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Investigator Award

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Reeves New Investigator

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Session A Ticks and Tickborne Diseases

Session B Operations and Applications

Session A Pesticides and pesticide use

Session A Vectors and Vertebrate Vectors

Session A 100 Years of responding to plague in humans, animals and fleas in California

Session B Inspection and enforcement of unmaintained swimming pools

Session B New techniques and innovations in operations

Session B Community outreach

William Walton Poster Competition

MVCAC Poster session

William C. Reeves New Investigator Award

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

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

Year	Award Winner	Title of Paper
1988	Vicki L. Kramer	A comparison of mosquito population density, developmental rate and ovipositional preference in wild versus white rice fields in the Central Valley
1989	Truls Jensen	Survivorship and gonotrophic cycle length in *Aedes melanimon* in the Sacramento Valley of California
1990	Gary N. Fritz	Polytenes, isozymes and hybrids: deciphering genetic variability in *Anopheles freeborni*
1991	David R. Mercer	Tannic acid concentration mediates *Aedes sierrensis* development and parasitism by *Lambornella clarki*
1992	Darold P. Batzer	Recommendations for managing wetlands to concurrently achieve waterfowl habitat enhancement and mosquito control
1993	Jeffery W. Beehler	The effect of organic enrichment and flooding duration on the oviposition behavior of *Culex* mosquitoes
1994	Merry-Holliday-Hanson	Size-related cost of swarming in *Anopheles freeborni*
1995	Margaret C. Wirth	Multiple mechanisms cause organophosphate resistance in *Culex pipiens* from Cyprus
1996	No award	
1997	John Gimnig	Genetic and morphological characterization of the *Aedes* (*Ochlerotatus*) *dorsalis* group
1998	Yvonne Ann Offill	A Comparison of mosquito control by two larvivorous fishes, the stickleback (*Gasterosteus aculeatus*) and the mosquitofish (*Gambusia affinis)*
1999	Parker D. Workman	Adult spatial emergence patterns and larval behavior of the “Tule Mosquito,” *Culex erythrothorax*
2000	Jason L. Rasgon	Geographic distribution of *Wolbachia* in California *Culex pipiens* complex: infection frequencies in natural populations
2001	Christopher Barker	Geospatial and statistical modeling of mosquito distribution in an emerging focus of La Crosse virus
2002	No award	
2003	Laura Goddard	Extrinsic incubation period of West Nile virus in four California *Culex* (Diptera: Culicidae) species
2004	No award	
2005	Troy Waite	Improved methods for identifying elevated enzyme activities in pyrethroid-resistanct mosquitoes
2006	Lisa J. Reimer	Distribution of resistance genes in mosquitoes: a case study of *Anopheles gambiae* on Bioko Island
2007	Carrie Nielson	Impact of climate variation and adult mosquito control on the West Nile virus epidemic in Davis, California during 2006
2008	John Marshall	The impact of dissociation on transposon-mediated disease control strategies
2009	Win Surachetpong	MAPK signaling regulation of mosquito innate immunity and the potential for malaria parasite transmission control
2010	Tara C. Thiemann	Evaluating trap bias in bloodmeal identification studies
2011	Sarah S. Wheeler	Host antibodies protect mosquito vectors from West Nile virus infection
2012	Brittany Nelms	Overwintering biology of *Culex* mosquitoes in the Sacramento Valley, California
2013	Kimberly Nelson	The effect of red imported fire ant (*Solenopsis invicta* Buren) control on neighborhoods in Orange County, California
2014	Thomas M. Gilbreath, III	Land Use Change and the Microbial Ecology of *Anopheles gambiae*
2015	Jessica M. Healy	Comparison of the efficiency and cost of West Nile virus surveillance methods in California
2016	Mary Beth Danforth	The impacts of cycling temperature on West Nile virus transmission in California’s Central Valley
2017	Nicholas A. Ledesma	Entomological and Socio-behavioral Components of Dog Heartworm (*Dirofilaria immitis*) Prevalence in Two Florida Communities
2018	Kim Y. Hung	House Fly (*Musca domestica L.)* Attraction to Insect Honeydew
2019	Matteo Marcantonia	Revising alkali metals as a tool for mark-recapture studies to characterize patterns of mosquito (Diptera: Culicidae) dispersal and oviposition
2020	Adena Why	Semiochemicals associated with the Western mosquitofish, *Gambusia affinis*, and their effect on the oviposition of *Culex tarsalis*
2021	Vanessa Hill	Evaluation of residential property types for *Ades aegypti* habitats in Placer County, California
2022	Phurchhoki Sherpa	Asian longhorned tick, *Haemaphysalis longicornis* (Ixodida: Ixodidae), and optimal collection methods for the tick in the Northeast United States
2023	Mark Dery	Effect of Bed Bugs (*Hemiptera Cimicidae*) aldehydes efficacy of fungal biopesticides

The President's Remarks – The past, present and future of the Association: Innovative mosquito control approaches for the future

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Post-Conference Summary: The 91st Mosquito and Vector Control Association was held from January 29 through February 1, 2023 at the Disneyland Hilton Hotel in Anaheim, California. The conference theme was innovative mosquito control approaches of the future, highlighting the key interests of the association membership, especially the current regulatory challenges. The three plenary speakers covered the background of new innovations in mosquito control, using the screwworm fly sterile male strategy as proof of concept for current innovations for mosquito control. Following the plenary session was the Reeves New Investigator Award competition, the inaugural session of the William Walton poster competition, and the symposium on innovations in mosquito control. The conference attracted more than 600 presenters and guests from throughout California and the country. There were a total of 99 talks, 1 panel discussion, and 22 poster presentations. Other major activities serving the interests of our association included the trustee training session, the 5k run and the Munzie Golf tournament. The 2023 conference was the second in-person conference held after the Covid-19 pandemic. The feedback during and after the conference indicated it was a great success for the association and its membership.

Mosquito control in California

Background: In 1930, mosquito control districts in California came together to form the California Mosquito Control Association. In 1976, the name was changed to California Mosquito and Vector Control Association to accommodate the expanding scope of membership responsibility. In 1995 the current name of the Mosquito and Vector Control Association of California was adopted (MVCAC 2021). The first annual conference of the Association was held December 15-16, 1930 in Berkeley, California under President Nobel Stover. Since then, the Association has continuously held its annual conference every year, except in 1942 and 1943 during the Second World War. The 1st (1930) through the 12th (1941) annual conferences were held in December, but after the pause of 1942 and 1943, the annual conferences from 1944 to the present have been in held in the months of January, February or March, and rarely in April (MVCAC 2023). The association has published its annual proceedings consistently since its inception; however, copies of the first 11 issues covering 1930 through 1940 currently are unavailable.

Enduring Strengths: Since the Association's founding, the MVCAC and its partners the California Department of Public Health and the University of California System have consistently exhibited several strengths throughout its 93 years of existence that include: (1) strong and visionary leadership, (2) financially stable, self-governing local organizations, forming a strong advocacy network for public health professionals throughout the state, and (3) constituent education, disseminating information on best practices in a coordinated message through it's annual conferences.

Some of the best innovations from member agencies and the Association over the years have included the development of tools involved in the reduction of mosquito sources, reducing mosquito populations, and sampling mosquitoes. In addition, partnering with University of California scientists developed innovative technologies that primarily targeted specific vector species, exploiting the biology of mosquitoes or specific chemistries to reduce their survival, and impair their ability to carry or transmit specific pathogens or diseases. This innovative streak has been fostered and sustained by ideas from the diverse individuals attracted into the profession. Such innovations have not been limited to new mosquito control products or chemistries, but included new tools for control or sampling mosquitoes, formulations and application methods. In addition, member agencies have pioneered improvements in safety standards for mosquito control workers during insecticide applications, and provided administrative structures to share or spread risk/liability among member agencies (Malamud-Roam 2017, Personal Comm.).

Challenges: The member agencies that form the Association are charged with addressing new threats from emerging and re-emerging diseases as well as the current and potential introduction of exotic vectors capable of transmitting exotic pathogens previously not spread locally. Over the past 30 years, the member agencies have experienced challenges associated with the invasion of West Nile virus, the emergence of new strains of St. Louis encephalitis virus and the discovery of a variety of tick-associated pathogens. The invasion of California by aggressive day-biting *Aedes aegypti* and *Ae. albopictus* has led to increasing demands for control as well as increasing risk of the transmission of several travel-associated viruses including yellow fever, dengue, chikungunya, and Zika.

In response, the industry has steadily developed new products effective for controlling mosquitoes and other vectors that are environmentally safe and efficacious. This effort has been undermined over the years by the development of insecticide resistance by the vectors and a change in how regulations that bring new products to market are promulgated and implemented. The process of bringing new chemical products to market requires a large capital investment and a sustained effort by knowledgeable people.

Despite great achievements over the past six decades, the major challenge that the industry faces currently is the changing regulatory climate. Regulators that traditionally followed science to determine the efficacy of chemical products before they were brought forth now have adopted a non-committal stance on legitimate requests for evaluation and approval − overlooking science-based decision-making thereby increasing uncertainty in the approval process for new products, and re-registration of existing products. The approval for research authorization that previously took 2-3 months has slowed currently to taking a year or longer, even in the post pandemic environment. The industry is keenly aware of this situation, and we urge our membership to refocus our efforts through advocacy to convince regulators to address requests, focusing on scientific consensus rather than unscientific and more vocal voices. The association must remain vigilant to counter growth of complacency and the rise of a "know-nothing mindset" opposing sound, science-based innovations that have sustained our industry.

History of Innovative Effort: The enactment of the Mosquito Abatement Law in 1915 was pioneered and championed by innovators who envisioned a future with less mosquitoes and the diseases they transmit. The industry has undergone several paradigm shifts in the controlling and management of mosquitoes. The first paradigm shift was characterized by the "pursuit of the magic bullet" that ushered in DDT and other chemistries of the 1940s through 1960s whose benefits were 'blunted' by the rapid rise of pesticide resistance, some of which appeared within a few years of new products coming to market.

The second paradigm shift started about 1970 and focused on bringing environmentally safe products into the mosquito control toolbox that included methoprene, microbials such as *Bacillus thuriengiensis Israelensis*, and spinosid. In addition, this period has altered new formulations of existing chemistries to improve efficacy under field conditions (Mulla 1994). Furthermore, the industry has focused on extending the life of these products by maintaining or improving their efficacies through proper management against the evolution of pesticide resistance (Reeves 1992).

A third paradigm shift involves the innovative shift to the use of 'green products' as summarized in the March 1980 issue of *California Agriculture* published by the University of California, Agriculture and Natural Resources. This issue brought together scientists from across all campuses of the University of California, the California Department of Public Health, and the Mosquito Abatement Districts within the state to highlight the status and breadth of mosquito control research in the state. Two articles authored by Sister Monica Asman and others in that issue laid the scientific groundwork of some of the innovative control products of the future highlighted at this conference and summarized within the MVCAC proceedings. One of the articles was on genetic manipulation of mosquitoes and the other was the use of sterile male releases to reduce mosquito populations (California Agriculture, 1980).

The 91st annual conference theme was "innovative mosquito control products of the future", showcasing current products that could be on the market within a few years, and eventually dominate the industry in decades to come. These products include the perfection and wide use of the sterile male release technique for control of *Aedes* mosquitoes. Reports of large-scale deployment of SIT-based technology in a Brazilian city in 2020 through 2022 resulted in the significant reduction of a dengue outbreak (Castro Poncio et al. 2022) and gives a hopeful future for our member districts. Currently, a coalition of several districts in southern California have embarked upon an ambitious program to use sterile male releases to control invasive *Aedes aegypti* and *Ae. albopictus* (Orange County MVCD and Greater LA MVCD; Laura Young, Susanne Kluh 2023, Personal Comm., respectively) and perhaps *Culex* species in the future. The use of incompatible strains of *Wolbachia* species in both *Culex* and invasive *Aedes* is at an advanced stage, and was recently approved by USDA/EPA for use in controlling malaria in endangered bird species in Hawaii (Office of the Governor of Hawaii 2023). The genetic approaches employing CRISPR, precision genetic procedures, and other technologies being developed by scientists at University of California and elsewhere stands a great chance to be available for trials and eventual use in the coming years. Transgenic mosquitoes developed to be refractory to infection of disease causing agents such as malaria and arboviruses pioneered in the 1970s (California Agriculture 1980) currently being developed under the University of California malaria initiative are technologies that may eventually pave way to controlling local mosquito-borne diseases (UCI Magazine 2017). This is particularly important with recent reports of recent locally acquired malaria cases in Florida and Texas (CDC 2023).

Conclusion: The Association must always pay attention to new innovations while understanding our past and the efforts of our members over the decades. We must forge ahead with diplomacy, advocacy and the education of our regulators and the public at large to convey that our efforts are grounded in science, with the goal of improving the quality of life. Our innovative efforts are intended to safeguard the well-being of our citizenry, the environment, and the future.

Epilogue: J. Wakoli Wekesa is the District Manager of the East Side Mosquito Abatement District in Modesto, Stanislaus County, and is organized as a "Pest Abatement District," one among a handful of districts in the State under Chapter 8, Division 3 of the California Health and Safety Code Section 2800 et Seq. The District was formed on June 28, 1939, for the sole purpose of mosquito control, and is governed by six trustees appointed by the County Board of Supervisors. The first 16 years, Chester Robinson, a graduate of UC Davis, managed East Side MAD. Gordon Smith, a graduate of UC Berkeley recruited from Kern County MAD succeeded him in 1956. After a stellar career, Smith retired in 1973. Paul Gieke, who left in 1980 after Proposition 13 reduced the District's revenues, succeeded him. The aftermath of this revenue squeeze saw the Districts' full time staff reduced from 25 to only three; 13 positions were converted into seasonal staff, and the rest were laid off. Claude Watson, who was the 1989 President of the MVCAC, managed the District from 1980 to 2003, when Lloyd Douglas took over as District Manager. Wakoli Wekesa succeeded Lloyd Douglas July 1, 2019.

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Population modification of mosquito vectors for malaria control

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Introduction

As much as 80% of the reduction in the global malaria burden during the first 15 years of the 21st century has been attributed to vector control (Bhatt *et al*., 2015). This amounts to ~500 million cases averted and is a major step forward in global eradication plans. Unfortunately, this decline slowed recently, and disease incidence and prevalence remain high (WHO 2022). Cost-effective vector control tools, including insecticide-treated bed nets and indoor residual spraying, are still effective in many regional malaria elimination programs, but these methods are limited in others by the emergence of insecticide resistance (Hemingway, 2018). Along with new prophylactic and therapeutic drugs and robust vaccines, novel vector control technologies are expected to play a major role in the ongoing fight against malaria.

A genetics-based vector control strategy designated 'population replacement/modification' involves the introduction of genes that confer refractoriness to the parasites into a target mosquito species. These anti-parasite effector genes when present at sufficient frequencies should prevent parasite transmission and lead to reduced malaria morbidity and mortality (Eckhoff *et al*., 2016).

We adapted Cas9/guide RNA (gRNA)-based biology to develop gene-drive systems that move potent anti-parasite effector molecules based on single-chain antibodies (Isaacs *et al*., 2011, 2012) into the African malaria vectors, *Anopheles gambiae* and *An. coluzzii* (Carballar-Lejarazú *et al*., 2023). Laboratory-based trials of efficacy and efficiency and subsequent modeling were used to determine whether the developed strains, AgTP13 and AcTP13, respectively, meet criteria to qualify for advancement to field trials.

Methods

Empirical techniques and modelling used to evaluate AgTP13 and AcTP13 include gene-drive plasmid design and construction, embryo microinjection and screening procedures, molecular validation of gene-drive integration and effector molecule integrity, antimalarial effector expression validation, gene-drive mating, maternal-effect analyses, experimental introgression by mating from *An. gambiae* into *An. coluzzii*, small cage trials, life-table parameters, *Plasmodium falciparum* infection assays, and statistical analyses and modeling (Dong *et al*., 2009, 2011, 2020; Pham *et al*., 2019; Adolfi *et al*., 2020; Carballar-Lejarazú *et al*., 2020, 2021, 2022, 2023; Sanchez *et al*., 2020; Wu *et al*., 2021).

Results and Discussion

Generating the AgTP13 line was efficient and highly-accurate, consistent with previous experience with Cas9/gRNA-mediated integrations of transgenes (Gantz *et al*., 2015; Adolfi *et al*., 2020; Carballar-Lejarazú *et al*., 2020, 2023). Introgression of the gene-drive construct through interspecific matings of *An. gambiae* to *An. coluzzii* also was straightforward. The initial Cas9/gRNA-mediated germline conversion of mosquitoes from hemizygotes to homozygotes was high, 100% in males and >95% in females, consistent with previous studies (Gantz *et al*., 2015; Pham *et al*., 2019; Adolfi *et al*., 2020; Carballar-Lejarazú *et al*., 2020, 2023).

Life-table parameters can identify transgene-mediated fitness loads (insertion and/or expression effects) that may affect gene-drive mosquitoes. With the exception of a competitiveness challenge in homozygous AgTP13 males, and similar to previous studies, no life-table parameter alone appears to have a significant effect on drive dynamics (Pham *et al*., 2019; Carballar-Lejarazú *et al*., 2020, 2023).

Parasite prevalence, intensities-of-infection and reductions in human incidence are some of the key metrics of *P. falciparum* transmission dynamics (Smith *et al*., 2012). Literature on target performance thresholds includes modeling that predicts that reductions of oocyst prevalence ≥32% in three successive transmission cycles could result in malaria elimination if infectious mosquito bites are low (Blagborough *et al*., 2013). Experiments in model rodents and meta-analyses of data from human and animal parasite infection studies showed that levels of <10,000 sporozoites in mosquito salivary gland decreased significantly the probability of subsequent disease (Aleshnick *et al*., 2020; Graumans *et al*., 2020). An expert panel recommended reductions of

20-50% in human incidence as the target for a population modification strain to be eligible for field testing (James *et al*., 2020). In the absence of a straightforward way to test empirically these thresholds in human challenge trials, robust modeling with laboratory-derived parameters provide a practical alternative (Mondal *et al*., 2022). Experimentally-derived impacts of the effector genes on parasite prevalence and mean-intensities-of-infection in hemi- and homozygous AgTP13 and AcTP13 following mosquito challenge assays met acceptable prevalence- and intensity-reduction thresholds (Carballar-Lejarazú *et al*., 2023). Field release modeling combining the laboratory-derived data with characteristics of a representative island field site show that releases of a locale-specific strain could have epidemiologically-significant impact by reducing human prevalence and incidence.

Conclusions

The coupling of gene-drive systems to anti-parasite effector genes can produce laboratory-generated data for anopheline strains that can be used to inform complex epidemiological modeling of their potential impact on malaria incidence and prevalence. The TP13-based strains meet many of the minimally-acceptable transmission thresholds and are eligible for further studies in anticipation of field trials.

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Autocidal approaches for suppressing populations of *Aedes aegypti* and *Ae. albopictus* in California

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Abstract

For more than a decade, MosquitoMate has worked with mosquito abatement districts throughout the USA to develop and test novel autocidal control technologies against invasive *Aedes* mosquitoes, including multiple field trials in California that targeted *Aedes albopictus* (Skuse) and *Aedes aegypti* (L.). Autocidal technologies are 'self delivering' and deploy the 'mosquito against itself.' Similar to the traditional Sterile Insect Technique (SIT), the *Wolbachia* approach is based upon repeated, inundative releases of cytoplasmically incompatible male mosquitoes, with the goal of decreasing the number of viable eggs (Crawford et al. 2020). The Auto-Dissemination Augmented by Males (ADAM) approach uses repeated, inundative releases of non-biting males as vehicles to deliver small doses of a potent insect juvenile hormone analogue (pyriproxyfen; 'PPF') into the small, cryptic containers in which *Ae. aegypti* and *Ae. albopictus* often breed (Mains et al. 2015).

In 2017, the Environmental Protection Agency (EPA) approved the MosquitoMate 'ZAP' males, which are *Ae. albopictus* males artificially infected with the *w*Pip *Wolbachia* from *Culex pipiens* (Dobson et al. 2016). However, the federal permit was time limited to five years and restricted to 20 states and the District of Columbia. In response, MosquitoMate applied for additional Experimental Use Permits (EUPs) and performed additional field trials in other states, in the hope that the EPA would remove the geographic restriction and allow ZAP males in all infested states. The latter data was submitted to the EPA in 2020. Although the ZAP registration was granted a five year extension at the end of 2022 (less than three weeks before registration expiration), the EPA has not yet allowed ZAP application in any additional states. Uncertainties resulting from the expiration and late extension have proven to be a substantial complication for MosquitoMate's ongoing operations.

In April of 2020, MosquitoMate submitted an application for *w*AlbB *Wolbachia* infected *Ae. aegypti* as a mosquito suppression tool ('WB1' males) to the EPA. An application to the California Department of Pesticide Regulation (CDPR) was also submitted for WB1 males. As of the submission of this article, the WB1 application remains under review at both the EPA and CDPR, and there is no defined timeline for completion. While stakeholders actively seek new control tools for mosquitoes of public health importance, MosquitoMate's regulatory experience may give pause to others considering a foray into the development of new public health tools.

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Sterile Insect Technique (SIT) a success story: The history of the primary screw worm fly and the cattle industry

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Abstract

The history of the sterile insect technique (SIT), also known as the sterile male technique, was first conceptualized by Dr. Edward F. Knipling in 1937. The proof-of-concept studies began in the early 1950s targeting the primary screwworm fly, *Cochliomyia hominivorax* (Coquerel), an extremely devastating pest of cattle, other livestock and wildlife throughout the Americas. The large-scale field trials started with a 'bust' and ended with a 'bang', demonstrating the perseverance and determination of Knipling and his colleague Dr. Raymond C. Bushland. There is a recent renewed interest in SIT for mosquito and disease control as an alternative to chemical control throughout the United States as well as globally. A brief history of the success of SIT against the primary screwworm fly is presented to discuss where we have been and where we hope to be going. As one of three Plenary Session presentations covering a wide breadth of SIT theories and research developments, we as a science stand on the cusp of a paradigm shift projecting mosquito control into the future in this ever-changing world.

Introduction

The primary screwworm fly, *Cochliomyia hominivorax* (Coquerel) is an obligate parasite of living warm-blooded animals, most notably livestock and wildlife throughout the Americas. Originally it was found in tropical and subtropical areas from the southern United States to Chile until eradication programs using the sterile insect technique (SIT) eliminated this pest from the United States, Mexico and most of Central America. The historic economic losses to the cattle industry were staggering even in a 1950s economy and the losses to wildlife were immeasurable. This pioneering work was conducted by two USDA scientists, Dr. Edward F. Knipling and Dr. Raymond C. Bushland in a small laboratory located in Menard, Texas (Fig. 1). This is the amazing story of scientific discovery, perseverance, and the lasting impact on society.

Biology and Ecology

The female screwworm fly lays eggs in clusters of 100-300 at a time in wounds of warm-blooded animals at 2- to 3-day intervals, and she can produce up to 3000 eggs in her lifetime. The female fly will feed around the wounds of animals acquiring the necessary protein for the next batch of eggs. These wounds include lacerated or bloody areas associated with fighting, barbed wire, scratches, castration, dehorning, branding, tick bites, navels of newborn animals or body openings with fetid odors. The eggs hatch after 12- to 24-hours and the larvae feed head down in the living tissue of the host for 4- to 12-days before dropping from the host to pupate. The larvae pupate on the ground and pupation may take as little as 1-week or up to 3 months, depending on the season, before the adult fly emerges. After emergence, the female flies will mate, but only once, which is the key to success for the sterile male control technique. There is no diapausing stage and screwworms can't survive the winter in cool temperate climates (Williams, 2010).

Figure 1.—The USDA laboratory moved from Menard, TX to Kerrville, TX where E.F. Knipling and R.C. Bushland embarked on their pioneering work on the primary screwworm fly. Pictured are USDA scientists, Dr. Steve Skoda (l) and Dr. Beto Perez de Leon (r) of the Livestock Insect Laboratory, Kerrville, TX (photo by Dr. Bill Donahue).

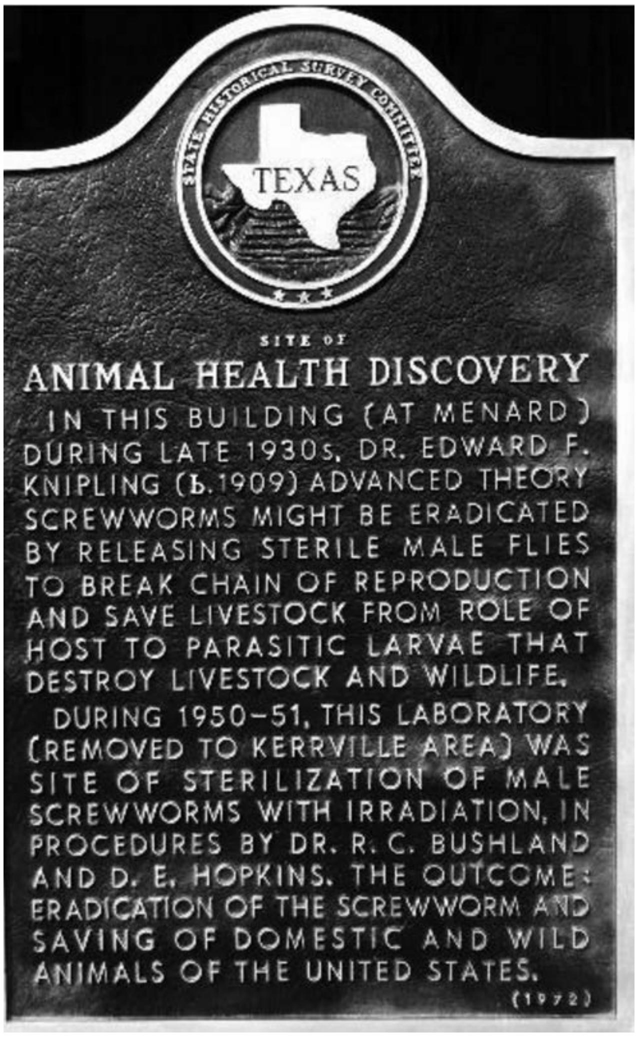

Figure 2.—Enlargement of the Texas State Historical Survey Committee Plaque in front of The USDA Laboratory now in Kerrville, TX.

Synthetic Insecticides – The Silver Bullet?

In 1962 Rachel Carson published her pivotal yet controversial book, Silent Spring (Carson, 1962). Much of the dialog is still being played out today with regards to human and environmental health associated with synthetic insecticides. Insecticide resistance was a focal point in insect pest population control as quoted by Carson, "The truth, seldom mentioned but there for anyone to see, is that nature is not so easily molded and that insects are finding ways to circumvent our chemical attacks on them." Carson went on to state that DDT and "The Age of Resistance" has led to many insects developing multiple resistant strains everywhere chemical control has been practiced. Robert van den Bosch, an entomologist with the University of California stated "Modern insecticides are biocides – the root cause of the insecticide treadmill". He agreed with Carson and was a huge proponent of biological control to manage insect pests.

In 1937, Dr. Edward Knipling first asked the question, what if I could mass rear screwworm flies, irradiate, and sterilize the males, and release them into the wild (Arnold, 2021)? He knew the females would not lay viable eggs thus dramatically affecting the reproductive potential of the pest population causing the numbers to continuously decline. One could continuously release sterile male flies to out compete the wild males and win the war of attrition! This method would harness the natural drive to reproduce without using noxious chemicals or insecticides. Knipling's theory was decades ahead of contemporary thinking regarding insect control and insecticide resistance.

Rachael Carson went on to state that "If Darwin were alive today the insect world would delight him with its impressive verification of his theories of the survival of the fittest". Certainly, with today's understanding of insecticide resistance, gene mutations, and population genetics we can understand how controlling pest insect species can be problematic. Carson states in the final chapter of Silent Spring, "The Other Road" that "Through all these new, imaginative, and creative approaches to the problem of sharing our earth with other creatures, there runs a constant theme, the awareness that we are dealing with life – with living populations and all their pressures and counterpressures, their surges and recessions."

C.F. Hodge back in 1911 calculated the reproductive potential of a pair of house flies and determined that from the first of April through the end of August, that under ideal conditions, the pair of flies would be the progenerators of over 191 quintillion adult flies, enough to cover the earth 2.5 feet deep (Hodge, 1911). I'm not certain of his model, however, this demonstrates the phenomenal reproductive potential of many pest insect populations. In a Science Special Section entitled "The Rise of Resistance", it was stated that "Breakthroughs in chemistry and molecular biology may provide many new pesticides and novel methods for pest control, but there is a considerable chance that the evolution of pest resistance will outpace human innovation." (Ash, 2018).

Because insecticide resistance is genetic-based and there are four iterations (metabolic, target site, reduced penetration and behavioral), the previous statement has a haunting tone. However, a new insect control strategy had to develop to 1) reduce the reproductive potential of the pest population, 2) 'break' the life cycle, 3) target the most vulnerable life stage, and 4) use a least toxic approach when selecting a control agent. Knipling figured this out in 1937, refining the sterile insect technique, laboratory and field testing, and eventual success in the 1950s. Rachel Carson discusses Knipling's success in the final chapter of "Silent Spring". Why didn't the Sterile Insect Technique (SIT) take-off?

The Single Original Idea of the 20th Century

Young Edward Knipling started his entomology career as a youngster, hand-picking screwworm maggots out of the wounds of the family cows, eventually landing a job at a rundown U.S. Department of Agriculture laboraory in Texas (Distallations 2022). He decided to study screwworm flies in detail – looking for any weakness that could destroy them. His work started in 1937 (Baumhover, 2002';

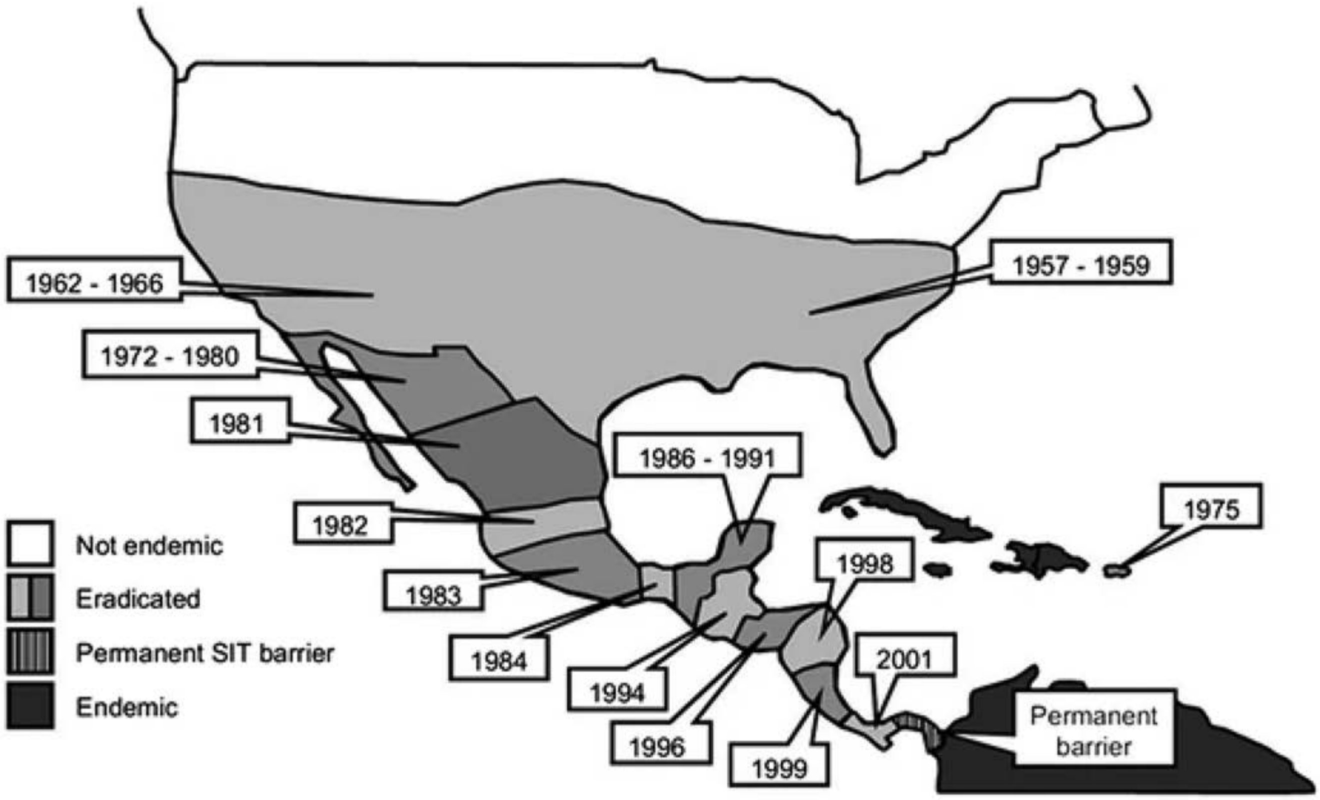

Figure 3.—Primary screwworm fly distribution map showing the progressive eradication efforts from 1957 through 2001.

Distillations 2022), however, from 1939 through 1945 his efforts were diverted due to the Dust Bowl and World War II, screwworms would have to wait.

Knipling decided not to request any Federal Funding for his research, knowing that taxpayer dollars would open his work up to public scrutiny. Feelings that know-nothing members of Congress absolutely loved to pounce on 'pointy-headed' scientists whom they assumed were wasting government cash. The era of the Golden Fleece Award would bring to light research that was deemed wasteful and Knipling's work seemed ripe for ridicule, "what kind of pervert studies screwworm sex?"

In early 1950s, Bushland produced the first viable sterile male screwworms using a nearby hospitals X-ray machine. He would smuggle tubes of adult male screwworms in and out of the hospital, bringing them back to the laboratory to evaluate viability and vigor. By 1951 the team led by Knipling and Bushland was ready to begin field testing. The site chosen was Sanibel Island off the coast of Florida and the field testing began. By the end of the season, it was deemed a complete flop, what went wrong? Wildlife reservoirs, not enough isolation, too few sterile males, not enough sterile males released, drop frequency too long, what? As they pondered their failure, Knipling was very happy that he kept his mouth shut!

Knipling and Bushland had perseverance and faith in their work. In 1954 a new test was planned for the island of Curaçao, Netherlands Antilles near Venezuela. They began to mass rear screwworms for sterilization estimating they would need 170,000 per week for the duration of the testing period. An artificial diet was formulated (lean ground beef, blood, water, and 2% formaldehyde) that accomplished the task, but the rearing facility needed to be down wind and far enough away from people due to the obnoxious odor emanating from the facility. They would deploy these sterile flies using a fixed wing aircraft and flying precise grids to ensure accurate dispersal. This time the results were stunning! The screwworm population collapsed within 9-months. The total cost of the project was a mere $20,000 and no pesticides were used in the control program.

After this stunning success, Knipling felt confident enough to go public with his ideas about the sterile male technique and the control of the primary screwworm fly. The timing was perfect, screwworms were overrunning several southern states devastating whole herds of cattle plus wildlife. Ranchers were desperate for help, "so much that the know-nothings in Congress swallowed hard and gave the screwworm sex guy some cash." Given the vast difference in size between Curaçao and the southern United States, Knipling's team needed to build a much larger rearing facility to mass produce 200 million sterile flies every week! Again, aircraft were used to deploy the sterile male screwworm flies and the smell was so obnoxious that some pilots refused to fly for the program. Once the logistics were worked out the screwworm eradication program soared and by 1966 the primary screwworm fly was eradicated from the United States (Fig. 3).

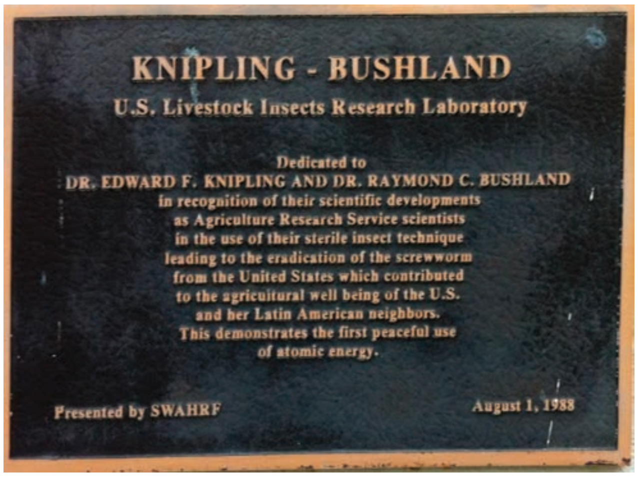

Figure 4.—Recognition of the scientific achievements of Dr. Edward F. Knipling and Dr. Raymond C. Bushland and the peaceful use of atomic energy.

Efforts continued to push the screwworm fly from Mexico through Central America to the Darien Gap and the Caribbean region, with a new rearing facility located in Panama that continues operations today. The facility cost was $77 million and took one and a half years to build and continues to release sterile males to prevent a resurgence of this pest back into Central America and North America.

In 1968 Dr. Edward Knipling was invited to an international conference entitled Insects Potentially Controlled by SIT, Sterile-Male Technique for Eradication or Control of Harmful Insects, International Atomic Energy Agency and Food and Agriculture, Vienna, 27-31 May 1968. The first presenter was E.F. Knipling, USDA-ARS, and his talk outlined the status of SIT for the potential control of quite a few pest insects on which research information was available and which were ready for field testing. These species included 10 species of fruit flies including the Mediterranean fruit fly, as well as tsetse fly, onion fly, horn fly, human bot fly, house fly, boll weevil, coddling moth, Japanese beetle, hornworm, cabbage looper, tobacco bud worm, cotton boll worm, rice stem borer and mosquitoes – *Aedes aegypti, Ae. scutellaris, Culex pipiens,* and *Anopheles gambiae*. The question begs to be asked, if Rachel Carson and the participants and attendees of the International Atomic Energy Agency and Food and Agriculture conference and other scientists around the world saw the incredible success and potential of SIT, why didn't this scientific breakthrough (Fig. 4) become more mainstream?

However, the battle of the screwworm fly continues as there was an outbreak in 2016 in the Florida Keys affecting the endangered Florida Key deer (Entomology Today 2018). Efforts to mobilize a team of scientists were immediate and USDA-ARS scientists spearheaded the effort, including Dr. Steve Skoda (Fig. 1) of the Kerrville Laboratory who was mentioned earlier in this presentation.

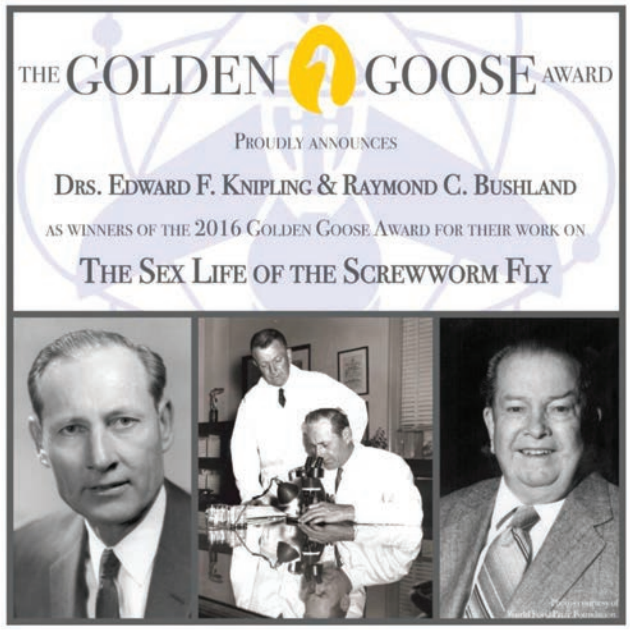

Figure 5.—Despite decades of ridicule for the focus of their work, two researchers behind the study of the sex life of the screwworm fly were saluted at a September 2016 award ceremony.

The Golden Goose Award

The Golden Goose Award (Fig. 5) honors scientists whose federally funded research may have been considered silly, odd, obscure when first conducted, but resulted in significant benefits to society as stated in a press release dated 22 June 2016. "Screwworm research may sound like a joke, but it isn't. It saved the livestock industry billions and is giving us a way to fight Zika," said Rep. Jim Cooper (D-TN), who first proposed the Golden Goose Award. "We should trust our scientists more than our politicians when it comes to research priorities." From an obscure theory in 1937 to the recognition of their brilliance and perseverance in 2016, Dr. Edward F. Knipling and Dr. Raymond C. Bushland finally got the recognition they deserved!

