, which are subjected to different abiotic conditions that can influence mosquito abundance, behavior, and ecology.

Acknowledgments

We want to acknowledge and thank the SYMVCD Urban Control Operations crew, who assisted with securing the eight surveillance sites for the study: C. Reyes, D. Zepf, and A. Barrera. We would also like to extend our sincerest thanks to the residents of South Natomas for their participation in this study.

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Contra Costa Mosquito & Vector Control District versus *Aedes aegypti*, a tale of two cities

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Introduction

The invasive mosquito species, *Aedes aegypti*, was first detected in California in 2013 and has rapidly spread and become established in many parts of the State, including southern California and the Central Valley as far north as Shasta County (Vector-Borne Disease Section, California Department of Public Health, 2023). There have been sporadic finds in the Coastal Region in San Mateo and Alameda counties, but these populations were apparently transitory. Since 2022, the Contra Costa Mosquito and Vector Control District (District) has detected and responded to finds of *Ae. aegypti* in three cities in Contra Costa County with different outcomes in each case.

Methods and Results

The first detection of *Ae. aegypti* in Contra Costa County occurred in 2022, when a single adult female was collected during a backyard inspection in response to a service request in the city of Martinez (Schutz et al., 2023). Following extensive door-to-door inspections and trapping with BG Sentinel and BG-GAT traps, it was determined that the infestation was limited to a $\frac{1}{4}$ mile radius of the initial detection (Fig. 1). Subsequent trapping and follow-up inspections in the area and in other locations in the County in 2023 and 2024 did not yield any additional specimens (CCMVCD, 2025).

On 27 Aug 2024 a single adult female *Ae. aegypti* was collected in a carbon-dioxide baited EVS trap in North Concord (on District property) in the course of routine surveillance for West Nile virus. Follow-up trapping with BG Sentinel traps produced 3 additional specimens, all within 500 feet of the original find (Fig. 2). Door-to-door inspections of neighboring businesses and a cemetery failed to locate any larval sources. Surface treatments with Suspend SC® were performed at several locations, including the cemetery, and no additional adults were collected after 5 Sep. We hypothesized that the adults we collected represented individuals that were accidentally transported from another location from either within or outside our County.

On 26 Sep 2024, an adult female *Ae. aegypti* was collected during a residential backyard inspection in the City of Antioch. This triggered extensive trapping and

door-to-door inspections in the neighborhood, including weekend inspections to increase the likelihood that residents would be home to permit entry to their properties. Because it was already late in the season and we wanted to quickly determine and hopefully contain the extent of the infestation, we did not send letters to homeowners in advance of our inspection attempts, which may have led to a fairly high proportion of refused inspections or failures to respond to our personnel and to door hangers that were left after inspection attempts. A community event was held at a neighborhood park, which did yield some additional inspections, and BG Sentinel trapping was conducted in an expanding radius around the initial find. Two wide-area larvicide (WALS) treatments (Williams, Ramirez and Lesser, 2022) with VectoBac® WDG and several Suspend® treatments were conducted. A variety of larval sources were found during our inspections, including a hanging plant that yielded > 100 larvae, yard drains, and assorted rain barrels, buckets, plant saucers and other containers. In all, 464 adults were collected at 64 locations (Fig. 3), and 272 larvae were collected at 28 locations (Fig. 4). We collected adults within at least a 3 mile radius of the initial find, indicating a much larger infested area than we dealt with in Martinez in 2022. Based on a density map of adult mosquito collections that showed the greatest density in the southeastern corner of the infested area, gradually decreasing towards the north and west (Fig. 5), our hypothesis was that the initial point of introduction was in the southeast corner. We were unable to complete door-to-door inspections of the entire area, due to cooling weather and difficulty obtaining entry to some properties. We were not confident that we were successful in containing this infestation, and inspections and trapping will resume in spring 2025.

Discussion

Based on the limited extent of the 2022 *Ae. aegypti* infestation in Martinez, we believe it was detected before the population had time to spread. Therefore, we were able to contain and effectively eradicate it through door-to-door inspections, source reduction and treatments, as needed, of every property in the infested area. In Antioch, the population had already spread extensively before we became aware of it,

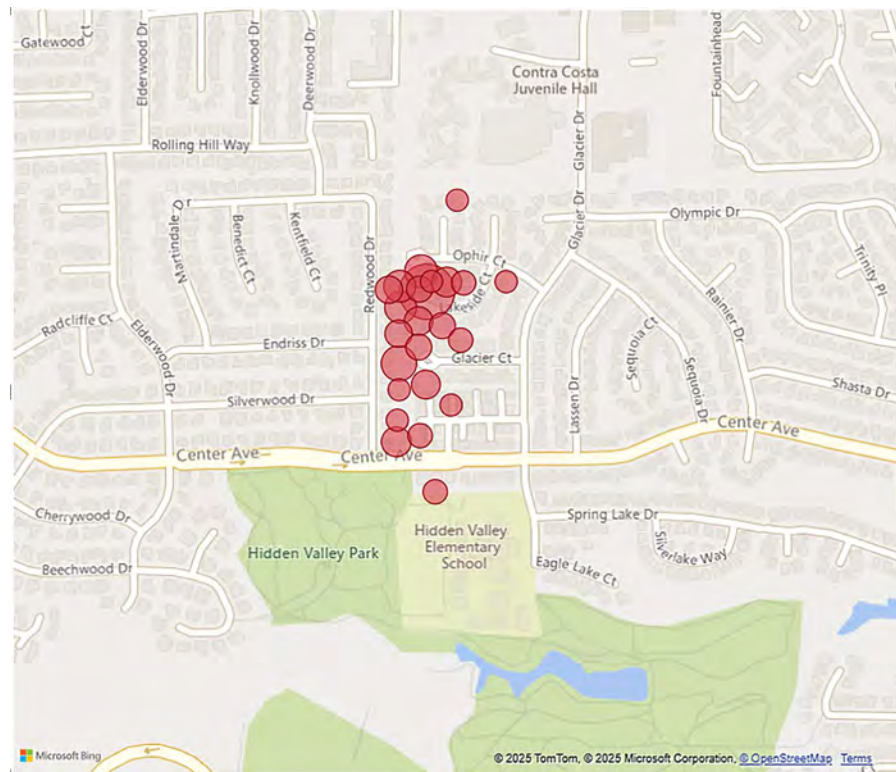


Figure 1.—Locations where adult *Ae. aegypti* were collected in BG Sentinel traps, Martinez, CA, 2022 (note: all figures were drawn to the same scale).

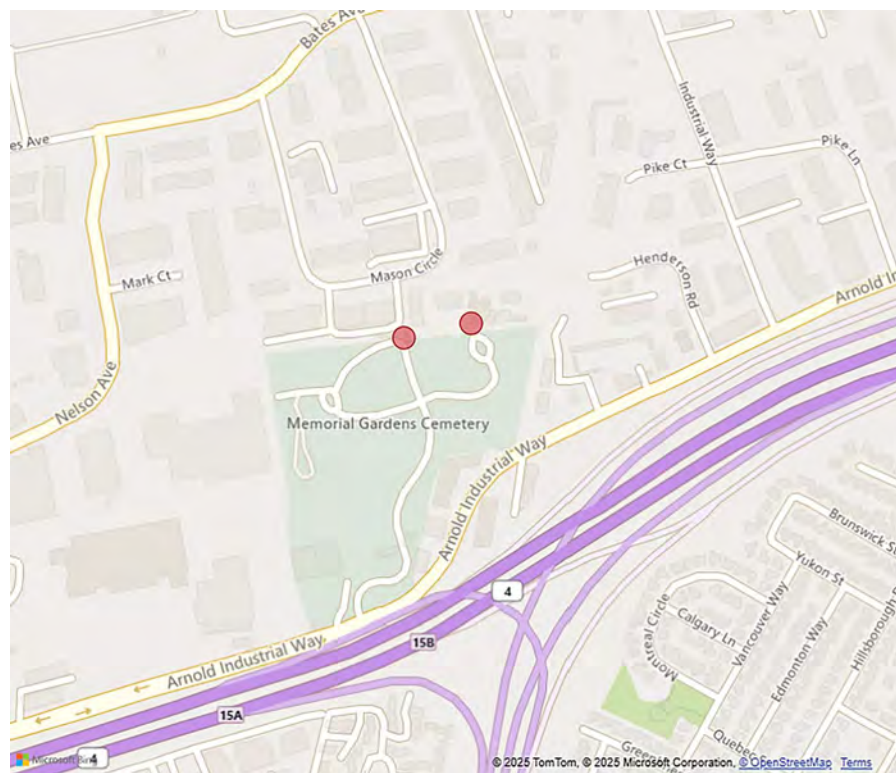


Figure 2.—Locations where adult *Ae. aegypti* were collected in BG Sentinel traps, Concord, CA, 2024.