The Future

My entomology career has spanned 44 years and during that time I have been travelling "The Other Road" as Rachel Carson described in the final chapter of Silent Spring. Whereas many inroads have been achieved in insect control, many of our efforts have gone back to repeating previous mistakes. Insecticide resistance remains a serious problem especially with arthropod pests that transmit disease and misery to people and animals. Environmental and health issues are magnifying in an era of climate change, so what are the options? The hope for a silver bullet has faded, but the sterile insect technique as postulated and proven by Knipling and Bushland just might be the Holy Grail of insect control strategies. Certainly, the sterile insect techniques offer a proven record of success over the last 75 years and have a tremendous potential for the future of a wide range of insect control strategies. Endangered wildlife such as the Florida Key deer and Hawaiian honey creepers need our attention now and we must use all our resources and knowledge to keep them and ourselves from extinction.

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Effect of bed bug (Hemiptera: Cimicidae) aldehydes on efficacy of fungal biopesticides**

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Introduction

Bed bugs (Hemiptera: Cimicidae) are among the most difficult to control of the urban pests. Insecticide resistance is widespread within bed bug populations, with pyrethroid resistance commonly detected in field populations (Romero 2018). The entomopathogenic fungus *Beauveria bassiana* (Bals. – Criv.) Vuill. (Hypocreales: Cordycipitaceae) has been introduced as one possible solution for the management of bed bugs, *Cimex lectularius* L. (Hemiptera: Cimicidae). Currently, one biopesticide is registered for bed bug control in the United States - Aprehend (ConidioTec LLC, Centre Hall, PA, USA). This product contains a 2% suspension of *B. bassiana* spores in oil and has been found to be effective (>94% mortality) against both insecticide resistant and susceptible bed bug populations (Barbarin et al. 2017). Additionally, *B. bassiana* can be transferred horizontally from exposed to unexposed conspecifics (Barbarin et al. 2012, Aak et al. 2018).

Bed bugs produce four volatile aldehydes [(*E*)-2-hexenal, 4-oxo-(*E*)-2-hexenal, (*E*)-2-octenal, and 4-oxo-(*E*)-2-octenal] that function as alarm and aggregation pheromones (Siljander et al. 2008, Gries et al. 2015, Choe et al. 2016, Ulrich et al. 2016). Two of these aldehydes, (*E*)-2-hexenal and (*E*)-2-octenal, inhibited the growth of *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) in culture and resulted in a reduction of mortality in bed bugs exposed to conidia (Ulrich et al. 2015).

The objective of our study was to determine if the presence of bed bug aldehydes impact the efficacy of *B. bassiana*. First, two commercial biopesticide formulations containing *B. bassiana* were used to determine if the presence of bed bug aldehydes (exuviae or synthetic blend) impacted the effectiveness of *B. bassiana* for bed bug control. Second, the synthetic aldehyde blend was used to determine if exposure to these compounds impacted the rate of growth of *B. bassiana* in culture. This study extends our knowledge of interactions between *C. lectularius* and *B. bassiana* by investigating the possible defensive function bed bug aldehydes may have against entomopathogenic fungi.

Methods

To determine the effect of the bed bug aldehyde blend on the effectiveness of *B. bassiana* for bed bug control, the following experiments were conducted. First, groups of ten adult bed bugs (*C. lectularius*, ≈10 d postbloodmeal) were assigned to one of the following four treatments: (1) fungus only, (2) fungus + exuviae, (3) fungus + synthetic aldehydes, and (4) untreated control. Petri dishes (60 mm diameter) were lined with filter paper (Whatman #1). To these plates, one of two commercial products containing *B. bassiana*, Aprehend or BotaniGard 22WP (Laverlam International, Butte, MT, USA), was applied to achieve a concentration of 3.9×10^5 spores/cm^2 (BotaniGard 22WP) or 2.3×10^7 spores/cm^2 (Aprehend). One milliliter water was applied to the filter paper disc for the untreated control. The treatment dishes were kept uncovered and allowed to dry. Each group of bed bugs placed into the treatment dish for one hour and then transferred to a 20-ml scintillation vial containing a folded piece of clean filter paper (50 × 10 mm), which served as a resting platform. For treatment #2 (fungus + exuviae), the vial contained exuviae (≈50) of mixed age and stage (3rd to 5th instar) collected from a *C. lectularius* colony vial. For treatment #3 (fungus + synthetic aldehydes), the vial contained a 50 exuviae equivalent aldehyde blend dose (based on the amounts found in freshly shed 5th instar exuviae of *C. lectularius*; Dery et al. 2020) dissolved in 40 µl acetone. The synthetic aldehyde blend was applied to a disk of absorptive matting attached to the underside of the vial cap. Mortality in each group was recorded daily for 15 d. Nine replications were conducted for each of the two formulations of *B. bassiana*.

To determine the effect of bed bug aldehydes on fungal growth, the rate of hyphal growth in culture was measured using a method adapted from Inglis et al. (2012). Potato dextrose agar plates (100 × 15 mm) were inoculated in the center of the plate with 1 µl of a 4.95×10^7 spores/ml *B. bassiana* suspension in 0.01% Tween-80. Each of the plates were treated with 100 or 25 exuviae equivalent amounts of the *C. lectularius* aldehyde blend dissolved in 40 µl acetone [based on the amount of aldehydes found in 5th instar exuviae; Dery et al. (2020)]. The aldehyde blend was applied to a sterile filter paper disc (16 mm diameter) on the inner lid of the inverted Petri dish. The control group received the spore suspension and clean acetone. The plates were sealed and stored at 26°C in the absence of light. The radial growth was measured daily for 15 days by averaging two perpendicular measurements from the center of the plate to the edge of visible hyphae. Ten replications were completed for each group.

Results and Discussion

The presence of bed bug aldehydes reduced/delayed mortality following exposure to *B. bassiana*. This effect was especially apparent when bed bugs were exposed to BotaniGard, a formulation of *B. bassiana* used for the control of garden/agricultural pests. On day 15, these bed bugs experienced 61–62% mortality when aldehydes (exuviae or synthetic blend) were present, a significant reduction (log-rank test, $P < 0.001$) compared with 97.7% mortality in fungus exposed bed bugs without additional aldehydes. When bed bugs were treated with Aprehend, we observed a significant difference between the two sources of bed bug aldehydes. The presence of bed bug exuviae in the vial did not affect the mortality trend of the treated bed bugs that resulted in 100% mortality within 7 days. However, when a synthetic aldehyde blend was present in vials, the fungus-treated bed bugs experienced delayed mortality. Bed bugs exposed to Aprehend alone reached 100% mortality by day 6, while those exposed to the synthetic aldehyde blend experienced 63% mortality by day 6. These bed bugs eventually reached 94% mortality at day 15. This difference may have been a result of a lower concentration of aldehyde in the exuviae vials. Exuviae of unknown age were used as an aldehyde source, which volatilize from the exuviae over time (Choe et al. 2016). As a result, there may have been a lower amount compared with the synthetic aldehyde blend, which was based on freshly shed exuviae. Our results might provide some insight into earlier reports on fungal biopesticides and bed bugs. For example, Aak et al. (2018) found a lack of correlation between the level of bed bug aggregation and mortality caused by the horizontal transfer of *B. bassiana*. Our results support suspicions by Aak et al. (2018) that this could be a result of a higher concentration of aldehydes within larger aggregations, offsetting a greater chance of fungal transfer among bed bugs within a larger aggregation.

The growth of *B. bassiana* in culture was reduced or delayed by the presence of the bed bug aldehydes blend. At day 7, the diameter of *B. bassiana* was significantly reduced (Dunn's Multiple Comparisons, $P < 0.05$) when exposed to both low and high aldehyde concentrations compared with the control. However, the diameter of the fungus at the lower dose aldehyde treatment (0.168 mg; 25 exuvia equivalents) had reached an equivalent diameter as the control group by day 15, whereas the fungal growth was still significantly reduced in the higher dose aldehyde treatment (0.673 mg; 100 exuvia equivalents). This finding indicated that the antifungal effect of the aldehydes on fungal growth was dose-dependent. Ulrich et al. (2015) reported that the growth of *M. anisopliae* in culture was completely inhibited when >0.5 mg of (*E*)-2-octenal or (*E*)-2-hexenal were present, but fungal growth occurred at lower aldehyde concentrations (<0.5 mg). The lack of complete inhibition of *B. bassiana* in our experiments indicated that the aldehyde blend did not completely prevent the spores from germinating at the concentrations tested.

The presence of bed bug aldehydes may suppress or interfere with the effectiveness of *B. bassiana* in the field. The impact of aldehydes on *B. bassiana* may even be a plausible mechanism of resistance, if this fungus is widely used for bed bug control. The effects of the aldehydes on the efficacy of fungal biopesticides could be largely reduced if used before large bed bug infestations are established. The early detection and management of bed bugs would prevent the formation of large aggregations and the production/accumulation of high amounts of antifungal aldehydes in the harborage sites, minimizing the potential effect of aldehydes on the efficacy of the fungus.

Conclusions

Our results show that aldehydes associated with bed bugs can impact the effect of *B. bassiana* on bed bugs. The presence of bed bug aldehydes in the headspace reduced or delayed the resulting mortality following exposure to *B. bassiana*. The varied impact of bed bug aldehydes on *B. bassiana* was dependent on how the fungus was formulated. Additionally, the presence of synthetic aldehydes in the headspace slowed the growth of the fungus in culture. Further work under field settings is warranted to investigate the effect of bed bug aldehydes on the performance of fungal biopesticide products under a more realistic setting.

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Modeling mosquito abundance near rice fields in Placer County

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Abstract

In Placer County, *Culex tarsalis* is one of the main vectors of West Nile virus (WNV). Climate conditions, such as temperature, and ideal larval habitats (standing freshwater), increase the abundance of this species. Rice cultivation provides *Cx. tarsalis* a suitable environment for larval development. An analysis was conducted to examine whether temperature and the acreage from rice fields predict *Cx. tarsalis* abundance using geospatial mapping throughout Placer County. Data were collected from CO_2 traps dispersed throughout the county and buffers were mapped to show the distribution of rice acreage over 13 years. The strongest relationship between rice acreage and *Cx. tarsalis* abundance was found with combined conventional and organic rice field acreage within a 4-mile radius buffer of the CO_2 trap. These results illustrate how the cultivation of crops can affect mosquito abundance.

Introduction

As the earth warms due to anthropogenic activities, there is widespread concern for increasing vector populations and vector-borne diseases (IPCC 2018). Weather can influence the rate of development of mosquitoes and the rate of transmission of viruses (Paz 2015). As climate warming has become more evident, with elevated levels of carbon dioxide associated with increasing temperatures, California is at greater risk for the invasion of non-native insects (Trumble and Butler 2009). There are limited resources which discuss the relationship between climate change and the incidence of vector-borne diseases such as West Nile virus (WNV). A connection between temperature patterns and the spatiotemporal patterns of WNV has been described in California (Hartley et al. 2012).

Currently, there are over 170 mosquito species that have been identified in the United States and over 30 mosquito species identified in Placer County (AMCA 2022, PMVCD 2020). Mosquito-borne pathogens recorded in Placer County include WNV, Western equine encephalomyelitis virus, dog heartworm, St. Louis encephalitis virus and malaria, with WNV being the most predominant (PMVCD 2022). In the United States, there are 65 mosquito species that have been found infected with WNV (Nasci et al. 2013). *Culex tarsalis*, *Cx. pipiens*, *Cx. quinquefasciatus* and *Cx. stigmatosoma* are the primary vectors of WNV in California (Barker et al. 2003).

Rice cultivation in Placer County fluctuates with rice value, urban development, water availability, and other influences. In 2018, there was a 25% increase in crop value, with rice acreage ranked highest among crops (County of Placer 2019). There is an increase in mosquito abundance during the early stages of rice cultivation (Mogi and Miyagi 1990), which decreases as rice plants mature (Diuk-Wasser et al. 2006). In Placer County, the proportion of positive WNV infected mosquito pools was greater near conventional rice fields compared to organic rice fields (Saretha and Sorensen, unpublished). In Florida, temperature, humidity, and precipitation were found to significantly predict mosquito abundance when lagged by 1–3 weeks (Steck et al. 2022). The goal of our study was to determine whether temperature and humidity at the time of trapping or with a lag of 1–3 weeks can predict female *Cx. tarsalis* abundance when combined with trap location and adjacent rice acreage.

Methods

Placer Mosquito and Vector Control District (PMVCD) datasets between 2009 and 2021 were used to calculate the acres of organic and conventional rice fields within a ½, 1, 2, 3, 4, 5, 6, 7, and 8 mile radius of CO_2 mosquito trap sites for each year. The datasets included a list of all routine CO_2 trap sites throughout the county that were operated weekly for approximately 38 wks during the mosquito season. Abundance herein was defined as the number of female *Cx. tarsalis* per trap per night. Additional traps were placed at other areas per request to monitor mosquito activity.

The dataset included the number of rice fields, rice acreage, and the method of cultivation (organic or conventional) as determined by visual cues and by talking to rice growers in Placer County. With the land use datasets provided, geospatial files were imported onto ArcGIS Online software (ArcGIS, Environmental Systems Research Institute, Inc., Redlands, CA) for the years 2009 to 2021. The map was filtered to only display the

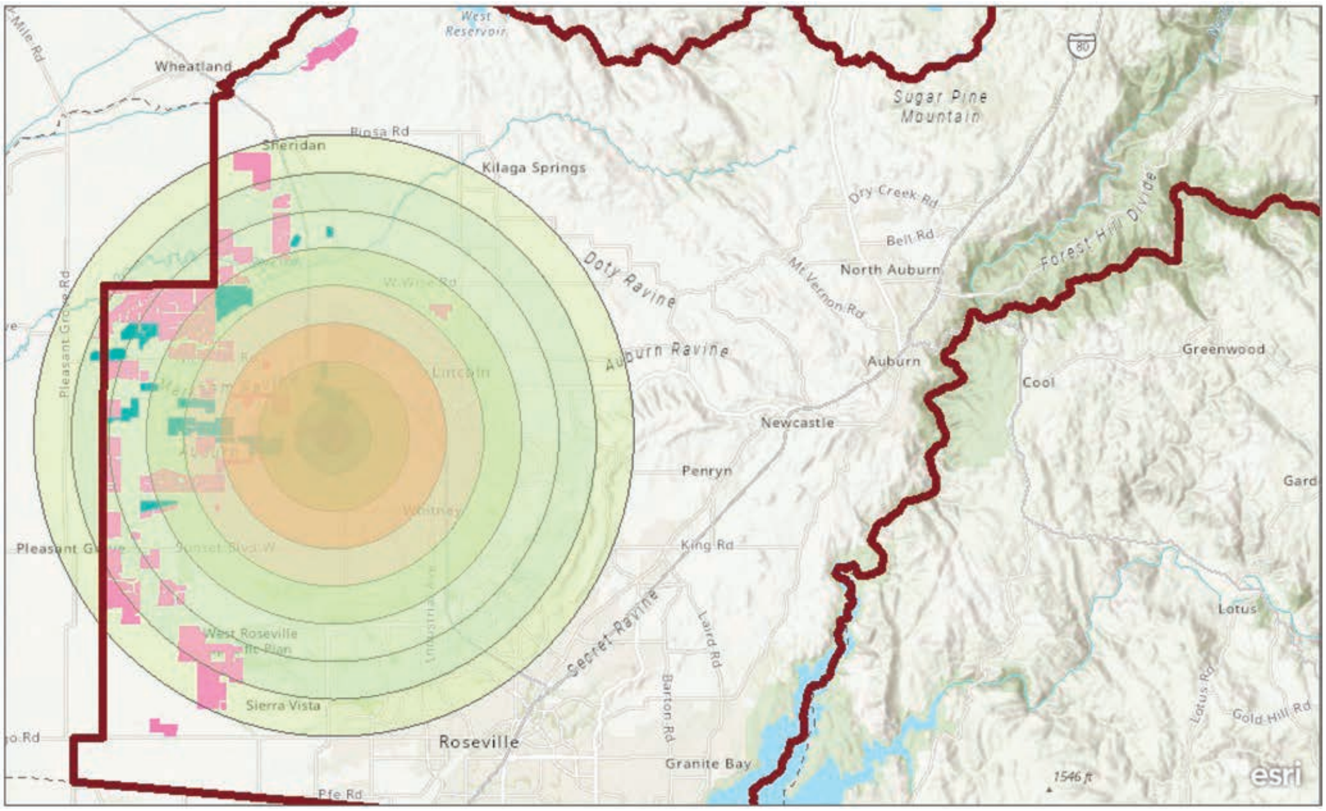

Figure 1.—Conventional (pink) and organic (blue) rice fields in Placer County from one example year (2020). Circles depict nine different radii around an example trap site (½-and 1-8-mile radii). The acreage of conventional and organic rice fields was calculated for each combination of trap site and buffer distance.

conventional and organic rice fields and their represented acreage per year. Similarly, the geospatial data for the CO_2 traps were imported for the same years. The rice area then was calculated in acres within ½, 1, 2, 3, 4, 5, 6, 7, and 8 mile radii from each trap site (Figure 1).

Weather data recorded every 20 minutes at Lincoln Airport, located in Placer County, were aggregated into daily and weekly average, maximum, and minimum temperature and humidity for the years 2009-2021. Precipitation was not included due to insufficient data.

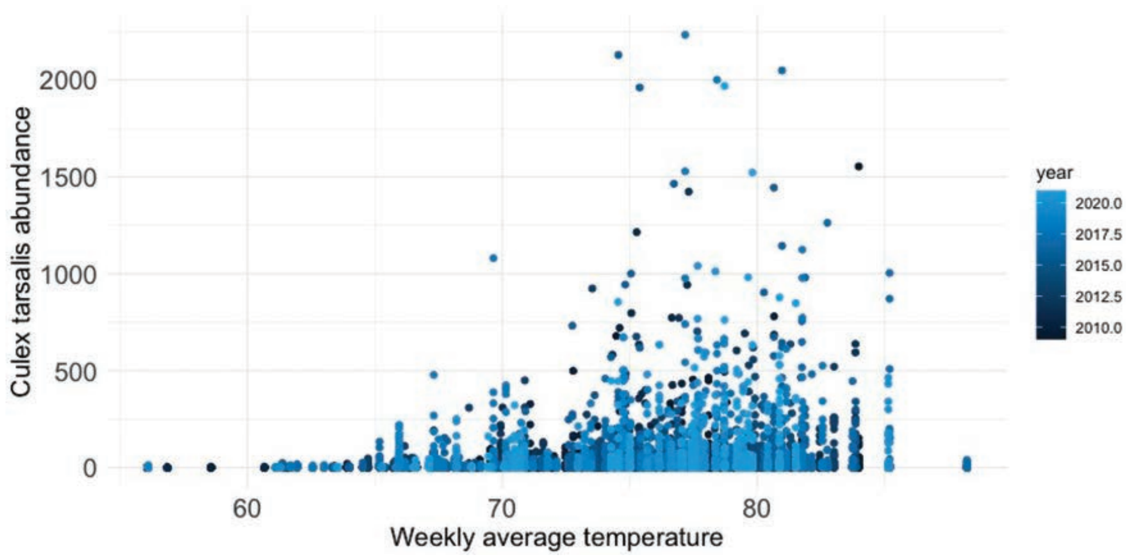

Figure 2.—Scatterplot displaying *Cx. tarsalis* abundance in Placer County, CA, plotted as function of weekly average temperature, in °F, from 2009-2021. The data point shade of blue becomes lighter for the most recent years.

Table 1.—Linear Regression Model (LM) results for the abundance of *Cx. tarsalis* as a function of conventional, organic, or combined rice field acreage within increasing buffer radii.

Mile Radius	Rice Field Crop	P-Value	Adjusted R-Squared
0.5 Mile Radius	Conventional	8.32E-08	0.0414
	Organic	<2e-16	0.1038
	Conventional + Organic	1.47e-07	0.1395
		<2e-16	
1 Mile Radius	Conventional	<2e-16	0.1883
	Organic	<2e-16	0.1497
	Conventional + Organic	<2e-16	0.2722
		<2e-16	
2 Mile Radius	Conventional	<2e-16	0.2667
	Organic	<2e-16	0.1509
	Conventional + Organic	<2e-16	0.2721
		0.0156	
3 Mile Radius	Conventional	<2e-16	0.3474
	Organic	<2e-16	0.1832
	Conventional + Organic	<2e-16	0.3477
		0.259	
4 Mile Radius	Conventional	<2e-16	0.3933
	Organic	<2e-16	0.1852
	Conventional + Organic	<2e-16	0.3924
		0.91920	
5 Mile Radius	Conventional	<2e-16	0.3733
	Organic	<2e-16	0.1961
	Conventional + Organic	<2e-16	0.3779
		0.0163	
6 Mile Radius	Conventional	<2e-16	0.3611
	Organic	<2e-16	0.1768
	Conventional + Organic	<2e-16	0.3708
		0.000902	
7 Mile Radius	Conventional	<2e-16	0.3274
	Organic	<2e-16	0.1643
	Conventional + Organic	<2e-16	0.3458
		1.19e-05	
8 Mile Radius	Conventional	<2e-16	0.2508
	Organic	<2e-16	0.2152
	Conventional + Organic	1.93e-10	0.2612
		0.00143	

Additional variables were created to lag antecedent weather data for 1, 2, and 3 weeks. *Culex tarsalis* immature development can take as few as 10 days from larval hatch to adult emergence and is temperature dependent (Reisen 1995). Including time lagged temperature and humidity may distinguish the delayed effects of temperature and humidity on abundance through faster or slower development (Mordecai et al., 2019).

Restrictions on the data were made to remove mosquito trap locations that had less than 5 collections annually or collections outside of the May through September rice growing season. The remaining months were not included because there ws no rice grown and temperatures were too low for mosquito development.

All analyses were conducted in R, version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). A multiple linear regression model was conducted to determine the relationship between *Cx. tarsalis* abundance and weather data (which included the average, maximum, and minimum of daily, weekly, and 1-3 lagged weeks of temperature and humidity) and the acreage of rice within ½, or 1–8 mile radii of conventional, organic, and both rice fields combined. A forward stepwise regression model was conducted for variable selection. Rice acreage was tabulated yearly.

Results

Each predictor was examined independently, and a forward stepwise regression was used to add variables which significantly improved the fit of the model for the data. Weather variables at the time of trapping or lagged 1 to 3 weeks and adjusted for trap site and surrounding rice acreage were not strong indicators of *Cx. tarsalis* abundance. Linear regression modeling showed a weak positive relationship between weekly average temperature and *Cx. tarsalis* abundance (Figure 2; $R^2 = 0.019$, $P < 2.2e^{-16}$, df = 13,881).

Because the model did not explain much of the variability for *Cx. tarsalis* abundance, the season was filtered into two sections, the first half, which included epiweeks 18-26 (~May – June) and the second half,

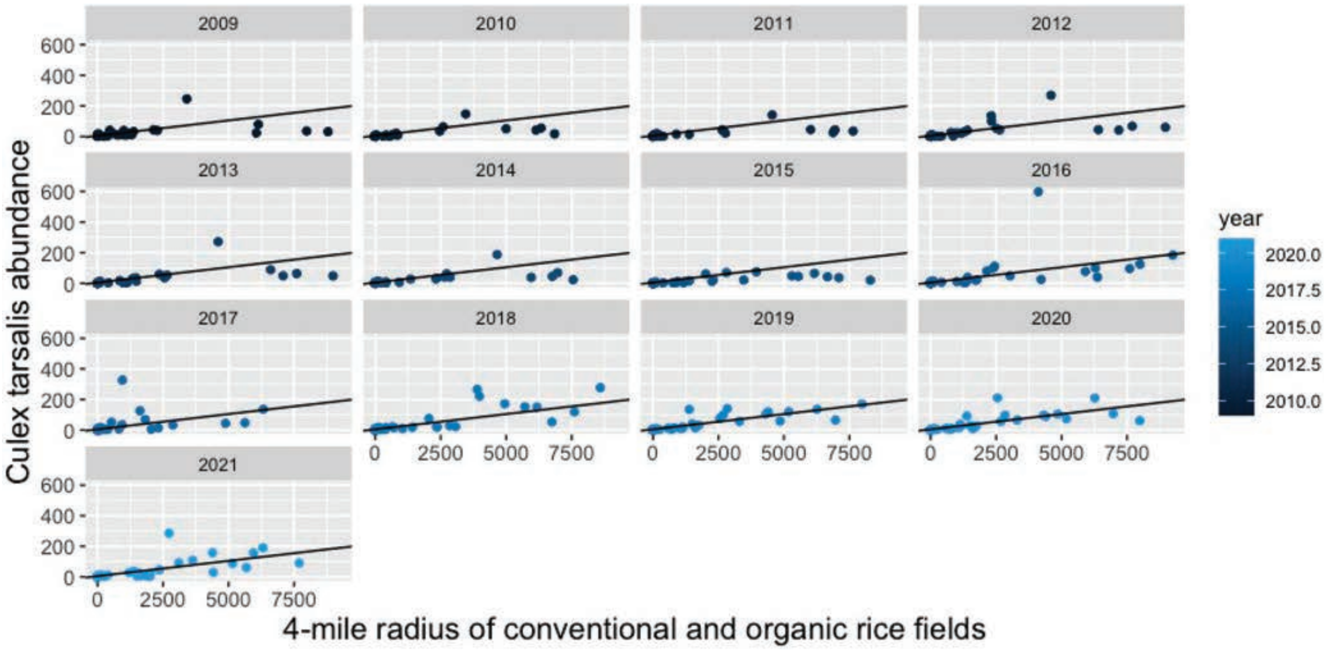

Figure 3.—Regressions of *Cx. tarsalis* females per trap night as a function of rice acreage within a 4-mile radius of a trap site, from 2009-2021.

epiweeks 27-40 (~July − September). The most significant relationship occurred during the first half of the summer between daily average temperature and *Cx. tarsalis* abundance ($R^2 = 0.027$, $P < 2.2e^{-16}$, df = 14,113).

When running the analysis for the rice fields, originally ½-, 1-, or 2-mile radii buffers were used to determine a relationship between rice acreage and *Cx. tarsalis* abundance. The adjusted R-squared value was small but increased as the buffer radii increased in size. Additional mile buffers were added to determine if there would be a stronger relationship for larger buffers. As anticipated, there was a stronger correlation of *Cx. tarsalis* abundance plotted as a cumulative function of rice field acreage as the radii increased from a half a mile to 4 miles (Table 1, Figure 3). When the model tested the 5-mile buffer, the adjusted r-squared values showed no significant difference and continued to steadily decrease for the remaining distance buffers (Table 1).

The same analyses were done for *Anopheles freeborni*. Similar to the *Cx. tarsalis* analysis, the models predicted that both 4- and 5-mile buffers of combined conventional and organic rice fields were the most predictive distances (adjusted $R^2 \sim 0.23$, $P < 2e^{-16}$, df = 657). Conventional rice fields acreage was the best independent predictor, however, combining conventional with organic rice fields slightly improved the model fit for both mosquito species.

Conclusion

A 4-mile buffer of combined conventional and organic rice fields best explained differences in *Cx. tarsalis* abundance among traps. Combining conventional and organic rice fields in the modeling analysis was a stronger predictor compared to conventional or organic fields considered separately. However, when comparing conventional to organic rice for *Cx. tarsalis* and *An. freeborni*, models indicated that conventional rice fields were the better predictor for the abundance of both species. A possible explanation for this could be that conventional farming methods and maintenance (the use of fertilizers and pesticides) resulted in more ideal mosquito habitat. Alternatively, effects of conventional rice may have been greater due to the overall greater number of acres of conventional rice grown in Placer County.

Because combined conventional and organic rice field acreage within a 4-mile radius was a strong predictor for *Cx. tarsalis* and *An. freeborni* abundance, both mosquito species may disperse 4-miles distance from their emergence sites (Figure 4). Operational implications for this finding include increased focus on surveillance, treatment, and pathogen detection within a 4-mile radius from rice fields.

Although the rice field acreage was a strong predictor for *Cx. tarsalis* abundance, temperature and humidity data showed a weak relationship with *Cx. tarsalis* abundance. The non-significance of the lagged and non-lagged weather variables was unexpected, as it was anticipated that there would be a significant relationship between temperature data and *Cx. tarsalis* abundance because of faster generation times, increased reproduction, or increased survival. Possible reasons weather variables were not significant could be that the relationships between *Cx. tarsalis* abundance and weather variables were non-linear, or that other factors were more important determinants of mosquito abundance. Additionally, it could be that humidity was not a predictor for *Cx. tarsalis* host-seeking

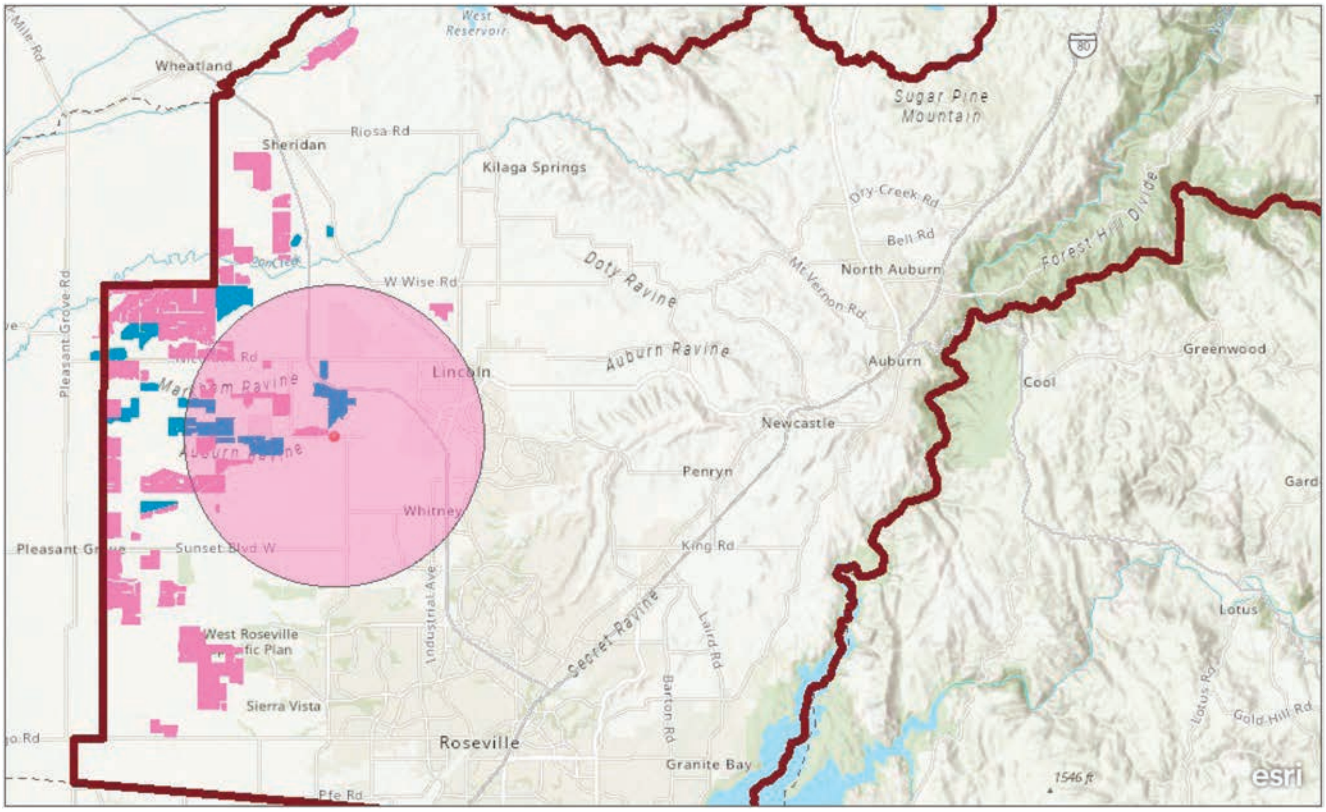

Figure 4.—An example mosquito trap site in western Placer County, CA, showing acreage of rice fields within 4-mile buffer of the trap site in 2021.

abundance because elevated humidity is not common in Placer County. Additionally, variation in humidity at the location where temperature and humidity was measured (Lincoln Airport) likely differed from humidity in and around the wet rice fields. In Florida, where elevated humidity is very common, a similar study found that humidity was a strong predictor of the abundance of several mosquito species (Steck et al. 2022). Our finding of a stronger correlation with weather and abundance earlier in the season rather than later in the season could be because once it's late in the season, the mosquito abundance could have reached its upper threshold for population abundance, temperatures could be higher than ideal, or other factors could be stronger drivers of mosquito abundance.

Our conclusion that the abundance of host seeking *Cx. tarsalis* mosquitoes is best predicted by rice acreage within a 4-mile radius has important implications for day-to-day operations of mosquito control professionals. This finding suggests that surveillance, control, and public outreach efforts could be targeted on this radius around rice habitat. These results add to the existing body of literature showing how certain crops can increase the abundance of certain mosquito species, which can impact the transmission of mosquito-borne diseases. Assessing CO_2 trap placement and mosquito abundance is important for estimating regional outbreak potential and to implement strategies to control for *Cx. tarsalis* abundance.

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Socioeconomic and environmental factors associated with the risk of dengue fever incidence in Guatemala

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Introduction

Dengue fever is one of the most prevalent mosquito-borne diseases, with about half of the world's population at risk of dengue virus infection (WHO, 2022). dengue fever is a mosquito-borne illness that infects 390 million people annually (Bhatt et al. 2013). dengue is widespread in Central America including in Guatemala (Signor et al. 2020). One of the principal vectors for dengue virus is the mosquito *Aedes aegypti*. *Aedes aegypti* was considered eradicated from Guatemala in 1959 but has been reintroduced, and since then dengue outbreaks have occurred.

Dengue fever outbreaks first reemerged in Guatemala in the late 1970s (Ponciano et al. 2019, Signor et al. 2020). The second outbreak did not occur until the rainy season of 1987 in Escuintla, Southern Guatemala. Since then, outbreaks have been occurring more often and at increased incidence rates with the largest dengue outbreak occurring in 2010 (Ponciano et al. 2019). In California, the vector *Ae. aegypti* has been introduced relatively recently (Gloria-Soria et al. 2014) so now some regions of California could be at risk for dengue fever outbreaks. One reason to investigate dengue cases in Guatemala is to understand factors associated with dengue in a relatively nearby region where both *Ae. aegypti* and dengue fever are widespread. The objectives of our study were to investigate socioeconomic and environmental variables associated with dengue fever incidence in Guatemala.

Few studies have been conducted on dengue in Guatemala, yet there are typically tens of thousands of cases per year (Wilson et al. 2002). The objectives of this study were to investigate socioeconomic and environmental variables associated with dengue in Guatemala. Few studies have been conducted on dengue in Guatemala, yet there are typically tens of thousands of cases per year. This study differentiates itself from previous studies by exploring the interplay of climatic factors (temperature and precipitation) and socioeconomic factors (Maya population, economic activity, literacy, attending school, population density, urban population, indoor plumbing, and internet use) as risk factors at the municipality level. Specifically, the relationships between Maya population and dengue fever incidence, as well as between internet use and dengue fever incidence have not been explored. Additionally, this is the first study to assess elevation as a predictor of the zero-inflation in dengue fever incidence in Guatemala. Central American countries are relatively close to the United States, and understanding factors associated with the dengue cases in a country in close geographic proximity can help us prevent dengue in California. Therefore, knowing the variables that best explain dengue cases in Central America may offer relevant information for other neighboring regions like the United States where dengue cases could emerge.

We hypothesized that population density, absence of indoor plumbing, urban population, temperature, and precipitation would be positively associated with dengue cases, while school attendance, internet use, literacy, Mayan population, economic activity, and elevation would be negatively associated with dengue cases. Furthermore, we hypothesized that elevation would be a significant predictor of the zero-inflation in dengue fever incidence in Guatemala. We also hypothesized that modeling an increase in temperature of Guatemalan municipalities by 1°C−2°C could increase dengue fever incidence in those municipalities.

Methods

Socioeconomic data were obtained from the Guatemala census from 2018, the most recent census (National Institute of Statistics, 2018). In 2018, the population of Guatemala was 14,901,286. Socioeconomic factors included population density, literacy, use of the internet, Mayan language speakers, economic activity, and attending school. Environmental variables included the elevation at the head of the municipality (county seat), minimum yearly temperature (°C), and total annual precipitation (mm). Latitude and longitude of the county seat were also obtained. Total dengue cases were obtained from the Ministry of Health of Guatemala for each of the 340 municipalities for 2017 and 2018, and these data were combined for the two years. Cases were classified as mild (dengue sin signos de alarma, classic) or severe (dengue grave, hemorrhagic fever) by clinical diagnosis, and only dengue (classic/sin signos de alarma, not hemorrhagic)

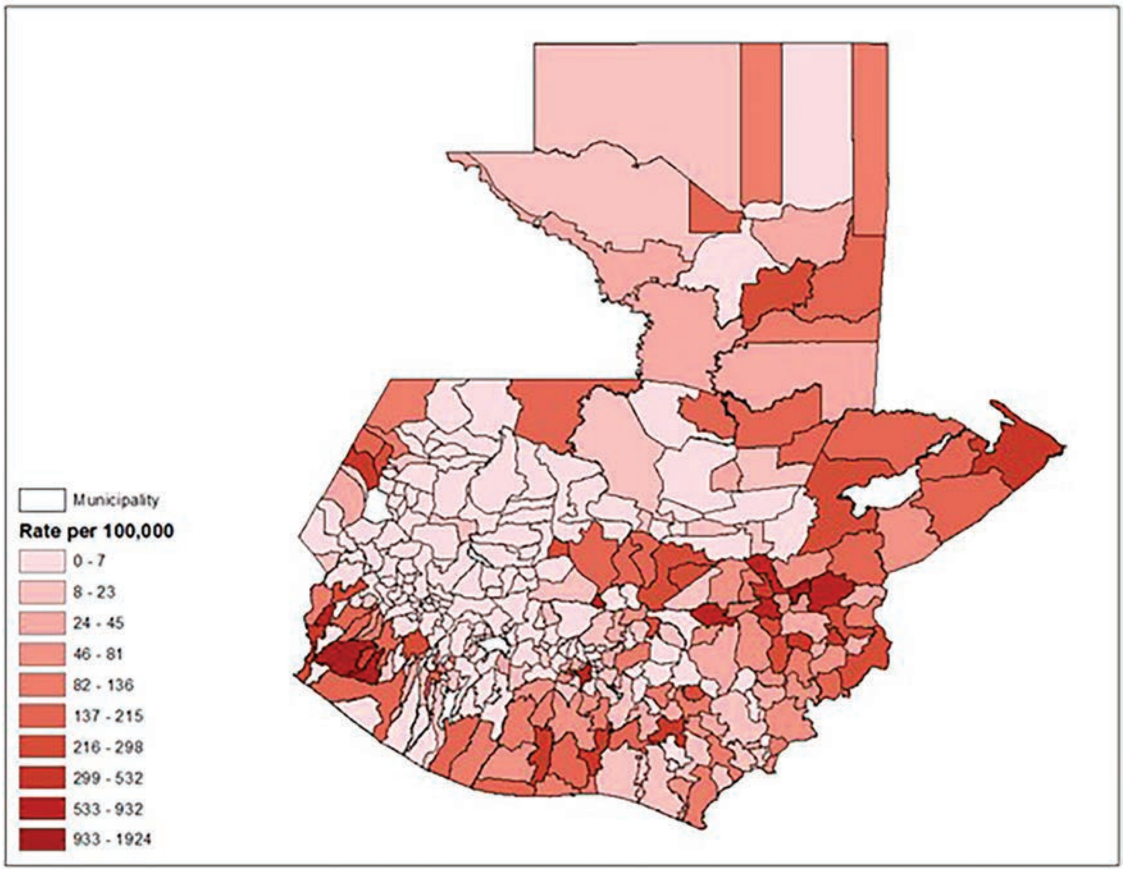

Figure 1.—Dengue fever Incidence Rates per 100,000 people in Guatemala by Municipality for the Combined Years of 2017 and 2018.

were included. Dengue hemorrhagic fever (DHF) is extremely rare and individuals with DHF were not included because they were likely to previously have had classic dengue fever. This was done to avoid overcounting of the same individuals leading to possibly voiding our assumption of independent data. Cases were reported from small clinics and hospitals and made available for each municipality for the years 2017 and 2018.