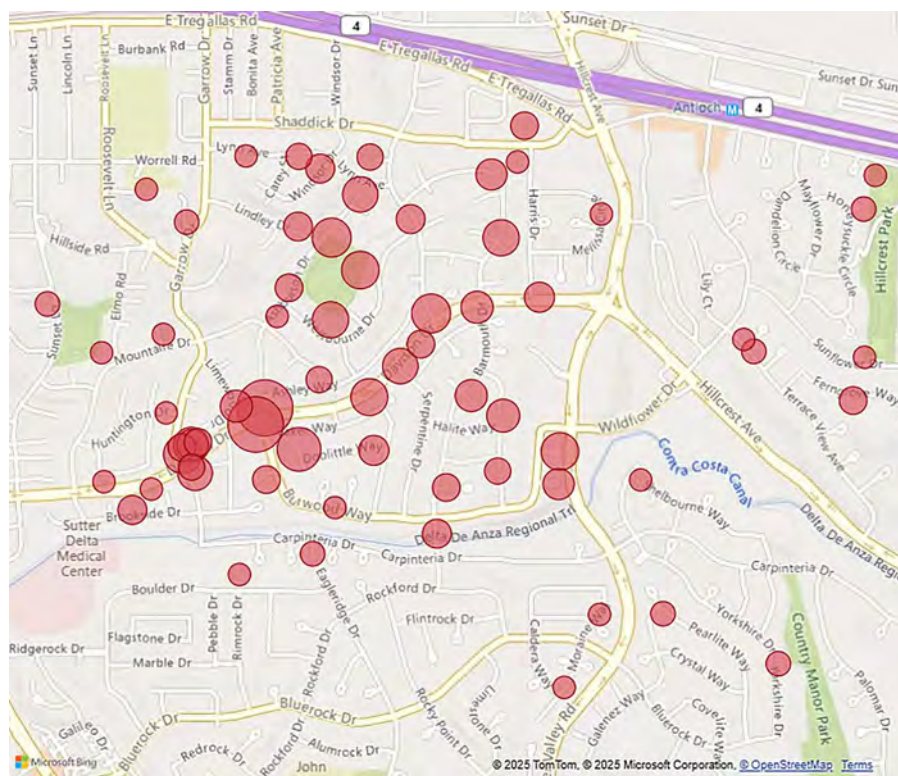


Figure 3.—Locations where adult *Ae. aegypti* were collected in BG Sentinel traps, Antioch, CA, 2024.

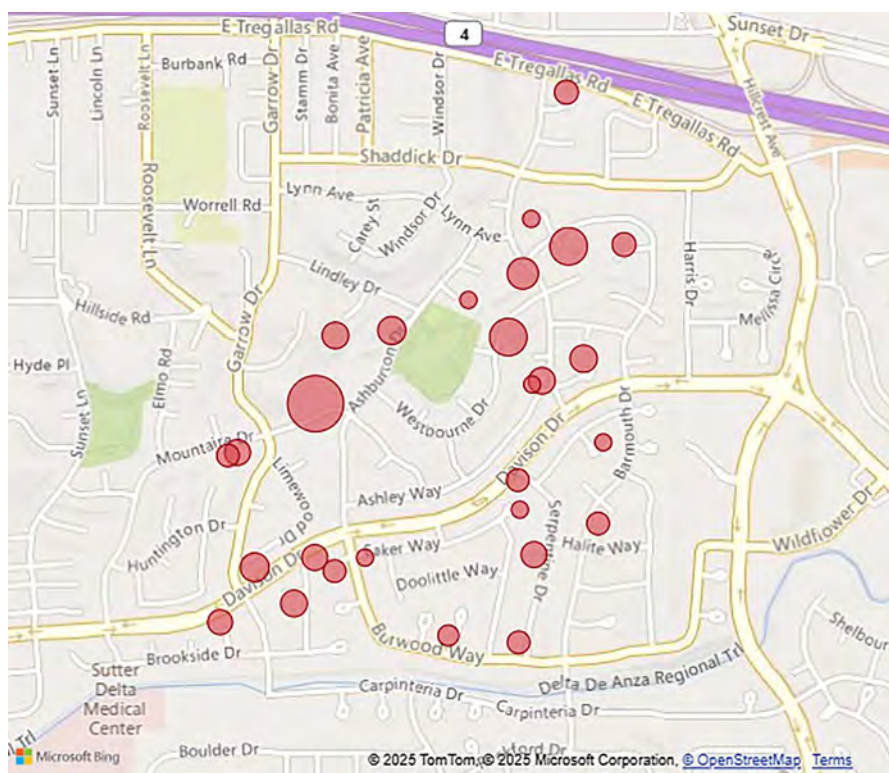


Figure 4.—Locations where *Ae. aegypti* larvae were collected during property inspections, Antioch, CA, 2024.

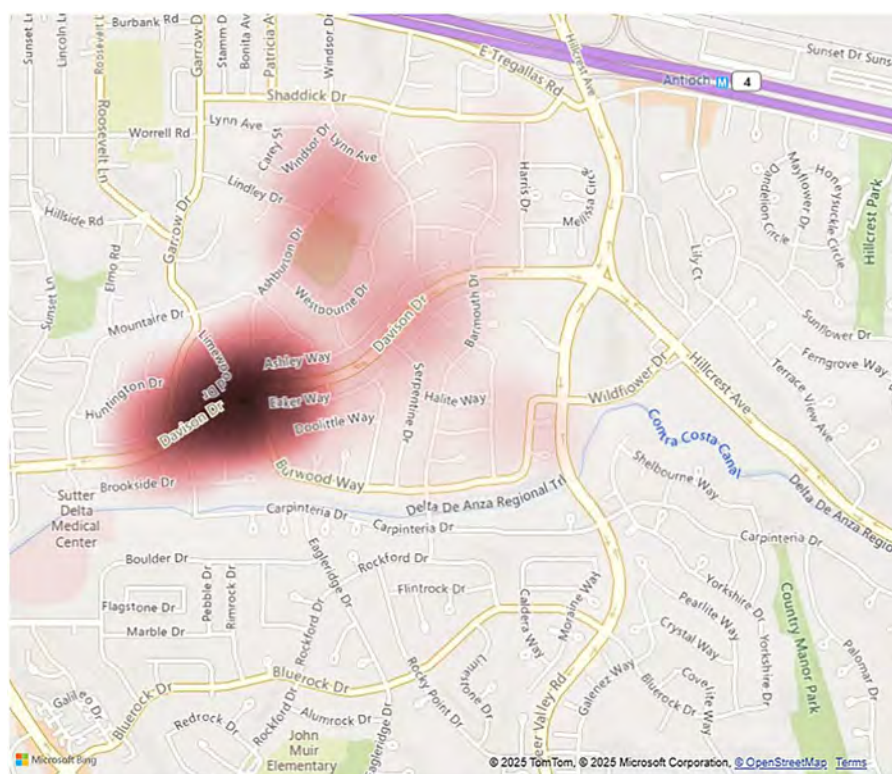


Figure 5.—Density map of adult *Ae. aegypti* collections, Antioch, CA, 2024.

and because the detection occurred fairly late in the season and covered a much larger area, we were not able to complete the process of delineating and containing it before the end of ‘mosquito season’. We were not confident that we were successful in containing this infestation, and inspections and trapping will resume in spring 2025. Engaging the public and encouraging them to report mosquito issues promptly remains a critical factor in detecting, preventing, or at least slowing, the spread of this invasive species.

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Evaluation of A1 efficacy on larval breeding cups in front and back yards following *Aedes aegypti* detections in San Jose

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Abstract

A small, local infestation of *Aedes aegypti* was detected in East San Jose in March of 2024. A combination of door-to-door inspections and treatments were implemented, before beginning wide area larvicide (WALS) treatments applying VectoBac WDG with an A1 sprayer. A field efficacy trial was conducted by recruiting 17 residents, who allowed us to place containers in their front and back yards. Ten sites were within the treatment zone and seven were outside (controls). Empty cups were placed in pairs in four different habitat types (open air, light foliage cover, dense foliage cover and under house eaves) in front and back yards. Cups were then seeded with 20 larval *Culex pipiens* (L2-L3) and monitored for 48-hour mortality in the laboratory. The treatment significantly increased larval mortality even under dense foliage cover, causing 66% mortality in treatment cups vs 7% mortality in control cups. We observed significantly elevated mortality in front yards compared to back yards in the treatment area. WALS treatments were repeated, but had modest impact on adult trap counts in the *Aedes* detection zone and failed to prevent further spread of the infestation.

Assessing the efficacy of Zenivex E20 against *Aedes* and *Culex* mosquitoes: Insights from field trials and lessons learned

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Introduction

The continued spread of *Aedes aegypti* in California presents growing challenges for vector control programs. In response, the Sutter-Yuba Mosquito and Vector Control District evaluated Zenivex E20, a pyrethroid-based adulticide with the active ingredient etofenprox, during the summer of 2024. The trials aimed to assess its performance against *Aedes* and *Culex* species and compare it with Permanone 31-66 which contains permethrin.

Methods

Three field cage trials were conducted on separate dates using wild-caught *Aedes melanimon*, laboratory-reared *Ae. aegypti* (sourced locally in Yuba City, CA), and colony *Culex tarsalis* (KNWR 2002 strain). Application rates were 0.51 oz/acre for Zenivex E20 and 0.29 oz/acre for Permanone 31-66. Each trial consisted of 12 cages total—6 per species—with approximately 20 mosquitoes per cage. Cages were arranged in two linear transects, with placement at 100 ft, 200 ft, and 300 ft from the spray line to simulate operational ULV applications and evaluate drift in open fallow fields. The first trial was conducted in the evening under wind speeds of 4.5 mph and included only *Ae. aegypti* and *Ae. melanimon*. Trials two and three were conducted in the morning with lower wind speeds (1.6–2.4 mph) and included *Ae. aegypti* and *Cx. tarsalis*.

Results and Discussion

In Trial 1, Zenivex E20 achieved 93% mortality in *Ae. melanimon* and 55% in *Ae. aegypti*; *Cx. tarsalis* was not included in this trial. In Trial 2, Zenivex E20 showed limited efficacy against *Ae. aegypti* (14%) and moderate efficacy against *Cx. tarsalis* (55%). Permanone 31-66, also tested in Trial 2, produced 96% mortality in *Ae. aegypti* and 100% in *Cx. tarsalis*. In Trial 3, Zenivex E20 achieved 66% mortality in *Ae. aegypti* and 100% in *Cx. tarsalis*, whereas Permanone 31-66 maintained high efficacy with 92% mortality in *Ae. aegypti* and 100% in *Cx. tarsalis*. These results emphasized the influence of environmental conditions, such as wind speed and application timing on adulticide efficacy. Although Zenivex underperformed compared to Permanone with *Ae. aegypti*, it demonstrated consistent control of *Cx. tarsalis* and *Ae. melanimon*. Given its favorable safety profile and utility in resistance management, Zenivex merits further evaluation.

Conclusion

Zenivex E20 demonstrated potential as a rotational adulticide, particularly for *Cx. tarsalis* and wild *Ae. melanimon*. Its lower mammalian toxicity supports use in residential environments. Additional field trials—especially involving wild *Cx. tarsalis* and *Anopheles freeborni*—are recommended to refine understanding of its operational value and performance variability.

Evaluation of larviciding as an *Aedes* control strategy

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Introduction

Aedes aegypti mosquitoes have been routinely detected in California since 2011 and in the Coachella Valley since 2016. Although the Coachella Valley Mosquito and Vector Control District (District) was created in 1928 and has been working to actively reduce mosquito populations and the viruses that they can transmit since 1955, the establishment of *Ae. aegypti* has greatly impacted the routine work of the District through increased mosquito bites reported by residents, increased trap and collections, and increased treatments. The local transmission of dengue in the nearby cities of Phoenix, Pasadena, and Long Beach further highlights the challenges that this species brings where residents a decade prior often questioned the need for mosquito control.

Since 2016, the District has made a series of wide area larvicide applications (WALS) to neighborhoods to control *Ae. aegypti*. With nine years of applications (Table 1), the District program has changed.

Discussion

In deciding when to make applications, programs should consider two themes. First, define how to measure application effectiveness. For liquid applications, water-sensitive cards can be used to determine if droplets reached the target area. Cups can be deployed to collect product. Cups can contain water and larvae, or in the case of Bti, cups can be placed in the field dry and then have water and mosquito larvae added after the application to measure potential efficacy. The primary result sought is a reduction in the adult mosquito population.

The second theme to consider is how to communicate the results of the work. We propose that there are three audiences to consider. For the residents within the treatment areas, questions arise on how to know that the property was treated and when they should expect to see a reduction in

Table 1.—Summary of applications made for *Aedes* control using the Wide Area Larvicide system to treat large areas.

Year	Aerial application	Ground application
2016	Coachella	
2017	Indio; Palm Springs	
2018	Mecca	Indio; Palm Springs
2019	Palm Springs	Cathedral City; Palm Desert
2020	Palm Desert	La Quinta
2021	Palm Springs	Cathedral City; Coachella
2022	Palm Desert	Palm Springs
2023	Palm Springs	Rancho Mirage
2024		Coachella; Indio

adult mosquitoes. For people outside of the treatment areas, some may ask for results to understand why their residence was not selected. Others may ask questions about drift of the product or ask what other impacts might result from the applications. The third group to consider is how to address requests not to be treated. At our District, we are not able to accommodate requests to not be treated when making aerial applications. For ground applications, we first inspect the residence to determine if mosquitoes are present. We have found that the majority of requests have been rescinded once we inspect the property and discuss with the resident that mosquitoes were present and the activity of the pesticide being used. For instances where there have been no mosquitoes or habitat present on the premises, we have agreed to not make a treatment to the residence but still treat the neighboring properties.

Acknowledgements

The authors acknowledge that the work described herein is possible thanks to the entire District team, as success comes from everyone contributing to reducing mosquito populations.

In2Care stations, control, and community outreach

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Abstract

Creating an effective IPM program means utilizing the available tools in a manner that promotes maximum benefits to residents by reducing mosquito populations. Our District utilizes In2Care stations as a part of our IPM program as a means of reducing mosquitoes. Arguably the largest part of any successful IPM program is public education and trust. We find that In2Care stations play a dual role. Not only do we find significant and repeatable mosquito population reductions with the stations, but they also provide a window of opportunity for our technicians to gain access to properties, educate the residents on yard hygiene, and provide a consistent reminder that our District is working to protect public health.

Progress and Challenges: First year of *Aedes aegypti* releases in a Sterile Insect Technique (SIT) pilot program in Los Angeles County

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Abstract

Aedes aegypti (L.) is an aggressive daytime biting mosquito and the primary vector for arboviruses including dengue, chikungunya, and Zika. The difficulties in controlling this mosquito species through traditional integrated vector management strategies has led the Greater Los Angeles County Vector Control District (District) to explore the implementation of the Sterile Insect Technique (SIT) as an additional control method. This pilot program involved the weekly release of sterile male *Ae. aegypti* throughout the mosquito season in two 50-acre intervention areas, with the goal of suppressing the local *Ae. aegypti* population. Although there were some challenges in releasing the intended numbers of sterile male mosquitoes, these releases demonstrated a significant population reduction of the wild adult *Ae. aegypti* population and significantly reduced hatch rates of eggs collected in the intervention areas compared to the control area. Challenges with the rearing and release methods need to be overcome to be able to implement this program throughout the District; however, the current study illustrated the promise of population suppression using SIT in conjunction with other integrated vector management strategies.

Introduction

Aedes aegypti has rapidly expanded its range since its initial detection in Los Angeles County in 2014, establishing itself as a persistent public health concern (Metzger et al. 2015). Global trade and climate change enable both the spread and reintroduction of this invasive species (Kraemer et al. 2019). This mosquito thrives in urban environments, exploiting cryptic water sources in residential areas (Wilke 2020). Compounding this challenge is its growing resistance to commonly used chemical pesticides, which limits the effectiveness of traditional control strategies (Liebman et al. 2019).

Aedes-borne arboviral diseases such as dengue, chikungunya, and Zika, although not endemic to Southern California, regularly infect visiting and returning travelers. The first locally transmitted cases of dengue in Los Angeles County were confirmed in 2023 and a subsequent increase in 2024 has underscored the urgent need for alternative and complementary control methods for use in integrated vector management (IVM) programs (CDPH 2025).

In the midst of these developments, the District launched a pilot program utilizing the Sterile Insect Technique (SIT), a species-specific and environmentally friendly method that involves mass releases of sterile male mosquitoes to suppress wild populations. Unlike pesticides, SIT does not contribute to chemical resistance, nor does it pose risks to non-target organisms. Male mosquitoes, which do not bite or

transmit arboviruses, actively seek out and mate with wild females, thereby reducing the number of viable offspring over time (Morreale et al. 2024).

The current pilot study evaluated the first year of sterile male releases and the effects these releases had on the wild *Ae. aegypti* population in the Sunland-Tujunga neighborhood of Los Angeles. Here, the progress made and the operational challenges encountered during this first year were examined, evaluating the efficacy of SIT in reducing both egg viability and adult mosquito abundance under real-world conditions.