The relationship between our environmental and socioeconomic predictor variables and the dengue cases outcome variable was initially evaluated through chi-square tests of independence and one-way ANOVA. Chi-squared tests were used to examine whether or not each variable was related to dengue cases. One-way ANOVA was used to examine whether each socioeconomic or environmental variable was significantly related to a low, medium, or high number of cases of dengue in each municipality. Because dengue cases had a Poisson-type distribution, and many municipalities had zero cases, dengue cases were examined using zero-inflated negative binomial regression models (Sokal and Rohlf 1981). Three zero-inflated models were considered, which included the following: one model was for the relationship of socioeconomic variables and dengue, the second model was to examine environmental variables and dengue cases, and the third model combined the socioeconomic and environmental variables. For all models, elevation was considered as a predictor of zero-inflation. In addition, predicted rates of dengue fever incidence and adjusted confidence intervals were calculated after increasing minimum yearly temperature by 1°C and 2°C. The predicted rates were done for three municipalities with the lowest minimum temperatures, and three with the highest minimum temperatures. In addition, predicted rates of dengue were run for Guatemala municipalities, which contains Guatemala City, the capital with a high population density.

Results

There were 4,210 dengue cases for the year of 2017, and 7,414 dengue cases for the year of 2018, with a grand total of 11,624 dengue cases for the two-year combined period in Guatemala. The incidence rate of dengue in the municipalities of Guatemala for the two-year period (2017−2018) ranged from a low of 0 cases/100,000 individuals to a high incidence of 1,923 cases/100,000 people. Figure 1 illustrates the spatial pattern in the incidence rates. The municipalities with higher rates were more common on the periphery of the country, which tend to have lower elevations. The inland portion of the country has higher elevation and had lower to zero incidence rates.

Significant variables from the socioeconomic and environmental models were included in a combined model. For the combined model, population density, individuals

Table 1.—Zero-Inflated Negative Binomial Regression Model Including Socioeconomic and Environmental Variables, and Incidence Rate Ratios of the dengue Cases for Each Municipality (2017−2018). N = 340 municipalities; * p<0.05

Variables	IRR (95% CI)
Population Density	0.71 (0.59, 0.85)*
% Attending School	0.78 (0.62, 0.99)*
% Internet use	2.11 (1.44, 3.10)*
% Economically Active	0.97 (0.71, 1.34)
% Homes without Indoor Plumbing	0.90 (0.73, 1.11)
% Mayan	0.78 (0.61, 0.99)*
Min Temperature	4.11 (2.90, 5.84)*
Precipitation	1.14 (0.95, 1.36)
Intercept	0.0003 (0.0002, 0.0004)*
Inflate	**OR (95% CI)**
Elevation	15.96 (3.55, 71.85)*
Intercept	0.027 (0.004, 0.18)*
Model Fit	**Coefficient**
Alpha	2.17 (1.79, 2.63)

* p < 0.05, N=340.

attending school, internet use, and Mayan were found to be significantly associated with the incidence of dengue fever cases from 2017 to 2018 (Table 1). In addition, the environmental variable minimum temperature was significant and elevation was found to be a significant predictor of zero-inflation (Table 1).

Several factors increased the risk of dengue fever incidence. An increase of one SD in the percent of people using the internet in a municipality increased the risk of dengue fever incidence in the municipality by a factor of 2.11. The risk of dengue incidence in a municipality increased by a factor of 4.11 for every one SD increase in minimum yearly temperature in a municipality.

In addition, several factors decreased the risk of dengue. An increase of one SD in population density in a municipality reduced the risk of dengue fever incidence by a factor of 0.71. For every SD increase in the percent attending schools in a municipality, the dengue incidence decreased by a factor of 0.78. Finally, for every SD increase in the percent of Mayan population, the risk of dengue fever incidence in that municipality was reduced by a factor of 0.78.

The interactions between Mayan population and attending school were not significantly associated with dengue fever incidence and it was determined that the ZINB combined model (Table 1) without the interaction term was the best fitting model (lowest AIC/BIC).

Predicted Values and Adjusted Confidence Intervals

Predicted rates of dengue and corresponding confidence intervals were calculated for seven representative municipalities of the final model; this was done by increasing the temperature of those municipalities (Table 2). Predicted values and their corresponding confidence intervals were first calculated for when the all the variables as seen in Table 7 (including temperature) were fixed at their sample values (Table 2). The municipalities with the lowest minimum temperatures, San Jose Ojetenam, Concepción Tutuapa, and Sibilia all had incidence rates (IR) of 0 for dengue fever for the fixed temperature scenario; similarly, there was a less than or near 1 IR after the temperature adjustments (Table 2).

For the three municipalities with the highest minimum temperature, El Estor, Iztapa and Panzós, an increase in temperature did lead to a prediction of increased cases of dengue fever. El Estor, Iztapa, and Panzós had dengue fever IRs of 111.46, 618.92, and 79.64, respectively, for the fixed temperature scenario and had higher rates of dengue fever after increasing temperature by 1°C (145.36, 807.17, 103.86), which increased further after increasing the temperature by 2°C (189.57, 1052.67, 135.44). Similarly, the municipality of Guatemala had a dengue fever IR of 21.17 in the fixed temperature prediction scenario and an increased dengue fever IR for the 1°C increase scenario (27.61) and even further for the 2°C prediction scenario (36.01).

Discussion and Conclusion

The aim of this research was to contribute to understanding how environmental and socioeconomic factors influenced the distribution of dengue in Guatemala. This was initially evaluated through chi-square tests of independence and one-way ANOVAs between environmental and socioeconomic predictor variables and dengue cases, and then again through zero-inflated negative binomial regression models. Environmental and socioeconomic

Table 2.—Prediction Values and Adjusted Confidence Intervals, for the three municipalities with the lowest and highest minimum temperatures, and for Guatemala. * IR = incidence rate. * Fixed temperature is the average minimum temperature

Municipality	Dengue fever IR/100,000	Current Minimum Temp	DF IR per 100,000 with Fixed Temp	DF IR per 100,000 After 1°C Increase	DF IR per 100,000 After 2°C Increase
Concepción Tutuapa	0	4.78°C	0.083	0.129	0.200
San Jose Ojetenam	0	5.03°C	0.125	0.193	0.299
Sibilia	0	5.10°C	0.781	1.21	1.87
El Estor	229.11	22.56°C	104.22	161.36	249.85
Iztapa	98.14	22.60°C	554.48	858.54	1329.35
Panzós	1.39	22.78°C	82.79	128.19	198.49
Guatemala	87.94	14.44°C	23.47	36.34	60.80

* Fixed temperature is the average minimum temperature in that municipality from 2017−2018.

factors were both associated with dengue incidence in Guatemala.

When all variables were combined, the significant variables which were positively associated with dengue included internet use and minimum temperature. On the other hand, attending school, population density, and Mayan population were negatively associated with dengue fever. This is the first study to find a significant relationship between Maya population and internet use with dengue fever incidence at the municipality level in Guatemala. Elevation has not before been considered as a predictor of zero-inflation for dengue fever incidence in Guatemala, to the best of our knowledge. These findings might inform future vector control strategies in Guatemala.

Additionally, the prediction scenarios in our study found that dengue fever incidence could increase in Guatemala with even a modest 1−2°C increase in temperature. In some municipalities, that increase in temperature would be sufficient to improve the suitability for *Ae. aegypti*. This finding might support the suggestion that the ideal temperature for *Ae. aegypti* survival is 20 to 30°C (Tun-Lin et al. 2000). Others have additionally found that dengue fever incidence increased steeply as temperature increased from 22 to 29°C (Fan et al. 2015).

Future studies should explore how some of the significant factors, such as internet use, relates to dengue cases. In addition, the Mayan population at high elevations may have some protection from dengue, yet these municipalities may be more vulnerable than others with a modest temperature increase. Perhaps most important is the finding that temperature increases may lead to steep increases in dengue cases. This may impact numerous regions globally including California.

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Is Texas relevant to California? A summary of prior *Wolbachia* field trial results against *Aedes* mosquito populations

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Abstract

Aedes aegypti and *Aedes albopictus* are invasive mosquitoes and of public health concern because of their aggressive day-biting behavior and their ability to spread medically important pathogens (e.g., Zika, dengue, and chikungunya viruses). Despite the use of conventional chemical pesticides to manage these species, both species have colonized much of the USA and continue to expand their range. In this presentation, the prior work of MosquitoMate in Houston, Texas, will be summarized in relation to potential future work in California. Of direct relevance, localized regions of both California and Texas are dually-infested with both *Ae. aegypti* and *Ae. albopictus*. Similar to the traditional Sterile Insect Technique (SIT), the *Wolbachia* approach is based upon repeated, inundative releases of incompatible male mosquitoes, with the goal of decreasing the number of viable eggs. This presentation summarized the regulatory status of the *Wolbachia* technologies in the USA Environmental Protection Agency and results from open field trials used to examine for efficacy under field conditions.

Temperature and *Wolbachia* infections in *Culex pipiens* mosquitoes: implications for novel mosquito controls

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Introduction

A number of novel mosquito controls are being investigated and used for reduction of mosquito populations, to protect public health through prevention of infectious disease such as dengue. *Wolbachia*-infected *Aedes aegypti* males (and *Aedes albopictus* males) can be deployed through the incompatible insect technique (IIT) technique and mate with wild females, with no viable offspring produced (Dobson 2021). *Aedes aegypti* populations also can be replaced by *Wolbachia*-infected *Ae. aegypti* males and females leading to a reduction in cases of dengue (O'Neil et al. 2019). Mosquitoes infected with *Wolbachia* generally follow the trend of having lower titers of arboviral infection, and therefore a lower vector competence. The sterile insect technique (SIT) which has long been used to control agricultural insect pests is also being developed for mosquito control (Sánchez-Aldana-Sánchez et al. 2023). In addition, genetically modified mosquitoes have been successfully deployed to reduce mosquito populations (keysmosquitoproject.com). There are numerous possible novel techniques being tested.

In California, *Culex pipiens* is one of the primary vectors of West Nile virus (WNV), and it is typically infected with *Wolbachia* (Rasgon and Scott 2004, Torres et al. 2020). *Culex pipiens* occurs in N. California, *Cx. quinquefasciatus* in S. California, with hybrids found in the Central Valley. *Culex quinquefasciatus* with higher levels of *Wolbachia* was found to have lower WNV titers and vector competence (Glaser and Meola 2010). The bacterium *Wolbachia* is sensitive to high temperatures, and laboratory and field studies have shown that increasing temperatures can reduce *Wolbachia* levels in insects (Ross et al. 2020).

In the Central Valley of California, summer temperatures can exceed 40°C (104°F). On September 6, 2022, a peak temperature of 116°F (46.67 °C) was recorded. Climate extremes such hot summer temperatures might reduce the density of *Wolbachia* in *Cx. pipiens* in the field, which could result in *Cx. pipiens* having higher titers of WNV. Few studies have investigated how field temperatures impact *Wolbachia* abundance in mosquitoes.

Materials and Methods

Culex pipiens will be collected during the WNV surveillance season from May through September using EVS traps in San Joaquin and Merced counties to investigate variation in *Wolbachia* relative abundance under natural field conditions. Field sites with historically high and low WNV detection rates in mosquitoes will be included. Temperature will be recorded at field sites. DNA will be extracted from mosquitoes and screened for *Wolbachia* density using qPCR. Relative abundance of *Wolbachia* in samples will be determined.

Results and Discussion

The expectation is that *Wolbachia* relative abundance will be lower as temperature increases through the season and will be lower at sites with historically high WNV detection rates. Preliminary data have been obtained and have found variation in *Wolbachia* density. This project will continue through 2023. Results will be discussed in the context of *Wolbachia*-related mosquito control.

Acknowledgements

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Generation and mass production of a universally incompatible *Culex quinquefasciatus* line for *Wolbachia*-based sterile insect technique

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Introduction

Birds not mosquitoes (BNM, https://www.birdsnotmosquitoes.org/) is a multi-agency partnership, urgently working to save the native honeycreeper birds of Hawaiʻi from extinction driven by the transmission of avian malaria (*Plasmodium relictum*) by the invasive mosquito *Culex quinquefasciatus*. As part of this effort, the *Wolbachia*-based incompatible insect technique (IIT) was selected as an intervention aimed at reducing *Cx. quinquefasciatus* populations in the wild and interrupting the malaria transmission cycle.

Debug, based at Verily Life Sciences in California, has partnered with BNM to develop an IIT system for suppression of *Cx. quinquefasciatus* in Hawaiʻi. Over the past seven years, Debug has developed automated rearing, sex sorting and release solutions to enable the implementation of mosquito IIT at scale, with demonstrated success in suppressing of *Aedes aegypti* across many locations (https://blog.debug.com/).

Culex mosquito populations naturally harbor a number of wPip *Wolbachia* forms (clades I-V), which display complex cytoplasmic incompatibility (CI) interactions. This natural incompatibility among *Culex* from different geographic locations is how *Wolbachia* and CI were first discovered. Debug planned to utilize demonstrated natural incompatibility between wPip IV present in a colony of *Cx. quinquefasciatus* from Istanbul, Turkey, with the major Hawaiian wPip clade V. However, initial CI testing demonstrated inconsistent CI between Istanbul wPip IV and Culex from Hawaiian populations.

Methods

With an aim of generating a universally incompatible *Culex* line, Debug performed *Wolbachia* transinfections of *w*AlbB *Wolbachia* from *Ae. albopictus* into *Cx. quinquefasciatus* previously cleared of endogenous wPip. The DQB (Debug *Cx. quinquefasciatus* wAlbB) line has been tested for phenotypes pertinent to population suppression via release of sterile (*Wolbachia*-infected) males. Automated mass rearing and sex separation pipelines have been developed to support mass production and release of DQB males.

Results and Discussion

Three independent DQB lines were generated via transinfection (DQB1-3). Testing for bidirectional CI was performed by crossing DQB males with Hawaiʻi origin *Cx. quinquefasciatus* females (Maui and Oahu colonies) and Hawaiʻi origin males with DQB females. DQB3 males demonstrated 99% or greater CI when mated with Hawaiian WT *Cx. quinquefasciatus* females, and >99.9% CI when DQB3 females were mated with Hawaiian males. DQB3 was therefore selected as the leading line for IIT intervention in Hawaiʻi.

Debug have optimized automated larval mass rearing, pupae and adult sex sorting pipeline for *Cx. quinquefasciatus* and have developed packing and shipping protocols to support transport of DQB males from Verily California to release sites in Hawaiʻi (Maui and Kauai). The mountainous and densely vegetated habitat of the native Hawaiian Honeycreeper requires that the aerial release of mosquitoes will be necessary for deployment of DQB males. Debug and partners are currently optimizing aerial release systems using compostable packets of DQB males.

Conclusion

After the recent acquisition of an EPA Section 18 Emergency Use Permit for the release of DQB3 males on Hawai, initial mark-release-recapture and aerial release testing will commence in Hawaiʻi in the coming months.

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The University of California Malaria Initiative: Genetically engineered *Anopheles* malaria vectors for malaria eradication in Africa

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Abstract

The University of California Malaria Initiative (UCMI) is a collaborative program that includes researchers from four University of California campuses (Irvine, Davis, San Diego, Berkeley) and Johns Hopkins University, who are dedicated to the elimination of malaria. The current goal is to genetically modify mosquito populations to prevent them from transmitting the parasites that cause malaria. The aim is to collaborate with malaria-endemic countries to develop new tools to add to existing malaria control programs. If a country determines that the genetically engineered mosquito (GEM) is an appropriate malaria control strategy, they will co-develop the GEM and the implementation strategy required for a release. UCMI is currently working with the government of São Tomé and Príncipe, an island nation off the coast of western Africa, investigating the potential of using GEMs for malaria elimination. This presentation reviewed the technology and the ongoing program in São Tomé and Príncipe.

Preparation for targeted sterile insect technique to control invasive mosquitoes in California: mosquito colonization, gender emergence ratio and separating the males

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Abstract

The urban-adapted, daytime-biting *Aedes aegypti* is the primary mosquito vector of dengue and has the potential to transmit several other arboviruses, including Zika, chikungunya and yellow fever. In California, *Ae. aegypti* has spread to over 300 cities within 22 central and southern counties in less than a decade. Due to its cryptic larval habitat, control efforts have not been successful. Therefore, there is a need for innovative biological tools such as the Sterile Insect Technique (SIT) to effectively control this invasive mosquito thereby expanding the existing integrated pest management (IPM) program. Here, we provide a summary of laboratory experiments conducted to establish an *Ae. aegypti* colony and examine gender emergence ratios and strategies to separate males for SIT. The use of pressure operated flasks with yeast yielded >80% egg-hatching success in 1.5 hr while hatching without yeast resulted in <50% hatching success. Pupae were monitored for adult emergence, and males were manually separated immediately after emergence. Among freshly emerged adults, the proportion of males was highest during the first 24 hrs (78%) and then dropped to 13% by Day 6. Implications of these findings and future research ideas were discussed.

Southern California SIT Joint Pilot Project: Partnering for The Future of Mosquito Control

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Introduction

Insect population control using the Sterile Insect Technique (SIT), by means of ionizing (X-ray) irradiation, is not a new method in pest management. This SIT strategy has been successfully used for decades on a variety of pests across the globe. It is, however, a novel approach for use in mosquito control. Projects by groups in Florida, Texas, Brazil and other parts of the world have paved the way for mosquito control agencies to investigate the use of this technology on a local scale. The Orange County Mosquito and Vector Control District (OCMVCD) and the Greater Los Angeles County Vector Control District (GLACVCD) initiated a partnership in late 2021 to conduct a SIT Joint Pilot Project. The projected 5-year pilot project aims to study the effects of releasing irradiated adult male *Aedes aegypti* (L.) on established *Ae. aegypti* populations in urban/suburban areas of southern California. This discussion highlights the steps involved in starting an inter-district joint project, outlines the study goals and objectives, and provides an overview of the project status.

Invasive *Aedes* mosquitoes have plagued California for more than a decade now. *Aedes albopictus* (Skuse), *Ae. Aegypti*, and *Aedes notoscriptus* (Skuse) appeared in Los Angeles County in 2011, 2013, and 2014, respectively, while *Ae. aegypti* and *Ae. albopictus* were detected in Orange County in 2015, followed by *Ae. notoscriptus* in 2017 (Metzger et al. 2017, 2022). Since that time, their presence has changed the quality of human life in southern California. Collectively the districts serve over nine million residents spanning a combined 2,100 square miles. These invasive mosquitoes are commonly referred to as invasive *Aedes*, ankle bitters, or zebra mosquitoes. These mosquitoes' pestiferous behavior, adaptable and prolific nature, documented resistance to conventional pesticides, and vector competency make them not only problematic, but a serious public health risk. Combined, these three species are known to transmit the disease-causing agents of dengue, chikungunya, Zika, yellow fever and dog heartworm. Vector control programs are eager to explore alternative strategies, like SIT, to combat these tenacious invasive pests.

Advantages of Partnership

Facing high volumes of public complaints, increased disease risk, and the financial implications of control efforts to address invasive *Aedes* mosquitoes, the OCMVCD and GLACVCD partnered to conduct a pilot project to study the efficacy of SIT to suppress the region's most abundant and threatening invasive mosquito, *Ae. aegypti*. Results reported from a seminal mosquito SIT project by the Lee County Mosquito Control District, in Fort Meyer, FL, indicated they were able to achieve a statistically significant reduction of the *Ae. aegypti* population on Captiva Island, FL. The OCMVCD and GLACVCD Boards of Trustees established a formal cooperative agreement in late 2021 to jointly purchase a small irradiator (Rad Source 1800 Q Unit, Buford GA, USA) and collaborate on a bi-jurisdictional SIT study aimed at examining the effects and efficacy of releases of X-ray sterilized *Ae. aegypti* males in urban/suburban residential areas of Orange and Los Angeles counties. The advantages of partnering on the project included cost sharing of equipment and materials, inter-agency collaborative study with a large spatially scaled study design, implementation, and analysis, and the expanded opportunity for innovation and ingenuity by a large diversified team.

Study Goals and Objectives

The broad goal of the SIT Joint Pilot Project is to suppress wild populations of *Ae. aegypti* using X-ray sterilized adult male *Ae. aegypti* to numbers below purported disease transmission thresholds, thereby lowering the disease risk to the community and consequently reducingbiting pressure. Most published work on irradiation-based SIT using mosquitoes has examined irradiation of *Ae. aegypti*, (or other species) in the pupal stage. This project aims to irradiate adult male *Ae. aegypti*, as it is reported in the literature that irradiation in the adult stage allows for a longer timeframe to optimally administer the irradiation dose to the mosquito, allowing flexibility in the logistics of releasing mosquitoes and sharing an irradiator between county agencies. Specific objectives of the study call for establishing protocols for irradiating the male mosquitoes in the adult stage, testing irradiated mosquito fitness parameters (longevity, flight ability, mating) after each manipulation (chilling, compaction, irradiation, dusting) in successive cumulative combination. To inform and prepare for project releases in 2024, we plan to conduct a series of mark-release-recapture (MRR) studies in the selected treatment areas simultaneously in both districts during late summer/early fall of 2023. Information from the single-point and multiple-point MRRs will allow us to determine the dispersal capabilities and minimum treatment area size, the proper sterile male to wild male release ratios, and total male numbers needed for weekly releases. We will also develop a distribution model. The two Districts will evaluate the efficacy of the SIT program in residential neighborhoods concurrently, comparing treatment areas to nearby non-treatment control areas, across multiple seasons and years. If shown to be effective, we plan to expand irradiated mosquito production and releases into new areas and explore the potential for releasing irradiated males to control additional mosquito species. We view this work as the foundation for a future regional program.

SoCal SIT Next Steps

The next steps for the project involve initiating public education campaigns in the respective cities and neighborhoods where the treatment releases are planned. The Joint Project team will continue to develop and test protocols, equipment, and standard operating procedures for all steps involved in blood feeding, mass rearing, sex sorting, irradiating, and preparing the adult sterile male mosquitoes for release trials. There are currently multiple laboratory and semi-field studies in progress at both District facilities. Adult mosquito trapping and oviposition bucket collections will continue for the second year beginning in the spring of 2023 and MRR studies will begin in late 2023. Monitored (florescent powder) sterile male mosquito releases are estimated to begin in late summer/early fall of 2024.

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Precision-guided Sterile Insect Technique (pgSIT) – a novel biotechnology platform for safe and efficacious mosquito control.

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SIT and *W*IIT rely on releasing inundative numbers of sex-sorted and sterile adult male mosquitoes, but these methods generally have failed due to inadequate production related to high costs, low fitness of released males, and logistical challenges. Newer GE approaches such as female-specific Release of Insects of carrying a Dominant Lethal (fsRIDL) by Oxitec or CRISPR-homing gene drives are facing strong regulatory barriers or are not successful at large scale due to natural selection.

To address these challenges, a new method called precision-guided SIT (pgSIT) has been developed (Kandul et al. 2019, 2022, Li et al. 2021), which combines the precision of GE with the stability of traditional SIT and *W*IIT. In pgSIT, two CRISPR components are separated into two GE mosquito strains, each expressing Cas9 or multiple guide RNAs. A genetic cross between the two strains produces pgSIT eggs that develop into sex-sorted and sterile male mosquitoes, which can be released into the environment as eggs using egg-to-adult boxes. The ability to store and then release pgSIT eggs decreases deployment costs, enables global logistics, and improves mosquito suppression efficacy. Because of its precision-guided genetics, pgSIT consistently leads to mosquito male sterility while preserving male fitness, longevity, and competitiveness in the environment.

To scale sex-sorting of mosquitoes for genetic crossing for mass pgSIT egg production, a new technology called SEPARATOR has been developed. SEPARATOR uses large particle flow cytometry to high-throughput sex-sort mosquito larvae using harmless fluorescent proteins. This approach also may be used for sex-sorting male larvae for radiation-based SIT or WIIT, and the *Ae. aegypti* SEPARATOR strain is available for licensing from Synvect, Inc. (www.synvect.com).

Overall, the development of pgSIT and SEPARATOR technologies offer a promising solution to the challenges of controlling the spread of invasive mosquito species such as *Ae. aegypti*. By preserving the fitness, longevity, and competitiveness of the released males, pgSIT enables more efficient and cost-effective mosquito suppression. These technologies have the potential to be applied to multiple mosquito species and can be deployed globally to reduce the risk of deadly mosquito-borne diseases. In conclusion, the integration of pgSIT and SEPARATOR technologies, called the next-generation SIT, represent a significant step forward in the fight against invasive mosquitoes and could have a major impact on public health worldwide.

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Field application and results of the Oxitec self-limiting gene technology against *Aedes aegypti* in Florida

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Abstract

The mosquito *Aedes aegypti* is a globally invasive species outside of its native range in eastern Africa. This mosquito is an important vector of the viruses that cause dengue, yellow fever, Zika, and chikungunya. This species feeds almost exclusively on humans, and feeds and oviposits close to human habitations, especially in cryptic natural and artificial containers. Throughout its range, *Ae. aegypti* populations are resistant to pyrethroids, the class of insecticides most commonly used to control adult mosquitoes. For these reasons, *Ae. aegypti* is a difficult mosquito to control by traditional larviciding, adulticiding, and larval site disruption. Here we described discuss public outreach and the operational feasibility of Oxitec self-limiting gene technology to control this invasive species in the Florida Keys.

Collaborative efforts with Oxitec and Delta Mosquito & Vector Control District in the Central Valley of California

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Introduction

The yellow fever mosquito, *Aedes aegypti* (L.), is an invasive species in California. Since 2013, *Ae. aegypti* has spread and become established in 25 counties and 300 cities and towns in California, posing unique challenges to mosquito control because of its diel periodicity, proximity to people, and use of cryptic harborages and larval sources. Delta Mosquito and Vector Control District (DMVCD) invited Oxitec to evaluate their self-limiting gene technology against invasive *Ae. aegypti* within its District, pending state regulatory approval from the California Department of Pesticide Regulation Office. This presentation focused on the community and outreach efforts by Delta MVCD and Oxitec and the widespread, enthusiastic public support in the Central Valley for this cutting-edge innovative technology using self-limiting gene male mosquitoes from Oxitec.

Methods

The Delta MVCD collaborated with Oxitec in sharing resources and best practices using a complimentary mix of experience, technical expertise, working hand-in-hand, and local knowledge to carry out this project with local communities in the District. The collaborative community engagement involved participating in community events such as Visalia Rawhide Baseball games, classic car shows, farmers and flea markets, school events, and city events, conducting webinars, presenting at the Visalia Lions Club, and the California Environmental Health Association. A biweekly meeting of the leadership of Delta MVCD and Oxitec personnel helped guide policies and District activities.

Results and Discussion

Presented was the collaborative roles of Delta MVCD and Oxitec in the Central Valley discussing the history of *Ae. aegypti* in the District, public assessment and approval, co-pilot study format, transparency, and equity of the joint team. In addition, the next steps were discussed such as future project planning and field release site selections, continued outreach and public engagement, and federal and state regulatory review processes.

Conclusion

The Delta MVCD was successful in the on-going collaborative efforts with Oxitec to prepare and engage in a future field study to evaluate Oxitec's self-limiting gene technology against invasive *Ae. aegypti* in the Central Valley of California to protect the public from its mosquito-bites and possible transmission of dangerous mosquito-borne pathogens such as yellow fever, dengue, chikungunya, Zika, and dog heartworm.

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The Who, What, and Where of California's Environmental Tick Surveillance Program

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Abstract

The Vector-Borne Disease Section (VBDS) of the California Department of Public Health maintains a comprehensive statewide environmental tick surveillance program. With multiple tick species and tick-borne pathogens of human health importance documented in California, VBDS performs surveillance for these pathogens, such as (but not limited to) *Borrelia burgdorferi* and *Rickettsia ricketsii*, both as routine surveillance and in response to human cases. This presentation addressed the vectors, pathogens, and countywide prevalence of these pathogens in tick vectors throughout the state, summarizing five years of collection and testing data, from 2018-2022.

Tick surveillance in Marin and Sonoma counties: past, present, and future

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Abstract

Tick surveillance has long been an important component of the Marin/Sonoma Mosquito and Vector Control District (the District) laboratory program. Over the years, the program has surveyed 32 parks and collected thousands of *Ixodes pacificus* adult and nymphal ticks to test for *Borrelia burgdorferi* sensu lato and *Borrelia miyamotoi*. Because tick-bite prevention is a primary goal of this program, data transparency has increased in recent years with the use of interactive maps. These maps include collection and testing data to help the public better understand where ticks and pathogens have been found. The District has also modified and enhanced surveillance to keep up with a changing world; implementing sentinel tick surveillance, conducting surveillance in wildfire burn areas, and utilizing new technologies to enhance the collection of tick abundance data. The District continues to research and implement new methods as surveillance protocols, techniques, and technologies evolve.

Program overview

One of the main objectives of the Marin/Sonoma Mosquito and Vector Control District's (the District's) tick surveillance program is to determine where the public might encounter ticks and tick-borne pathogens in parks in Marin and Sonoma counties. This is accomplished by surveying for ticks on publicly accessible trails. Collected ticks are identified to species, sex, and life stage, and processed as described in the following section. *Ixodes pacificus*, commonly known as the western blacklegged tick, are tested for two human pathogens: *Borrelia burgdorferi*, the bacterium that causes Lyme disease (Johnson et al. 1984), and *Borrelia miyamotoi,* a bacterium that can cause a relapsing fever-type illness (Platonov et al. 2011). Results of these collections and laboratory assays are published at the end of each year, allowing the public to see where ticks and pathogens have been found (Marin/Sonoma Mosquito and Vector Control District 2023a).

In addition to testing for *B. burgdorferi* and *B. miyamotoi*, the District collaborates with the California Department of Public Health (CDPH) to test *I. pacificus* for other pathogens, including *Anaplasma phagocytophilum*. In recent years, Marin County has seen an increase in the number of human anaplasmosis cases (California Department of Public Health 2021), leading to additional testing by CDPH. This collaboration has been integral to the District's tick surveillance program, as in-house *A. phagocytophilum* laboratory testing has not been possible.

Public outreach and education are the District's main form of tick control. Due to the ecology of ticks as well as the toxicity of acaricides, chemical and physical control of ticks on public lands are typically not viable options for the District. Educating residents about tick habitat, life cycle, and tick-bite prevention is the most effective way to prevent tick-borne pathogens. The District has cultivated a plethora of brochures and web content to help inform residents (Marin/Sonoma Mosquito and Vector Control District 2023b). In addition, the education specialist teaches K-8 students about the biology and ecology of ticks.

In recent years, the District has introduced a sentinel surveillance project, which includes repeated sampling at set locations to determine changes in *I. pacificus* abundance over time (Eisen et al. 2018). This project has led to additional observations on the effects of wildfires on tick populations. By incorporating new equipment for recording tick locations, this study will help the District better understand the effect of uncontrolled wildfires, as well as forest management and controlled burns, on tick populations.

Collection and testing

The District conducts tick surveillance year-round. Although most adult and nymphal *I. pacificus* are captured from November through July, adults and/or nymphs have been collected during every month of the year (Figure 1). Additionally, other tick species such as *Dermacentor occidentalis* (the Pacific Coast tick) and *Dermacentor variabilis* (the American dog tick) are found throughout the year. Collection sites are chosen each year from a list of state, regional, and local parks with appropriate tick habitat. District staff obtain necessary permits and contact park staff prior to each collection.

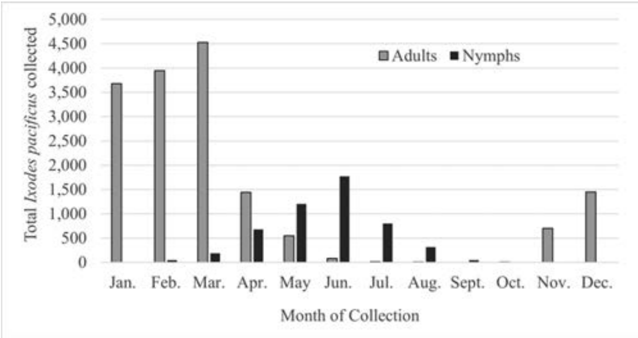

Figure 1.—Total *Ixodes pacificus* adults and nymphs collected by month, 2008−2022.

The District uses a tick collection method known as flagging (Eisen et al. 2018). Flags consist of a 1-meter squared piece of white fabric attached to a metal pole with binder clips. The flags are dragged along the ground at the side of the trail over vegetation, leaf litter, rocks, downed logs, and other substrates. After approximately 10 meters, they are checked for ticks. All ticks are identified by species, sex, and life stage and placed into polystyrene containers (Fisher Scientific, Santa Clara, CA, USA).

The species, sex, and life stage of the ticks are confirmed in the laboratory. *I. pacificus* are placed into SPEX SamplePrep 800 series mixer/mill tubes with two 5-mm glass beads (Fisher Scientific, Santa Clara, CA, USA) and stored at −20°C in preparation for DNA extraction. From 1 to 5 adult *I. pacificus* are pooled based on collection date, park, trail, and sex. From 2008 - 2016, nymphal ticks were pooled for testing. However, since 2017, nymphs have been tested individually. DNA extractions are conducted using the MagMAX-96 Viral RNA Isolation Kit (ThermoFisher Scientific, Waltham, MA, USA).

Since 2016, the District has used an adapted version of the Barbour et al. (2009) duplex real-time polymerase chain reaction assay to identify *B. burgdorferi* sensu lato and *B. miyamotoi* bacteria in extracted DNA. The 25 ul reaction mixture contains 12.5 ul 2X TaqMan Universal Master Mix II (ThermoFisher Scientific, Waltham, MA, USA), 1.25 ul primer/prob stock solution, 6.25 ul PCR-grade water, and 5 ul DNA template. Final primer and probe concentrations are 900 nmol/L and 250 nmol/L, respectively. Amplification consists of 95°C for 10 minutes, followed by 44 cycles of 95°C for 15 seconds and 60°C for 1 minute.

Summary of findings and data transparency

Since 2008, the District has visited 32 parks, and collected and tested 16,406 adult and 5,067 nymphal *I. pacificus* for *B. burgdorferi* sensu lato. The overall minimum infection prevalence (MIP) is 1.76% for adults and 4.14% for nymphs. The MIP is a statistic used when testing pools of arthropods for a pathogen. Testing in pools is an efficient and cost-effective way to screen for pathogens when the prevalence of the pathogen is relatively low. If a pool tests positive, the MIP assumes that only one tick within the pool was carrying the bacterium. The definition of MIP for the District's ticks is:

$$\mathrm{MIP} = \frac{\text{total positive pools}}{\text{total ticks tested}} \text{ X } 100$$

Since 2016, the District has collected and tested 5,076 adult and 1,614 nymphal *I. pacificus* for *B. miyamotoi,* with an overall MIP of 1.00% for adults and 1.11% for nymphs.

Tick surveillance results are published annually in the Marin/Sonoma Mosquito and Vector Control District Vector Surveillance Report (Marin/Sonoma Mosquito and Vector Control District 2023c). This document provides collection and testing data by park and trail. The cumulative surveillance efforts for each park are published on interactive maps on the tick surveillance webpage (Marin/Sonoma Mosquito and Vector Control District 2023a). These maps provide information for each park that has been sampled for ticks. Information includes sampling effort (total visits), total adults and nymphs collected, and adult and nymphal MIPs. Providing this information to the public allows them to assess the risk of potential exposure to ticks in specific locations, as well as demonstrating the variability in prevalence of the two bacteria. These maps also show how widespread *I. pacificus* are in the two counties and help remind the public of the importance of tick-bite prevention.

Outreach

The best way for the District to prevent tick-borne diseases is to educate the public about tick-bite prevention. Each year, the District produces a unique advertisement for billboards and bus shelters highlighting ticks. These campaigns begin in the spring when the risk of encountering an *I. pacificus* nymph increases. Previous slogans include "Be Tick Smart" and "All it takes is one bite". These typically receive about one million impressions in the months that they are active.

Web-based materials are also among the District's most visible forms of outreach. Webpages devoted to tick surveillance, tick-safe landscaping tips, tick-bite prevention, and *I. pacificus* ecology (Marin/Sonoma Mosquito and Vector Control District 2023d) receive approximately 200 views per month and are among the most visited pages on the District's website.

In-person events and classroom visits allow District staff to engage directly with the public and provide additional information about ticks and tick safety. Participation in community events such as county fairs and local festivals provide the District a direct link to the public. Branded items given out at these events, such as magnets, pencils, and collapsible dog bowls, spread the District's message and contact information. One of the District's most requested collateral materials is the tick packet, which contains a tick identification card, brochures about ticks and Lyme disease, and a tick remover. These items are provided to civic organizations, local and state parks, and at events.

New surveillance techniques

Over the past several years, the District's tick surveillance program has adapted and evolved with new technologies in the laboratory and field. Most recently, the introduction of a sentinel surveillance project has spurred several interesting advancements in the program. Beginning in 2018, the District chose short transects at three parks to conduct sentinel surveillance. This protocol allows for the comparison of tick population abundance over time, with the hope of being able to answer the question: Is this a big tick year? According to Kramer et al. (1993), repeated bi-weekly sampling at a specific site will negatively impact the population of *I. pacificus* ticks. Therefore, to accurately assess abundance, ticks collected on sentinel trails are returned to the environment. The protocol for this project was developed in accordance with the recommendations put forth in Eisen et al. (2018). In short, District laboratory staff conduct monthly surveillance as described above at pre-designated trails. Instead of collecting the ticks after 10 meters of flagging, the number of each species, life stage, sex, and the side of the trail on which the collection was made are recorded, and the ticks are returned to the environment to limit the impact of sampling.

Throughout this project, several changes have occurred at the sentinel transects. Uncontrolled wildfires burned the vegetation at two of the three sites. In the aftermath of these events, the District has been able to use the monthly abundance data to see changes in the *I. pacificus* populations at these sites. The third site, which had remained unaltered until 2022, underwent significant management in which vegetation was removed and burned in several prescribed burns over the course of a year. The introduction of new technologies will help the District understand how these changes affect the *I. pacificus* population.

Beginning in 2021, the District implemented a new tool to record where ticks were being found. The Trimble TDC600 handheld units (Trimble, Westminster, CO, USA) use the TerraFlex application to record a GPS point when ticks are collected. This point is associated with information collected about the ticks, and uploaded to the Trimble Connect website, where it can be downloaded and utilized in Excel and Tableau platforms for analysis and data visualization. As wildfires and forest management alter the ecosystem of the sentinel trails, this GPS information will help the District better understand the impact of these changes on the tick population.

Adapting and expanding tick surveillance programs with the use of new technologies and laboratory techniques is critical to identifying emerging tick-borne diseases, monitoring changes in tick distribution, and increasing awareness and prevention efforts.

Acknowledgements

The Marin/Sonoma Mosquito and Vector Control District's tick surveillance program is maintained by dedicated staff, including lead biologist Sarah Brooks, biologist Kristen Holt, public information officer Nizza Sequeira, and education program specialist Casey Richter. Former scientific programs managers Piper Kimball and Angie Nakano, former vector ecologist Ron Keith, and former education program specialist Eric Engh, all contributed significantly to the development and advancement of the program. Without these individuals, the District's program would not be what it is today.

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Tick borne disease surveillance in Alameda County in 2021

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Introduction

The Western black-legged tick (*Ixodes pacificus*) is the primary vector of Lyme disease caused by the bacterium *Borrelia burgdorferi,* and of tick-borne relapsing fever (TBRF) caused by the bacterium *Borrelia miyamotoi*. Both pathogens are primarily transmitted to humans through the bite of an infected Western blacklegged tick.