Methods

Ethical Statement

GLACVCD is a public health agency that is enabled and empowered through legislation incorporated in the California State Health and Safety Code to counter the threat of mosquito-borne diseases. Mosquitoes reared in insectary conditions do not carry disease and due to sex-sorting, resulting in very few females released; there was no risk of disease transmission from the District's SIT efforts. The District also involved the Los Angeles County Board of Supervisors, Los Angeles City Council, and the Sunland-Tujunga Neighborhood Council for input on the proposed project prior to the study. A knowledge, attitude, and perceptions (KAP)

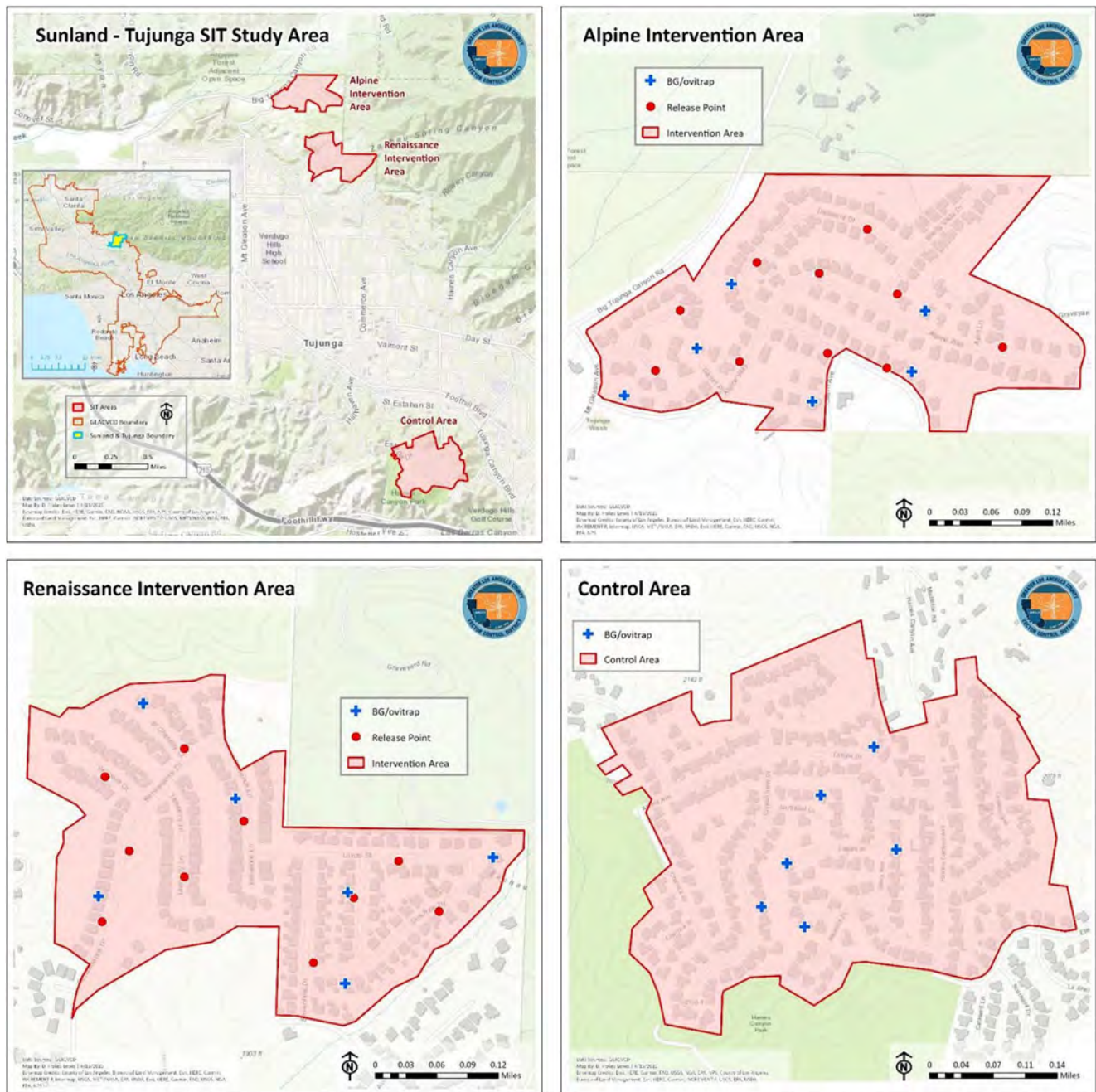


Figure 1.—Map of SIT study areas outlined in red in the Sunland-Tujunga neighborhood in the Crescenta Valley, Los Angeles, California with inset showing GLACVCD boundaries within the County of Los Angeles. (top left). Alpine intervention area (top right), Renaissance intervention area (bottom left), and the control area (bottom right). BG/ovitraps marked with blue crosses and release points marked with red dots.

survey was conducted door-to-door in the intended release areas.

Study Areas

Study areas were identified according to the following criteria: the presence of *Ae. aegypti* without the presence of other invasive *Aedes* species, an area comprising 50 acres, and the presence of barriers which would prevent immigration of *Ae. aegypti* into the study area. SIT pilot

releases were conducted in the Sunland-Tujunga neighborhood of Los Angeles, California within the Crescenta Valley (Figure 1). The three selected semi-isolated areas were mostly bounded by hills, barriers that would inhibit the migration of *Ae. aegypti* in and out of the areas. The region has a semi-arid climate with hot, dry summers and mild winters. A 50-acre subdivision located in the Verdugo Mountains in the southwestern part of the valley was selected for the non-intervention area (control area).

Table 1.—Total sterile male mosquitoes released each week in intervention areas during the pilot project in 2024. Week numbers 27 and 30 had no releases. Release ratios represent the number of recaptured males to captured wild males and are shown as a decimal. Release ratios where no wild males were captured are shown as “NA”. Adult traps and ovi traps were collected the week following each release; hatch rates, recaptures, and release ratios are shown the week after their release. Hatch rates were averaged for each week in the intervention areas (SIT) and control area. Blanks represent no eggs collected.

Month	May			June				July				
Week No.	20	21	22	23	24	25	26	27	28	29	30	31
No. Released	23,858	24,824	12,329	21,524	35,464	33,378	34,711	0	35,187	17,529	0	33,010
Recaptures		361	56	62	92	163	53	138	3	65	19	2
Release Ratio		361.0	56.0	NA	92.0	NA	26.5	69.0	1.5	21.7	19.0	NA
Hatch Rate SIT Area					16.7%	0.0%	24.5%		84.0%	75.0%		99.6%
Hatch Rate Control							90.0%	98.3%	96.0%	100.0%	100.0%	100.0%

Two 50-acre subdivisions in the foothills of the San Gabriel Mountains on the northeast side of the valley were selected as intervention areas (Alpine and Renaissance intervention areas).

Oviposition and Adult Abundance Monitoring

Monitoring of mosquito populations in the South Tujunga and Alpine areas began in August of 2022. Monitoring of the Renaissance area began in August of 2023. Oviposition activity and adult abundance were monitored weekly during the mosquito season (April–October) at 18 trap sites, six in each study area (Figure 1). BG-Sentinel 2 traps (BG, Biogents, Regensburg, Germany) baited with BG-lures and CO₂ in the form of dry ice were used to monitor adult abundance. Each week, BG traps were set overnight, five days after each SIT release. To monitor oviposition activity, oviposition traps (ovitrap) were placed near the BG traps at each site. The ovi-traps consisted of black plastic, one-gallon buckets, lined with seed germination paper and filled with tap water. Ovitrap papers were collected and replaced weekly. Ovitrap papers with eggs were retained and allowed to dry and cure in a humidity-controlled container for up to three days. The humidity-controlled container consisted of a 48 qt Igloo cooler with three, 60 g 2-way humidity packs (Boveda, 84% RH). The quantity of eggs and subsequent hatch rate (eggs hatched/total eggs) was recorded at each site. The egg papers were cut into smaller pieces and placed into a 16 oz deli cup containing brewer’s yeast and 300 mL of aged tap water. After four days, the numbers of hatched larvae were counted.

Mass-rearing and Sex Sorting

To establish a local colony of *Ae. aegypti*, eggs collected from the study areas were sent to MosquitoMate, Inc., (MosquitoMate) Louisville, Kentucky. The colony was established and maintained in Kentucky. MosquitoMate then produced and shipped monthly predetermined amounts of egg capsules (each containing approximately 2,000 eggs) required for releases to the rearing facility located at the District headquarters in Santa Fe Springs, California. Egg capsules were flooded in one pan each and mosquitoes were reared *en masse* following MosquitoMate’s prescribed feeding regimen and insectary conditions (Dobson et al.

2023). The insectary was heated to maintain pan water temperature at 25.0°C ± 0.5°C. About 200 eggs were removed from several rearing pans at random two days post-hatch. The number of hatched and unhatched eggs were compared to assess the hatch rate.

During the pupal stage, male pupae were sorted from female pupae and remaining larvae using Fay-Morlan glass-plate pupal separators (Fay and Morlan 1959). Female pupae and remaining larvae were discarded. Male pupae were placed in two 6 in. × 4 in., 24 oz plastic food storage containers filled with 12 oz of bottled drinking water (hereafter referred to as eclosion containers) and placed in 12 in. × 12 in. Bug Dorm cages (Forestry Suppliers, Jackson, MS). Cages were stocked with 10% (w/v) sucrose solution *ad libitum*.

Irradiation, Marking, and Transportation

Two days following sorting, eclosion containers and any remaining pupae were removed from cages. Cages were then transported to the irradiation facility located at the Orange County Mosquito and Vector Control District (OCMVCD) in Garden Grove, California. Cages were promptly placed in a 4°C cold room for 15 min to immobilize the males prior to handling. First, 100 mosquitoes were counted and weighed to achieve a standardized weight per mosquito. Using this value, the total weight needed to meet the target release numbers and to evenly distribute mosquitoes were calculated. The total weight of the males from each cage was recorded for quality control purposes, then redistributed into compaction chambers. Mosquitoes were compacted to a density of 100 mosquitoes/mL to prevent damage to them by immobilization as well to maintain a consistent dose during irradiation (Chung et al. 2018). Compaction chambers were developed by OCMVCD and consisted of a 350 mL disposable syringe cut into 80 ml pieces with 3D printed dual plungers covered on one side with insect screen (unpublished).

Compaction chambers were moved from the chill room and into the X-ray irradiator (RS-1800, Rad Source Technologies, Buford, GA, USA). Chambers were arranged symmetrically in a circle using a 3D printed plate. An ion chamber (10X6.06-3 High Dose Rate Chamber, Radcal,

Table 1.—Extended.

August				September				October				
32	33	34	35	36	37	38	39	40	41	42	43	44
21,850	28,693	4,896	23,681	12,285	49,784	34,927	34,133	25,648	15,861	18,952	22,945	28,056
59	58	131	74	156	1	239	189	346	135	18	56	3
19.7	14.5	26.2	24.7	13.0	0.3	79.7	47.3	28.8	45.0	NA	NA	NA
32.4%	0.0%	33.6%	40.7%	51.3%	22.4%		0.0%	0.0%	0.0%	50.0%		
94.8%	73.3%	98.2%	100.0%	75.6%	88.1%		93.3%	97.5%	79.4%	95.0%		

Monrovia CA, USA) was used to measure the dose received in real-time. Mosquitoes received a 35 Gy dose and then compaction chambers were inverted and placed opposite their original position from center to maintain a consistent dose between mosquitoes. The mosquitoes then received a final dose of 35 Gy for a total dose of 70 Gy to achieve 99% sterilization.

Compaction chambers were brought back to the cold room for immobilization. Sterilized mosquitoes were removed from compaction chambers and placed in release containers made from 2.5 qt cylindrical plastic mixing containers (Home Depot, Atlanta, GA, USA). The lids were modified to have a 4.75 in. circular hole covered with insect screen and a small “X” cut on the plastic edge to facilitate marking with fluorescent dye. The inside walls of the release containers were sanded with low grit sandpaper to provide a non-slick resting space for mosquitoes. Release containers were removed from the chill room and then marked with fluorescent DayGlo Eco powder (Cleveland, OH) via an insufflator inserted into the “X” cut into the lid. Fluorescent powder colors were rotated weekly to distinguish male mosquitoes from different release batches.

Prior to transportation to release sites, paper towels cut and folded into 3.25 in. by 3 in., 8 ply rectangles (WypAll Brand, L30 12.5 in. by 12 in. heavy cleaning towels) were soaked in a 10% (w/v) sucrose solution and placed on top of the release containers lids to provide sugar solution *ad libitum* during recovery and transportation. Release containers were loaded into a climate-controlled vehicle for transportation to the Sunland-Tujunga release sites 50 miles away.

Releases

Releases of sterilized marked males occurred weekly from 16 May to 31 Oct 2024. Ten release sites were selected in each of the intervention areas. Release points were evenly distributed throughout each area, with a slight bias toward the southwest of each area to account for the prevailing wind direction (Figure 1). During releases, container lids were removed, allowing mosquitoes five min to exit freely before being re-capped. Care was taken to avoid placing containers near temperature extremes or near potential predators. Mosquitoes remaining in release containers were brought

back to the laboratory, counted, and subtracted from the total release number.

Statistical Analysis

Data collected from adult mosquito and oviposition cup trapping were analyzed using R (via RStudio). Generalized Linear Mixed Models (GLMMs) were used to assess adult counts, egg counts, and egg hatch rates. Due to overdispersion in the adult and egg count data, negative binomial distributions were applied. Hatch rate analyses used binomial distributions. Additionally, a high proportion of zero counts in the datasets warranted the use of zero-inflated GLMMs to account for excess zeros.

The ovitrap index was calculated as the proportion of traps positive for eggs whereas egg density was the mean number of eggs per trap each week (Balatsos et al. 2024, Gato et al. 2021). Decrease in egg densities between intervention and control areas were calculated with a standard percent change equation, $(E_{SIT} - E_{cont})/E_{cont}$, where E_{SIT} is the mean egg density in the intervention areas and E_{cont} is the mean egg density in the control area. Abbott’s equation was used to calculate induced sterility, $1 - (Eh_{SIT}/E_{SIT}) (E_{cont}/Eh_{cont})$, where Eh_{SIT} is the number of hatched eggs in the intervention areas and Eh_{cont} is the number of hatched eggs in the control area (Bellini 2013, Gato et al. 2021).

Adult population suppression was calculated using Mulla’s formula for control, $100 - (C_1/T_1) (T_2/C_2)$, where C_1 and T_1 are the average mosquito populations for the season before releases, C_2 and T_2 are the average mosquito populations for the season that releases occurred in the control and intervention areas, respectively (Mulla et al. 1971).

Results

Releases

A total of 594 egg capsules were hatched with the intention of rearing and releasing approximately 1,144,000 males over the span of the season in the intervention areas. In practice, 854,465 mosquitoes were reared and irradiated. Of these, 250,936 mosquitoes failed to exit their release containers, ultimately resulting in an estimated 603,529

sterile male mosquitoes released. Weekly release numbers varied due to operational challenges in the insectary and inconsistent emergence from release containers. No releases were conducted on week number 27 or week number 30 in July. Detailed release data are provided in Table 1.

By the end of the season, 0.42% of released males were recaptured. The seasonal average ratio of recaptured sterile males to captured wild males was approximately 38:1. This release ratio is well above the recommended 10:1 ratio (World Health Organization 2020). However, release ratios varied with each release depending on the number released and the wild male population (Table 1). All but two weeks were over the 10:1 ratio. Weeks number 27 and 30 in July had no release which led to lower sterile male recaptures the following week. Release ratios could not be calculated for weeks where no wild males were captured.

Ovitrap

Although differences in ovitrap egg counts were observed between all areas during the 2024 treatment year, the majority of ovitraps were negative for *Ae. aegypti* oviposition (Table 2). As a result, there was no statistically significant difference in the average number of eggs collected per trap among the two treatment areas and the control in 2024 (Alpine: $p = 0.44$, Renaissance: $p = 0.49$). Similarly, comparisons of egg density between 2023 and 2024 within each treatment area showed no significant changes (Alpine: $p = 0.244$, Renaissance: $p = 0.311$). No significant differences were found between the number of eggs collected per ovitrap between Renaissance and Alpine in either 2023 ($p = 0.873$) nor 2024 ($p = 0.94$). Induced sterility, calculated from hatch rate and egg density averaged

Table 2.—Egg counts by trap averaged over the release period. The average is shown for each study area.

	Egg Counts
A1	6.0
A2	5.6
A3	1.4
A4	3.2
A5	2.0
A6	0.0
Average	3.0
R1	14.6
R2	10.3
R3	1.5
R4	2.2
R5	2.2
R6	3.5
Average	5.7
C1	21.2
C2	1.0
C3	14.5
C4	2.2
C5	1.4
C6	4.6
Average	7.5

67.1% in Alpine and 49.0% in Renaissance during the release period.

Eggs collected in Alpine had an average hatch rate of 28.6%, while Renaissance had an average hatch rate of 46.1%. In contrast, the control area exhibited a significantly higher hatch rate of 92.5%, representing a decrease of 69.5% in Alpine and a decrease of 46.4% in Renaissance (Table 3). Hatch rates in both intervention areas were significantly lower than the control ($p < 0.001$). No releases occurred during the weeks of 4 and 25 July; the following week, hatch rates in the intervention area rose to 84.0% and 99.6%, respectively (Table 1).

Adult Suppression

District-wide, *Ae. aegypti* abundance was lower in 2024 compared to 2023. Similarly, the control area experienced a significant reduction in adult abundance between 2023 and 2024 ($p < 0.05$). During the 2024 release period, seasonal average adult abundance in both Alpine and Renaissance was significantly lower than in 2023 and also significantly lower than the control area ($p < 0.001$) (Figure 2). Using Mulla's formula *Ae. aegypti* populations were reduced by 87.6% in Alpine and 75.8% in Renaissance (Table 3).

Discussion/Conclusion

Conducting an operational evaluation of the different components of a small-scale SIT program in a real-world context is essential before designing, planning, and successfully expanding to a large-scale SIT program. First, it bridges the gap between proof-of-concept studies and large-scale implementation by identifying logistical and environmental challenges, such as water quality variability, sanitation, material selection, mosquito robustness, and workflow. Second, it provides empirical evidence on how SIT performs under specific conditions within the District, informing strategies on future release numbers, release site selection and buffer zones. Furthermore, by quantifying adult abundance reduction and induced sterility, it builds confidence in the program among residents and other stakeholders.

Operational challenges in the insectary led to a reexamination of rearing procedures. Early hatch rates of egg capsules fell short of expectations, prompting the addition of a small amount of larval food 24 hours prior to hatching eggs from capsules in rearing pans, an adjustment

Table 3.—Summary of significant results. Percent decrease in hatch rates of collected eggs from ovitraps compared to the control area during releases in 2024. Adult reduction is the result of Mulla's formula for control which compares the intervention areas to the control area considering the year before and after treatment. The SIT column represents the average of from both intervention areas (Alpine and Renaissance).