Dermacentor occidentalis and *Dermacentor variabilis,* both can carry spotted fever caused by *Rickettsia spp.* bacteria. According to California Department of Public Health records, *D. occidentalis* is second only to *I. pacificus* in total numbers of tick attachments to humans. The Pacific Coast tick (*D. occidentalis*) and the American dog tick (*D. variabilis*) may be found year-round in Alameda County but are most abundant late spring-early summer.

For over 25 years, Alameda County Vector Control Services District (ACVC) has conducted a tick and tick-borne pathogen surveillance program at regional parks, city parks, and public open spaces along heavily used walkways and trails. The current paper summarizes the results of surveillance efforts during 2021.

Methods

ACVC staff drag square meter tick flags along vegetation alongn designated trails for intervals of 30 minutes. Collected ticks were stored in 95% ethanol and transported to the laboratory where they were identified to species and enumerated. The ticks were then pooled into groups of 5 adults or 2 nymphs by species, date and trail for testing via real-time PCR for *Borrelia* and *Rickettsia spp.*

Results and Discussion

During 2021, 2,747 *Ixodes pacificu*s, 1,572 *Dermacentor occidentalis*, 41 *Dermacentor variabilis*, 5 *Ixodes spinipalpis*, 2 *Ixodes auritulus*, and 4 *Haemaphysalis leporispalustris* ticks were collected (Table 1). The largest number of ticks (1,021) was collected in Redwood Regional Park. Anthony Chabot Regional Park had the greatest tick species diversity (5 species).

Overall, 1,422 *I. pacificus* (825 adults, 552 nymphs, and 45 larvae) were tested for infection with *Borrelia sensu lato* and *B. miyamotoi* using real-time PCR (Table 2). County-wide, *Borrelia sensu lato* and *B. miyamotoi* were detected at a minimum infection prevalence (MIP) of 2.3% and 1.8% in adult and 4.3% and 0.9% in nymphal *I. pacificus*, respectively. These MIP values recorded in 2021 were typical for Alameda County.

Temporal analysis showed that the MIP varied in adult ticks between 0 and 5.7% and in nymphs between 0 and 7.4% for *Borrelia sensu lato*. The MIP for *B. miyamotoi* ranged from 0 to 2.7% in adults and from 0 to 2.1% in nymphs (Table 3). *Borrelia sensu lato* and *B. miyamotoi* infected adult and nymphal *I. pacificus* were collected in Alameda County in

Table 1.—Number of ticks collected along trails in Alameda County.

Location	*Ixodes pacificus*	*Dermacentor occidentalis*	*Dermacentor variabilis*	*Ixodes spinipalpus*	*Haemaphysalis leporispalustris*	Total
Anthony Chabot Regional Park	211	318	20	1	3	553
Del Valle Regional Park	219	48	0	0	0	267
Garin Regional Park	98	5	0	0	0	103
Tassajara Creek Regional Park	9	147	2	0	0	158
Pleasanton Ridge Regional Park	248	53	1	0	0	302
Redwood Regional Park	410	608	2	0	1	1,021
Sunol Regional Park	64	0	0	0	0	64
Joaquin Miller Park	526	149	4	4	0	683
Augustin Bernal Park	113	10	0	0	0	123
Open Spaces, Sunol	600	24	0	0	0	624
Open Spaces, Pleasanton	107	21	0	0	0	128
Open Spaces, Dublin	98	159	10	0	0	267
Open Spaces, Oakland	32	3	0	0	0	35
Open Spaces, Berkeley	12	27	2	0	0	41
Total Per Tick Species	2,747	1,572	41	5	4	4,369

Table 2.—Minimum infection prevalence (MIP) of *Ixodes pacificus* from Alameda County for *Borrelia sensu latu* (Bbsl) and *B. miyamotoi* (B. miya).

I. pacificus Life Stage	# of Ticks	Pooled Samples	Bbsl Positive Pools	B. miya Positive Pools	Bbs lMIP, %	B. miya MIP, %
Adult	825	183	19	15	2.3	1.8
Nymph	552	277	24	5	4.3	0.9
Larvae	45	1	0	0	0	0
Totals	1422	461	43	20		

Table 3.—Monthly changes in the MIP of *I. pacificus* from Alemeda County for *Borrelia sensu latu* (Bbsl) and *B. miyamotoi* (B. miya).

	I. pacificus, Adults			*I. pacificus*, Nymphs		
Month	# of Ticks	Bbsl MIP, %	B. miya MIP, %	# of Ticks	Bbsl MIP, %	B. miya MIP, %
January	149	2	2.7	0	0	0
February	253	1.2	1.6	1	0	0
March	158	5.7	1.9	19	0	0
April	92	33	1.2	140	4.3	2.1
May	159	0.6	1.9	212	1.9	0.9
June	14	0	0	176	7.4	1.1
July	0	0	0	4	0	0
August	0	0	0	0	0	0
September	0	0	0	0	0	0
October	0	0	0	0	0	0
November	0	0	0	0	0	0
December	0	0	0	0	0	0

April and May, with the highest MIP in adults (5.7%) in March and the highest MIP in nymphs (7.4%) in June. The *B. miyamotoi* MIP was 1-2% during the tick season.

In total, 1,569 *D. occidentalis* and 41 *D. variabilis* adult ticks were collected in regional parks, city parks and open spaces (Table 4). During 2021, 402 *D. occidentalis* and 26 *D. variabilis* were tested in pools for the presence of *Rickettsia* spp. pathogens using real time PCR. Overall, 16 *D. occidentalis* pools and 6 *D. variabilis* pool were infected with *Rickettsia* spp. Sequencing positive samples showed that one *D. occidentalis* pool from Joaquin Miller Park in Oakland was infected with *Rickettsia philipii*, a human pathogen. This was the second detection of *R. philipii* infected ticks from trails in Alameda County. In addition, *Rickettsia bellii* and *Rickettsia rhipicephali* were present in *D. occidentalis* and *D. variabilis* ticks collected in parks and open spaces in Oakland, Dublin and Pleasanton. To date, neither *R. bellii* nor *R. rhipicephali* have been associated definitively with disease in humans or animals.

Table 4.—Numbers of *Dermacentor occidentalis* and *Dermacentor variabilis* collected along hiking trails in Alemeda County.

	Dermacentor occidentalis		*Dermacentor variabilis*		
Location	Female	Male	Female	Male	Total
Anthony Chabot Regional Park	162	155	10	10	337
Del Valle Regional Park	25	23	0	0	48
Garin Regional Park	2	3	0	0	5
Tassajara Creek Regional Park	87	60	2	0	149
Pleasanton Ridge Regional Park	28	25	1	0	54
Redwood Regional Park	306	361	0	2	669
Joaquin Miller Park	80	68	1	3	152
Augustin Bernal Park	6	4	0	0	10
Open Spaces, Sunol	10	14	0	0	24
Open Spaces, Pleasanton	11	10	0	0	21
Open Spaces, Dublin	74	85	6	4	169
Open Spaces, Oakland	2	1	0	0	3
Open Spaces, Berkeley	12	15	0	2	29
Total	805	824	20	21	1,670

Conclusion

Although the overall tick surveillance effort in 2021 increased, the estimated MIPs for different pathogens were similar to previous years. In 2022, we doubled the number of ticks collected compared to 2021 and expanded our sampling to include more trails. We also have begun experimenting with different flag materials to enhance sampling effectiveness, while reducing thorns and burrs from attaching to the fabric. We look forward to changing to distance-based tick collection transects and moving away from time-based methods.

Acknowledgements

Thanks to the biologists and management at ACVC for their work in conducting the tick collecting, without which the program would not be possible.

The past, present, and future of ticks at the San Mateo County Mosquito and Vector Control District

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Abstract

The San Mateo County Mosquito and Vector Control District (SMCMVCD) has conducted surveillance for ticks in San Mateo County since 2001. Since then, SMCMVCD has built a robust, multi-faceted program of collection, pathogen testing, control experiments, and public education and outreach on ticks. A summary of the evolution of SMCMVCD's tick collection and testing methods, research, tick data, and current and prospective tick projects was presented.

Tick surveillance and testing methods used to evaluate tick-borne pathogen risk in Sacramento and Yolo Counties

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Introduction

Tick-borne pathogens including *Borrelia burgdorferi*, the causative agent of Lyme disease, are a public health concern in California. *Ixodes pacificus*, also known as the Western blacklegged tick, is the primary vector of *B. burgdorferi*, is distributed throughout the Pacific coast of the United States, and is established in 56 of the 58 counties in California (California Department of Public Health 2023). Ticks have four life stages: egg, larvae, nymph, and adult. The larval and nymphal stages of the tick life cycle require a single bloodmeal before molting (Padgett and Lane 2001). *Ixodes pacificus* feed on a wide variety of hosts (CDC 2022, McVicar et al. 2022) that can potentially dilute the opportunity for the tick to either transmit or acquire pathogens during blood-feeding (MacDonald et al. 2017). The ability of the tick to transmit pathogens is dependent on host competency, therefore a higher diversity including less competent host species can lower potential pathogen transmission (Ratti et al. 2021). *Borrelia burgdorferi* is acquired when *I. pacificus* feed on an infective host. The Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) conducts surveillance for tick and tick-borne pathogens to establish the potential risk of exposure to the public. The various aspects of this program are discussed, and surveillance results are presented herein.

Methods

Site selection

Several factors are considered when selecting sites for tick surveillance including presence of hosts, habitat, humidity, and human activity. *Ixodes pacificus* is vulnerable to desiccation so sites were selected that provided a shaded understory and/or were found in close proximity to a riparian corridor. Lastly, surveillance locations coincided with high-use hiking trails where people had a greater chance of encountering ticks. Within Sacramento and Yolo Counties there were a total of twenty tick surveillance sites. Each surveillance site was flagged once per month from October through May for a total of one hour, after which collected ticks were returned to the laboratory for processing.

Tick collection

Prior to tick collection environmental data including wind speed, wind direction, percent humidity, dew point, and temperature was collected using a Kestrel 5500 weather meter (Kestrel Instruments; Boothwyn, PA). Data were recorded onto a field data sheet along with the technician's names, site code, and any notes on the condition of the site.

Ticks were collected using a dragging technique (Carroll and Schmidtmann 1992, Tälleklint-Eisen and Lane 2000). The flag consisted of a one-meter square flannel cloth connected to a PVC pipe handle. Technicians walked at a slow steady pace along the trail while gently dragging the flannel square over the trail foliage. The function of the flag was to simulate a passing host and to gather questing ticks off the tops of foliage. During dragging, the flags were checked frequently for attached ticks. When ticks were found, they were removed with forceps and placed into vials containing 70% isopropyl alcohol. The alcohol prevented ticks from escaping and provided an extra safety measure for technicians. Once the flagging was complete collections were returned to the laboratory for processing.

Tick processing and testing

Technicians matched field datasheets with the collection vials and processed the ticks by moving the collection into a petri dish and pouring off the alcohol. Ticks were then placed on a Kimwipe (Kimberly-Clark Professional, Roswell, GA) to remove excess alcohol. Ticks were identified to species, counted, and all *I. pacificus* were pooled into groups of up to five individuals per vial by sex and location. Each pooling vial contained two 5mm glass beads (Walter Stern Inc., Port Washington, NY) to aid with homogenization. The pooled ticks were stored at -20° C prior to testing.

To test the pools for *B. burgdorferi*, 500ul of the MagMax Lysis/Binding Buffer (Life Technologies, Carlsbad, CA) was added to each vial, then they were homogenized using a Spex Sample Prep 8000D (Spex Certiprep; Metuchen, NJ) for 3 minutes. Total nucleic acid was extracted using 5x MagMax Viral kit and the MagMax 96-Express magnetic particle processor (Life Technologies; Carlsbad, CA). The extracted DNA samples were tested for

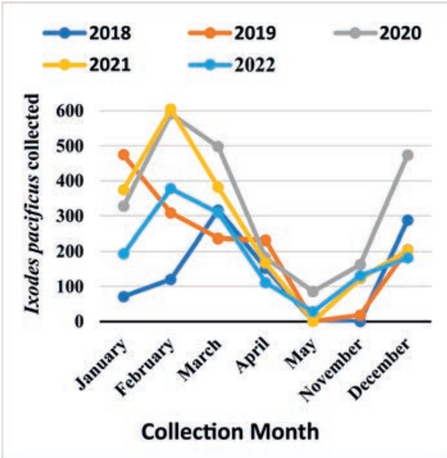

Figure 1.—Total number of *Ixodes pacificus* collected monthly in Sacramento and Yolo Counties from 2018-2022; sampling effort remained relatively consistent over time; Tick surveillance is not conducted June-October.

the presence of *B. burgdorferi* by quantitative polymerase chain reaction (qPCR) using a QuantStudio 5 Real-time PCR system (Thermo-Fisher Scientific, Waltham, MA) with the TaqMan Fast Virus 1-step master mix (Life Technologies, Carlsbad, CA), and a previously published primer set (Heilig et al. 2010) (F- CAA ACC AAG ATG AAG CTA TTG CTG TA , R- CTT CCT GTT GAA CAC CCT CTT GAA) and probe- (FAM-CAG CCT GAG CAG TTT GA) specific for the flagellin gene of *B. burgdorferi* (GenBank accession no. X15660). Reactions took place in a 96-well plate. All plates included both a positive and a negative extraction control, non-template control, and a positive *Borrelia burgdorferi* PCR control.

Data analysis

Statistical analyses were conducted using R v 4.2.1 (R Core Team 2022). The minimum infection prevalence (MIP) was calculated overall by year and by location.

$$\text{MIP} = (\text{total number of positive pools} \div \text{total number of ticks tested}) \times 100$$

Minimum infection prevalence was compared among years and collection sites using chi-square tests of independence.

Results and Discussion

From 2018 – 2022 the highest abundance of ticks was observed between December and March (Fig. 1). During this five-year period, 80% *burgdorferi* positive *I. pacificus* collected and tested by SYMVCD were from Sacramento County. Although the overall annual MIP varied from year to year (Table 1), there was no significant difference in the minimal infection prevalence rate among years ($X^2 = 7.8$, df = 4, P = 0.09).

Table 1.—Overall minimum infection prevalence (MIP) of *Borrelia burgdorferi* collected in Sacramento and Yolo Counties from 2018 – 2022; MIP was calculated by both collection location and collection year.

MIP by Collection Location			
Sites	*B. burgdorferi* positive pools	Total *I. pacificus*	MIP
Cache Creek Trail	1	605	0.2
Sacramento Bar Park	1	289	0.3
Putah Creek #3	1	262	0.4
Sailor Bar park	1	237	0.4
Lower Sunrise Park	1	167	0.6
Snipes-Pershing Park	7	982	0.7
Black Miners Bar Park	9	898	1.0
East Lake Natoma Park	11	1003	1.1
Upper Sunrise Park	4	282	1.4
Mississippi Bar Park	8	399	2.0
Nimbus Dam Overlook	7	320	2.2
Willow Creek Park	37	1477	2.5
MIP by Year			
Year	*B. burgdorferi* positive pools	Total *I. pacificus*	MIP
2018	9	947	1.0
2019	21	1472	1.4
2020	18	2316	0.8
2021	18	1851	1.0
2022	22	1329	1.7

Overall, *B. burgdorferi* was detected at 12 of the 20 tick surveillance sites across the five-year study period (Fig. 2). The MIP values for the 12 positive sites ranged from 0.17 at Cache Creek Trail to 2.51 at Willow Creek Park (Table 1). We found a significant difference in the minimal infection prevalence among locations ($X^2 = 36.4$, df =11, P $<$ 0.001).

With the increased potential for transmission of tick-borne diseases, there is need to continue to improve both tick surveillance methods and public outreach efforts. To better standardize the tick collecting process, SYMVCD will modify tick collection effort to a distance travelled instead of timed collection periods (Salomon et al. 2020, Eisen and Paddock 2021). By changing to a measured distance, the tick abundance and infection prevalence calculations provide a more accurate evaluation of risk.

Increasing public awareness about tick-borne disease and guidelines on how the public can protect themselves from ticks is the primary way SYMVCD protects the public from ticks and tick-borne disease. To broaden public awareness, colorful educational signage about ticks were recently posted along high traffic walking pathways and trails where tick surveillance was conducted. A forthcoming outreach measure will include upgrades to the SYMVCD website to include tick and *B. burgdorferi* surveillance data. The SYMVCD will continue to conduct surveillance for tick abundance and *B. burgdorferi* infection with the goal of raising awareness of this vector-borne disease threat.

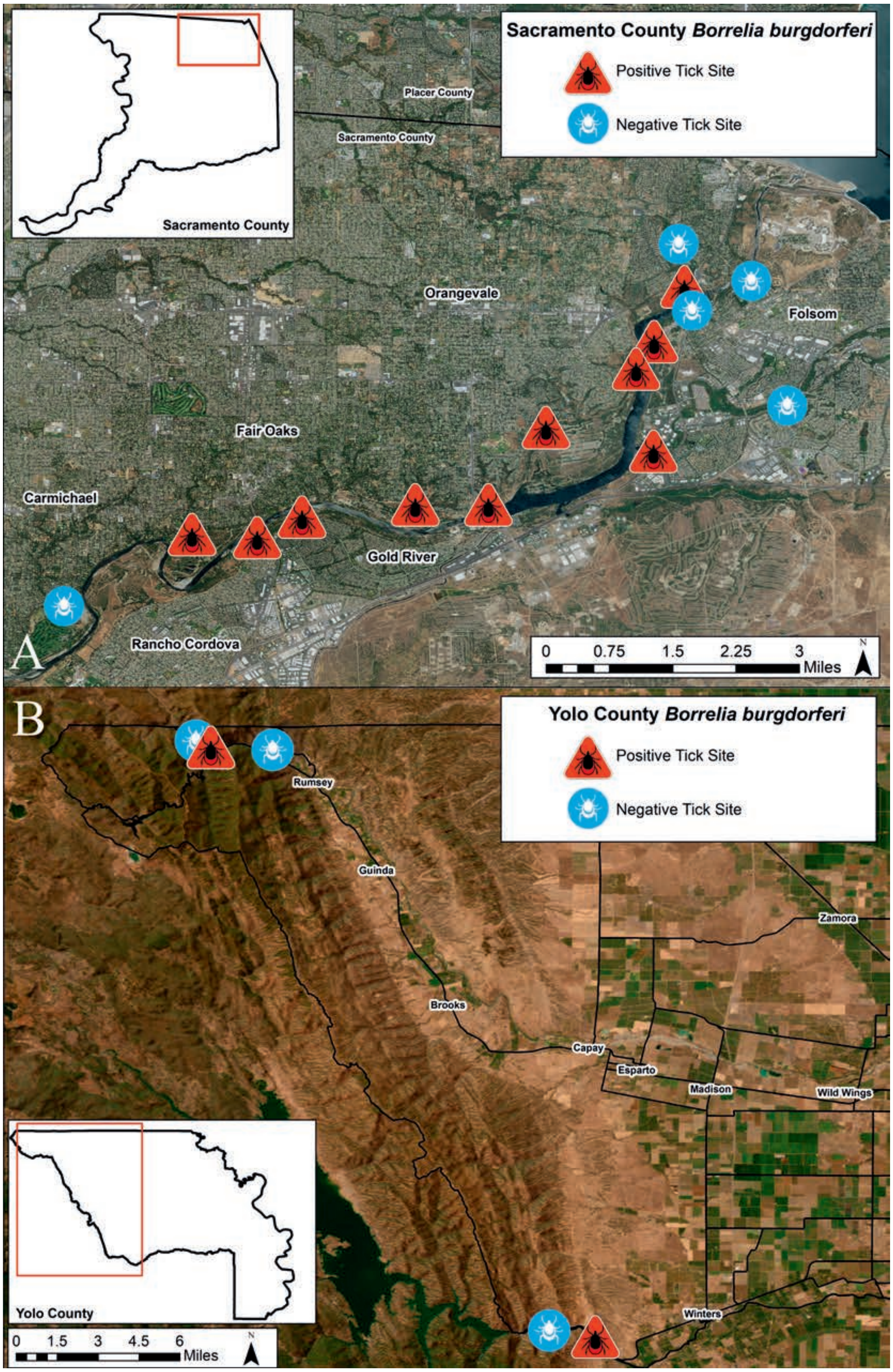

Figure 2.—Tick collection sites in Sacramento (A) and Yolo (B) Counties from 2018 -2022; location symbology represents whether *Borrelia burgdorferi* was detected in *Ixodes pacificus.*

Acknowledgements

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Tick surveillance, testing, and outreach by the San Diego County Vector Control Program

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Introduction

The County of San Diego Vector Control Program (VCP) Tick program started in 2004 after an outbreak of wild rabbit deaths due to the tick-borne pathogen *Francisella tularensis* (tularemia) was detected by the San Diego County Animal Disease and Diagnostic Laboratory (ADDL). This led to collaboration between the VCP and ADDL to collect *Dermacentor occidentalis* tick samples and rabbit roadkills throughout the County for tularemia testing by polymerase chain reaction (PCR). The following years expanded the VCP tick surveillance to identify and test two other major tick species found in the County, *Ixodes pacificus* and *Dermacentor variabilis* [*similis*]. The ADDL was integrated into the County VCP in 2010 and became the Vector Disease and Diagnostic Laboratory (VDDL), focusing on vector borne diseases. In 2011, *Borrelia burgdorferi*, the causative agent for Lyme disease, was detected in *Haemaphysalis leporispalustris* ticks removed from rabbits showing that Lyme disease was present in certain ticks in San Diego County. The VCP Tick program continued to evolve over the next decade with new technologies, methods, and more information being obtained about the status of tick-vectored pathogens throughout the county. Through tick surveillance, disease testing, and community outreach, the VCP has worked to protect the public health from tick-borne diseases in the San Diego County.

Tick Surveillance Methods

San Diego County is 4,300 square miles with hundreds of hiking trails and recreational areas where ticks can be found. Tick collection is focused on areas where ticks pose a higher risk of infesting people. Determinants include population density around a trail, the popularity of a trail/recreational area, and the abundance and species of tick found in the area. Ticks are collected by "tick flagging," a method that uses a 36 × 36-inch square white canvas flag connected to a wooden dowel. Tick flags are dragged across brush and vegetation to capture questing ticks. This procedure captures almost entirely adult ticks, which are then sorted by species and sex in the field before being submitted to the vector ecologists to verify species identity and pool specimens into samples of 10 ticks. Ticks are also submitted for identification when they are removed from rodents in the rodent surveillance program. Tick flagging is still used as the main method for adult tick surveillance with nymph capturing methods potentially in the future of the VCP tick surveillance program.

Tick Pathogen Testing

Tick pathogen testing was completed by the County of San Diego Department of Agriculture, Weights, and Measures ADDL from 2004-2010. Tick tularemia detection was initially performed with a single target 16S PCR assay; however, due to the close genetic similarities of *F. tularensis* and other incompletely characterized *Francisella*-like tick endosymbionts, a multi-target, real-time TaqMan PCR assays were implemented in the mid-2000s to increase testing specificity and sensitivity (Kugeler et al. 2005). The integration of the ADDL into the VCP in 2010 led to an increase in testing for vector-borne pathogens including tularemia, Lyme, and spotted fever group rickettsia surveillance during the following years. The laboratory continues to perform routine *F. tularensis* and *B. burgdorferi* surveillance testing through the cooler weather months including assays for *F. tularensis* type A and type B differentiation, *B. burgdorferi* sensu lato, *B. burgdorferi* sensu stricto, and *Borrelia miyamotoi*. The VCP also monitors the tick populations for Spotted Fever Group (*Rickettsia philipii*, *Ricketssia typhi*, and Rocky Mountain spotted fever (RMSF) *Rickettsia rickettsia*) with *Rickettsia* spp. testing through in-house batch testing and sequencing through our collaborative partners.

Public Outreach

The VCP Outreach group provides public health information on vectors and vector-borne diseases to residents in the county. At the start of the program, it provided in press releases and information booths at fairs. Current outreach methods expanded with a larger team and the development of new communication technologies to also include presentations, social media posts, website updates, and pamphlets (www.sdvector.com).

Public outreach has developed into a critical component of the tick program, communicating information and data gathered from surveillance and disease testing and using it to educate the public about the public health risks of ticks and the pathogens they may carry.

Summary

The County continues to improve its methods of surveillance, diagnostic testing, and community outreach. As technologies improve and methodologies are updated, the VCP continues to adapt to emerging diseases to protect the public health from tick vectors and the diseases they may carry.

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2021 Survey of tick-related services offered by the Mosquito and Vector Control Association of California member agencies

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Introduction

California's Mosquito Abatement Districts Act (AB 1590) was enacted in 1915, and provided for the formation, organization, and financing of mosquito abatement districts. In 1935, legislation authorizing the creation of pest abatement districts was passed, which allowed agencies to address other public health vectors. As a result, some agencies were formed to address solely the growing mosquito control issues, whereas others incorporated other vectors into their mission. Lyme disease was recognized in ticks in California in 1985. Some vector control agencies in California began incorporating information about Lyme and other tick-borne diseases in their public education programs, and some agencies started conducting tick surveillance. The Lyme Disease Advisory Committee (LDAC) was formed in 1999 (SB 1115; California Health and Safety Code 104191) and is composed of members representing the California Lyme Disease Association, Lyme disease support network, university researchers, the Mosquito and Vector Control Association of California (MVCAC), the California Medical Association, California Conference of Local Health Officers, and the California Department of Public Health (CDPH).

A survey was initially conducted by Robert Lane, University of California, Berkeley, in 2005, to determine the level of engagement of MVCAC member agencies in tick surveillance, education, and control. Results were presented at the 4th International Congress of Vector Ecology that year. In 2011, Peavey et al (2012) conducted another survey to determine the extent to which MVCAC agencies engaged in tick control and what services were offered, and compared these results to those presented by Lane in 2005. In 2021, during a meeting of the LDAC, the question as to how many MVCAC member agencies still performed any tick-related activity, and how that differed from previous results from Lane and Peavey arose. Another survey was conducted in the end of 2021 to answer this question.

Methods

At the time of this survey, there were 61 agencies which were members of MVCAC. The survey was sent via email to all MVCAC member agencies and consisted of questions to determine the number of agencies providing tick-related services, the number of agencies providing surveillance, testing, education and control of ticks, and additional details on the services provided. For agencies which responded that they did not provide any tick-related services, a question was asked to determine the reason for not offering those services. The MVCAC Public Relations Committee attempted to follow up by email and phone with all the agencies which did not respond to the initial survey. Another follow up was done by the author by email and phone to ensure most agencies responded to the survey.

Results

Of the 61 MVCAC member agencies surveyed, 26 agencies responded to the initial survey sent by email. After the follow up from the MVCAC Public Relations Committee and by the author, a total of 47 agencies responded (Fig. 1).

When asked whether they provided any tick-related services, 25 (53.2%) of the 47 responding agencies answered yes and 22 (46.8%) answered no (Fig. 2). Agencies not providing any tick-related services stated that was due to inadequate funding or resources (6 agencies, or 27.3%), lack of tickborne disease activity (6 agencies, or 27.3%), lack of interest from their Board (3 agencies, or 13.6%), and the fact that they are a mosquito abatement district only (no other vectors, 13 agencies, or 59.1%).

All 25 agencies which responded that they provide tick-related services responded that they do provide tick identification and 10 (40%) provide tick testing. When asked which ticks they test, all 10 responded they test *Ixodes pacificus*, 3 (30%) test *Dermacentor variabilis*, 3 (30% test *Dermacentor occidentalis*, and 2 (20%) test *Rhipicephalus sanguineus*. When asked for which pathogens they test, the 10 testing agencies varied in their responses, which overall included *Borrelia burgdorferi*, *Borrelia miyamotoi*, *Borrelia bissettii*, *Borrelia sensu latu*, *Rickettsia*, and *Francisella tularensis*. When asked who the agency provided testing for, 7 (70%) stated they provided tick testing for in-house data collection only, 1 (10%) provided tick testing for the public only, 1 (10%) provided tick testing for both in-house data collection and the public, and 1 (10%) provided tick testing for in-house data

Figure 1.—Location (county) of MVCAC member agencies which replied to the survey.

collection and sent ticks from the public to a county public health laboratory for testing.

When asked if they provide tick control services, 24 (96%) agencies responded they do not;only one agency responded they provide control, but clarified that the control method used was vegetation management, mainly brush clearing along trails used by the agency for access to perform mosquito control.

Figure 2.—Location (county) of MVCAC member agencies which provide tick-related services.

When asked if they provide public outreach related to ticks, 22 (88%) responded they do, and listed distribution of tick-related materials, posting notices on areas with ticks, social media outreach, education at schools, information on website, informational booths and tables at community events, and targeted advertising as some of the methods used.

The responses from the survey conducted in 2021 were compared to those provided in 2011 and 2005 (Table 1). In 2021, 47 agencies responded to the survey, whereas in

Table 1.—Comparison of the responses to surveys regarding tick-related services offered by MVCAC member agencies.

Year	Agencies Responding	Tick Control	Disease Agent Survey	Tick ID for the Public	Tick Testing for the Public	Public Education
2005	10	0	3 (30%)	7 (70%)	1 (10%)	6 (60%)
2011	56	3 (5%)	8 (14%)	30 (54%)	4 (7%)	23 (41%)
2021	47	1 (2%)	10 (21%)	25 (53%)	1 (2%)	22 (47%)

2011, 56 responded. Although more agencies responded that they provided tick identification in 2011 than in 2021, more agencies responded that they perform tick testing in 2021.

Conclusions

Results from the survey conducted in 2021 showed that the MVCAC member agencies continue to be a significant local resource to their residents regarding ticks and tick-borne disease prevention. The lower response rate observed during the survey in 2021 may be due to survey-fatigue, as agencies were receiving multiple surveys to respond to at the time. Many agencies that did not respond to the survey were agencies limited by their mandate or mission as a mosquito abatement district only, and may not have responded because they assumed the survey did not apply to them.

In areas of the Sierra Nevada, the coastal region, and southern California, where residents may encounter more ticks, MVCAC member agencies usually provide some tick-related service. Tick information and related educational materials were also provided by agencies in areas where ticks are not very prevalent or are rare.

Acknowledgements

Nola Woods, Public Affairs Director for the Contra Costa Mosquito and Vector Control District and 2021 Chair of the MVCAC Public Relations Committee, and the committee members for their work on following up with non-responding agencies in their regions. Conlin Reis, District Manager for the Fresno Westside Mosquito Abatement District, for the help in creating the survey template.

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Sentinel surveillance for ticks and tickborne pathogens in China Camp State Park, 2019-2022

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Abstract

In 2019, the California Department of Public Health initiated a long-term study in China Camp State Park, Marin County, to determine tick phenology and to monitor seasonal trends in pathogen prevalence. Ticks were surveyed monthly, and adult ticks counted and returned except for the month of January in 2020, 2021, and 2022 when adult *Ixodes pacificus* were tested for pathogens, including *Borrelia burgdorferi* sensu lato, *Borrelia miyamotoi* and *Anaplasma phagocytophilum*.

Overall, 76.8%, 22.3%, 0.6%, and 0.3% of ticks collected ($n = 8{,}758$) were *I. pacificus*, *Dermacentor occidentalis*, *Dermacentor variabilis*, and *Haemaphysalis leporispalustris*, respectively. Of 1,415 adult *I. pacificus* collected during the collection month (January), 3.2%, 1.9% and 1.5% were positive for *B. burgdorferi* s.l., *B. miyamotoi* and *A. phagocytophilum*, respectively. Of 953 *I. pacificus* nymphs tested, 12.2% and 5.2% were positive for *B. burgdorferi* s.l. and *B. miyamotoi*, respectively. Overall *A. phagocytophilum* prevalence in *I. pacificus* nymphs was 2.7% (24/880). Adult *I. pacificus* ticks were active from October to May with peak activity in January, while nymphs were active from February to June with peak activity in April. *D. occidentalis* adult ticks were active from November to July with peak activity in April. *D.occidentalis* nymphs were encountered in July and August. *H. leporispalustris* nymphs were active in April and May.

In summary, *I. pacificus* was the predominant tick species collected in China Camp State Park. The highest risk for adult tick bites is in January, while the risk for nymphal bites peaked in April when questing tick numbers were the highest. Peak prevalence of *B. burgdorferi* s.l. in nymphs occured two months later in June.

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

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Abstract

The increasing use of prescribed burns to mitigate invasive species spread and restore historical landscapes has significant potential to influence tick abundance. In Santa Clara County (SCC), park staff are utilizing prescribed fire at Joseph Grant Park to control the spread of invasive plants (i.e. *Avena* sp.) in grassland habitats. The Santa Clara County Vector Control District, in collaboration with SCC parks, is conducting a two-year study to assess the effects of these prescribed fires on tick abundance. Three sampling plots have been selected within the park encompassing land that has been burned three times in five years, burned for the first time in Summer 2022, and undisturbed with no record of burn in the last ten years. Tick collection was conducted monthly using tick dragging and CO_2 baited dry ice traps. Explanatory variables, such as temperature, humidity, and plant composition, were recorded in each plot. We hypothesize a reduction in tick populations 2 years post burn as a result of larval and nymphal die off in the initial fire.

Leveraging host community ecology to understand and control vector-borne disease

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Introduction

Vector-borne diseases emerge from complex community interactions among arthropod vectors, their bloodmeal hosts, and other interacting species (Swei *et al*., 2020). Understanding the transmission dynamics and risk of vector-borne diseases requires extensive ecological studies to understand host-vector interactions at the community, organismal, and cellular level. Tick-borne diseases, such as Lyme disease, are especially difficult to study, because ticks feed on a wide variety of species with highly variable roles in pathogen transmission and vector maintenance (Castro and Wright, 2007). However, detailed field studies to characterize the community interactions that are involved in the transmission and maintenance of Lyme disease can lead to revealing interactions between host community diversity and vector-borne disease risk (LoGiudice *et al*., 2003; Ostfeld and Keesing, 2012).

Hard ticks (family Ixodidae) are responsible for transmitting the greatest number of vector-borne zoonotic pathogens in the United States. These ticks feed on a variety of vertebrate bloodmeal hosts which, in turn, interact with each other and influence pathogen transmission. At the same time, vertebrate hosts exist on a landscape that is becoming increasing fragmented in ways that affect the movement and spread of animals important in enzootic tick-borne transmission cycles (Allan *et al*., 2003; Brownstein *et al*., 2005; Zolnik *et al*., 2015). Using a longitudinal dataset collected from habitats endemic for *Ixodes pacificus*, the vector of Lyme disease in the western United States, we propose to use community ecology data to better understand how to interrupt the Lyme disease transmission cycle to reduce zoonotic disease risk.

Methods

To better understand the influence of host composition and landscape features on tick-borne disease transmission, we established a network of field sites in oak woodland habitat to empirically measure the composition of vertebrate hosts and the influence of habitat fragmentation on host and tick abundance and infection prevalence. Field sites were established and monitored from 2016 to 2021 in the San Francisco Bay area. A total of 14 sampling plots were established from habitats that spanned a gradient of patch areas. At each site small mammals, lizards, and larger vertebrate populations were assessed by direct live capture or indirect wildlife camera analysis. Questing *I. pacificus* ticks were sampled by drag sampling. Small mammal ear punch tissues and nymphal ticks were tested for infection with *Borrelia burgdorferi*, the Lyme disease pathogen. Generalized linear models were used to evaluate the relationship between host community diversity and habitat landscape features and entomological measures of Lyme disease risk such as the density of nymphs (DON) and density of infected nymphal ticks (DIN).

Results and Discussion

Over the course of six years of surveillance, we captured a total of 954 rodents, 295 reptiles, and identified 2,321 individual animals by wildlife camera trapping. We collected and tested 1,003 nymphal *I. pacificus* ticks for *B. burgdorferi* infection. We found that the density of nymphal ticks (DON) was positively predicted by habitat patch size and rodent species richness (Fig. 1). Density of infected nymphs (DIN) was also positively predicted by rodent species richness (Fig. 2). These results indicated that vertebrate diversity, and rodent reservoir host species richness, in particular, was important for maintaining both the total density of nymphs as well as infected nymph populations. Applied disease ecology can use these host and landscape interactions to target and interrupt ecological interactions that are important for ticks and pathogen transmission. Host targeted acaricide treatment as well as reservoir-targeted vaccines are two ways to potentially reduce both the density and infection prevalence of ticks with *B. burgdorferi* and thereby reduce zoonotic disease risk.

Conclusions

These investigations have revealed insights into the functional role of host diversity and composition on the risk of Lyme disease. These critical interactions can be leveraged in applied vector management strategies such as targeting important reservoir hosts with topical or systemic acaricides or with host targeted vaccines that focus on the most important pathogen reservoirs (Gomes-Solecki *et al*., 2006; Hinckley *et al*., 2016).

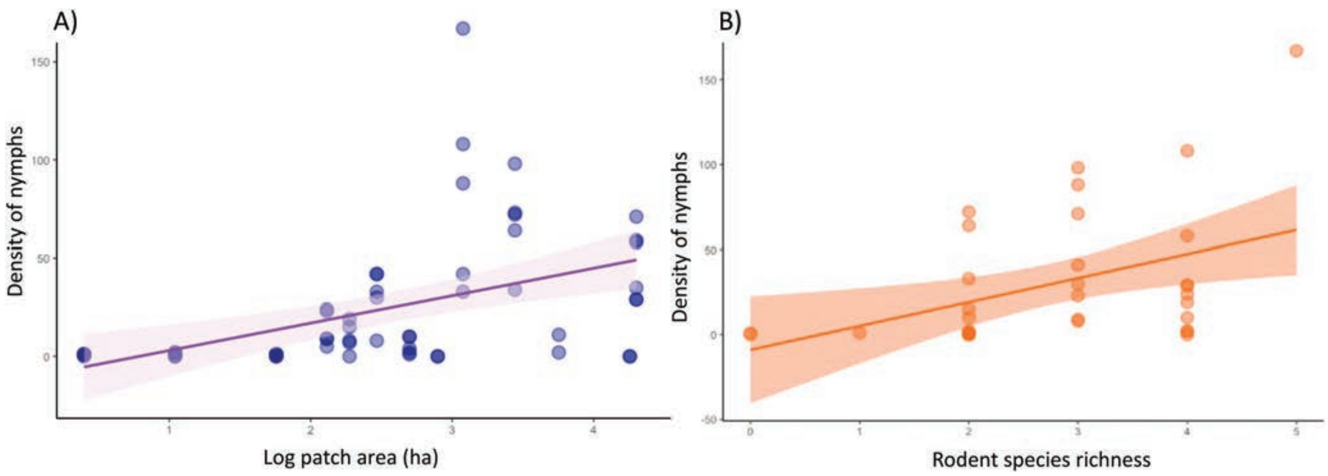

Figure 1.—Generalized linear mixed effect model plots showing density of nymphs response to (a) habitat patch area (estimate = 0.89, $p < 0.01^{**}$) and (b) rodent species richness (estimate = 0.30, $p < 0.001^{***}$).

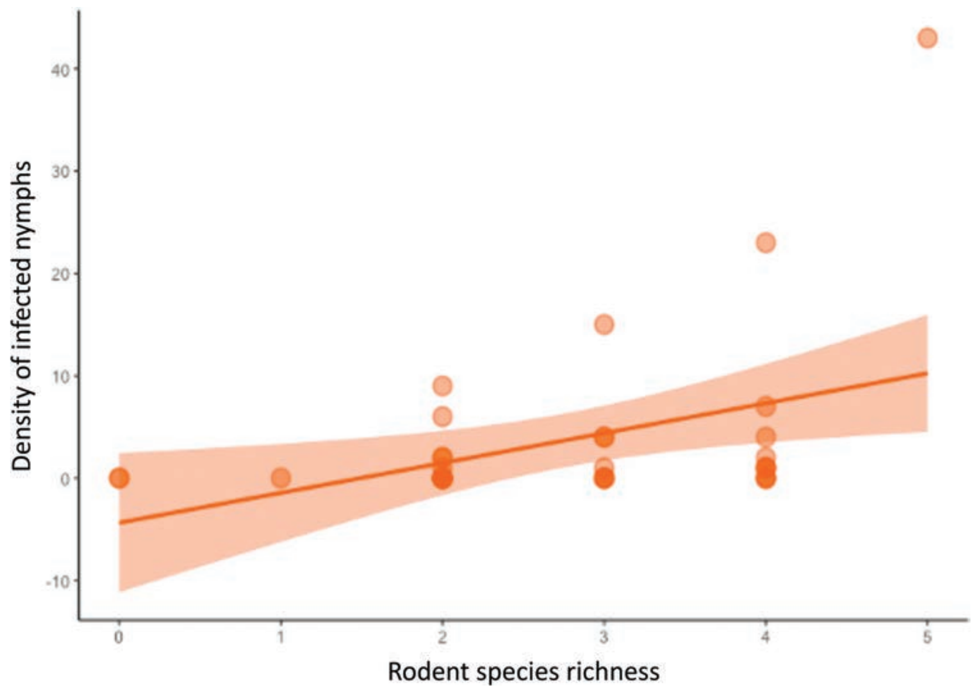

Figure 2.—Generalized linear mixed effect model plot of density of nymphal *I. pacificus* infected with *B. burgdorferi* response to rodent species richness (estimate = 0.5, $p = 0.01^{*}$).