	Alpine	Renaissance	SIT
Decrease in Hatch Rate $p < 0.001$	69.5%	46.4%	61.1%
Adult Reduction $p < 0.001$	87.6%	75.8%	81.7%

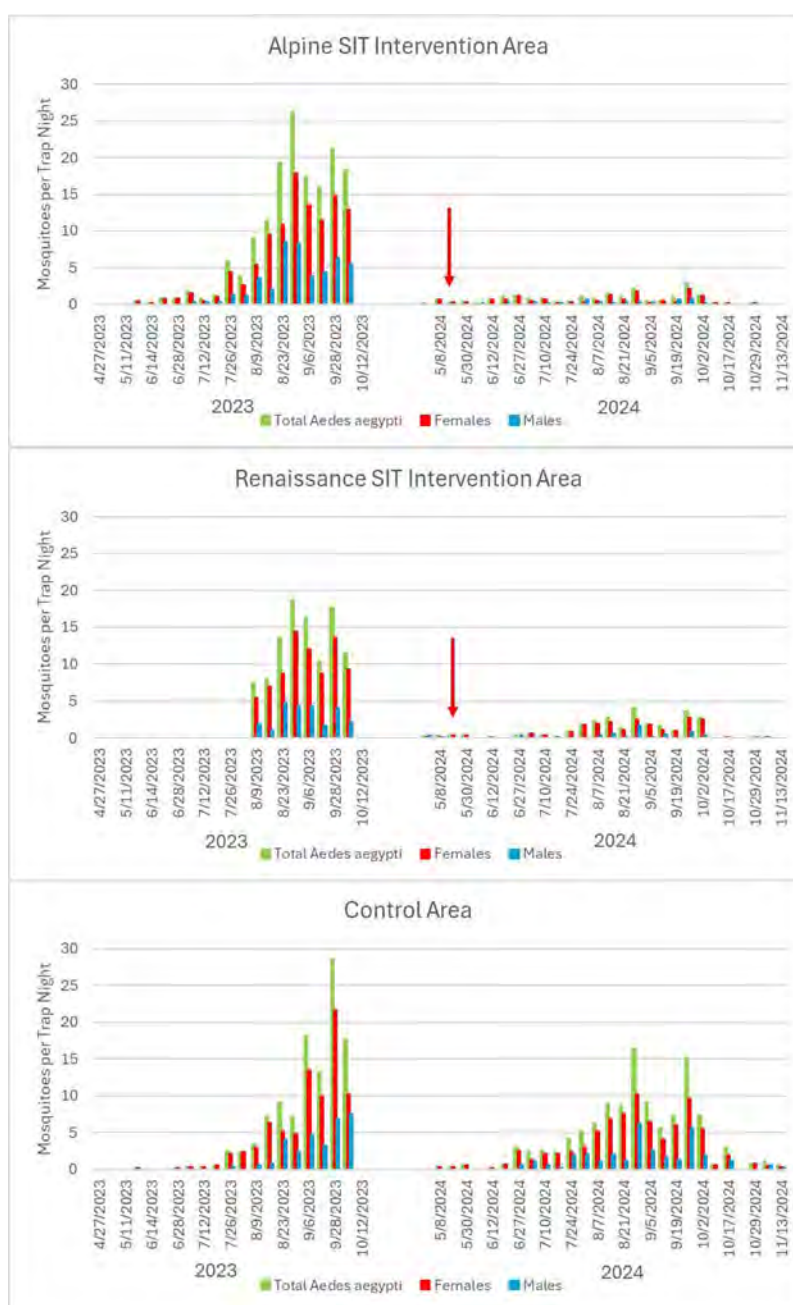


Figure 2.—Clustered column chart showing the total *Ae. aegypti* collected (green), female *Ae. aegypti* collected (red), and male *Ae. aegypti* collected (blue) in 2023 and 2024 in the SIT study areas. Red arrows on the Alpine and Renaissance SIT intervention area graphs indicate when releases began. Monitoring of the Renaissance SIT intervention area began in August of 2023. Trapping concluded in early October of 2023 and in early November in 2024.

recommended by MosquitoMate. This resulted in improved synchrony during sorting; more larvae had pupated at the time of sorting. Further discussions with MosquitoMate led to a more robust cleaning and sanitization routine, sterilizing rearing pans and eclosion chambers for 10 min in a 10% bleach solution.

Despite these measures, third-instar larvae mortality was observed in the rearing pans. Fearing a possible mold or pesticide contamination, biosafety controls were implemented around the insectary. Protocols included

restricting field staff access to parts of the building, to prevent pesticide contamination in the insectary. Additionally, instead of reusing tap-filled jugs for filling larval pans with water, bottled purified water was purchased for rearing and sorting. When mortality persisted, it was discovered that the “low-splash” bleach being used contained glycerin, which might negatively affect immature mosquitoes. Replacing this with standard 7.55% sodium hypochlorite bleach restored hatch rates of egg capsules and reduced larval mortality in rearing pans.

Table 4.—Hatch rates and male yields comparing the use of tap water and bottled water during one rearing cycle in the insectary. 10 pans were tested with tap water and 20 pans with bottled water. Males produced per pan show the number of males produced on average over all pans and the percentage is based on an expected yield of 2,000 males per pan.

	Tap Water (n = 10)	Bottled Water (n = 20)
Hatch Rate	64.40%	86.70%
Males produced/pan	238 (11.9%)	1747 (87.4%)

Subsequent tests confirmed that pans with tap water experienced high mortality (Table 4), suggesting changes to water quality; therefore, water from a reverse-osmosis filter will be used to fill pans for all future rearing.

With each release, a proportion of mosquitoes failed to exit the release containers. Though few were dead, most of these mosquitoes that did not exit were lethargic and would not fly. From May to July, the rate of males that did not exit containers ranged from 1.6% to 11.15%. In August, the rate increased drastically, hovering between 13.5% and 89.2%. Many factors were evaluated including chilling duration, cage density, and handling times without finding a causal factor. Unexplored hypotheses included possible effects of water quality when preparing the sucrose solution and 24-hour illumination from a newly installed bug zapper. This has been shown to affect the activity of ovipositing insectary mosquitoes (Bibbs et al. 2024). It is possible that the light from the bug zapper affected the activity rhythm of the male mosquitoes, causing them to stay in the release containers. Despite lower than intended release numbers, most release ratios were still above 10:1 and the adult population was successfully suppressed. Few weeks had a less-than-ideal release ratio. Week number 28 after there was no release had a low release ratio. Week 31, also followed a week with no release, but no wild males were collected to calculate a release ratio. Notably, those weeks still had recaptures from prior releases. Week number 36 in September experienced a heatwave; the temperature during release was 41.1°C. The collection the following week had very few recaptures, resulting in a less-than-ideal release ratio.

The predominance of zero-egg ovitrap collections led to statistically insignificant differences in egg density between the treatment areas and the control area, even using a zero-inflated GLMM. However, the highly significant hatch rate suppression in the intervention area compared to the control area, provided evidence that the released sterilized males located and mated with wild females, even if ovitraps failed to detect oviposition. Hatch rates rebounded immediately when releases paused, and fell again once they resumed, and weeks with lower release numbers corresponded to smaller hatch rate reductions, underscoring the importance of maintaining a large overflooding male ratio.

Applying Mulla's formula for estimation of control allowed us to account for pre-treatment difference between the control and intervention areas. The Renaissance area had significantly more mosquitoes than the control area prior to

intervention. A simple percent change calculation from Renaissance to control would not have shown an accurate suppression rate if pre- and post-years were not considered in the calculation as they were with Mulla's formula.

In both the hatch rate and suppression rate, Renaissance had lower control as compared to Alpine. This may be attributed to the geographical isolation of Alpine. Renaissance had adjoining neighborhoods to the southwest and southeast. Immigration of *Ae. aegypti* from those neighborhoods, with help from the prevailing winds from the southwest, could have contributed to the decrease in control in Renaissance. Studies have shown that adding a buffer area with additional release points can help to achieve better control in intervention areas (Le Goff et al. 2024, Morreale 2024).

The District plans on continuing with sterile male releases during the 2025 season. Learning from the challenges encountered in 2024 with rearing in Santa Fe Springs, improvements to processes and a new rearing facility closer to the study area will be implemented. Issues with males failing to exit their release containers remains unresolved. Lethargic males can drastically reduce the number of sterile males released. A buffer zone outside of the Renaissance intervention area is planned to also increase the effectiveness of SIT in the Renaissance intervention area. Although changes in egg densities from ovitraps were not significant, the hatch rate data was significant and quickly identified the effectiveness of releases. Overall, the SIT pilot program successfully reduced hatch rates and suppressed the adult population in the intervention areas. SIT has proven to be a viable option for IVM programs at mosquito control agencies.

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Dengue in our backyard: Insights and innovations from 2024

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Abstract

In 2024, the San Gabriel Valley Mosquito and Vector Control District (SGVMVCD) confronted a significant public health challenge with a surge in locally acquired dengue cases in Baldwin Park and El Monte. By October 15, the Los Angeles County Department of Public Health had confirmed eight cases, highlighting the increasing threat of dengue secondary transmission and the urgent need for rapid, coordinated intervention.

SGVMVCD responded by activating its Enhanced Neighborhood Support (ENS) strategy, a comprehensive framework aligned with dengue response recommendations from the California Department of Public Health. This approach is designed to address potential mosquito-borne disease threats through enhanced surveillance, mosquito control, and public outreach, ensuring a rapid and effective response to contain the outbreak and prevent further transmission. ENS efforts included sustained mosquito trapping and testing, door-to-door property inspections, truck-mounted pesticide treatments, and targeted public education. To ensure complete coverage, the District utilized inspection warrants to access properties where residents were unresponsive or denied entry, addressing potential larval sources across the affected areas.

This presentation explored the operational challenges and key insights gained from the 2024 dengue response. Attendees will learn how the ENS strategy integrates collaborative partnerships, public engagement, and flexible operating procedures to manage mosquito-borne diseases effectively. As local dengue transmission becomes a more frequent reality, this talk offers actionable strategies for outbreak investigation, control, and long-term public health management.

Locally acquired dengue response in Panorama City, California

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Abstract

Since the initial detection of *Aedes albopictus* and *Aedes aegypti* in Los Angeles County in 2011 and 2014, respectively, these invasive mosquito species have become established in every city and community within the Greater Los Angeles County Vector Control District's (GLACVCD) service area. Each year, the GLACVCD receives numerous reports of travel-associated dengue and chikungunya cases from the Los Angeles County Department of Public Health, providing the risk for local transmission of these arboviruses. Notably, the first cases of locally acquired dengue in California were recorded in Pasadena and Long Beach in 2023. In September 2024, the GLACVCD received reports of its first locally acquired dengue case in Panorama City. This presentation addressed the District's rapid response efforts and lessons learned from these developments.

The Scientific Legacy of Jan O. Washburn (1952–2024)*

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Introduction

L. Philip Lounibos and Eric Haas-Stapleton

Jan Washburn, who passed away on January 22, 2024, contributed to mosquito control in California in multiple capacities. He was Editor of the Proceedings of the MVCAC from 2009 to 2015. In Alameda County, he was a member of the Board of Trustees of Alameda County Mosquito Abatement District in California for 30 years (1993 to 2023) and Board of Trustees President in 1995 and 2012. He was Interim General Manager at the Alameda County Mosquito Abatement District for two brief periods during 2015 and 2017. Previously, as a postdoctoral fellow and specialist in the Department of Entomology at the University of California Berkeley, his research on the community ecology of the treehole mosquito *Aedes sierrensis*, with particular attention to parasites and predators of this pest species, led to multiple publications in refereed scientific journals, most notably concerning the ciliate *Lambornella clarki* which transforms from free-living to parasitic form in the presence of *Ae. sierrensis*, as described by Jan in a 1988 paper in *Science* (Washburn et al. 1988a).

As Jan Washburn devoted so much of his life to the appreciation, research, education, and conservation of insects, a three-hour symposium was organized to honor his scientific legacy at the Annual Meeting of the Entomological Society of America in Phoenix AZ in November 2024. Presenters were scientists and colleagues, family, friends, mentees, or advisors who knew Jan personally and his accomplishments (Fig. 1). Libe Washburn, an oceanographer, described his brother's natural history interests growing up in rural West Virginia and their later collaboration on a publication in *Science* on the dynamics of aerial dispersal of

flightless scale insects. Gordon Frankie, Jan's PhD mentor at UC Berkeley, focused on his community-level contributions to Frankie's project on native bees in northern Costa Rican (Province of Guanacaste) dry forest. Two former Entomology graduate students with Jan, Dave Egerter and Dave Wagner, collaborated to describe Jan's research on the parasites and predators of the treehole mosquito, *Ae. sierrensis*. Phil Lounibos detected a thread of Jan's research relevant to biological control, beginning with Jan's Masters of Science project on gall wasps at the University of Delaware through his treehole mosquito research at UC Berkeley; Lounibos also drew attention to Jan's other contributions on the ecology of container mosquitoes. Taro Ohkawa used his likeness cartoons to portray Jan's leadership roles on the 'bug' side of the baculovirus laboratory of Loy Volkman at UC Berkeley, before describing aspects of his own research with videos on cellular motility in the nucleopolyhedrovirus system. The final two presentations, by former graduate students working with him, described Jan's contributions to mosquito control (E. Haas-Stapleton) in California, especially in Alameda County, and Jan's multifarious contributions to citizen science, natural history education, and conservation as a docent, volunteer, guest lecturer, field guide, and adjunct faculty at University of California at Santa Cruz (E. Lyons). During the Symposium's intermission, examples of Jan's natural history artworks, mostly paintings and line drawings, were displayed from a digital file on to a projection screen.

Growing up with Jan and our collaboration about aerial dispersal of minute wingless arthropods

Libe Washburn

As one of Jan Washburn's brothers, I discussed his early development as a naturalist and entomologist. Jan was born in Wilmington, DE, but grew up in a small WV town called Vienna during the 1950s and 1960s. Jan and I were very

* This paper summarizes presentations given at a Member Symposium at the 2024 Entomological Society of America meeting at Phoenix, AZ, and is published here in recognition of Dr. Washburn's long affiliation with the Mosquito and Vector Control Association of California.



Figure 1.—Presenters in the Entomological Society of America Symposium in Phoenix AZ, 2024, to honor the scientific legacy of Jan O. Washburn: L to R: D. Wagner, L. Washburn, T. Ohkawa, P. Lounibos, E. Haas-Stapleton, E. Lyons, G. Frankie.

close. Our home was near diverse habitats including hills, valleys, deciduous forests, wild and cultivated fields, streams, and the Ohio River. Jan, I, and neighborhood friends spent a lot of time in these areas, especially in summer when we were “turned loose” by our parents. Having these habitats nearby was important in Jan’s development as a naturalist. These were great areas to observe insects, spiders, reptiles, amphibians, and birds. It was in these areas where Jan began his insect collecting, particularly butterflies. He was exposed to butterflies at a young age because we had framed butterflies around the house where our paternal grandmother resided. In addition to his butterfly collecting, Jan began drawing at an early age and developed into an excellent illustrator and artist.

When Jan was a graduate student in entomology and I was a postdoctoral scholar in oceanography, we collaborated on a study to understand dispersal behavior of a scale insect that infested freeway ice plant. Jan’s previous work indicated that the scale insects exhibited specific behaviors prior to dispersing into the air column. Two of our research questions to understand these behaviors were: (1) Why do the scale insects face downwind and rise up on their hind legs before dispersing? (2) Once airborne, do they exhibit behavior which tends to keep them in the air column? Due to their small size, we were unable to measure the air velocity profile surrounding the scale insects. Instead, to address question (1) we used the velocity profile derived from an analytical model of flow in a viscous boundary layer. This revealed that horizontal drag forces on the scale insects increased by about a factor of 4 when they faced down wind and rose up on their hind legs. We hypothesized that this drag increase pushed the insects into the air column. The down-wind orientation coupled with higher drag may also have eased release of the hind feet since the insects climb vertically in response to light; a downwind push by drag forces would tend to release feet made for vertical climbing. To address question (2) we hypothesized that any

behavior to keep the insects in the air column would reduce their descent in still air. To test this, Jan conducted an experiment to determine the terminal velocities of live and dead scale insects descending in calm air. The insects were dropped into a large, graduated cylinder where the terminal velocities were determined by measuring the time to fall a given vertical distance. The experiment clearly showed slower descent by the live insects. The insects slowed their descent by extending their legs horizontally and arching their bodies upward at both ends. Field studies with “sticky traps” on tall buildings captured scale insects exhibiting the body orientation observed in the laboratory. This study was published in *Science* (Washburn & Washburn 1984) and highlighted a rare opportunity for our interdisciplinary collaboration and a boost for Jan and me when we were early-career scientists.

Jan Washburn: Biologist, artist, environmental educator and friend to all who knew him

Gordon W. Frankie

Jan Washburn is well known for his scientific contributions as a student and later as a professional biologist. He is also known for his environmental education work and as an artist working with several media. Jan helped me and my colleagues in Costa Rica as we were establishing our bee research group in the small town of Bagaces in the Northwest Province of Guanacaste. We needed to solicit help from local people to conduct our research and above all we needed to let local residents know we were conservationists. Jan helped us with his famous artwork by producing line drawings of local animals, T-shirt designs, newsletter artwork, and a baseball hat with a conservation message. We used all of these artistic items to remain in good communication with local residents of Bagaces. This outreach effort was extremely effective in establishing ourselves as “Buena Gente” in the area. Even today we maintain contact with several of the residents from years ago.