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Brown dog tick and Rickettsiae outreach and surveillance

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Introduction

We describe a program of surveillance and education to engage partners near the southwestern US-Mexico border and on Arizona indigenous lands at high risk during an epidemic of Rocky Mountain spotted fever (RMSF), caused by the bacterium *Rickettsia rickettsii*. Our program is named BiTeRS, an acronym for Border Tick Rickettsial Surveillance. Leadership for BiTeRS is within the CDC-funded Pacific Southwest Center of Excellence for Vector-Borne Disease. The program began in 2021 and encompasses partners in both Arizona and California. In addition to dedicated partners, we began to accept arthropod samples (primarily ticks) directly from the public in 2023.

In addition to epidemic *R. rickettsii*, there are risks of infection with *R. parkeri, Rickettsia* 364D, *R. typhi*, *R. felis,* and *R. massiliae* transmitted via various tick and flea vector species in the border region (Shapiro et al. 2010, Abramowicz et al. 2011, Biggs et al. 2016). Although the case fatality of RMSF can be as high as 40% in highly epidemic and vulnerable areas (Alvarez-Hernandez et al. 2015), *R. massiliae* is an emerging human and canine pathogen with far lower but poorly documented pathogenicity (Vitale et al. 2006). We realize that the lay public may lack information that would allow them to protect themselves and pets, that physicians and veterinarians could potentially benefit from outreach and educational resources, and that data on vector and pathogen status could inform best practices to manage disease. Our partners also are empowered through their participation to be able to build better, evidence-based programs to protect Tribal members, the public accessing parks, citizens in certain counties, or other stakeholders of each partner. It is also important to build a workforce that is skilled and knowledgeable about disease, risk, and management.

Methods

The key goals of BiTeRS are to promote public health and help protect people and dogs from RMSF as well as other tick-borne pathogens that may circulate near the border region. We are doing this through: 1) applied research to document distribution, identity, and environmental risk factors of zoonotic tick-borne diseases along the entire US-Mexico border; 2) engagement and training of public health entomologists and other skilled workers to respond to ticks and tick-borne disease and to apply concepts and practices in One Health; and 3) building collaboration between CDC-funded Centers of Excellence (COEs), academic communities, public health organizations at federal, state, tribal, and local levels, and workers in animal care, lands management, and other related agencies.

BiTeRS is a "partner centered, customized program with a mission that doesn't just aim to promote *our* ideas and goals but rather to listen to our partners' goals and needs and help them customize data collection and practices appropriate for their goals. We ensure data privacy, anonymity when desired, and control over publication or other presentations of data. To add value and increase engagement, we have the ability to deliver canine wellness clinics that include rabies vaccines and other services clinics where we also bring ectoparasite sampling kits and educational resources; seminars, trainings, and "Lunch n Learn" programs; and hardcopy and online educational materials.

Results

To date, BiTeRS has collected and identified 1191 ticks, comprised of *Rhipicephalus sanguineus* (79% of all the ticks acquired), the brown dog tick is a primary RMSF vector; *Dermacentor similis* (13%), the American dog tick which was formerly named *D. variabilis* in the western US; *Ixodes pacificus* (2%), the western blacklegged tick, a vector of Lyme disease and anaplasmosis; and rarely *Haemaphysalis leporispalustris*, the rabbit tick which can act as vector for *Rickettsia* spp. but rarely bites humans; at least two species of *Argas* spp.; and *Otobius megnini,* the spinose ear tick. Among all the ticks, 11.7% have been PCR- positive for *Rickettsia* sp., none to date in the California and Arizona dataset have been positive for *R. rickettsii*. Among brown dog ticks, 6.3% are *Rickettsia* positive, with DNA sequencing confirming that they are infected with *R. massiliae, R. rhipicephali,* and endosymbionts.

Conclusions

The team considers that its successes have included increased awareness in a high risk region, increased resource availability, networking and communication, and improved understanding of tick and rickettsial diversity.

Key challenges are maintaining partner energy and efforts over time, staffing turnover at partner organizations, and targeting surveillance to the optimal time of year and location. We are looking ahead to further characterizing the diversity of rickettsial species, significance and ecology of *R. massiliae,* assessing intervention through modeling, spay/neuter, and dog demography study ahead of vaccination campaigns, more surveillance especially using dogs as sentinels, and more education and outreach.

Acknowledgements

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Modeling Rocky Mountain spotted fever transmission in dog populations to protect human health

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Introduction

Rocky Mountain spotted fever (RMSF), caused by the bacterium *Rickettsia rickettsii*, is a tick-borne disease causing ongoing and expanding fatal human outbreaks in western North America. Epidemics have occurred in Arizona and are ongoing in northern Baja California (Demma et al. 2006, Zazueta et al. 2021). To date, several cases have occurred in southern California, but epidemic level outbreaks have not been observed (Drexler et al. 2017, Kjemtrup et al. 2022). In the southwestern United States and northern Mexico, the brown dog tick, *Rhipicephalus sanguineus* sensu lato, is the primary vector that spreads the rickettsia to dogs and humans. Because dogs are the principal hosts for the brown dog tick and are amplifying hosts for *R. rickettsii*, predicting and monitoring the spread among dog populations is critical for protecting human health in this region. Outbreaks in Arizona and northern Mexico have been associated with dense dog populations and local tick infestations, but to this point, RMSF emergence has been difficult to predict, as it tends to appear, spread, and disappear again (Demma et al. 2006, Álvarez-López et al. 2021). To help address this deficit, we developed a compartmental (SIR) metapopulation model to describe the pattern of RMSF transmission in dogs in northern Baja California, Mexico, with the goal of informing surveillance and mitigation strategies for RMSF throughout the region.

Methods

We developed a compartment model in R describing transmission between *R. sanguineus* and domestic dogs in 14 communities and cities in northern Baja California, Mexico over 8 years. In this model, ticks were able to acquire the infection by feeding on dogs at each life stage, and transmit back to dogs in subsequent feedings. Transovarial transmission allowed female ticks to pass the infection on to their offspring. Dogs were infected according to the probability that they were fed upon by at least one infected tick that successfully transmited the pathogen.

A baseline model was parameterized using current best estimates for dog population density, tick population abundance, and transmission rates of *R. rickettsii* between ticks and dogs (Dantas-Torres 2010, Piranda et al. 2010, Garcia et al. 2018, López-Pérez et al. 2020). It was assumed that there were three tick generations per year, and that the number of ticks feeding on dogs varied seasonally, peaking in the summer. Dog reproduction rates and tick infestation rates were assumed to be the same in every patch across the region. Parameters with uncertainty and inherent variability were assigned appropriate distributions (either normal or uniform) and the model was run 1,000 times. Probability of epidemic occurrence, time to outbreak after introduction, and epidemic patterns across patches were assessed.

Results and Discussion

Introduction of a single infected dog into a large city resulted in regional establishment of RMSF in only 0.3% of model iterations in the baseline scenario, indicating that repeated introductions and convergence of optimal conditions were necessary for an epidemic to occur. In each case where an epidemic occurred, the lag time between introduction and emergence of cases was prolonged, with initial outbreaks occurring nearly two years after introduction. Individual patches—cities or towns—demonstrated variable outbreak patterns. Regardless of patch size or density, outbreaks were largest early in the epidemic, and were followed by a variable number of lower magnitude outbreaks as the proportion of recovered immune dogs stabilized. Although cases in dogs dropped to zero or near zero between outbreaks, the pathogen was maintained in the tick population through transstadial and transovarial transmission, causing cases to emerge when the ratio of susceptible to recovered/immune dogs increased within each patch.

Conclusions

The delay between introduction and epidemic emergence indicates that long-term and widespread surveillance may be needed to detect RMSF in a region where it has recently been introduced. In addition, although cases in dogs may not be occurring or be occurring at a low level, *R. rickettsii* persists in ticks, and cases may emerge sporadically. Although this model does not account for human risk, it indicates that once the pathogen is established, risk will persist for both dogs and humans.

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One Health, One World: The changing epidemiology of Rocky Mountain spotted fever in California

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Abstract

Rocky Mountain spotted fever (RMSF) is a life-threatening tick-borne disease. In California, RMSF is rare; nonetheless, changes over the last 40 years in the epidemiology and eco-epidemiology of the now known vector ticks, *Dermacentor* and *Rhipicephalus*, have impacted our understanding of risk of exposure to this disease. These ticks occur in completely different habitats (sylvatic and peridomestic, respectively) resulting in different exposure risks for humans. In this presentation, the demographic, exposure, and clinical aspects associated with the last 40 years of reported RMSF cases to the California Department of Public Health (CDPH) were presented. Cases of RMSF in California result from both autochthonous and out-of-state exposures. During the last 20 years, more cases reported exposure in Southern California or Mexico than in the previous 20 years. The driver of these epidemiologic changes is likely the establishment and expansion of *Rhipicephalus sanguineus* sensu lato ticks in Southern California and on-going outbreaks of RMSF in northern Mexico. Recent human cases and studies have demonstrated that the role of dogs as ther disease reservoir and the brown dog tick as vector need to be considered in designing prevention messaging.

A rare occurrence of the brown dog tick (*Rhipicephalus sanguineus*, Latreille, 1806) in Alameda County

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Introduction

Alameda County Vector Control Services District (ACVCSD) routinely conducts surveys for ticks. Three tick species are predominantly found within parks and green areas: the western blacklegged tick (*Ixodes pacificus,* Cooley & Kohls, 1943), the Pacific Coast tick (*Dermacentor occidentalis,* Marx, 1892), and the Western dog tick (*Dermacentor similis*, Lado et al., 2021). We received an unusual Request For Service from the public in early 2022, when an Oakland resident contacted our agency regarding ticks in their urban neighborhood backyard. The resident had been finding ticks for three months and adjacent green areas were nonexistent. Tick specimens were collected and identified as brown dog ticks (*Rhipicephalus sanguineus,* Latreille, 1806). Although this species has historically been found within Alameda County, it has only been limited to rare occurrences.

The Brown Dog Tick

The brown dog tick and kennel tick (*R. sanguineus*) is a species found circumglobally in areas where dogs reside. Dogs are the main host for this tick species, but they are known to occasionally bite humans (Goddard 1989, Demma et al. 2005). *Rhipicephalus sanguineus* are capable of carrying disease-causing pathogens such as *Rickettsia rickettsia*, the bacterium that causes Rocky Mountain spotted fever (Wickso et al. 2007). Some distinguishing features of this tick species are its hexagonal basis capituli, deeply cleft fore coxae, presence of festoons, eyes on the side of the scutum, and an anal groove (CDC 1978) (Figure 1). *Rhipicephalus sanguineus* has adapted well to surviving in urban areas with its endophilic behavior by finding shelter within walls and actively hunting for a host instead of solely questing for a blood meal (Dantas-Torres 2010).

Rocky Mountain Spotted Fever

Rickettsia rickettsia is a bacterial pathogen that *R. sanguineus* is known to transmit and causes Rocky Mountain spotted fever (RMSF). Fatality rates in humans can be as high as 20% if patients are left untreated (Demma et al. 2005). RMSF was first reported in California in 1903 and is principally transmitted by *Dermacentor spp*. ticks (Wickso et al. 2007, Demma et al. 2005). *Rhipicephalus sanguineus* ticks are the primary host for RMSF in Mexico but were discovered to be the vector in Arizona in 2003 and later in California (Phillips 2017, Wikswo et al. 2007). RMSF can be transferred transovarially from parent to offspring in ticks, which means that the offspring do not need to feed on an infected host to carry RMSF (Thorner et al. 1998). Currently the antibiotic doxycycline is used to treat RMSF disease in humans. Early detection of disease symptoms and treatment are necessary to ensure survival (Phillips 2017). With this information, it is important for ACVCSD to prevent potential pathogen transmission by responding to Requests For Service regarding tick concerns.

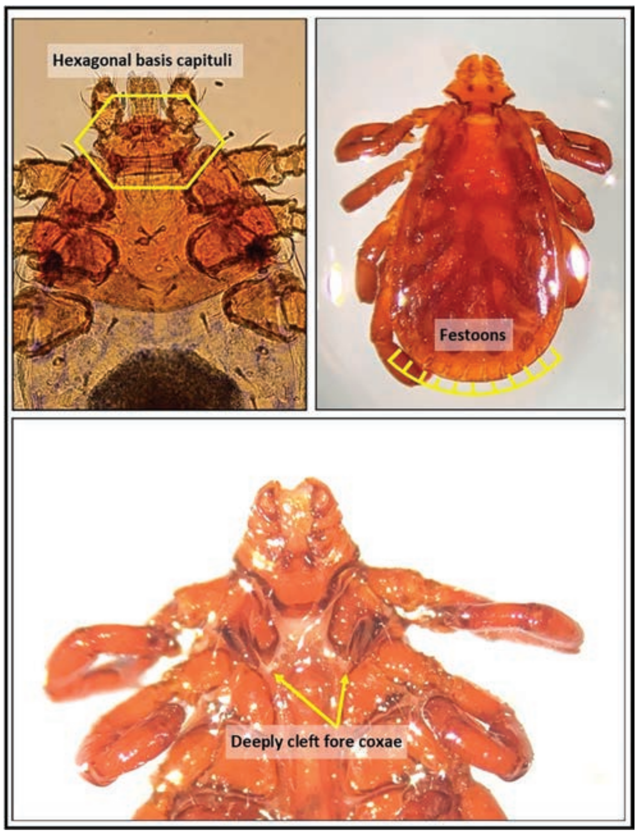

Figure 1.—Distinguishing features of *Rhipicephalus sanguineus* include hexagonal basis capituli, presence of festoons, and deeply cleft fore coxae.

City of Oakland Case Study

In early April 2022, an Oakland resident contacted our department regarding ticks in their yard. They had moved into their new home about three months prior and owned dogs that lived onsite. An initial inspection of the home and surrounding areas by Vector Control Biologist Augustine De Villa revealed small patches of grass. Much of the home was situated on ground covered with concrete and the nearest park with greenery was several blocks away. It was determined that the tick species in question was unlikely to be the exophilic *Ixodes* spp. or *Dermacentor* spp. Most ticks discovered were located on the front patio area near the base of the wall. Several tick specimens were collected from the property and were identified by Vector Ecologist Wade Lee as *R. sanguineus*.

After confirming the tick species, several recommendations for management were given to the resident for controlling *R. sanguineus*. At the time, the resident was administering a natural garlic-based formulated ectoparasite treatment tablet for their dogs which did not resolve the *R. sanguineus* infestation. ACVCSD recommended a visit the veterinarian to determine the best treatment for the dogs such as a systemic treatment with chewable tablets containing an acaricide. Further recommendations were also given for a licensed pest control company to treat the property and surrounding areas where ticks were found and likely to occur. Lastly, it was recommended for the resident to launder the pet bedding until ticks were fully eliminated.

Conclusion

With the ability of *R. sanguineus* to survive in urban areas where dogs are the primary host, there is potential to encounter an increased number of similar cases in the future within Alameda County. To prevent potential spread of disease, education and proper treatment protocols will be extremely important in eliminating brown dog tick infestations and the potential for RMSF transmission. Education should include discussing environmental modifications such as sealing cracks and crevices around the home, regular laundering of the pet bedding, and trimming vegetation to reduce any tick hiding areas. If there are dogs present, regular tick checks and proper veterinarian advice for medication would be recommended. Documentation of similar unusual tick-related Requests For Service can be used as a passive surveillance system to detect new infestations.

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Rice field mosquito control: challenges and strategies

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Abstract

Most varieties of rice require standing water for cultivation. The heavy clay soils of the Sacramento Valley are ideal for growing rice because they retain water and limit percolation, and therefore are one of the four primary regions where rice is grown in the United States. The standing water needed to grow rice provides ample habitat for the development of larval mosquitoes, predominantly *Anopheles freeborni,* an aggressive nuisance biter, and *Culex tarsalis,* a competent vector of West Nile virus. Because rice fields are often found near suburban and urban areas, the Sacramento-Yolo Mosquito and Vector Control District works to control both larval and adult rice field mosquito populations to protect the residents of Sacramento and Yolo County from bite pressure and West Nile virus infection. A range of integrated pest management strategies are utilized including: outreach to farmers, best rice management practices for reducing mosquitoes, application of larvicides, planting of mosquitofish, treatments for adult mosquito control, and a fall flooding program. Each aspect of this program were discussed in detail in a recently published programmatic review (Wheeler et al. 2022). Rice is an important crop for the Sacramento Valley and effective rice field mosquito management strategies are critical for protecting public health from mosquitoes and mosquito-borne disease.

Reference Cited

Wheeler, S. S., M. Reed, S. Ramos, G. Goodman, and S. Elkashef. 2022. Programmatic review of the mosquito control methods used in the highly industrialized rice agroecosystems of Sacramento and Yolo Counties, California. Wetlands Ecol. Manage. 30: 945–960.

Surveillance for mosquito-borne encephalitis virus activity in California, 2022

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Abstract

In 2022, 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 35 of 58 counties in California, and St. Louis encephalitis virus (SLEV) activity was reported from 12 counties. A total of 209 human WNV infections were reported, and non-human WNV activity was detected among horses, dead birds, mosquitoes, and sentinel chickens. Fourteen human cases of SLEV disease were identified in seven counties, and enzootic SLEV activity was detected in mosquitoes from nine counties.

Introduction

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

In 2022, 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 35 (60%) of 58 counties in California (Table 1), while SLEV activity was reported from 11 (19%) 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 received confirmatory testing using an SLEV IgM MAC-ELISA and plaque reduction neutralization tests (PRNT) at VRDL. Additional WNV infections were identified through screening performed by blood centers.

In 2022, a total of 209 symptomatic and 14 asymptomatic infections in blood donors with WNV were reported (Tables 1 and 3). Of the 209 symptomatic cases, 164 (78%) were classified as West Nile neuroinvasive disease (WNND) (e.g., encephalitis, meningitis, acute flaccid paralysis, or other neurologic dysfunction) and 45 (22%) were classified as non-neuroinvasive

Table 1.—West Nile virus activity in California by county, 2022. Humans include asymptomatic infections detected through blood bank and organ donor screening. NT = None tested

County	Humans	Horses	Dead Birds	Mosquito Pools	Sentinel Chickens
Alameda	1	0	0	0	0
Alpine	0	0	NT	NT	NT
Amador	0	0	NT	NT	NT
Butte	3	0	2	38	27
Calaveras	0	0	NT	NT	0
Colusa	1	0	NT	0	3
Contra Costa	1	0	1	2	5
Del Norte	0	0	NT	NT	NT
El Dorado	0	0	0	NT	NT
Fresno	33	0	2	296	NT
Glenn	1	0	NT	1	NT
Humboldt	0	0	0	NT	NT
Imperial	0	0	NT	1	NT
Inyo	0	0	NT	0	NT
Kern	23	3	0	99	NT
Kings	7	1	NT	105	NT
Lake	0	0	2	7	3
Lassen	0	0	NT	NT	NT
Los Angeles	68	1	49	578	33
Madera	3	0	2	132	NT
Marin	0	0	0	0	NT
Mariposa	0	0	NT	NT	NT
Mendocino	0	0	NT	NT	NT
Merced	7	0	2	20	16
Modoc	0	0	NT	NT	NT
Mono	0	0	NT	NT	NT
Monterey	0	0	0	NT	NT
Napa	0	0	0	1	NT
Nevada	0	1	1	NT	0
Orange	11	0	1	39	NT
Placer	2	0	5	89	NT
Plumas	0	0	NT	NT	NT
Riverside	0	0	5	125	NT
Sacramento	5	2	40	42	6
San Benito	0	0	NT	0	0
San Bernardino	4	0	7	100	NT
San Diego	3	0	0	0	NT
San Francisco	0	0	0	NT	NT
San Joaquin	5	1	1	208	NT
San Luis Obispo	0	1	0	0	NT
San Mateo	1	0	0	0	0
Santa Barbara	0	0	0	0	0
Santa Clara	1	0	26	23	NT
Santa Cruz	0	0	0	0	NT
Shasta	1	0	1	39	3
Sierra	0	0	NT	NT	NT
Siskiyou	0	0	NT	NT	NT
Solano	3	0	9	12	0
Sonoma	1	0	1	0	NT
Stanislaus	15	1	0	70	NT
Sutter	1	0	4	31	16
Tehama	3	2	NT	NT	6
Trinity	0	0	NT	NT	NT
Tulare	15	3	2	1030	10
Tuolumne	0	0	NT	NT	NT
Ventura	0	0	1	0	0
Yolo	3	0	24	65	4
Yuba	1	0	1	12	13
State Totals	223	**16**	**189**	**3,165**	**145**

Table 2.—St. Louis encephalitis virus activity in California by county, 2022. NT = None tested

County	Humans	Mosquito Pools[1]	Sentinel Chickens
Fresno	2	14	NT
Imperial	0	6	NT
Kern	3	12	NT
Kings	0	27	NT
Los Angeles	2	1	0
Madera	0	18	NT
Orange	1	0	NT
Riverside	0	57	NT
Sacramento	1	0	0
Stanislaus	1	1	NT
Tulare	4	16	0
Totals	**14**	**152**	**0**

[1] Positive mosquito pools included *Cx. tarsalis* (103 pools), *Cx. quinquefasciatus* (45 pools), *Cx. pipiens* (4 pools).

disease; 14 (6.7%) cases were fatal. Cases were residents of 28 counties, and 131 (63%) were male. In 2022, WNV disease incidence in California was 0.53 cases per 100,000 persons. Incidence was highest (4.61 cases per 100,000 persons) in Tehama County, whereas Los Angeles County reported the most cases (61, 29% of total) (Figure 1, Table 3). The median age of those with WNND was 65 years (range, 9 to 91 years), and among cases with non-neuroinvasive disease the median age was 60.5 years (range, 14 to 93 years). The median age of the 14 WNV-associated fatalities was 81 years (range, 45 to 88 years). Dates of symptom onset ranged from April 8 to December 11, with the peak occurring during epidemiological week 31 (July 31 - August 6), when 20 (10%) symptomatic infections were reported.

Fourteen symptomatic cases of SLEV infection also were identified in 2022. Eleven (79%) cases presented with neuroinvasive disease and one fatality was reported. Cases were residents of seven counties (Table 2) and six (43%) were male. The median age was 66 years (range, 36 to 83 years) and dates of symptom onset ranged from May 31 to September 1.

Mosquito Surveillance

In 2022, mosquito testing was performed at DART and 13 local mosquito and vector control agencies. A total of 1,261,191 mosquitoes (47,687 pools) collected in 38 counties were tested by a triplex real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) for SLEV, WEEV, and 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 3,165 mosquito pools from 26 counties (Tables 1 and 4), and SLEV was detected in 152 mosquito pools from 9 counties (Table 2). Statewide, the annual minimum infection rate (MIR-defined as the minimum number of infected female mosquitoes per 1,000 tested) of WNV in all mosquitoes

Table 3.—Reported West Nile virus human cases by county of residence and year, California, 2013−2022.

County	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2022 incidence per 100,000 person-years	Ten-year incidence per 100,000 person-years
Alameda	0	1	0	0	1	0	1	0	0	1	0.06	0.02
Alpine	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Amador	0	0	0	1	0	1	1	0	0	0	0.00	7.84
Butte	24	24	53	21	4	12	5	4	13	3	1.49	8.09
Calaveras	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Colusa	2	3	1	2	0	0	1	0	0	1	4.59	4.59
Contra Costa	5	5	1	4	4	4	1	4	2	1	0.09	0.27
Del Norte	0	0	0	0	0	0	0	0	0	0	0.00	0.00
El Dorado	1	0	0	1	0	0	0	1	1	0	0.00	0.21
Fresno	8	43	8	14	13	14	51	10	14	30	2.77	2.01
Glenn	9	10	19	6	0	2	0	1	2	1	3.48	17.39
Humboldt	0	0	0	0	0	1	0	0	0	0	0.00	0.07
Imperial	0	1	1	0	3	0	3	1	0	0	0.00	0.50
Inyo	0	0	0	0	4	0	0	0	0	0	0.00	2.11
Kern	25	11	11	17	30	13	28	8	8	22	2.42	1.00
Kings	1	4	0	8	5	0	3	2	8	7	4.60	2.50
Lake	0	1	2	1	0	1	0	2	0	0	0.00	1.04
Lassen	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Los Angeles	151	253	286	151	277	43	31	90	16	61	0.61	1.38
Madera	3	3	4	6	2	4	3	6	3	3	1.91	2.35
Marin	2	0	1	0	0	0	0	0	0	0	0.00	0.12
Mariposa	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Mendocino	0	1	2	0	0	0	0	0	0	0	0.00	0.33
Merced	0	1	1	0	10	2	10	12	6	7	2.46	1.72
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	0	0	1	0	1	0	0	0	0	0.00	0.05
Napa	1	0	0	0	0	1	0	0	0	0	0.00	0.15
Nevada	0	0	2	0	0	1	0	0	0	0	0.00	0.30
Orange	10	263	92	32	33	9	5	17	3	9	0.32	1.50
Placer	6	7	0	7	0	9	1	2	2	2	0.49	0.88
Plumas	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Riverside	35	14	127	11	32	15	12	10	3	0	0.00	1.06
Sacramento	11	10	4	25	6	15	4	7	6	5	0.32	0.59
San Benito	0	0	0	0	0	0	0	0	0	0	0.00	0.00
San Bernardino	13	21	54	8	57	9	7	3	1	4	0.18	0.81
San Diego	0	11	42	20	2	2	3	1	3	3	0.09	0.27
San Francisco	1	0	0	0	1	0	0	0	1	0	0.00	0.04
San Joaquin	8	9	2	13	14	14	7	2	7	4	0.51	1.02
San Luis Obispo	0	0	0	0	0	0	2	0	2	0	0.00	0.14
San Mateo	0	0	0	0	0	0	0	0	1	1	0.13	0.03
Santa Barbara	1	0	0	0	0	0	0	0	2	0	0.00	0.07
Santa Clara	2	10	8	1	0	1	1	0	2	1	0.05	0.14
Santa Cruz	0	0	0	0	0	0	0	0	1	0	0.00	0.04
Shasta	1	2	3	1	1	1	0	2	3	1	0.55	0.83
Sierra	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Siskiyou	0	0	1	0	0	0	0	0	0	0	0.00	0.23
Solano	1	5	1	4	1	0	1	1	2	3	0.67	0.43
Sonoma	0	0	0	0	0	0	0	0	0	1	0.21	0.02
Stanislaus	17	33	13	26	28	15	16	35	5	15	2.73	3.69
Sutter	10	8	2	12	3	1	1	1	0	1	1.01	3.93
Tehama	5	4	5	5	2	2	0	2	0	3	4.61	4.30
Trinity	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Tulare	5	21	13	10	12	8	24	7	8	15	2.95	2.57
Tuolumne	0	0	0	0	0	1	0	0	0	0	0.00	0.18
Ventura	2	1	6	7	1	2	2	0	0	0	0.00	0.25
Yolo	6	15	8	16	6	11	1	4	3	3	1.36	3.30
Yuba	13	6	10	11	1	2	0	0	0	1	1.22	5.35
Total WNV Cases	**379**	**801**	**783**	**442**	**553**	**217**	**225**	**235**	**128**	**209**	**0.53**	**1.01**
Asymptomatic Infections	54	91	77	41	47	26	18	28	19	14		
Total WNV infections	**433**	**892**	**860**	**483**	**600**	**243**	**243**	**263**	**147**	**223**		

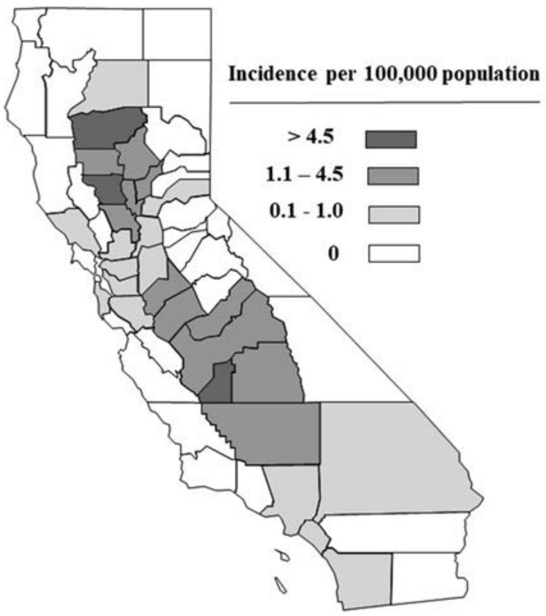

Figure 1.—Incidence of human cases of West Nile virus by county in California, 2022.

tested was 2.5. During California's peak transmission period (July – September) the statewide MIR in *Culex* mosquitoes was 4.5 and seven 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, 2022).

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

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

Chicken Serosurveillance

In 2022, 24 local mosquito and vector control agencies in 20 counties maintained 79 sentinel chicken flocks (Table 4). Blood samples were collected from chickens every other week and tested for antibodies to WNV, SLEV, and WEEV by an EIA at the CDPH Vector-Borne Disease Section laboratory and one local agency. Presumptive positive samples were confirmed by IFA or western blot.

Of 5,013 chicken blood samples tested, 145 seroconversions to WNV were detected among 35 (44%) flocks in 13 counties (Tables 1 and 4). Seroconversions to WNV occurred between July 1 and October 25, with the peak occurring during epidemiological week 36 (September 4 – September 10). No SLEV seroconversions were detected in 2022.

Dead Bird Surveillance

In 2022, the WNV and Dead Bird Call Center and website received a total of 4,996 dead bird reports from the public from 49 counties (Table 6). Tissue samples from dead bird carcasses or oral swabs transferred to RNA preservation cards (Fortius Bio, San Diego, CA) were tested at DART or at one of 13 local agencies by RT-qPCR. Of the 1,330 bird carcasses that were deemed suitable for testing, WNV was detected in 189 (14%) carcasses from 22 counties (Tables 1 and 6). Twenty different bird species tested positive for WNV: 54% were American crows, 13% were California scrub-jays, 14% were other corvids, and 19% were non-corvid or unknown species. Positive birds were detected from May 4 to November 9, with the peak occurring during epidemiological week 35 (August 29 – September 4).

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 2022, WNV infection was confirmed in 16 horses from 10 counties (Table 1). Five (31%) of the horses died or were euthanized because of their infection.

Discussion

In 2022, 35 (60%) of 58 counties reported WNV activity. A total of 209 human cases were reported from 28 counties, which was the third lowest number of cases reported in California since 2010 (Figure 3). The lowest number of cases reported in California since 2010 was observed in 2021 with 129 WNV cases. Los Angeles County reported the most cases (N = 61), but the incidence was highest in Tehama County (Table 3). Non-human WNV activity was reported from 32 counties and two of the environmental indicators were higher than the previous year. Compared to 2021, the percentage of WNV positive birds was similar, but the WNV MIR was greater than during the past 4 years (2018-2021). The percentage of WNV positive sentinel chicken flocks was also higher compared to 2021 and was the second highest percent overall since 2018 (Figures 4, 5 and 6; California Department of Public Health). Surveillance results documented WNV activity throughout most of the year, but the vast majority of detections occurred from June through October, with peak activity occurring in August.

Following the re-emergence of SLEV in California in 2015, SLEV has continued to co-circulate with WNV in

Table 4.—Results of mosquito and sentinel chicken testing for West Nile virus by county, California, 2022.

County	No. mosquitoes tested	No. mosquito pools tested	WNV + pools	No. flocks	No. chickens	No. WNV positive flocks	WNV + sera
Alameda	17,093	734		3	18	0	0
Butte	19,787	424	38	7	46	6	27
Calaveras				1	10	0	0
Colusa	450	9		1	10	1	3
Contra Costa	9,791	307	2	4	23	1	5
Fresno	60,708	1,848	296				
Glenn	852	18	1				
Imperial	2,852	164	1				
Inyo	699	15					
Kern	26,560	803	99				
Kings	10,065	232	105				
Lake	10,779	461	7	2	12	1	3
Los Angeles	140,115	3,800	578	21	120	9	33
Madera	35,088	880	132				
Marin	1,736	87					
Merced	10,787	549	20	8	47	4	16
Napa	1,803	117	1				
Nevada				2	12	0	0
Orange	132,610	4,347	39				
Placer	31,295	1,942	89				
Riverside	256,713	7,656	125				
Sacramento	39,563	3,719	42	3	19	2	6
San Benito	64	14		1	8	0	0
San Bernardino	43,136	2,707	100				
San Diego	24,839	1,929					
San Joaquin	69,031	2,338	208				
San Luis Obispo	1,374	59					
San Mateo	2,887	156		2	14	0	0
Santa Barbara	3,093	130					
Santa Clara	17,540	2,573	23				
Santa Cruz	2,697	125					
Shasta	15,851	580	39	5	34	1	3
Solano	13,281	480	12	3	20	0	0
Sonoma	4,253	192					
Stanislaus	38,719	1,216	70				
Sutter	9,194	282	31	5	35	4	16
Tehama				3	30	2	6
Tulare	172,580	4,917	1,030	1	10	1	10
Ventura	3,265	67		3	29	0	0
Yolo	23,389	1,625	65	2	14	1	4
Yuba	6,652	185	12	2	14	2	13
Total	**1,261,191**	**47,687**	**3,165**	**79**	**525**	**35**	**145**

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. This resulted in the identification of 14 human SLEV cases from seven counties; the highest number of SLEV cases reported since the re-emergence of this virus. A total of 152 SLEV-positive mosquito pools were reported from nine counties, including five of the counties with reported SLEV cases (Table 2). Although no SLEV seroconversions were detected in chickens, sentinel flocks were absent from almost all counties where SLEV was detected in mosquitoes (Table 2). In prior years, SLEV has been detected in flocks before SLEV was detected in mosquitoes collected within the county, highlighting the importance of utilizing all surveillance tools to detect arbovirus activity.

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

Conclusions

Activity of WNV was lower in 2022 compared to most prior years, but 2022 activity was higher relative to 2021.

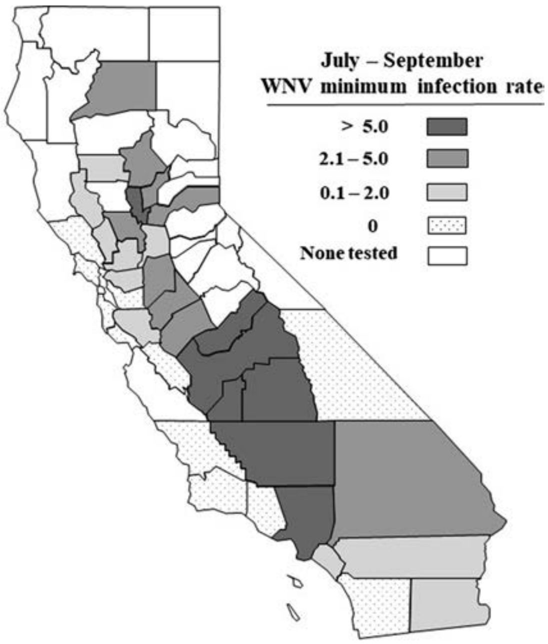

Figure 2.—West Nile virus minimum infection rate in *Culex* mosquitoes, by county, California, July − September, 2022. Minimum infection rate is defined as the minimum number of infected female mosquitoes per 1,000 tested.

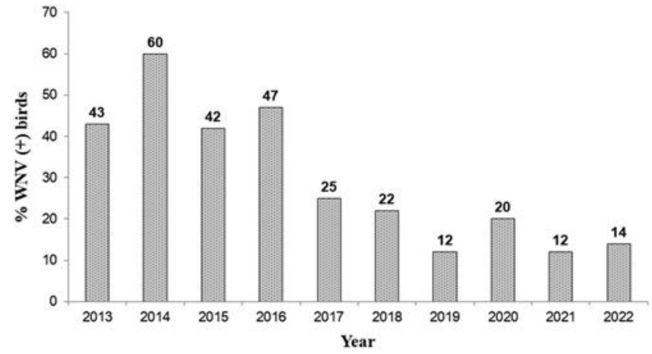

Figure 4.—Percentage of dead birds positive for West Nile virus in California, 2013−2022.

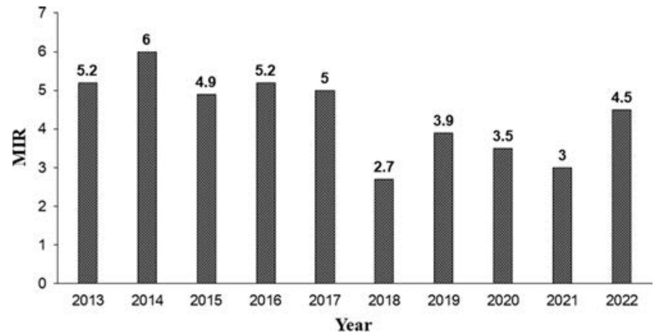

Figure 5.—Minimum infection rate (MIR) in *Culex* females per 1,000 tested for West Nile virus in California, July − September, 2013−2022.

WNV remains the greatest vector-borne disease threat in California, with over 7,000 cases and more than 300 fatalities reported since its invasion in 2003. Human cases of WNV disease were higher than in 2021 and SLEV human disease cases were the highest reported since the re-emergence of SLEV in CA, highlighting the importance for ongoing surveillance and awareness of potential human disease risk. Environmental detections of both viruses 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.

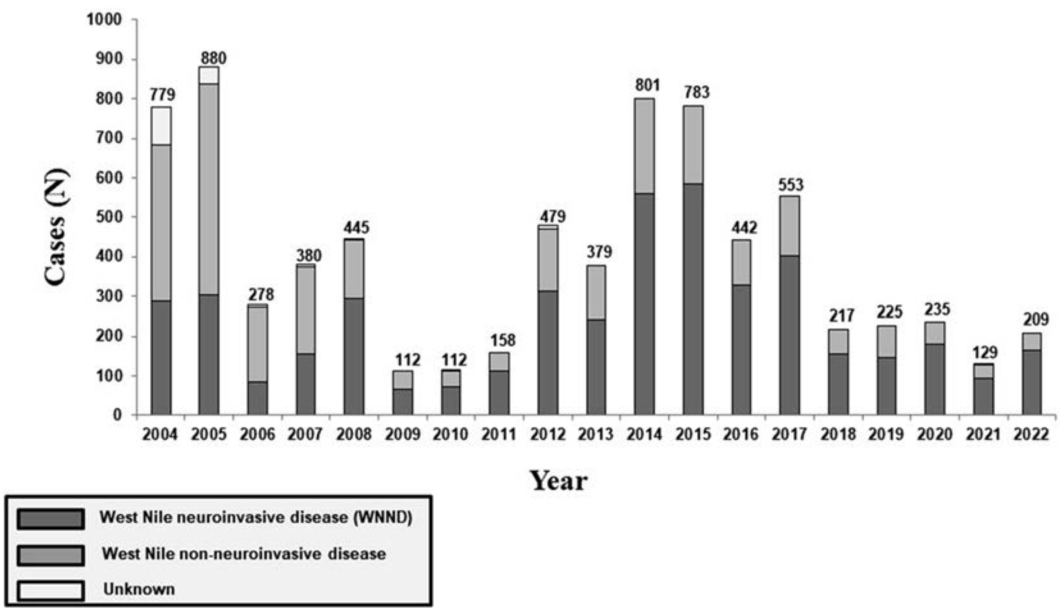

Figure 3.—Human cases of West Nile virus in California, by year, 2004−2022.

Table 5.—Mosquito species tested for West Nile virus, California, 2022.

Culex species	No. Pools	No. mosquitoes	WNV +	MIR
Cx. erythrothorax	1,607	54,020	2	0.0
Cx. pipiens	8,415	119,518	181	1.5
Cx. quinquefasciatus	20,719	628,053	2,059	3.3
Cx. restuans	4	10	0	0.0
Cx. stigmatosoma	454	4,403	31	7.0
Cx. tarsalis	15,415	441,665	888	2.0
Cx. territans	2	43	0	0.0
Cx. thriambus	55	99	1	10.1
Culex species	2	4	0	0.0
All *Culex*	**46,673**	**1,247,815**	**3,162**	**2.5**
Anopheles species	No. Pools	No. mosquitoes	WNV +	MIR
An. franciscanus	143	3,801	0	0.0
An. freeborni	3	72	0	0.0
An. hermsi	3	26	0	0.0
All *Anopheles*	**149**	**3,899**	**0**	**0.0**
Aedes species	No. Pools	No. mosquitoes	WNV +	MIR
Ae. aegypti	543	4,200	2	0.5
Ae. melanimon	17	381	0	0.0
Ae. nigromaculis	9	316	1	3.2
Ae. squamiger	1	28	0	0.0
Ae. taeniorhynchus	5	161	0	0.0
Ae. vexans	12	370	0	0.0
All *Aedes*	**587**	**5,456**	**3**	**0.5**
Other species	No. Pools	No. mosquitoes	WNV +	MIR
Culiseta incidens	243	3,443	0	0.0
Culiseta inornata	15	149	0	0.0
Culiseta particeps	16	340	0	0.0
Psorophora columbiae	3	39	0	0.0
Unknown	1	50	0	0.0
All other	**278**	**4,021**	**0**	**0.0**

Table 6.—Dead birds reported, tested, and positive for West Nile virus, California, 2022.

County	Reported	Tested	Positive	Percent
Alameda	230	38		
Alpine	0			
Amador	6			
Butte	38	7	2	29
Calaveras	3			
Colusa	4			
Contra Costa	373	42	1	2
Del Norte	0			
El Dorado	27	9		
Fresno	124	8	2	25
Glenn	4			
Humboldt	5	1		
Imperial	0			
Inyo	0			
Kern	23	1		
Kings	11			
Lake	12	6	2	33
Lassen	1			
Los Angeles	783	115	49	43
Madera	8	3	2	67
Marin	56	4		
Mariposa	0			
Mendocino	5			
Merced	44	7	2	29
Modoc	0			
Mono	0			
Monterey	18	4		
Napa	20	9		
Nevada	11	4	1	25
Orange	411	284	1	
Placer	93	47	5	11
Plumas	3			
Riverside	93	21	5	24
Sacramento	617	270	40	15
San Benito	3			
San Bernardino	101	24	7	29
San Diego	127	47		
San Francisco	44	8		
San Joaquin	124	29	1	3
San Luis Obispo	16	2		
San Mateo	262	64		
Santa Barbara	25	5		
Santa Clara	463	83	26	31
Santa Cruz	57	10		
Shasta	28	6	1	17
Sierra	0			
Siskiyou	1			
Solano	143	33	9	27
Sonoma	98	19	1	5
Stanislaus	121	5		
Sutter	47	13	4	31
Tehama	5			
Trinity	0			
Tulare	33	9	2	22
Tuolumne	3			
Ventura	67	10	1	10
Yolo	177	73	24	33
Yuba	28	10	1	10
Totals	**4,996**	**1,330**	**189**	**14.2**

Acknowledgements

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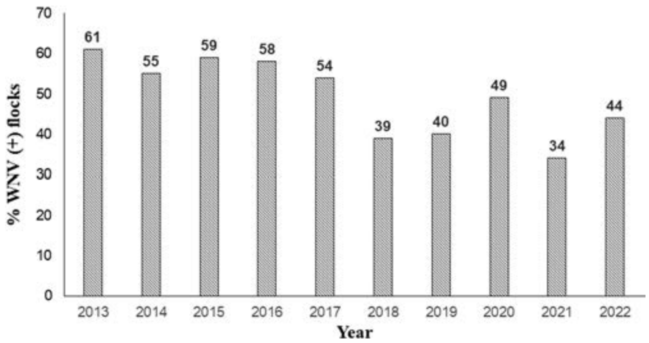

Figure 6.—Percentage of sentinel chicken flocks in California with one or more birds positive for antibodies to West Nile virus, 2013–2022.

(especially Ervic Aquino, Christian Irian, Margaret Kerrigan, Mary Joyce Pakingan, Erin Trent, and the WNV Call Center staff).

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Measuring and distributing workload in mosquito abatement

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Introduction

The Alameda County Mosquito Abatement District (ACMAD) is an independent special district with headquarters in Hayward, California, that is tasked to improve the quality of life and prevent mosquito-borne diseases in Alameda County (Alameda County Mosquito Abatement District, 2020). A large part of the field work is completed by vector biologists and seasonal technicians who work within service zones that were established in 2013. These service zones organized staff and enabled them to meet the needs of ecologically and geographically diverse service areas (Figure 1A). ACMAD sought to study three items related to the current work zones: (1) evaluate existing distribution of work to ensure workload equity across zones, recognizing the diverse work tasks and conditions that differ across zones and the importance of equitable service delivery (Estus et al., 2022), (2) determine a methodology and criteria for how best to define work boundaries as there is currently no established criteria at ACMAD, and (3) make a recommendation for how new boundaries should be defined.

Methods

This project involved three phases as part of the analytical approach: discovery, analysis, and recommendations for development. This section outlines what work was completed as part of these three phases and the subsequent sections will provide relevant findings.

Personnel interviews

ACMAD staff were individually interviewed and surveyed about their respective work zones or responsibilities, experiences with ACMAD and mosquito abatement generally, and how best to tackle evaluating work output and developing work zone recommendations. Additionally, five external mosquito and vector control professionals were interviewed: the Supervising Public Health Biologist from the California Department of Public Health, the District Manager from San Mateo County Mosquito & Vector Control District, the Program Manager I from County of Santa Clara Vector Control District, and the District Manager from Shasta Mosquito and Vector Control District.

Workload data analysis and rezone recommendations

Mosquito abundance, pesticide application and service request data were analyzed and visualized using a variety of software, including: MapVision (Leading Edge Technologies), QGIS (QGIS Development Team), Tableau (Tableau Software), Power B.I. (Microsoft), and Excel (Microsoft). Following this, ACMAD leadership were invited to offer reflections and input on potential map reconfigurations in group meeting settings. ACMAD field staff were invited to draft their own maps by drawing on a map of the ACMAD service area after being sent maps of work output data from the last four years. Following the contributions from staff and leadership, two alternatives were developed based on findings from the discovery and analysis phases. These alternatives were then analyzed to determine how altering the boundary lines would change the distribution of work among field staff.

Results And Discussion

Summary of interview responses

Defining service zone by city boundaries can help with interagency communication, for example, when engaging with code enforcement, or public works and parks departments. Regular reassessment of service zones is crucial with habitat changes and population growth or redistribution. Equipment needs and commuting challenges complicate the equitable distribution of work. The commute time between ACMAD headquarters and service work zones should be minimized. Specialized mosquito control equipment (e.g., heavy machinery) should not be transported to service requests in urban settings. The equipment that can be used should be considered when assessing the magnitude of insecticide applications as greater quantities can be applied with machines (e.g., truck-mounted larvicide mist blower) relative to what can be accomplished by hand. Regions with higher socioeconomic status will generate high numbers of service requests compared to less affluent or communities with a higher proportion of Black or Brown residents. Sites with higher mosquito abundance (e.g., marsh habitats), unmaintained swimming pools or West Nile virus burden need a higher frequency monitoring and insecticide applications. Zones with the most marshes should be amended to be more manageable for one person, in part, because depending on

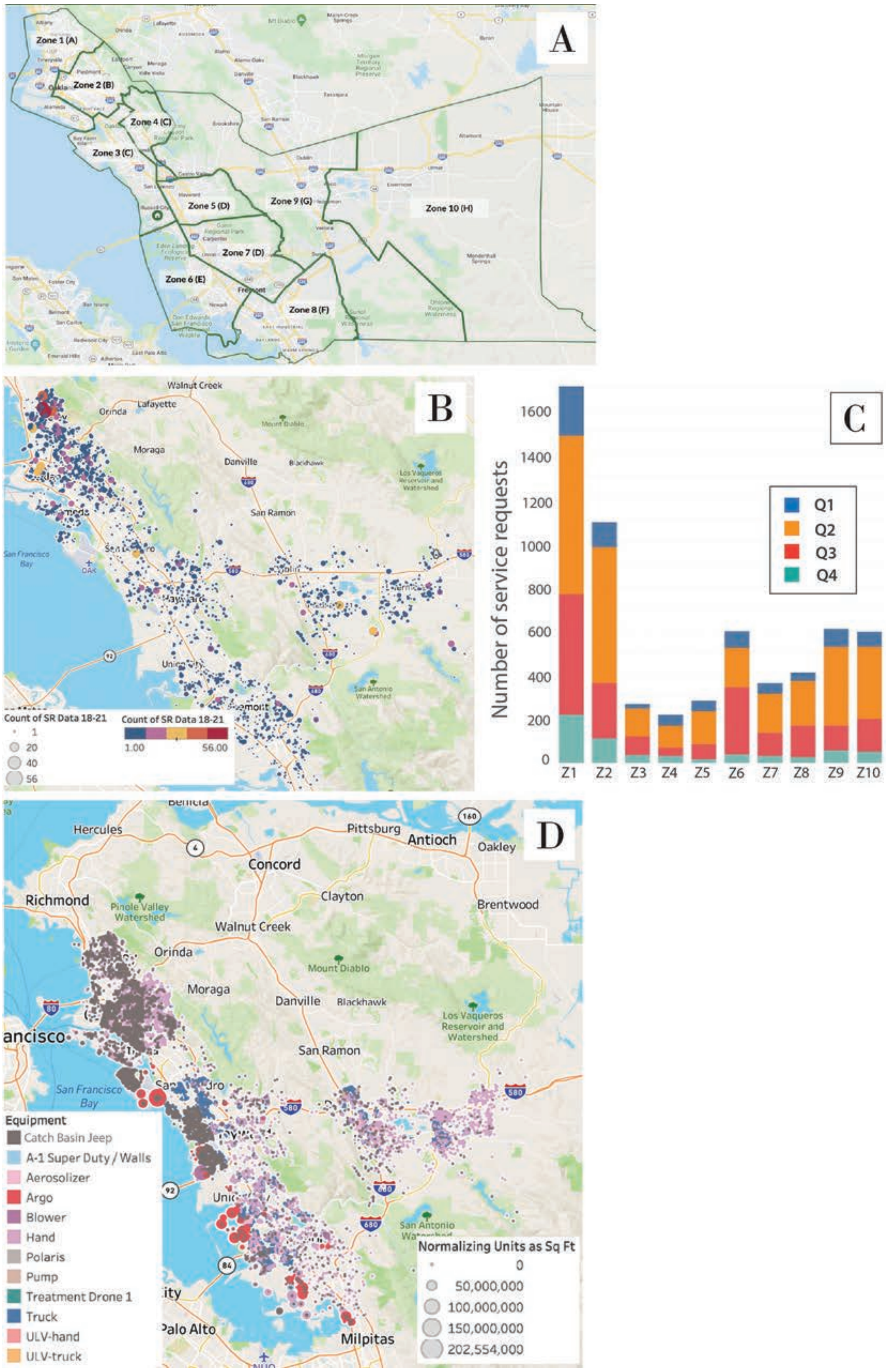

Figure 1.—ACMAD Zones and work outputs from 2018 - 2022. (A) Service zone boundaries at ACMAD from 2013 - 2022. (B) Cumulative count of service requests from 2018-21. Colors and dot size indicate the number of service requests completed in that area. The warmer colors and larger dots indicate more service requests completed, compared to cooler colors and smaller dots. This map excludes service requests that were associated with unmaintained swimming pools. (C) Stacked bar graph shows the cumulative count

the support of other staff for assistance can be logistically challenging. Once *Aedes aegypti* arrives to the county, the work type and load will likely change substantially. Rather than rotating staff through zones annually, as is done by some districts, staff and management reported preferring to keep the zone-lead model that is currently in place while continuing to share work duties. Many field staff advocated strongly for ACMAD to expand hiring seasonal technicians or add another full-time team member.

Workload data for 2018 - 2021

Data from four calendar years (January 2018 − December 2021) was used to understand how work ebbs and flows and the state of work in ACMAD at the time of this analysis. A review of service request data found what staff shared in interviews to be true, the highest concentration of service request activity was in the northern part of the county, currently Zones 1 and 2 (Figure 1B).

One of the issues raised during interviews was the seasonality of ACMAD's work. While most service request activity was completed in Quarters 2 and 3, there were concentrations of high activity in Zones 1 and 2 throughout the year (Figure 1C).

The geospatial distribution, quantity, and frequency of insecticide applications by full-time staff were visualized by converting the area treated to square feet (Figure 1D). The results showed that large surface areas were treated in central and southern regions of the county that abut the San Francisco Bay and that these relied upon heavy equipment such as an ARGO (red ellipses) or truck-mounted A-1 Super Duty mist blower (light blue ellipses), and backpack larvicide blowers (purple ellipses). Higher frequency, low area insecticide applications by hand (light purple ellipses) or using catch basin Jeep (grey ellipses) predominated in the northern regions of the county. Larvicide applied in the eastern region of the county relied predominantly on hand or backpack larvicide blower. Ultralow volume (ULV) applications of adulticide were rare. ACMAD leadership emphasized that the goal of applying mosquito control products was not to cover the most area, but to cover the right amount of area given mosquito risks.

Alternate zone boundaries

After reviewing the data, it was clear that while there was not a serious maldistribution of work, rezoning the work boundaries to better reflect the needs of staff, management, and the evolving landscape in Alameda County would be a reasonable next step. To understand additional parameters for rezoning, ACMAD staff and management were offered a short survey to assess the importance of city boundaries, highways and major streets, land use (e.g., wilderness, marsh and suburban), and daily work task diversity (e.g., service requests vs insecticide applications). The consensus opinion was that considering land use was important. A key difference that emerged between staff and management was the importance of city boundaries versus a diverse mix of work. Field staff prioritized having work task diversity over aligning zones to city boundaries. In contrast, ACMAD leadership and other management cited effective communication with local jurisdictions as important for using city boundaries.

Table 1.—The four criteria and supporting factors used to guide service work area rezoning.

Criteria	Factor
Equitable Workload Distribution	Service Requests (SR)
	Treatments by Surface Area
	Swimming Pool SR Data
	Staff and management perspectives
Task Diversity	Mix of land uses
	Seasonality of work tasks
Geographic Features	Land features
	City boundaries
	Highways & major streets
Implementation Feasibility	Commute time
	Equipment needed
	Best estimation of seasonality, impact of climate
	Staff and management perspectives

Analysis of internal and external interviews, surveys, and the review of quantitative data led to a long list of possible criteria for guiding work boundary decisions that was reduced to four criteria (Table 1). The following notable criteria were not considered: mosquito abundance data, population demography, and a quantitative assessment of transit time from the District office to the zone.

Two alternative zone boundary distributions were developed using information from the interviews, surveys and quantitative data. The first (ALT 1) focused primarily on moving boundaries to align with highways, major streets, city boundaries, and land features (not shown). Consequently, it did not substantially alter the work effort or distribution from the zone boundary plan that had been in place during 2013 − 2022, and this was not considered further other than to serve as a comparison to the original zone distribution and other alternatives. The second (ALT 2) sought to better align eastern zones with city boundary lines while also redistributing coastal zones to have greater task diversity (Figure 2A). The intent for AL2 was to serve as a starting

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service requests in Zones (Z) 1-10 from 2018-2021. Color blocks represent the four quarters (Q1, Q2, Q3, and Q4) of the year during which service requests were completed. In every zone Quarters 2 and 3 were the top two busiest periods for service requests. (D) Cumulative count of square feet treated from 2018-21 without the support of seasonal employees. Colors indicate what equipment was used and the larger the dot the larger the surface area treated. This map excludes mosquitofish treatments and ditching.

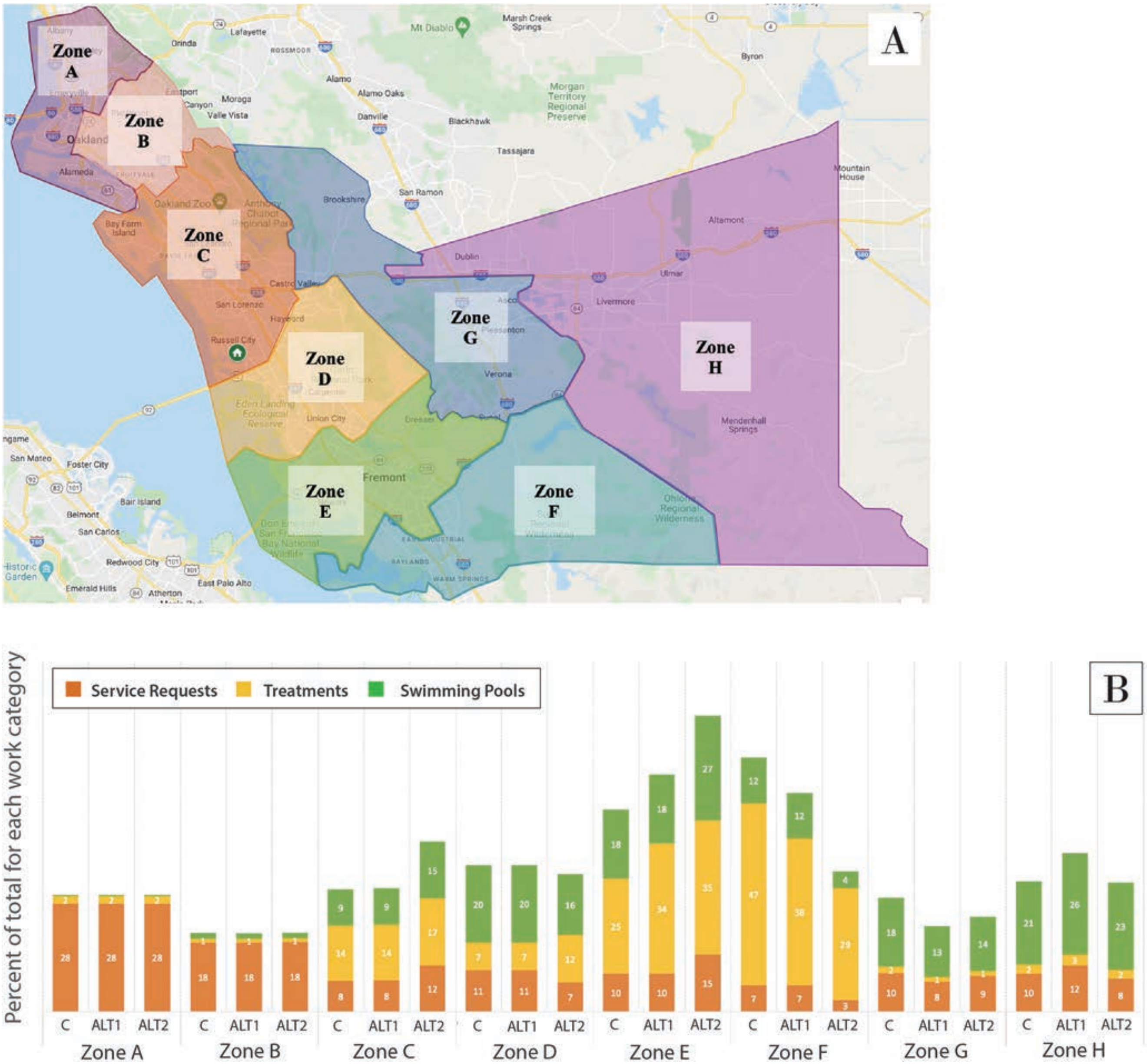

Figure 2.—Proposed ALT2 rezoning plan and analysis. (A) Map of ALT1 zones. (B) Workload distribution for the current (C), ALT1, and ALT2 zone boundary distribution. The proportion of service requests, insecticide applications (treatments) and unmaintained swimming pool work in each zone was visualized for the original, ALT1 and ALT2 zone boundary distributions.

point for supporting conversations with field staff and management, while providing another comparison to the original zone distribution plan.

Comparing the original zone distribution with ALT1 and ALT2

Because ALT1 was closely aligned with the original zone distributions, we focused on understanding the differences between ALT1 and ALT2 (Figure 2B). Although ALT2 was effective at reducing the workload, particularly the treatment workload of Zone F (previously Zone 8), it increased the work for neighboring Zone E (previously Zone 6). This outcome is precisely what staff and management shared as a concern when discussing the distribution of the marsh. Although there were moderate changes in the workload in other zones (e.g., C, G, and H), the largest change occurred for Zones E and F. However, Zones A and B were relatively unchanged from the original Zones 1 and 2, in part, because of the higher share of service request work. Although the conclusion of the workload analysis suggested that ALT2 was not an ideal alternative to the original distribution, it provided information for how another alternative might be crafted. For example, closely reviewing these data offered opportunities to rethink how Zones E and F could be distributed and how those redistributions to neighboring zones might lead to changes in more northern or eastern zones.

Discussion

This analysis provided a foundation for future discussions on how best to distribute and measure work in ACMAD's service area. There were no clear, easily quantified ways to do justice to the complex work effort of ACMAD field staff, but there were ways to frame and better understand the 'apples to oranges' comparisons that were necessary to assess workload equity. A cautionary feature of this analytical approach was the reliance on data related to completed work as a measure for anticipating appropriate workloads across the service area in the future.

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Interviews By The Author

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WNV resource allocation in the age of *Aedes*: Timing West Nile virus and *Aedes* control efforts to maximize staff deployment and efficiency

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The Challenge

How do you maintain an effective control program to address West Nile virus (WNV) with the growing burden of three invasive mosquito species? Endemic WNV annually represents a significant threat to our resident's health. At the same time, now that *Aedes aegypti* is firmly established in Los Angeles County, imported cases of Zika, dengue and chikungunya pose a continuing risk for a local outbreak, although we have not had any evidence of local transmission. Adding additional staff to adequately address both issues is an ongoing challenge and represents significant additional costs to the Greater Los Angeles County Vector Control District .

Greater Los Angeles County Vector Control District

The GLACVCD is a public health agency that is enabled and empowered by legislation incorporated in the California State Health and Safety Code to provide ongoing mosquito and vector control for its residents. The GLACVCD was formed in 1952 under the name of Southeast Mosquito Abatement District through a citizen petition aimed at controlling mosquitoes emanating from the Los Angeles River and to protect residents from vector-borne disease, specifically at that time St. Louis encephalitis.

The District has evolved over time and now provides mosquito, non-biting midge, and black fly control services to nearly six million residents in 36 cities and unincorporated portions of Los Angeles County, totaling an area of 1000 square miles. GLACVCD services are funded by *ad valorem* property and special assessment taxes on each parcel. The assessment is based on land use and parcel size.

Current treatment program

West Nile Virus has driven the need for effective mosquito control and outreach for the GLACVCD over the past 20 years. Since the introduction of the disease in 2003 (Kwan et al. 2010), our program has adjusted to address the many challenges represented by the WNV transmission cycle between the primary vector *Culex quinquefasciatus*, birds and humans. WNV is endemic and currently represents the greatest risk of disease and death from a vector borne illness in Los Angeles County.

Intense larviciding *Cx. quinquefasciatus* control efforts begin as soon as the winter rains have finished, typically early March, and slows by the end of June, as water sources dry up and backyard swimming pools are put into service for the summer season.

At the heart of our program are our Vector Control Specialists (VCS). VCS are generalists working in the various elements of our control program. Their efforts are augmented by seasonal Mosquito Control Technicians (MCT). Operations has divided the GLACVCD service area into functional units called zones. Each zone is assigned to a VCS whose tasks include the response to public generated service requests (SR), inspection and larviciding treatment of known sources, such as, unmaintained swimming pools, roadside ditches, water impoundments, and other standing water sources on residential, commercial, and public properties.

In addition to the VCS and their zone work, the Underground Storm Drain (USD) team was developed to address the large number of *Cx. quinquefasciatus* produced within underground storm drains and stormwater treatment devices (BMP). The potential of these underground stormwater conveyance systems that span many hundreds of miles below Los Angeles County had long been suspected and originally confirmed after a trapping program was started in 1999 (Kluh et al. 2005). Because *Culex quinquefasciatus* do not present much of a nuisance as they prefer biting birds and had not been implicated as a major disease vector, their control had not been a priority. In anticipation of the arrival of WNV, the potential importance of *Cx. quinquefasciatus* was realized and the USD program established. We have since documented the presence of *Aedes aegypti* in these underground systems.

Street gutters are inspected and treated by seasonal MCTs. Water run-off from residential and commercial properties is a chronic source of habitat for mosquitoes, especially in our suburban and urban neighborhoods due to poor street maintenance and gutter uplift from tree roots. Two person assignments, composed of a VCS and an MCT, handle sources difficult to control by one technician such as

V-ditches, drainage ditches and the Los Angeles River unimproved sections.

For stormwater flood control channels, a specialized Jeep was developed with solid rubber filled tires to handle the various sharp objects and debris found in the flat-bottomed concrete channels. Besides the larviciding equipment, such as a hand-held wand, and spray nozzles mounted to the back bumper, the Jeep has been fitted with a lightweight plow blade and cable wench to facilitate source reduction by pushing debris out of the waters path.

Aedes introductions

Our first introduction to *Aedes albopictus* came in 2001 (Metzger et al. 2017) when the California Department of Agriculture found mosquitoes emerging from shipping containers originating from China containing Lucky Bamboo. Samples were sent to Dr. Mir Mullah at U.C. Riverside and identified. An aggressive control program was implemented, nurseries and warehouses were inspected and treated, surveillance and trapping were conducted, and the Centers for Disease Control placed a temporary embargo on the importation of Lucky Bamboo. Finally, Lucky Bamboo stalks were treated prior to shipping and hydrous gel was used in the shipping containers instead of water. Further surveillance was conducted and no evidence of *Ae. albopictus* was found.

Ten years later in September 2011, an infestation of *Ae. albopictus* was discovered by the San Gabriel Valley Mosquito and Vector Control District. GLACVCD worked collaboratively with the neighboring agency to conduct intensive surveillance and control efforts. The initial infestation zone incorporated approximately 18 square miles in the cities of South El Monte, El Monte, and portions of unincorporated LA County, but despite our efforts the infestation spread quickly throughout San Gabriel Valley and adjacent cities within our service area (Metzger et al. 2017).

In 2014, two additional invasive mosquitoes were identified from cities under GLACVCD jurisdiction: *Aedes aegypti* and *Aedes notoscriptus*. Unfortunately, both species, but especially *Ae. aegypti,* have rapidly expanded their distributions throughout Los Angeles County (Metzger et al. 2017, Metzger et al. 2021). Increasing global travel and commerce, as well as changing environmental conditions are bringing increased vector-borne disease risks to the Los Angeles region.

Aedes Control

As mentioned above, in 2011 GLACVCD launched an aggressive control campaign to eliminate *Ae. albopictus*. Several agencies on the East Coast already dealing with the invasive *Aedes* were consulted for recommendation. The consensus at the time indicated education, door to door property inspections, and source elimination was the most effective strategy, despite being very time consuming. Using this strategy, GLACVCD had a reduction in service requests and trap counts, but the amount of personnel and resources required put too great a strain on our WNV control program to be maintained long term.

The detection of *Ae. notoscriptus* and *Ae. aegypti* in 2014 followed by our first imported cases of Zika provided ample justification to add more staff and create an *Aedes* response team. Currently, GLACVCD employs 9 fulltime staff dedicated to the program, but their efforts are at times augmented by our zone staff during peak *Aedes* activity starting in June and continuing into November. Over the past seven years, *Aedes* have spread throughout the County of Los Angeles and continuously increased the burden on our limited resources. GLACVCD follows the CDC recommendations for investigation and control around imported cases of Zika (Zika- CDC Interim response plan), dengue and chikungunya.

Shifting resources

To stem the tide of ever-growing staffing needs to accomplish both WNV prevention and *Aedes* control efforts over the course of the year, we divided the year into two periods and shifting staff activities as needed between both efforts.

Early season, January to June, activity focuses on *Culex* control to suppress WNV amplification as temperatures warm to minimize public encounters with virus infected mosquitoes in summer. During this time, our zone VCS perform source reduction, and encourage residents to drain non-functioning swimming pools or restore the swimming pools to a clean and operational state. VCS also work with residents, businesses, and agencies to eliminate standing water. Service requests are generally light during this period. As the season progresses and rainwater generated sources dry , VCS shift their priority to service requests and routine inspection and treatment activities of permanent breeding sites.

Later in the season, as nuisance biting from the *Aedes* mosquitoes becomes more prevalent, requests for service related to this issue rise. Prior to the arrival of invasive *Aedes* species, service requests were mostly driven by information campaigns and news stories concerning WNV. Residents in Los Angeles were able to sit outdoors, especially during the day, and enjoy their leisure activities without mosquito biting. Aggressive biting pressure from *Aedes* mosquitoes across the GLACVCD jurisdiction has led to a tremendous surge in service requests forcing changes in the way service requests are processed and, in the messaging, relayed to our residents. Prior to *Aedes*, most residents were content to remove standing water and use repellents in the early evening. Adulticiding to control *Culex* populations was not viewed favorably. After the arrival of the invasive *Aedes* species, residents now become angry when GLACVCD did not treat their yard for adults, and we are accused of not doing our job. As a District, we decided that 'comfort spraying' with adulticide was not effective, sustainable, or environmentally responsible, and would contribute to pesticide resistance in mosquito populations, potentially hampering much needed adult control efforts during a major disease outbreak. The Districts focus has changed to more robust public outreach, *Aedes* larval control in public spaces and businesses, as well responding to imported cases of Zika, dengue and chikungunya. Nevertheless, the *Aedes* related workload mid-summer and fall is intense. Fortunately, Zone staff can shift their attention to *Aedes* related control efforts as their WNV responsibilities are reduced at this time.

Conclusions

The GLACVCD control program was originally built to address diseases like Saint Louis Encephalitis and its primary vector *Culex tarsalis* in the few remaining wildlife habitats within and around Los Angeles County and then was shifted to a more urban approach and *Cx. quinquefasciatus* in anticipation of the arrival of WNV. The introduction and expansion of three *Aedes* species has created a new significant burden on the District's resources. While the addition of fulltime and staff and the creation of the *Aedes* program has made a significant difference, the explosion of service requests related to the aggressive biting behavior of the invasive *Aedes* and the response to imported cases of *Aedes* transmitted viruses requires additional resources late in the season. Shifting control activities between WNV and *Aedes* response as well as the extensive use of approved overtime has maintained reasonable response times for service requests without further expanding staffing. An early season push to address *Culex* sources frees time to support *Aedes* related service requests.

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Stopping the spread – A district wide response to preventing a local outbreak of dengue, Zika and chikungunya

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The Greater Los Angeles County Vector Control District (GLACVCD) responds to numerous domestic or travel-associated cases of arboviral infections every year. GLACVCD considers domestic cases to be the result of transmission that has occurred within district boundaries, whereas transmission of travel-associated cases have not occurred within district boundaries. Invasive *Aedes albopictus* and *Aedes aegypti* have the capability of transmitting arboviruses such as dengue, Zika and chikungunya. *Aedes albopictus* and *Ae. aegypti* have been confirmed to present within GLACVCD's boundaries since 2011 and 2014, respectively. This abstract will focus on travel-associated human cases. In most situations the individual traveled to a different part of the world where the disease is prevalent and has now returned home where *Aedes* are abundant. This abstract will detail the planning, preparation and execution of our district wide response.

The Los Angeles County Public Health notifies GLACVCD of suspected, probable, and confirmed travel-associated cases. GLACVCD has a response plan when responding to travel-associated cases primarily consisting of door-to-door surveillance and control efforts within a 150-meter radius of where the infected individual resides. Each response differs slightly depending on the neighborhood and location. Densely populated areas with multiple units require more time and resources than single family dwellings due to an increase in properties that need to be inspected. Typically, GLACVCD utilizes five teams for each door-to-door campaign, each of which is comprised of two staff members certified in California Department of Public Health Category A – Pesticide Application and Safety Training for Applicators of Public Health Pesticides and in Category B – The Biology and Control of Mosquitoes in California. Four teams conduct the door-to-door surveillance, leaving the fifth logistics support team to provide all treatments, handle all resident concerns, and be the point of contact for all teams in the field.

After the initial door-to-door operation is complete, GLACVCD management discusses the outcome and decides if further action is required. If the neighborhood in question has a large abundance of *Aedes* activity and access was not granted in a significant number of homes, then GLACVCD will conduct a second attempt to inspect missed properties. Surveillance will continue until the district is satisfied with the number of properties inspected.

In 2022 GLACVCD responded to twenty-five travel-associated cases, 92% of which were dengue cases. None of the mosquitoes collected were found to be positive for any of the viruses listed. These door-to-door campaigns resulted in hundreds of property inspections, countless sources removed, and many newly educated residents. As of 2022 California has not had any local transmissions reported from travel-associated cases of dengue, Zika, or chikungunya. The hard work and dedication GLACVCD has shown in 2022 and in the past years have better prepared each staff member in response efforts in case a local outbreak ever occurs.

Efficacy of wide-area truck mounted larval and adult mosquito control in urban Orange County, CA, 2021

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Introduction

Since Orange County Mosquito and Vector Control District's (OCMVCD) 2015 initial detection of *Aedes aegypti* (L.) in the city of Anaheim, this invasive mosquito has spread to all cities and census-designated areas in the county. This species is an important vector of dengue, chikungunya and Zika viruses, and possesses a risk for autochthonous transmission in southern California. Eradication and suppression of this species using traditional measures proved to be difficult and forced an interest to explore alternative treatment strategies. The current study determined the utility and efficacy of a truck mounted, wide-area larvicide system (WALS®) with VectoBac® WDG (*Bacillus thuringiensis israelensis,* Bti, strain AM65-52) against *Ae. aegypti* and simultaneously, OCMVCD's most important West Nile virus (WNV) vector, *Culex quinquefasciatus* Say, in a residential neighborhood of Orange County, CA in 2021.

Methods

During phase I, Reiter-Cummings gravid and dry-ice baited Biogents® sentinel traps were paired at eight locations within the treatment (1.3 km^2) and non-treatment (control area, 1.3 km^2) areas weekly to evaluate the effects of eight consecutive weekly Bti treatments on wild *Cx. quinquefasciatus* and *Ae. aegypti*. Following a sufficient three-week "washout" period between studies, phase II was initiated with two additional weekly Bti applications in the original treatment area and a combination treatment using WALS followed immediately by an ultra-low volume (ULV) DeltaGard® (2% deltamethrin) truck mounted adulticide application in the former non-treatment (control) area. During phase II mosquito abundance in the two treatment areas was compared to the abundance from the surrounding area routine trap locations, which served as the new non-treatment control area. Treatment and control sites were selected using ArcGIS hot spot analysis. A county wide grid of 1.3 km^2 units was used to delineate areas with 1) the highest levels of *Ae. aegypti* activity, based on mosquito service requests (2016-2022), and 2) the highest human WNV infection incidence, based on historic human case (2004-2020) data and 2020 census tract population data. The differences in mosquito abundances before and after treatments were compared using a generalized Linear Model (GLM) fitted Generalized Estimating Equations (GEE), using a Gaussian-distribution for the dependent variables, with alpha set at 0.05. A larval and adult mosquito field-assay was conducted on the first night of phase II where a combination larvicide and adulticide treatment was applied. Larval assays were conducted by placing empty plastic cups along a 50 m interval transect up to 300 m from the truck route. Two replicate cups were set in both the front (~50 m) and backyards (~100 m) of three single family homes within the treatment area. Cups were collected 30 min after the application and returned to the laboratory where 2nd and 3rd instar of a pyrethroid susceptible laboratory strain *Cx. quinquefasciatus* (collected from Chino Hills and Montclair, CA in 2006) and deionized water were introduced into each cup. Sentinel cages, each holding 25 of the same strain of pyrethroid susceptible *Cx. quinquefasciatus* adults, were placed on wind vein assisted self-orienting tripod stands at the same aforementioned locations in the park and homes. Mortality over time was recorded for both assays.

Results

The eight weekly, phase I WALS® treatments resulted in a statistically significant reduction of *Ae. aegypti* abundance (62.0%, Wald $\chi^2 = 9.16$, $d.f. = 1$, $P < 0.001$), but not in *Cx. quinquefasciatus* abundance (8.3%, Wald $\chi^2 = 0.76$, $d.f. = 1$, $P = 0.38$) when compared to the control group. After the phase II combination Bti and ULV treatments, a significant reduction in *Ae. aegypti* abundance (34.5%, Wald $\chi^2 = 4.8$, $d.f. = 1$, $P = 0.03$) was observed, but not in *Cx. quinquefasciatus* abundance (25.9%, Wald $\chi^2 = 0.87$, $d.f. = 1$, $P = 0.36$), when compared to the control area. Additionally, a significant decrease in *Ae. aegypti* abundance (17.9%, Wald $\chi^2 = 4.63$, $d.f. = 1$, $P = 0.03$) was found in the combination treatment area, but not for *Cx. quinquefasciatus* abundance (−0.82%, Wald $\chi^2 = 0.69$, $d.f. = 1$, $P = 0.41$) when compared to the Bti only treatment area. *Cx. quinquefasciatus* larval mortality within cups placed at the park site, six hours post larval introduction, was 96%, while mortality for the adult assay was 100% in all cages at 24 hours post spray. Average larval mortality in the urban residential neighborhood was 94% and 91% in front and backyards, respectively. Adult mortality at the same locations was 74% and 98% in the front and backyards, respectively.

Conclusion

In conclusion, the eight consecutive WALS® treatments significantly reduced *Ae. aegypti* abundance in the treatment area compared to the non-treatment control, but the same effect was not observed for *Cx. quinquefasciatus*. In a fourth of the time required to achieve 62% reduction in *Ae. aegypti* abundance over an eight-week treatment program, we detected a 34.5% reduction in *Ae. aegypti* when the treatment consisted of two weekly WALS® applications and a one time, three consecutive nights of ULV adulticiding. It was reasonable to suspect, although the treatments were shorter duration, that the combination larvicide and adulticide treatments were more efficient in suppressing *Ae. aegypti* than WALS applications alone. Our field evaluations demonstrated that during low wind conditions ($<$2 mph), typical in Orange County, CA during nighttime (12am-4am), both the WALS® and ULV adulticide applications were delivered effectively to the intended target area and killed mosquitoes. Although eradication of *Ae. aegypti* using conventional treatment strategies is likely unachievable, our study findings support the use of the combination of truck-mounted larvicide and adulticide application approach to efficiently suppress *Ae. aegypti* populations, and possibly to a level that may be amenable to breaking a disease transmission cycle during a local outbreak.

Acknowledgements

We would like to thank all the seasonal and full-time staff that helped make this work possible, including members of the operations, communications, and laboratory departments. We thank all the department managers for their support and the District Manager (during the study), Rick Howard, for his encouragement to pursue new strategies for mosquito control in our district.

Evaluation of In2Care® Mosquito Traps for area-wide control of *Aedes aegypti* in Orange County, California

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Abstract

The Orange County Mosquito and Vector Control District (OCMVCD) evaluated the effectiveness of In2Care® Mosquito Traps from June through December 2022. Traps were deployed at the label rate of 10 traps per acre (1 device/400m^2) at a 5-acre commercial property (50 traps serviced for six months) with known high *Aedes aegypti* abundance. Adult mosquito abundance was monitored bi-weekly with 6 BG-Sentinel traps in the treatment area and compared to the average abundance of *Aedes aegypti* from 9 BG-sentinel trap locations in high mosquito-borne disease risk areas in the County. Mosquito larvae were sampled monthly during the time of In2Care® service to determine species presence/absence. The percentage of In2Care® traps with all mosquito larvae species present peaked in October with 96% of traps positive for mosquitoes. The percentage of traps positive for *Ae. aegypti* peaked in September at 83.3% and declined to 9% of traps in December. *Culex quinquefasciatus* was detected in 58% of In2Care® devices in September and October and 97% of traps in November. The Henderson-Tilton equation was used to measure the change in pest density over time in the treatment and control areas. The *Ae. aegypti* population in the treatment area trended lower than the control areas not receiving trap intervention during the six-month study. In2Care® traps set at the rate of 10 traps/acre showed 17% suppression of the *A. aegypti* population as compared to a control area not receiving the trap intervention. Based on these results, OCMVCD found there is a limited role for area-wide In2Care® trap intervention in OCMVCD's Integrated Vector Management Program.