Predator and Parasite Interactions in Treehole Mosquito Communities

David E. Egerter and David L. Wagner

After completing doctoral research revealing the surprisingly active dispersal ability of scale insect pests (Washburn & Washburn 1984), Jan spent the next nine years in the laboratory of UC Berkeley Professor of Entomology John R. (‘Andy’) Anderson, as a postdoc (1984–1987) and then research specialist (1987–1993). Supported primarily by National Institutes of Health research grants written with Anderson, Jan’s work focused on the ecology of the western treehole mosquito, *Aedes sierrensis*, most notably its relationship with the ciliate *Lambornella clarki*. Assisted by protozoologist Michael Gross, Jan designed and performed experiments showing that, when threatened with predation by *Ae. sierrensis* larvae, free-living trophonts of *L. clarki*

transformed into parasitic theronts that attack, parasitize, and kill their larval predators. This was the first discovery of an ecological strategy involving a trophic shift by a free-living organism to a parasitic existence and the first example whereby facultative parasitism appears to have evolved as an antipredator strategy. Based on work performed during this time, Jan authored with Anderson and others 14 peer-reviewed publications, two of these as reports in *Science* (Egerter et al. 1986, Ganz & Washburn 2006, Norton et al. 1992, Mercer et al. 2010, Washburn & Anderson 1986, 1993, Washburn & Hartmann 1992, Washburn et al. 1986, 1988ab, 1989, 1991ab, 1992). Jan's illustration of the ciliate's life cycle was additionally selected as the cover art for that issue of *Science* (Washburn et al. 1991). By the age of 40, Jan had first-authored three reports in *Science*, on two distinct research subjects, a remarkable achievement as many scientists would consider authorship on one such report in *Science* the pinnacle of their careers.

Contributions of Jan Washburn to Biocontrol and Mosquito Ecology

L. Philip Lounibos

The time period for this conspectus of Jan's academic contributions roughly corresponds to the interval from his Master of Science research at the University of Delaware through his doctoral research at UC Berkeley to his postdoctoral scholar positions studying parasites and predators of the Western treehole mosquito; it does not include his many contributions in baculovirus research, despite their pertinence to biological control.

Three publications on different insect-plant systems are mentioned for their indirect relevance to biocontrol: 1.) Surveillance of a cynipid gall-wasp population for two years in the NJ Pine Barrens led to the conclusion that gall wasp extinction was attributable to parasitism by chalcid and inquiline parasitoids (Washburn & Cornell, 1981). These observations support that classical biological control with introduced parasitoids is capable of leading to local pest extinctions. 2.) Monitoring the ice plant-scale insect system that was the focus of Jan's dissertation research (Washburn et al. 1983) showed that scale insect populations depressed by malathion spraying rebounded more slowly after insecticides than the resident parasitoids. This observation supports the compatibility of chemical insecticides and biocontrol in this system. Finally, in an application of island biogeographic theory to parasites and predators of the gypsy (aka spongy) moth, Washburn (1984b) contested the conclusion that this parasite-prey community was in equilibrium. If Washburn's evaluation was correct, there was niche space available for more biocontrols of *Lymantria dispar* in the USA.

Without doubt, Jan's most important discoveries related to biocontrol occurred during his postdoctoral research on the parasites and predators of the Western treehole mosquito, *Aedes sierrensis*. His first high-profile publication on this system demonstrated that free-living forms of the ciliate *Lambornella clarki* were induced in the presence of *Ae. sierrensis* larvae to transform into parasitic theront stages

which encysted upon, and typically killed, larval mosquitoes (Washburn et al. 1988a). A follow-up paper showed that this morphogenesis of the ciliate depended on larval resources: in resource-poor conditions, *L. clarki* was less likely to transform from free-living to parasitic form compared to conditions of abundant food (Washburn et al. 1991b). Jan was also part of the research team that showed high dispersal of *L. clarki* by infected female adult *Ae. sierrensis*, which deposited spores of the parasite during pseudo-oviposition events (Egerter et al. 1986). Washburn (1995) postulated that mosquito biocontrol in pool habitats would be more likely than in containers.

The field work conducted by Jan, especially on a suite of treeholes at the Hopland Field Station of UC Berkeley in Northern California facilitated tracking of *Ae. sierrensis* and their parasites from individual holes and correlated emerged adults with individual measures of fitness (Washburn et al. 1987). Jan also quantified how immature stages of this treehole *Aedes* were washed out by heavy rains (Washburn & Anderson, 1993), an underappreciated source of mortality for mosquito immatures. In laboratory simulations, he tested whether invasive *Aedes albopictus*, at that time not known in California, might successfully establish in California treeholes (Washburn & Hartmann, 1992), and contributed to research that determined that the tri-trophic interaction between *Ae. sierrensis* larvae, *L. clarki* ciliates, and bacterial prey were ecologically stable (Eisenberg et al. 2000).

Molecular Mechanisms in Caterpillar Baculovirus Infection

Taro Ohkawa

During our time together in the Loy Volkman laboratory, Jan Washburn directed the so-called "insect" side of the laboratory, while I ran the "cell" side of the laboratory. Jan's incredible knowledge of insects and the interaction between pathogens and insects was invaluable to the research direction of the laboratory, and our collaboration allowed many key insights about baculoviruses (e.g., Ohkawa et al. 2005, Zhang et al. 2005). Baculoviruses are double-stranded DNA, enveloped viruses which infect the larval stages of Lepidoptera. As such, they have been studied as potential biocontrol agents and are used worldwide for pest control for forest insects and crops such as cotton and soybean. Baculoviruses exhibit interesting pathology in infected caterpillars – e.g., they induce a walking behavior during infection, block molting, and liquefy the cadaver after death. At the cellular level, we found that baculoviruses (specifically *Autographa californica* M nucleopolyhedrovirus) manipulate the actin cytoskeleton of the host in striking ways. After entry into the cell cytoplasm, viral nucleocapsids utilize actin-based motility to propel them about the cell. Following nuclear entry, viral early genes cause numerous alterations to the host cytoskeleton, including an aggregation of actin around the cell periphery and nuclear translocation of soluble actin monomers. As the virus replicates, nuclear actin polymerizes into filaments, which contributes both to the structure of viral replication centers as well as allowing

virus escape from the nucleus, and ultimately from the cell, to spread the infection.

Contributions of Jan Washburn to mosquito control in California

Eric Haas-Stapleton and Ryan Clausnitzer

Our presentation highlighted the scientific legacy of Jan O. Washburn, emphasizing his significant contributions to mosquito control practices and governance with the Alameda County Mosquito Abatement District. Jan was instrumental in incorporating rigorous data-driven approaches into mosquito management strategies, enhancing district effectiveness and operational excellence. His key leadership roles, including Trustee for 30 years, twice as Interim General Manager, and multiple terms as Board President, instilled a culture of excellence and scientific rigor that inspired staff and colleagues. He guided strategic decisions by championing technologies, including quantitative RT-PCR and drone applications in mosquito control, long before they became mainstream in mosquito control. Jan had a state-wide impact through his role as Editor of the Proceedings and Papers of the Mosquito and Vector Control Association of California for six years from 2009 to 2015, furthering the spread of scientific knowledge and best practices in mosquito control across the state.

A notable aspect of Jan's leadership was his dedication to creating an equitable and inclusive workplace. He played a crucial role in championing domestic partner benefits for all employees at Alameda County Mosquito Abatement District, irrespective of their sexual orientation. His advocacy was particularly impactful during a time when legislative measures, notably the Defense of Marriage Act signed by President Bill Clinton the previous year, had restricted rights by explicitly defining marriage as a union exclusively between a man and a woman. This initiative was deeply personal for Jan, who felt compelled to "out himself" to the Board of Trustees to underscore the discrimination inherent in the existing policies and to advocate for change. His courageous stance not only led to significant policy improvements but also set a precedent for inclusivity and equality within the District.

The presentation honored Jan's legacy, which continues through the Dr. Jan O. Washburn Memorial Mosquito Laboratory at Alameda County Mosquito Abatement District, dedicated to inspiring future generations in their scientific endeavors and commitment to community service. This laboratory stands as a tribute to Jan's passionate journey in science and his enduring impact on public health and workplace equality.

Jan Washburn as Naturalist, Educator, and Mentor

Eric H. Lyons

Jan Washburn's career exemplified a profound dedication to insect natural history, education, and mentorship. Through

a variety of educational initiatives, Jan inspired students, friends and colleagues by cultivating a deep appreciation and understanding of insects, ecology, and the natural world. His extensive work at the University of California Botanical Garden as a docent and docent instructor involved engaging diverse audiences, from elementary school students to Master Gardeners across Northern California, providing them with practical and immersive ecological education. His lectures to Master Gardeners, spanning regions from San Jose to Mendocino, were particularly impactful, translating complex ecological and evolutionary knowledge into practical actions such as insect herbivory control and reintroducing pipevine swallowtails to local habitats.

At the University of California, Santa Cruz from 2000 to 2017, Jan taught general ecology to hundreds of undergraduate students. His approach to general ecology education emphasized hands-on experiences and public outreach, highlighting his belief that education could happen anywhere, from rivers to mountains to deserts. He consistently demonstrated his passion through activities designed to engage learners of all ages, creating a vibrant community around ecological discovery and conservation.

Jan's mentorship was characterized by a blend of rigorous scientific inquiry, adventure, humor, and genuine care for the well-being of his students and colleagues. He maintained that the joy of learning about nature and insects was amplified when shared openly, encouraging his mentees to explore alongside him with curiosity and enthusiasm. Jan's legacy is visible not only in the scientific community he nurtured but also in the broad public awareness and appreciation of insects and ecology fostered through his engaging and approachable style of teaching and mentorship.

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