Teamwork, tech, and taxes to tackle tidal mosquitoes

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Introduction

Aedes dorsalis is a tidal salt marsh mosquito, which if left untreated, can cause a major nuisance for surrounding residents. Over one-thousand acres of tidal marsh in Fremont, CA are managed by the United States Fish and Wildlife Service, so that ATVs, drones, surfactants, or adulticides are not permissible for use as mosquito treatment options. Wieland et al. (2022) presented the effectiveness of a truck-mounted mist-sprayer used to treat this tidal marsh and to save time and money, so our district has continued to use this method for the past three years. This current study is a cost-benefit analysis of this treatment strategy to prepare for invasive *Aedes*.

Methods

The three years prior (2017-2019) and the three years post-implementation (2020-2022) of the mist sprayer approach were compared. Significant differences in study parameters before and after using the mist sprayer were determined using Student's t-test and the data graphed using Prism Software (version 9.5.1; GraphPad, San Diego, CA) The total acres treated were a sum of the seven sites that comprise this study site. The product costs were for Vectobac 12AS, Vectobac G, Altosid Pellets, and Natular G30. The wage costs were calculated for the number of hours spent treating the site, using the same standard pay rate for each person. Larval abundance was the average larvae-per-dip measured before each treatment.

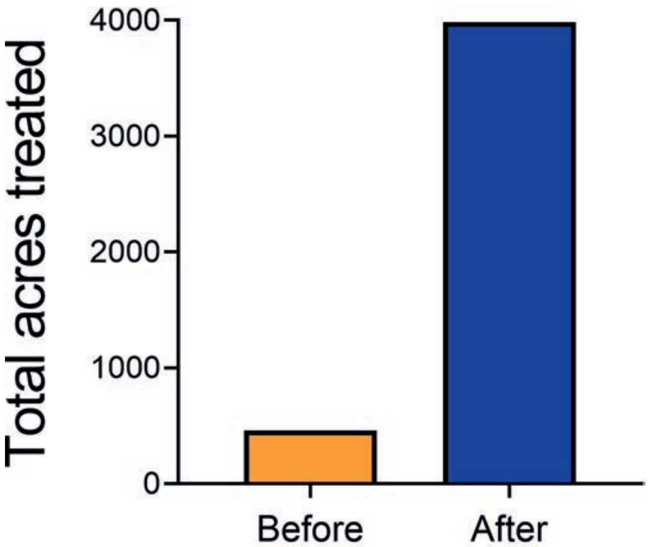

Figure 1.—The total number of acres treated at this site before (2017-2019) and after (2020-2022) implementing mist sprayer treatments (N = 2 sites).

Results and Discussion

There were marked differences in all parameters before and after implementing the new treatment method. The total acres treated increased from 462 acres to 3,985 acres (Figure 1). When we further analyzed the average number of acres treated per hour, the number increased significantly ($P < 0.001$) from 1.5 acres to 20.0 acres. (Figure 2). The total dollar amount spent on pesticides increased from $23,323.95 to $27,051.82 (Figure 3), but the average cost of pesticides per acre treated before and after, was not significantly different ($P = 0.15$) (Figure 4). When we

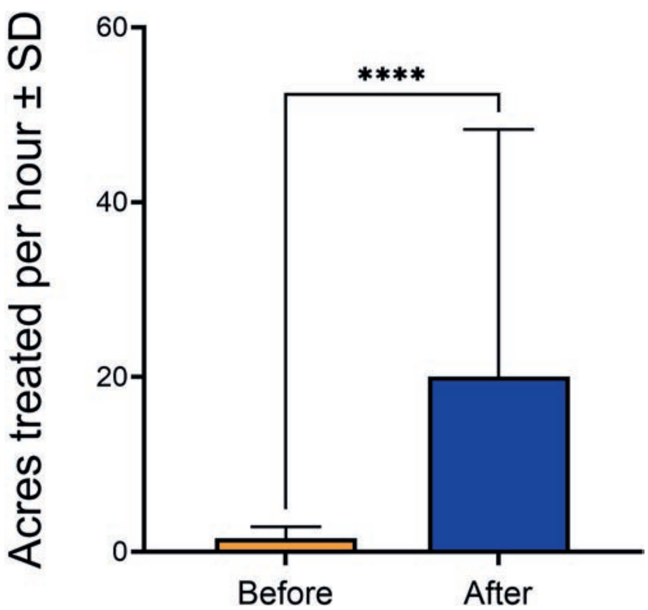

Figure 2.—The average number of acres treated per hour before (2017-2019; N = 92) and after (2020-2022; N = 197) implementing the mist sprayer. **** Means significantly different ($P < 0.001$).

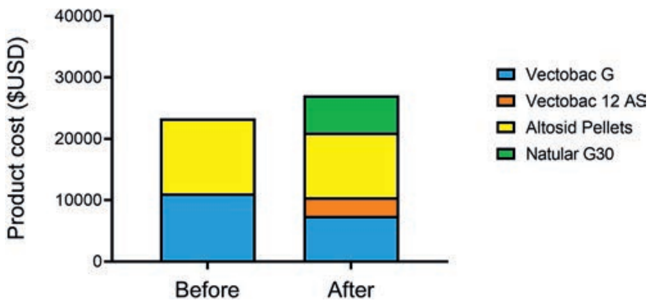

Figure 3.—The total cost spent on all pesticides before (2017-2019; N = 92) and after (2020-2022; N = 197) implementing the mist sprayer.

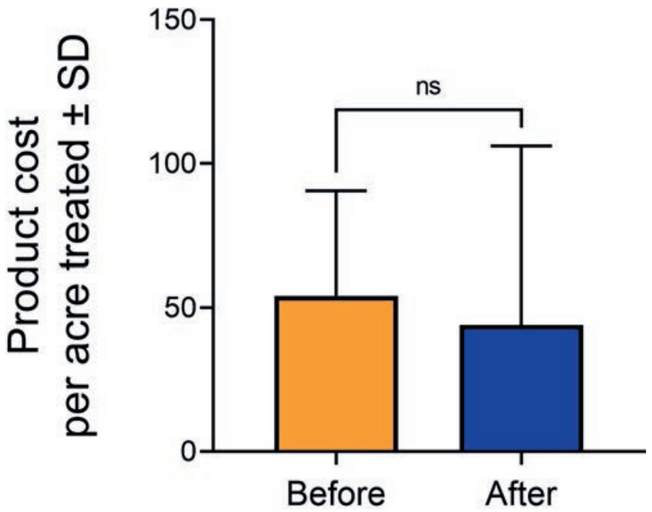

Figure 4.—The total cost spent on all pesticides per acre before (2017-2019; N = 92) and after (2020-2022; N = 197) implementing the mist sprayer. [ns]Means not significantly different ($P > 0.05$).

analyzed the average cost spent on staff wages to treat each acre, we found that it decreased significantly ($P = 0.03$) from $84.50 to $56.49, respectively (Figure 5). The major finding was that there were significantly fewer larvae per dip during pre-treatment inspections with the new treatment method than the old ($P < 0.001$), decreasing from 5.0 to 1.1 (Figure 6).

Conclusions

Using the truck-mounted mist-sprayer to treat tidal marshes on this United States Fish and Wildlife property we were able to save time and money which pleased nearby residents by reducing mosquitoes present, and District staff by reducing the physical labor needed for other treatment options. With the time and money saved, our District will be more prepared for invasive *Aedes* in the future.

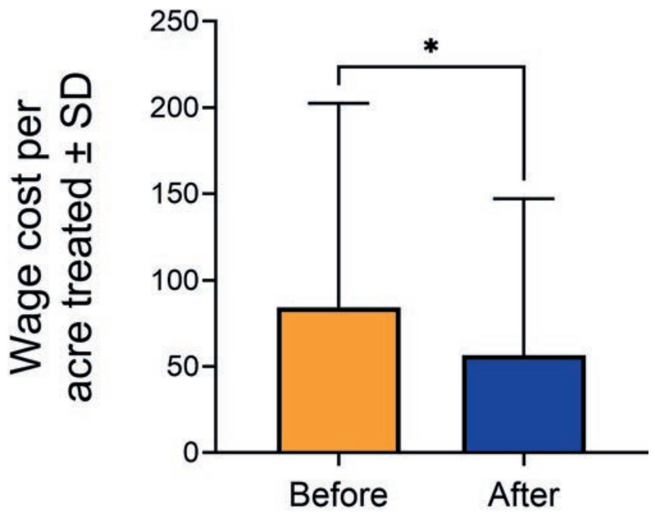

Figure 5.—The total cost spent on staff wages per acre at this site before (2017-2019; N = 92) and after (2020-2022; N = 197) implementing the mist sprayer. * Means significantly different ($P < 0.05$).

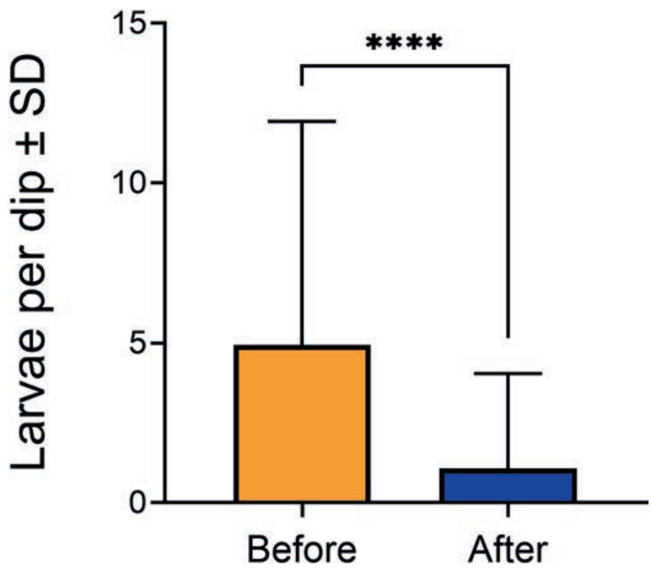

Figure 6.—Total averages of larvae per dip at this site before (2017-2019; N = 37) and after (2020-2022; N = 104) implementing the mist sprayer. **** Means significantly different ($P < 0.001$).

Acknowledgements

We thank our colleagues at Alameda County Mosquito Abatement District for impactful discussions that improved the outcome of the work described herein.

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Aedes taeniorhynchus in the coastal salt marshes of Los Angeles County

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Aedes taeniorhynchus is an aggressive day and dusk biting mosquito that targets humans and large mammals. They are commonly found in coastal salt marshes and inland alkaline areas. After initial detection in July 2009, *Ae taeniorhynchus* remained undetected for years. In September 2015, the District received four service requests from Island Village townhomes in the city of Long Beach for unusually high numbers of aggressive biting mosquitoes. Property inspections yielded no sources, but one of the residents had adult mosquito samples that were taken back to the laboratory for identification. The mosquitoes were identified as *Ae. taeniorhynchus*. At that time no one at GLACVCD was familiar with *Ae. taeniorhynchus* control methods, so we reached out to Orange County Vector Control for some assistance. Miguel Arias who was their *Ae. taeniorhynchus* expert showed us the potential larval habitats located in the Los Cerritos Wetlands. After identifying the source where they were laying their eggs and adults were emerging, Miguel Arias helped develop a comprehensive integrated vector management program to mitigate their presence. The program started with inspections when the lunar tides over 5.9 feet inundate eggs subsequently producing an emergence and adult abundance. If inspections of the tidal marshes detected larvae, treatments were conducted using a Maruyama backpack blower with a bacterial granule (e.g., VectoBac G or VectoMax FG).

After their initial detection there was limited control, because they were discovered late in the season. Much to the relief of the residents, the cooler late fall and winter months precluded subseqent adult emergence. The following year by combining inspections of the marshes and treatments when needed, there was limited adult activity and only small pockets of larval activity were found instead of the entire flooded region of the marsh as was found during the previous year. The District continues to monitor larvae after the high tides from April to November and makes pesticide treatments when necessary. Since the successful implementation of the *Ae. taeniorhynchus* program we have not received additional service requests due to their aggressive presence.

Acknowledgement

Special thanks to Miguel Arias at OCMVCD for his guidance and support and OCMVCD for their unwavering commitment to public health by continuing to assist GLACVCD in times of need.

Control efforts in marsh areas of the Salton Sea in the Coachella Valley

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Introduction

The Salton Sea was once a thriving area and a tourist destination, hosted speed boat racing, was popular for bird watching, and brought new real estate development to the area (Fig. 1). The sea was once sustained by flood irrigation run off and surplus irrigation water from the Imperial Irrigation District. Since then the shoreline has receded due to stricter laws on agricultural irrigation run-off and the excess irrigation water once discharged in the sea has been sold for drinking water to other areas of Southern California. Reduction in water input has led to a receding shoreline and has created large areas of marshland near outlets of agricultural run-off channels. It is important for the Coachella Valley Mosquito and Vector Control District (District) to control mosquitoes in this area as nearby rural communities as well as the western urban communities of the Coachella Valley can be affected by mosquito borne viruses transmitted primarily by *Culex tarsalis* produced in large numbers within these marshland habitats. Our environmentally sound approach to controlling this area not only benefits our residents, but also the environment and local wildlife inhabiting this area. The following provided an overview of our program that was designed to be efficient and effective in day-to-day operations.

Figure 1.—Salton Sea shoreline showing reduction of surface area over time.

Methods

The northern shore of the Salton Sea has been divided into 3 zones, each covered by one technician (Fig. 2). Two adjacent Agricultural zones are covered by one Technician each for a total of five Technicians servingthe Salton Sea shoreline. All five technicians assist one another to complete work in their assigned zones in a timely manner. Work in marsh habitats usually requires the use of Off-road equipment such as an Argo, ATVs, and UTVs. The terrain in this area is muddy and unstable, and there are many inaccessible areas due to heavy vegetation. Surveillance traps are set in these areas by laboratory staff bi-weekly to monitor mosquito abundance and virus activity. When either mosquito abundance increases to above average levels or work on the ground and shoreline becomes too difficult, aerial larvicide applications may be utilized with a local contracted helicopter pilot. A variety of larvicides with different active ingredients are used and rotated to prevent resistance. Technicians determine what label rate should be used on a given site. For example, a heavily vegetated area with high larval presence will require a higher application rate for control than an area that is easily accessible with low larval presence. To minimize impact of our activities on local wildlife, we use a fixed system of trails to travel to different areas along the shoreline (Fig. 3).

Results

Our operations have reduced mosquito abundance and virus activity. We work routinely with our laboratory staff to assess the efficacy of applications. Results of one evaluation of an aerial application over a 150 acre site using 14 water sensitive cards showed that the liquid treatment effectively was reaching all areas (Fig. 4). Results from area CO_2 traps also demonstrated a decline in local mosquito abundance after aerial larvicide applications in combination with control work done on the ground in nearby agriculture areas (Fig. 5).

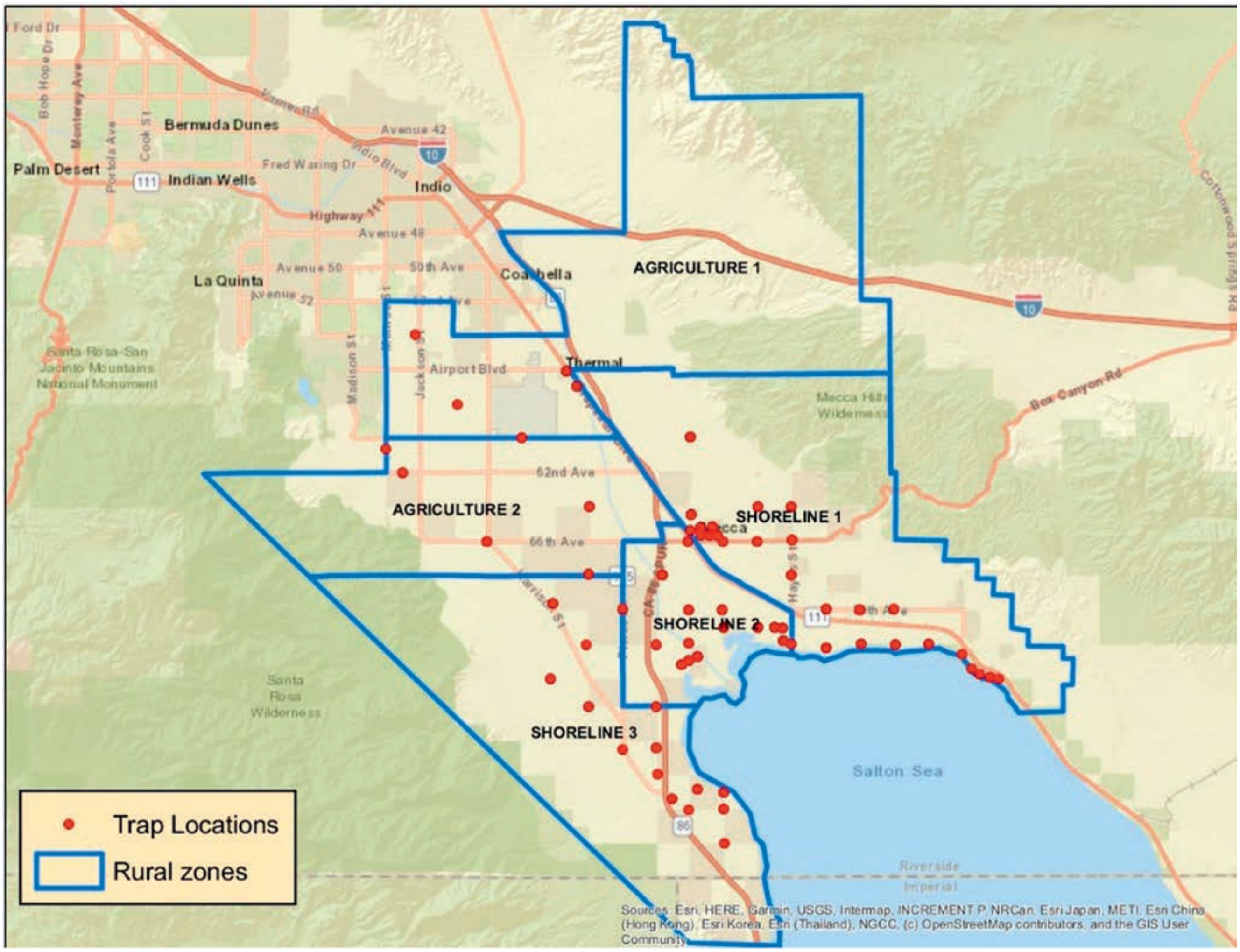

Figure 2.—Zone map and $C0_2$ trap locations near the Salton Sea marsh habitats.

Conclusion

Our surveillance and successful control efforts resulted in a decline in mosquito abundance. We expect the shoreline to continue to recede and the marsh area to grow in the following years.. New innovations in control will be developed and evaluated to evolve our program. The newest addition is the use of drone applications, which are currently under development and evaluation.

Acknowledgements

Thank you to our shoreline and agriculture teams. This area cannot be controlled by an individual and requires teamwork. The team consists of: Oldembour Avalos, Vincent Valenzuela, Jonathan Herrera, Jonathan Zamaniego, Marina Espejo and Iver Romero. Thank you to Gabriela Harvey and Greg Alvarado for the test result graphs and maps. Thank you to everyone in our operations department for assisting when needed.

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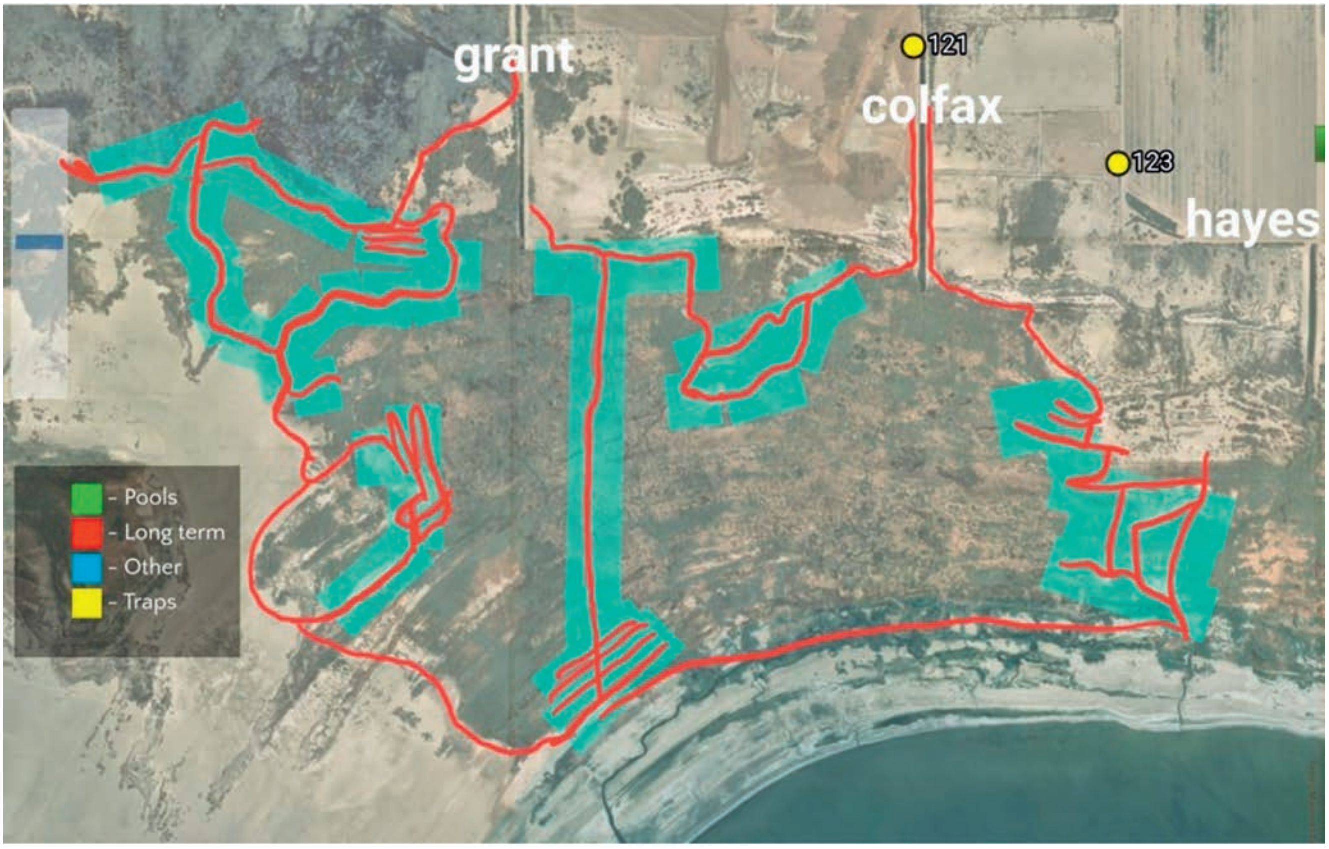

Figure 3.—Map of trails. Red indicates trail and blue shaded areas indicates water and common treatment areas.

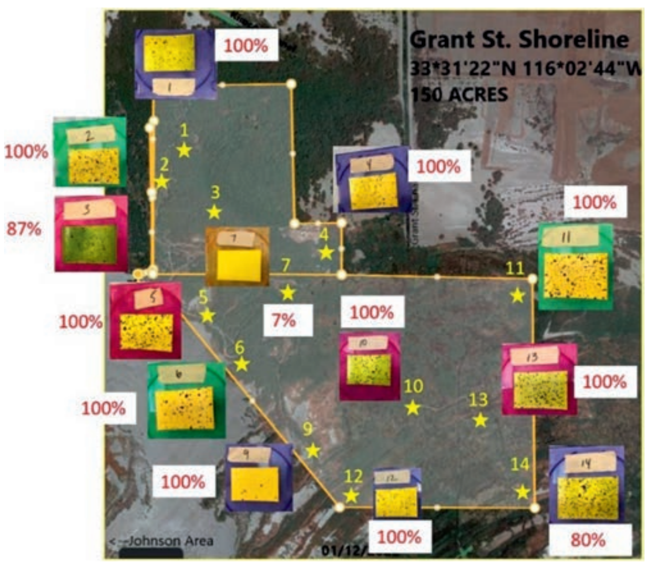

Figure 4.—Deposition of liquid larvicide droplets from a helicopter application on water sensitive cards deployed within the spray zone.

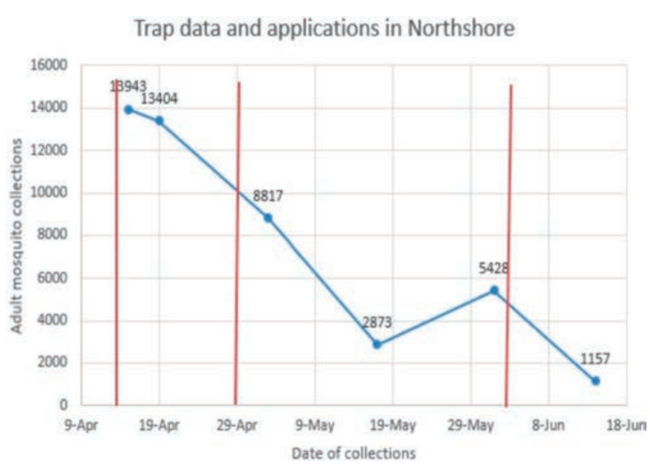

Figure 5.—CO_2 trap data showing reduction in mosquito activity after aerial and ground larvicide applications.

Larvicide applications with Unmanned Aircraft Systems in Placer County

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Abstract

The Placer Mosquito and Vector Control District (PMVCD) has been applying larvicides via unmanned aircraft systems (UAS) since 2019. PMVCD started by conducting trials to provide baseline data for safe and effective larvicide applications to incorporate this technology into day-to-day operations. Over 880 acres sources have been treated including: organic rice fields, large retention ponds, snowmelt pools, irrigated pastures, and wetlands. The DJI AGRAS MG-1S effectively treated mosquito sources that historically were treated with All Terrain Vehicles or smaller hard-to-access sources that were treated by technicians using hand-held equipment. UAS provides another effective tool, and we look forward to evaluating new, larger UAS in the future.

Comparison of rice field application efficacy of granular larvicide by fixed-wing aircraft and an unmanned aerial system

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Introduction

Rice is an important crop cultivated in the Sacramento Valley. Rice plants are grown in flooded fields that create large areas of suitable habitat for mosquito development. The Sacramento-Yolo Mosquito and Vector Control District (District) routinely applies mosquito larvicide products to rice fields by fixed-wing aircraft to control immature mosquito production. A complex of rice fields in Northern Sacramento County was identified as an area that historically required more larvicide treatments than the surrounding rice fields. We hypothesized that traditional fixed-wing applications were not applying granular larvicide evenly across these fields due to the shape of the fields and the need to avoid application to organic rice fields and infrastructure such as buildings and outdoor equipment storage. During the 2022 season, the District assessed the effect of product application method in the efficacy of larval mosquito control. Granular larvicide applications were made using both fixed-wing aircraft and quadcopter drone over the study site. Each application was evaluated for dispersal of larvicide granules in the targeted fields and the effectiveness of those treatments on reducing larval mosquito populations.

Methods

Larvicide applications were made using both a fixed-wing Air Tractor 502 (fixed-wing) operated by a contracted aerial applicator (Farm Air Flying Services, Sacramento, CA) and an unmanned aircraft system (UAS), Precision-Vision 35X (Leading Edge Aerial Technologies, Waynesville, NC), contracted for operation through Leading Edge Aerial Technologies. All applications were made using a mosquito larvicide formulated with *Bacillus thuringiensis israelensis*, Vectobac GS (Valent BioSciences, Libertyville, IL) at a rate of 5 lb per acre.

The rice field complex for this trial consisted of nine separate fields comprising 102 acres (Fig. 1) that were treated as a single complex for the purpose of this trial. Larval dipping was used to assess the density of larval mosquito populations (Knight 1964). We followed the District's rice field surveillance plan (Wheeler et al. 2022) which states that a rice field between 41 to 160 acres meets criteria for treatment when mosquito larvae (*Anopheles* or *Culex*) are detected in four out of forty dips. Overall, thirteen dipping stations were established across the study site (Fig. 1) and were sampled each week by a technician who dipped each station 4 times for a cumulative total of at least 40 dips. If criteria were met in the pre-application dipping, an aerial application by either fixed-wing or UAS was scheduled for the following day. Following each treatment, the study site was dipped again to assess the effect of larvicide application on larval densities. Application methods alternated between fixed-wing and UAS, for a total of 2 fixed-wing applications and 3 UAS applications.

Figure 1.—North Sacramento rice field complex where the trial took place is indicated by the red outline (101.73 acres). Larval sampling sites within the complex indicated by blue markers and granular collection buckets in the complex indicated by the yellow markers.

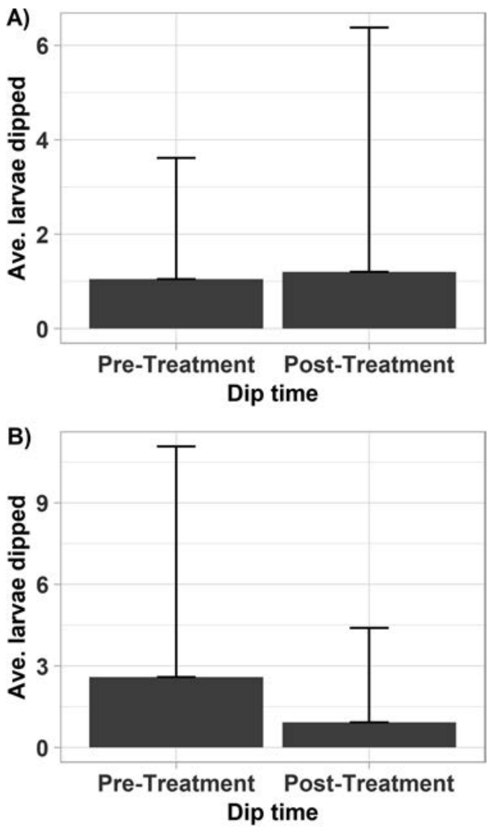

Figure 2.—Average larvae per dip (± SE) pre- and post-treatment for (A) fixed-wing and (B) UAS applications.

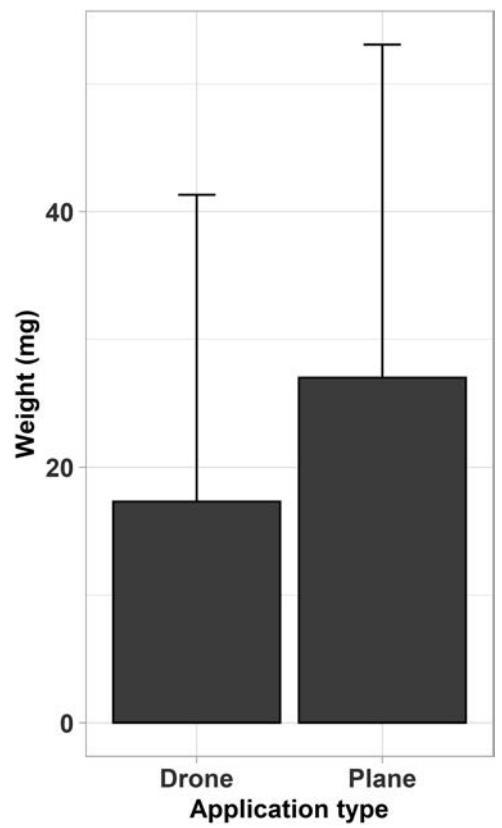

Figure 3.—Average VectoBac GS weight (± SE) per fixed-wing and UAS application; weight is in mg.

To assess the spread of larvicide granules across the field, 20 standard 5-gallon buckets with an opening diameter of 14.5 inches were placed throughout the rice field complex (Fig. 1). The buckets were placed on average 3 to 4 feet into the rice field adjacent to the surrounding road. Buckets were left in the field for the entirety of the trial with contents collected from each bucket within 24 hours of application. Contents collected from the buckets were transported back to the District where pesticide granules were separated from other organic material and weighed using an Ohaus Adventure Analytical balance model AX324 (OHAUS Corp, Parsippany, NJ).

Data analysis was carried out in R 4.2.1 (R Core Team 2022). A Wilcoxon-signed rank test was used to compare pre- and post-application larval counts and product deposition between UAS and fixed-wing treatments.

Results and Discussion

To assess the efficacy of treatments performed by UAS and fixed-wing, pre- and post- larval dip counts were analyzed for larval count reduction. For the fixed-wing application, no significant difference (Z= 680, P=0.26) was found between pre- and post-treatment larval counts, indicating that fixed-wing application had no effect on larval density (Fig. 2a). Conversely, for the drone application, we found a significant reduction in larval counts per dip (Z=598, P= 0.03) between pre- and post-treatment, indicating that the UAS applications were effective at reducing larval density (Fig.2b).

To assess product deposition, we compared the mean weight of larvicide granules collected in each bucket following all applications by either fixed-wing or UAS. The mean weight of larvicide granules following UAS and fixed-wind applications was 17.29 mg (SE=24.01) and 27.01mg (SE=26.06), respectively (Fig. 3). Overall, we found a significant difference in average Vectobac weight put out in the rice fields between UAS and fixed-wing application (Z=127, P=0.04). These results seemed to indicate that the fixed-wing applications deposited more material onto the rice field complex than the UAS. However, we only saw a significant larval reduction following UAS applications. When the overall application rate for each treatment method was calculated using the product deposited in sampling buckets, the UAS output was 2.5 lb per acre compared to the fixed-wing output of 3 lb per acre, however, both application rates were lower than the original target rate of 5 lb per acre.

Conclusions

The disparate findings with respect to deposited product and larval control may indicate a need for further investigation into the methods used for assessing product deposition. Fixed-wing aircraft fly higher and faster than

UAS, additionally the rotor wash from UAS can create ground-level air turbulence that may have interfered with deposition of granules into sampling buckets. The results of this trial indicated that the UAS applications were more effective at reducing larval mosquito populations; however, the reason for increased efficacy is not clear due to product deposition results. For this trial UAS were routinely used to apply larvicides to acreage previously flown only by fixed-wing aircraft. The larval reduction results indicated that smaller fields (<100 acres) surrounded by non-target sites may be good targets for UAS applications, and SYMVCD will continue to expand on UAS applications in the future.

Acknowledgements

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Naled (Dibrom) dispersal modeling, sampling, health impact assessments, and scoping literature review

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Abstract

Naled (Dibrom) is an organophosphate insecticide, used by the Salt Lake City Mosquito Abatement District to control adult mosquito populations through aerial ultra-low-volume (ULV) campaigns. Recently, advocacy groups have raised concerns about potential health impacts of exposure to Naled. This presentation provided findings from dispersion models to determine the dispersal of Naled within aerial spray blocks following ULV fixed-wing applications. The results identified hotspots attributable to each treatment area that would be suitable for in-situ sampling. Additionally, a systematic scoping literature review of peer-reviewed health impacts of Naled comprehensively synthesized the body of science on the topic and will be compared against both modeled and measured results.

Comparing two baits to attract gravid female mosquitoes

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Introduction

Routine mosquito surveillance is important for monitoring the species diversity and abundance of mosquitoes present in the Coachella Valley. In the urban areas, gravid traps are effective in capturing *Culex quinquefasciatus* females when they are ready to lay their eggs. McNamara and Healy (2021) compared two gravid bait attractants and found that a fish emulsion-based bait attracted more mosquitoes, primarily *Cx. quinquefasciatus*, compared to a hay-infused bait. The Coachella Valley Mosquito and Vector Control District (the District) uses a fermented infusion of ground rabbit chow and yeast as a bait to attract females seeking a water source. The District compared the routine gravid bait with the fish-emulsion based bait. The objective was to determine which bait provided larger collections with gravid traps.

Methods

Each week, the gravid baits were prepared and aged for 5 days. The District's routine formula bait was made with 8.5 oz (17 tablespoons) of ground rabbit pellets (Ace Hi Feeds) and 5 oz (10 tablespoons) of Brewer's Yeast Powder (Puritan's Pride) mixed thoroughly with water to make 121 liters (32 gallons). The fish fertilizer emulsion (Alaska) bait was made with 32 ounces (2 pints) of fish fertilizer mixed thoroughly with 121 liters (32 gallons) of water. The Reiter-Cummings modified gravid traps (Cummings 1992) were baited with 6 liters (1 ½ gallons) of bait and set late in the day and picked up the following morning (Fig. 1). For 8 weeks, between May 23rd and July 11th, 2022, the traps were set at 8 locations in the cities of La Quinta, Palm Desert, and Bermuda Dunes. At each location, two traps were placed approximately 6 meters (20 feet) apart, with the baits switched between sites each week. Adult mosquitoes in the collection chamber were identified and counted each day. Counts of *Cx. quinquefasciatus* female mosquitoes were analyzed using a two-way ANOVA.

Results and Discussion

Both attractants were effective in capturing *Cx. quinquefasciatus* females with no significant difference in catch size ($p = 0.70$; Fig. 2). For most weeks, the number of female mosquitoes collected were similar between baits. The District examined the cost and the time it took to prepare both baits and decided to continue to use the District's more cost effective gravid bait formulation. The District formulation costs $2.94 to make 121 liters (32 gallons) compared to $6.79 with the fish fertilizer emulsion.

Conclusion

Different gravid bait attractants are used for mosquito surveillance. Our results showed that both attractants provided similar *Cx. quinquefasciatus* trap collections.

Figure 1.—The Reiter-Cummings modified gravid trap. Traps were paired at each site and placed 6 meters (20 feet) apart and the bait positions were switched each week.

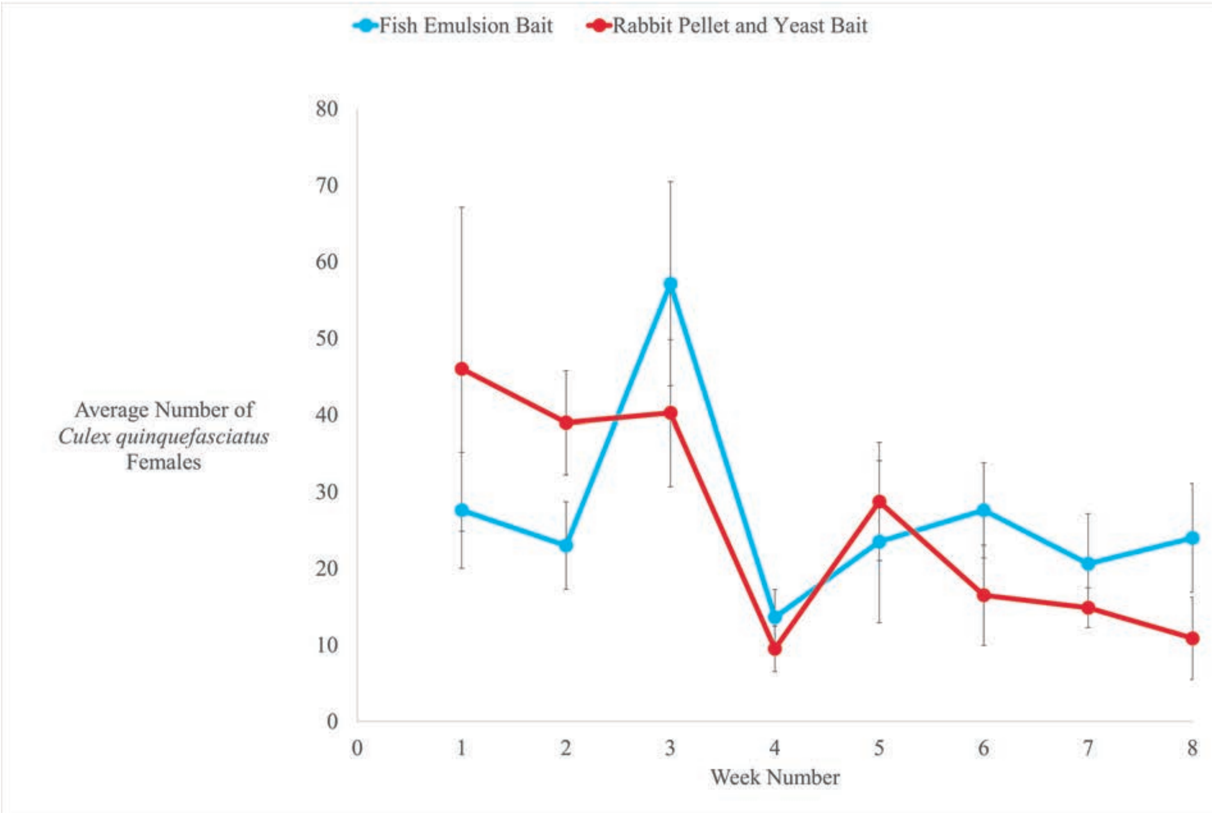

Figure 2.—The average number of female *Cx. quinquefasciatus* mosquitoes collected per trap night (+/- SE) using fish emulsion and rabbit pellet + yeast baits.

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The design and use of an "easy-to-construct" 12 V-powered CDC-style gravid mosquito trap for surveillance of West Nile virus in Orange County, California

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Abstract

Two of the most commonly-used gravid traps, the Centers for Disease Control and Prevention (CDC) and Reiter-Cummings box-style gravid traps, operate off 6 V DC battery-powered systems. In the CDC gravid trap, the suction motor (with its fan blades) is mounted within the inlet tube and positioned before the collection net. This configuration forces mosquitoes to pass through the spinning fan blades before capture in the net and often results in damaged specimens. In contrast, the Reiter-Cummings box-style gravid trap is designed with the suction motor located in a separate exhaust tube after the collection chamber, allowing for capture of mosquitoes before reaching the fan blades. Box-style gravid traps are advantageous in ensuring better survivability of the catch but are harder to construct compared to CDC-style gravid traps. Since the early 1990s, the Orange County Mosquito and Vector Control District has built and deployed hundreds of 6 V DC, battery-powered box-style gravid traps to monitor *Culex* populations and their arboviral infection rates. To increase airflow, simplify construction of gravid traps, and improve mosquito survival, we designed a CDC-style gravid trap with a more powerful motor (12 V DC computer fan motor) and positioned the motor outside the inlet tube on top of the collection chamber. The trap is mounted with cross supports over a small basin filled with attractant media. In addition to greater airflow, this 12 V DC-powered CDC-style gravid trap draws mosquitoes into the collection chamber without having to first pass through the motor's fan blades, similar to box traps. During a trap comparison study from September – October in 2020, the 12 V-powered, CDC-style and 6 V-powered gravid traps captured statistically equivalent numbers of *Cx. quinquefasciatus* females (57.1/trap-night and 47.3/trap-night, respectively). Of the 3,341 *Cx. quinquefasciatus* females collected in the study, 2,010 gravid mosquitoes were pooled (56 pools) and stored at −80° C. Mosquitoes were later removed and dissected into their respective body parts (abdomen, thorax, head, legs/wings), after which their abdomens were tested individually for arboviruses. Mosquitoes with positive abdomens had their body parts tested individually. In total, 18 gravid female *Cx. quinquefasciatus* from 13 pools tested West Nile virus-positive. Seven (43.8%) of the WNV-positive mosquitoes were found to have virus disseminated to their heads and legs/wings, suggesting that potentially infectious mosquitoes could be a large component of WNV-positive pools collected by gravid traps. The WNV infection rate (per 1,000) of the individually-tested mosquitoes was not significantly different from the maximum likelihood estimate based on pooled data. This easy-to-construct CDC-style gravid trap consists of relatively few, readily-available components, and could replace older, 6 V-powered gravid trap designs.

Introduction

Several types of battery-powered and passive gravid mosquito traps are used to collect mosquitoes for surveillance programs around the world. Gravid mosquito traps are used to capture female mosquitoes that have taken and digested at least one blood meal and are seeking an appropriate source of water or substrate on which to oviposit their eggs. Compared to other adult mosquito sampling methods (e.g., CO_2-baited traps, passive gravid traps), battery-powered gravid traps collect more post-blood fed female mosquitoes, thereby increasing the probability of recovering pathogen-infected mosquitoes. Since the introductions of the CDC gravid trap (Reiter 1983) and the Reiter-Cummings version (Cummings 1992), 6 V DC battery-powered gravid traps, coupled with an attractive oviposition media, have become the suggested arbovirus surveillance method for collecting *Culex pipiens*-complex mosquitoes in the U.S. (Reisen et al. 1999, CDC 2022). Two members of the complex, *Culex pipiens* L. and *Cx. quinquefasciatus* Say, are recognized as the principal vectors of St. Louis encephalitis and West Nile viruses (SLEV and WNV, respectively) in urban/suburban habitats of California (CDPH 2022). *Culex quinquefasciatus* is also a competent vector of filarial parasites in certain regions of the world and can be readily collected in gravid traps for studies of lymphatic filariasis (Irish et al. 2013). Gravid traps are showing efficacy at collecting invasive *Aedes* *aegypti* (L.) in Orange County. PCR tests for *Dirofilaria immitis* infections in *Ae. aegypti* from weekly gravid trap collections have provided insight into finding dog heartworm-risk areas in the county (Rangel et al. 2023, *in press*). In the current paper, we discuss the construction of a modified CDC-style gravid trap, and present the comparative results of mosquito collections and testing of individual mosquitoes for arboviruses from Reiter-Cummings box-style gravid traps and newly modified CDC-style gravid traps in Orange County, California, during 2020.

Materials and Methods

Study site

Trap studies were conducted at the headquarters of the Orange County Mosquito and Vector Control District (OC Vector) in Garden Grove, California (33.9208555° N, -118.3812558° W) in two distinct habitats (unvegetated and vegetated) within the District's approximately 2.1 ha (5.1 acres) property (Figure 1 A, B). The property is surrounded by commercial development and lies within Orange County's WNV high risk area (Nguyen et al. 2015). Two traps (one of each design) were set 50 m (165 ft.) apart in the unvegetated habitat where mosquitofish (*Gambusia affinis*) are held and reared in a series of fish ponds at the east end of the property. Two traps (one of each design) were set 50 m (165 ft.) apart in the vegetated habitat [ornamental trees and shrubs, principally coast live oak (*Querica agriflora*), orange trees (*Citrus sinensis*), California brittlebush (*Encelia californica*), Natal plum (*Carissa macrocarpa*)] that runs along an open concrete-lined, flood control channel. The center points of these distinct habitats are separated by approximately 150 m (500 ft.). Weather data for the study was obtained from a nearby weather station at Fullerton Municipal Airport (Fullerton, CA) from the website Time and Date©.

Trap types

Two Reiter-Cummings box-style gravid traps built previously by OC Vector were used in this study (Figure 2). Instead of operating each trap with four, 1.5 V D-cell DC batteries as described in the initial design (Cummings 1992), each trap was powered by a single 6 V DC 12 amp.-hr. rechargeable gel cell battery (PowerSonic Model PS-6100 F2, Power Sonic Corp., Reno, NV); batteries were recharged for each night's use. Two "easy-to-construct" 12 V-powered CDC-style gravid traps were made by OC Vector for this study (Figure 3). The design is similar in appearance to the commercially-available CDC gravid trap (John W. Hock Company Model 1712, Gainesville, FL).

For the 12 V trap, instead of internally-mounting a 6 V DC motor/fan in the 76.2 mm (3″) dia. ABS inlet tube, a 12 V DC 0.300 amp., 120 mm × 120 mm × 25 mm computer fan motor (CoolerGuys Model CG12025H12B2-3Y, https://www.coolerguys.com/collections/120mm-fans/) was placed on top of the plastic collection chamber (i.e., net) (Figure 3, A). The collection chamber was a modified

Figure 1.—Habitat types used in gravid trap comparison study, September − October, 2020, Garden Grove, CA. **A)** Unvegetated (near mosquito fish ponds). **B)** Vegetated (trees and shrubs).

1.9 L (2 qt.) food storage container (KaTom Restaurant Supply, Kodak, TN) that is used in box-style gravid traps supplied by BioQuip Products (Rancho Dominquez, CA) (Figure 3, B). The BioQuip-supplied collection chamber has an approximately 120 mm × 120 mm (4.75″ × 4.75″) fine-mesh wire screen built into its removable lid; the bottom is fitted with a 101 mm (4″) dia. coupler attached to a netted sleeve through which the inlet tube is inserted. When under power, the computer fan motor (positioned on the collection chamber's lid) draws in air through the inlet tube, and mosquitoes and other flying insects are retained in the collection chamber by the wire mesh lid as air is exhausted through the computer fan motor. The computer fan motor was powered by a 12 V DC 12 amp.−hr. rechargeable gel cell battery (PowerSonic Model PS-12120 F2, Power Sonic Corp., Reno, NV) (Figure 3, C); batteries

Figure 2.—6 V DC-powered Reiter-Cummings box-style gravid trap.

were recharged for each night's use. The length of the ABS inlet tube was approximately 16.5 mm (6.5″) long and was held in place by wood cross supports [51 cm (20″)] using two 14 cm (5.5″) - long lag bolts and their wing nuts for tightening and keeping the supports together.

Gravid traps were baited with a fermented alfalfa infusion (i.e., attractant media) used in OC Vector's gravid trap-based mosquito and arboviral surveillance program. During the "mosquito season" from May–October, OC Vector sets approximately 150 gravid traps per week in urban/suburban areas of the county (gravid trap density = 7 - 10 traps/100 km^2) (Cummings et al. 2016). The alfalfa infusion was mixed in a large 1,500 L (400 gal.) plastic holding tank with tap water and refilled twice/week at a mix rate described by Reiter (1983). No lactalbumin powder was added to the tank. Each infusion batch was fermented for 3-4 days under the sun before being dispensed through a tank spigot into plastic jugs for transport to the field. Traps were mounted on black restaurant dish boxes [51.4 cm × 39.4 cm × 12.7 cm (20.25″ × 15.5″ × 5″), KaTom Restaurant Supply, Kodak, TN] that were filled with 3.8 L (1 gal.) of freshly-dispensed media to a level approximately 5 cm (2 in.) below the bottom of the trap inlet tube (Reiter 1983).

Gravid traps were set mid-afternoon, twice a week, Wednesdays and Thursdays, and operated for approximately 20 h until retrieval the following mornings during September – October, 2020 (8 weeks total). Upon retrieval, collection chambers were held on dry-ice for several hours until the specimens were processed. Mosquitoes were identified by species and sex, and enumerated. Female mosquitoes of the primary arboviral vectors in the region (*Cx. quinquefasciatus*, *Cx. tarsalis* Coquillett, and *Cx. stigmatosoma* Dyar) (Resien et al. 1992, Resien et al. 1999, Molaei et al. 2010) were sorted into pools (1–50/pool) and stored at −80° C in uniquely numbered vials for arbovirus testing. Pooling of *Cx. quinquefasciatus* was limited to a maximum of 50 gravid females, with only one pool/trap-night per trap. Other insects (by-catch) were also counted and identified to taxonomic family, if possible.

Mosquito dissections

From May – October 2022 (after 1.5 years in storage), mosquitoes from each pool were removed from their vials and spread carefully across a small plate held on dry ice. Gravid mosquitoes were removed singly from the plate and each mosquito was placed on a fresh filter paper square for dissection under a microscope at room temperature. For each mosquito, wings and legs were cut off first using one end of a razor blade, followed by separation of the abdomen from the thorax with the rotated, unused end of the razor blade. Cuts on the appendages (legs and wings) were made distally from the coxa ("down leg") and distally from the membrane articulating cuticle of the wings ("down wing") to avoid accidentally adding thoracic tissue to a leg/wing specimen. The abdomen was separated from the thorax by cutting partially into the rear thoracic segments. This was done to ensure that tissue from the abdomen would not be included mistakenly in thoracic samples. Each cut per body region was made with a fresh razor edge to avoid potential cross-contamination with another body part. An individual mosquito's respective body parts (legs/wings, abdomen, thorax/head) were removed in the same sequence for each processed mosquito, placed in separate vials (3 vials per dissected mosquito) with discrete identification numbers, and stored at −80°C. Abdomens were tested first, and if an abdomen tested virus-positive, the mosquito head was later removed from the thorax and tested separately; the other mosquito body parts were then tested to determine if virus had disseminated from the abdomen. Because mosquitoes from a trap had been processed together, pooled, stored and re-

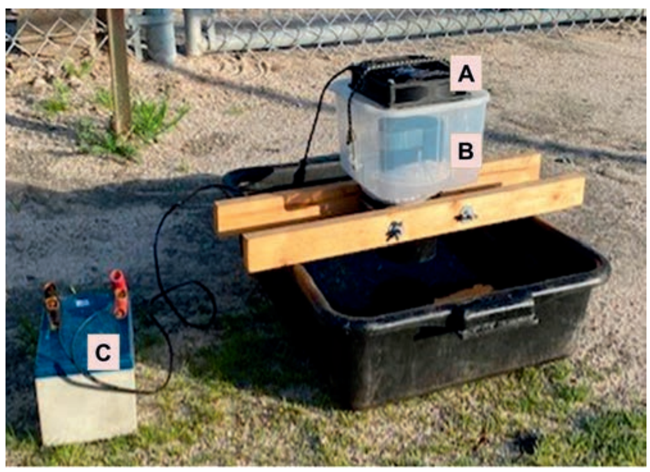

Figure 3.—12 V DC-powered CDC-style gravid trap with computer fan motor on top of collection chamber. **A)** 12 V DC computer fan motor. **B)** Collection chamber. **C)** 12 V DC 12 amp. -hr. rechargeable gel cell battery.

processed for dissection, many mosquitoes lacked a full complement of 6 legs attached to the thorax. Hence, many leg samples consisted of only 1- 2 legs per submission; in contrast, most wings were still attached. Care was taken to ensure that no loose or extraneous body parts from other mosquitoes were inadvertently included in the individual vials. Only body parts cut specifically from each mosquito were placed in the appropriately designated vials.

This aspect of the trap study was undertaken to determine the actual arboviral infection rate (IR) of the sampled mosquito population based on individual mosquito testing (Condotta et al. 2004, Gu et al. 2004). The arboviral infection rate is assumed to have a direct relationship to the human disease risk in a given area (Chiang and Reeves 1962). Testing of individual body parts of an infected mosquito also should provide insight as to the number of potentially-infectious mosquitoes in an arbovirus-positive pool.

Mosquito testing

Mosquito body parts were placed separately in 1.5 ml microcentrifuge skirted screw cap tubes, along with 8 small ZR BashingBeads (Zymo Research, Cat # SKU-S6003-50, Irvine, CA) and 100 µl of MEM Medium (Genesee Scientific, Cat # 25-504, San Diego, CA). The tubes were agitated using an 8000D Mixer/Mill (SPEX Sample Prep, Metuchen, NJ) for 45 sec. to macerate mosquito parts. Supernatant containing RNA was transferred into a MagMAX™ processing plate following viral RNA isolation steps per manufacturer recommendations for the MagMAX™ Express Magnetic Particle Processor-24 (ThermoFisher Scientific, Cat # AMB18365, Waltham, MA). Isolated RNA was immediately tested by real-time TaqMan multiplex RT-qPCR (ABI 7500, Applied Biosystems, Foster City, CA) using WNV, SLE, and WEE-specific primers (Lanciotti et al. 2000, Lanciotti et al. 2001, Brault et al. 2015), respectively) and testing was done in duplicates. For WNV, specimens with cycle thresholds (Ct) < 30 were considered virus-positive (Reisen et al. 2013); values from 30-40 were retested with primer/probes for the WNV NS1 region and confirmed WNV-positive at Ct values < 40 (Shi et al. 2001).

Experimental design

Four traps (two of each design, one per habitat) were used. The weekly positioning of box gravid traps and CDC-style gravid traps at the designated sites within each habitat was the same during the study. Traps were rotated between sites within each habitat on the second collection night of the week to avoid position bias. The study was conducted from 2 September − 23 October 2020 for 8 weeks (8 TN/week) for a total of 64 trap-nights.

Statistical analysis

Counts of mosquitoes and other collected insects were recorded and tabulated. Mean mosquito catch per trap-night (TN) (95% confidence interval, CI) and proportions were calculated for each trap and habitat type. The abundance of adult mosquitoes was calculated as the average number of females per trap/TN. Distribution normality of data was assessed and a non-parametric method was used for significance testing. The differences between trap types and collection venues were analyzed for *Cx. quinquefasciatus*, the dominant mosquito species, using the Kruskal-Wallis test.

The arboviral infection rates were calculated by three measures: the "true" infection rate (IR) was determined using the total number of arbovirus-positive individual mosquitoes × 1,000/total tested. The IR was then compared to calculated infection rates using pooled data, specifically the minimum infection rate (MIR) (Chiang and Reeves 1962, Walter et al. 1980) and maximum likelihood method (MLE) (Chiang and Reeves 1962, Gu et al. 2004, Biggerstaff 2009). We assumed that an individually-tested, arbovirus-positive mosquito would have produced an arbovirus-positive pool had it been tested in its original pool, based upon the high sensitivity/specificity of our multiplex TaqMan RT-qPCR assay. MIR and MLE calculations were based on this premise for assigning pools as "arbovirus -positive". All statistical analyses were performed using JMP software (JMP 16.0, SAS Institute, Inc.).

Results

Species composition

In total, 3,439 female mosquitoes of five species were collected during the 64 TNs (Table 1). *Culex quinquefasciatus* was the most frequently captured mosquito, comprising 97.2% (n = 3,341) of total females, followed by decreasing numbers of *Cx. stigmatosoma* (1.4%, n = 49), and *Cx. tarsalis* (1.2%, n = 40). Incidental counts of female *Ae. aegypti* (n = 8) and *Culiseta incidens* (Thompson) (n = 1) were also recovered, together amounting to 0.2% of the catch. Of the 3,341 *Cx. quinquefasciatus* females, 97.3% (n = 3,251) were gravid. All (100%) of the remaining females (n = 98) from the four other species were recorded as gravid. In addition, 189 male mosquitoes of two species were collected, with *Cx. quinquefasciatus* males representing the largest component (98.4%, n = 186), followed by three *Ae. aegypti* males (1.6%).

Weather and other insect data

The average daily temperature was approximately 21.7° C (daily range 16.1°- 41° C, min/max), and no rain occurred during the study. Other insects were also tallied in the collections and listed by taxonomic family (Table 2). Of these, plume moths (Pterophoridae) and a variety of small dipterans, such as fungus gnats (Mycetophilidae), moth flies (Psychodidae), and other small flies, were the most numerous.

Habitat and trap types

Significantly more *Cx. quinquefasciatus* females were collected in the unvegetated habitat near the fish ponds

Table 1.—Gravid trap mosquito collections by habitat, September – October, 2020, Garden Grove, CA.

	Unvegetated Habitat				Vegetated Habitat				Totals			
Mosquito species	Male	Female	Gravid	Tested	Male	Female	Gravid	Tested	Male	Female	Gravid	Tested
Cx. quinquefasciatus	99	2,159	2,110	1,217	87	1,182	1,141	898	186	3,341	3,251	2,010
Cx. stigmatosoma	0	41	41	14	0	8	8	8	0	49	49	22
Cx. tarsalis	0	37	37	16	0	3	3	0	0	40	40	16
Cs. incidens	0	1	1	0	0	0	0	0	0	1	0	0
Ae. aegypti	0	3	3	0	1	3	5	0	3	8	8	0
Totals	**99**	**2,241**	**2,192**	**1,247**	**88**	**1,196**	**1,157**	**906**	**189**	**3,439**	**3,348**	**2,048**

(64.6%, n = 2,159, 67.5/TN) than in the vegetated area (35.4%, n = 1,182, 36.9/TN) (Kruskal-Wallis^2 = 5.83, *d.f.* = 1, *P* = 0.016) (Figure 4, A). There was no significant difference between the catch of female *Cx. quinquefasciatus* for the CDC-style gravid trap (54.7%, n = 1,828, 57.1/TN) and the box-style gravid trap (45.3%, n = 1,513, 47.3/TN) (Kruskal-Wallis^2 = 1.10, *d.f.* = 2, *P* = 0.29) (Figure 4, B). Collections of other mosquito species and males were small and were not analyzed.

Mosquito infection rates

In total, 59.7% (n = 2,048 of 3,430) of the captured female *Culex* were tested for arboviruses (Table 1). No *Cx. tarsalis* (n = 16, 6 pools) tested arbovirus-positive; 1 pool of seven *Cx. stigmatosoma* females (n = 22, 6 pools) tested WNV-positive but was mistakenly not processed further. Individual processing of abdomens from gravid female *Cx. quinquefasciatus* (n = 2,010, 56 pools) resulted in 18 mosquitoes testing WNV-positive (Table 3). The WNV-positive *Cx. quinquefasciatus* mosquitoes were originally grouped into 13 pools ranging in size from 22 - 50 mosquitoes. Results showed that four pools held multiple WNV-positive mosquitoes (range 2 - 3): pool 9 (specimens 9.41, 9.43); pool 10 (specimens 10.08, 10.11); pool 40 (specimens 40.02, 40.06, 40.11); and pool 44 (specimens 44.22, 44.43).

Additional body parts from 8 of 16 of these abdomen positive mosquitoes were found WNV-positive. WNV RNA was detected only in the thorax of one mosquito; WNV RNA was found in the thorax and head but not the legs/wings in one mosquito; WNV RNA was found in the thorax, head, and legs/wings of 6 mosquitoes. Unfortunately, the stored body parts from two WNV-positive mosquitoes were mistakenly discarded. Of 16 WNV-positive mosquitoes that had their other body parts tested, seven (43.8%) had viral RNA detected in their heads (Table 3).

The overall WNV-infection rate calculations were as follows: IR = 8.96; MIR = 6.47; MLE = 7.46 (95% CI 4.17-12.53) (Table 4A). In the unvegetated habitat, 16 WNV-positive *Cx. quinquefasciatus* from 11 pools (IR = 13.50; MIR = 9.28; MLE = 11.49, 95% CI 6.14-20.23) were collected, and two WNV-positive *Cx. quinquefasciatus* from two pools (one each) were collected in the vegetated habitat (IR = 2.42; MIR = 2.42; MLE = 2.50, 95% CI 0.45-8.30). The CDC-style gravid traps captured 11 WNV-positive *Cx. quinquefasciatus* from eight pools (IR = 10.84; MIR = 7.88; MLE = 9.38, 95% CI 4.40-18.13), and the box-style gravid traps collected seven virus-positive *Cx. quinquefasciatus* from five pools (IR = 7.04; MIR = 5.03; MLE = 5.50, 95% CI 2.06-12.30) (Table 4B). The WNV-positive *Cx. stigmatosoma* pool was collected in the unvegetated habitat in a CDC-style gravid trap (MIR = 45.45). No mosquitoes tested positive for SLEV.

Discussion

Both gravid trap designs effectively captured female *Cx. quinquefasciatus* in relatively comparable numbers during the study. The stronger air flow generated by the more powerful 12 V DC computer motor in the CDC-style gravid trap did not make a significant difference in quantities of mosquitoes collected. Habitat differences accounted more for variations in trap counts than trap design. However, the 12 V DC CDC-style gravid trap was easier to construct, requiring the fabrication of only one ABS inlet pipe and two wood supports. No internal mounting of a motor within the inlet tube was required, as in the Hock version. Fortuitously, the computer fan motor fit exactly over the wire mesh of the collection chamber's lid. An elastic strap kept the motor tightly seated against the chamber, such that the motor did not draw in any "extraneous" air along its outside edges while positioned over the mesh screen. The open space of the collection chamber between the inlet tube and computer motor created a zone of stagnation that freed the mosquitoes from the incoming air stream. This simple configuration allowed for efficient flow of air through the inlet tube and the capture of undamaged mosquitoes, similar to box-style gravid traps.

One disadvantage of this modified CDC-style trap is that the catch is exposed to the elements. In addition, the 12 V DC battery is more expensive, takes longer to recharge than a 6 V DC battery, and is visible to the public. In box-style traps, the collection chamber and battery are enclosed

Table 2.—Gravid trap collections of other arthropods by habitat, September – October, 2020. Garden Grove, CA.

Other Arthropods	Unvegetated Habitat	Vegetated Habitat	Totals
Psychodidae (moth flies)	439	20	**459**
Mycetophilidae (fungus gnats)	232	68	**300**
Pterophoridae (plume moths)	1	40	**41**
Diptera (filth flies, small flies)	33	17	**50**
Spiders, ants, bees, others	37	33	**70**
Totals	**742**	**178**	**920**

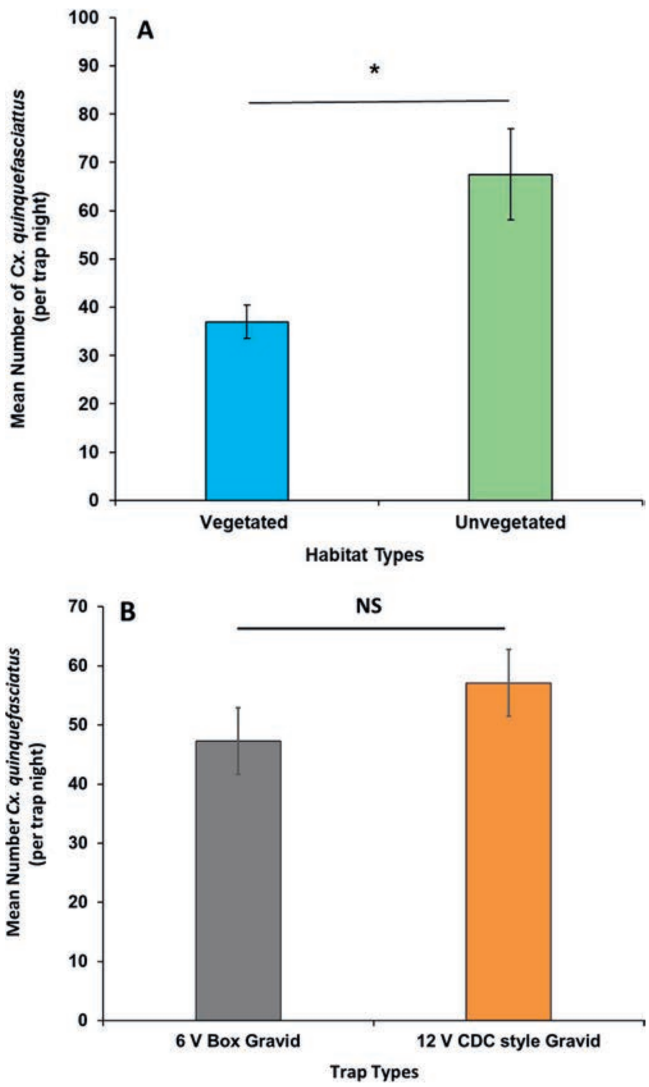

Figure 4.—**A)** Mean number of *Cx. quinquefasciatus* females collected in gravid traps by vegetated and unvegetated habitats. * Counts significantly different by habitat, $P = 0.016$, September – October, Garden Grove, CA. **B)** Mean number of *Cx. quinquefasciatus* females collected per trap-night in Reiter-Cummings box style and CDC-style gravid traps. NS: no significant differences between trap collections, $P = 0.29$, September – October, Garden Grove, CA.

inside the trap, protecting both the mosquitoes and the battery.

The study showed a significant difference in mosquito collections by habitat. The gravid traps set in the sparse, unvegetated habitat near OC Vector's fish ponds caught 64.6% of *Cx. quinquefasciatus* females and demonstrated that a large variability between mosquito counts in gravid traps can occur within a relatively small area [150 m (550 ft.) separation]. The water in the fish ponds of the unvegetated habitat might have been more appealing to ovipositing females than the shaded refugium in the vegetated area, in contrast to earlier studies where competing surface water sites were found to decrease gravid trap counts (Reisen et al. 1990, Reisen et al. 1991).

The actual number of WNV-positive mosquitoes in field-collected pools typically is unknown. Individual testing of *Cx. quinquefasciatus* produced a total of 18 infected mosquitoes (IR = 8.96), five more than what would be used when calculating the MIR for the 13 pools they represented (MIR = 6.47). However, because the MLE accounts for the possibility that more than a single mosquito is infected per tested mosquito pool, the IR and MIR were not significantly different from the MLE infection rate.

The WNV infection rate in gravid *Cx. quinquefasciatus* from a large adjacent area [50 km^2 (20 mi^2)] with comparable collection parameters and data [8 TNs/wk., N = 3,188 female *Cx. quinquefasciatus* (97 pools, 20 WNV-positive pools)] during September – October 2020 were similar (MLEs: study area 7.46, 95% CI 4.17-12.53 vs. adjacent area 7.04, 95% CI 4.45-10.70, respectively). Gu et al. (2004) estimated that arboviral intensity would have to be high for the IR from virus-positive individual mosquitoes to exceed MLE estimates when using variably-sized pooling, as was done in this study.

Most arboviral evaluations by mosquito control and health care agencies rely on whole body mosquito testing to determine infections and do not measure the actual status (infected and infectious) of mosquitoes in surveillance programs (Gu et al. 2008). Hence, the actual component of the mosquito population capable of transmitting viruses is unknown. Our finding of WNV RNA in the heads and leg/wings of seven WNV-infected mosquitoes (43.8% of positives) indicated that modest levels of potentially-infectious mosquitoes may be found in virus-positive samples obtained from gravid traps. The small amount of mosquito tissue in a leg/wing sample might have been a factor in not detecting WNV RNA in one mosquito's legs/wings (specimen 47.18), where its thorax and head tested WNV-positive but not the legs/wings (Table 4).

Virus contamination from infected to uninfected mosquitoes during processing and pooling may have resulted in more mosquitoes inadvertently testing WNV positive. We suggest that while possible, cross-contamination was unlikely to have occurred because of careful processing of individual specimens. Of the 13 WNV-positive pools, nine pools (341 mosquitoes) contained only one positive mosquito each, indicating no detection of viral cross-contamination in these pools. The four pools with multiple WNV-positive mosquitoes (2-3 each, 9 total) were large (49.5 mosquitoes/pool, 48-50 range, 198 total), and the potential presence of pools with more than one WNV-infected mosquito per pool was within the confidence interval of the MLE infection rate for the 56 pools (2010 mosquitoes). In addition, PCR test results for individual mosquito body parts from six of eight abdomen-positive mosquitoes from these four pools did not demonstrate the presence of WNV RNA in body parts other than the abdomens. [The body parts of two WNV-positive mosquitoes were lost, and one mosquito (specimen 10.11) had all its body parts test virus-positive, indicating a fully disseminated WNV infection]. Taken together, these factors suggest that all 18 individually-processed, WNV-

Table 3.—WNV critical thresholds (Ct) of individually-tested *Cx. quinquefasciatus* mosquito body parts.

Coll. Date	Pool ID No.	Pool Size	No. Mosq Pos.	Mosq. Specimen ID No.	WNV Critical Thresholds (Ct), *Cx. quinquefasciatus* Abdomen	Thorax	Head	Legs/Wings
10 Sep	8	36	1	8.31	26.79	-	-	-
11 Sep	9	50	2	9.41	26.49	-	-	-
				9.43	24.06	-	-	-
11 Sep	10	50	2	10.08	25.52	-	-	-
				10.11	25.87	18.45	19.41	22.97
18 Sep	17	50	1	17.13	25.84	18.82	13.51	22.46
1 Oct	32	45	1	32.04	22.22	17.39	19.54	27.71
2 Oct	37	48	1	37.21	24.94	23.90	-	-
2 Oct	40	50	3	40.02	25.09	-	-	-
				40.06	18.66	-	-	-
				40.11	20.19	-	-	-
8 Oct	43	24	1	43.19	27.98	-	-	-
8 Oct	44	48	2	44.22	18.57	lost	lost	lost
				44.43	15.44	lost	lost	lost
9 Oct	47	22	1	47.18	17.32	23.17	26.83	-
9 Oct	48	34	1	48.20	24.02	15.68	18.26	25.86
17 Oct	55	44	1	55.23	34.34	16.85	26.20	24.44
22 Oct	60	38	1	60.18	26.82	15.47	17.35	22.81

positive mosquitoes acquired their infections naturally and that inadvertent cross-contamination of virus through hemolymph leakage or foreign body parts was unlikely to have affected test results. Condotta et al. (2004) found that WNV-infection rates in pool size of five mosquitoes accurately paralleled the results of individual testing of host-seeking *Cx. pipiens* and *Cx. restuans* in Ontario, Canada. However, the amount of mosquito testing required

Table 4.—A) Collections of *Cx. quinquefasciatus* female mosquitoes in gravid traps and WNV infection rates by habitat (unvegetated and vegetated), September − October, 2020, Garden Grove, CA. B) Collections of *Cx. quinquefasciatus* female mosquitoes in gravid traps and WNV infection rates by gravid trap types (CDC and box-styles), September − October, 2020, Garden Grove, CA.

Table 4A

Cx. quinquefasciatus	Unvegetated Habitat Site 1	Unvegetated Habitat Site 2	Vegetated Habitat Site 1	Vegetated Habitat Site 2	Combined Sites/Habitat Unvegetated	Combined Sites/Habitat Vegetated	Totals
Mosquitoes collected by site	1065	1094	677	505	2159	1182	**3341**
Tested	572	613	477	348	1185	825	**2010**
No. of pools	14	14	14	14	28	28	**56**
WNV positive pools	6	5	1	1	11	2	**13**
WNV positive mosquitoes	7	9	1	1	16	2	**18**
WNV pos. mosquitoes/pos. pool	1.17	1.8	1	1	1.45	1	**1.38**
Individual Infection Rate (IR)					13.50	2.42	**8.96**
Minimum Infection Rate (MIR)					9.28	2.42	**6.47**
Maximum Likelihood Estimate (MLE)					11.49	2.50	**7.46**
95% Confidence Interval (CI)					6.14-20.23	0.45-8.30	**4.17-12.53**

Table 4B

Cx. quinquefasciatus	Unvegetated Habitat CDC-style	Unvegetated Habitat Box-style	Vegetated Habitat CDC-style	Vegetated Habitat Box-style	Combined Habitats/ Trap Types CDC-style	Combined Habitats/ Trap Types Box-style	Totals
Mosquitoes collected by trap	1268	891	560	622	1828	1513	**3341**
(Average/Trap-Night)	(79.3)	(55.7)	(35.0)	(38.9)	(57.1)	(47.2)	**(52.2)**
Tested	622	563	393	432	1015	995	**2010**
No. of pools	14	14	14	14	28	28	**56**
WNV positive pools	6	5	2	0	8	5	**13**
WNV positive mosquitoes	9	7	2	0	11	7	**18**
WNV pos. mosquitoes/pos. pool	1.5	1.4	1.0	0	1.38	1.40	**1.38**
Individual Infection Rate (IR)					10.84	7.04	**8.96**
Minimum Infection Rate (MIR)					7.88	5.03	**6.47**
Maximum Likelihood Estimate (MLE)					9.38	5.50	**7.46**
95% Confidence Interval					4.40-18.13	2.06-12.30	**4.17-12.53**

with such small sized pools would be impractical for most agencies tasked with testing thousands of mosquitoes each season for arboviruses (Chiang and Reeves 1962). In a mosquito infection simulation study with *Culex*, Gu et al. (2004) recommend variable pool sizes across a gradient of 1 - 50 mosquitoes to generate MLEs that more closely resembled true infection rates in mosquitoes, especially when arbovirus transmission is high. Both Gu et al. (2004) and Bustamante and Lord (2010) found that MIR and MLE calculations based on fixed, large size pooling (ca. 50/pool) greatly underestimated the true arbovirus infection rates in simulated infection studies for *Culex*.

OC Vector relies on accurate and timely assessments of WNV-infection rates in mosquitoes to guide its public education and mosquito control programs. Gravid traps increase the probability of recovering pathogen-infected mosquitoes within the *Cx. pipiens* complex and are the most sensitive and responsive element in OC Vector's arbovirus surveillance program. At times, OC Vector has encountered strong opposition from some of the public regarding adult mosquito control (adulticiding) with EPA-approved, ultra-low volume (ULV) insecticides (e.g., pyrethroids) in residential communities triggered by elevated WNV infection rates in mosquitoes (Cummings et. al. 2016). Members of the public have questioned the predictive accuracy of mosquito infection rate calculations [e.g., vector index (VI): Barker et al. (2009), Bowling et al. (2009), Kwan et al. (2012)], especially when MLEs (a component of the VI) are viewed in a minimized context of "just one infected mosquito" in a positive pool. In response to this line of inquiry, OC Vector has increased gravid trap densities and structured a risk-based surveillance grid [1 gravid trap/7.24 km^2 (2.75 mi^2)] in historically high WNV active areas (Krueger et al. 2020). Now, action thresholds for adulticiding are built on data from weekly mosquito collections and testing of variably-sized mosquito pools from a network of gravid traps over a broad area. Recognizing the inherently conservative nature of current infection rate estimates in a vector population is also helpful, as this small study demonstrated. Yearly refinements to OC Vector's arbovirus monitoring program have improved surveillance assessments and justification for ULV adulticide applications to suppress WNV outbreaks in the county.

Conclusion

This study demonstrated the effectiveness of an "easy-to-build" gravid trap in collecting substantial numbers of gravid *Cx. quinquefasciatus* in an urban habitat of southern California. The simplicity of this "economical" CDC-style trap allows for in-house fabrication of gravid mosquito traps and can help expand an agency's trapping capacity in surveillance programs. In addition, this field study was the first in the region to document the presence of multiple WNV-infected mosquitoes in the same pool and the occurrence of potentially infectious mosquitoes in WNV-positive pools. The study also demonstrated that WNV infection rates derived from MLEs are conservative in their estimation of the true number of infected mosquitoes in a local *Cx. quinquefasciatus* population, reinforcing OC Vector's data-driven approach on determining when to conduct ULV adulticide applications in the county.

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Quantifying and characterizing mosquito production in subsurface utility enclosures

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Abstract

The Consolidated Mosquito Abatement District identified subsurface enclosures as a major source of both *Culex* and *Aedes* mosquitoes in the summer of 2014. Over the past eight years the District has worked with the Pacific Gas and Electric Company (PG&E) to address mosquito production in subsurface utility enclosures. Over the years the program has gone through several iterations to address safety concerns with accessing enclosures and effective treatment of these enclosures. In 2022 the District collaborated with PG&E to treat and evaluate mosquito production in subsurface enclosures. This presentation summarized findings from the 2022 evaluation and outlined plans for evaluating enclosures in 2023.

Madera County Mosquito and Vector Control District – PG&E Substructure: Utility Vault Summary

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Introduction

The yellow fever mosquito, *Aedes aegypti,* is one of most important mosquitoes globally. It is a competent vector of several arboviruses of public health concern and is extremely adept to exploiting human-made water sources which has enabled its geographic range expansion to new areas, including the northern and central valley in California. After *Ae. aegypti* was first detected in Madera, CA. during the 2013 summer season, the Madera County Mosquito and Vector Control District (MCMVCD) set out to identify cryptic mosquito sources, particularly those caused by Pacific Gas and Electric (PG&E) utilities. Public utility vaults were identified as potential sources for mosquitoes in Madera County as early as 1972, but due to the difficulty of accessing the electrical transformer vaults, evaluating and quantifying the productivity of these mosquito sources has proven to be difficult. Since then, new studies have been carried out. Specifically, in 2022, the MCMVCD aimed to determine if utility vaults were still nuisance source by assessing a) the number of utility vaults holding water, b) how many showed mosquito larvae and emergence activity and c) whether exclusionary methods could be practical approach to limit mosquito access to these sources. Overall, this study illustrates the capacity for utility vaults to act as an important source for mosquito activity while exploring the effects of exclusionary methods in these potential mosquito sources

Methods

We initially carried out two preliminary surveys in April and November of 2022 to identify all known public utility vaults in the cities of Madera and Chowchilla, as well as to recording water levels, vault styles and mosquito status. For this project, 20 vaults located in the same neighborhood were chosen to determine if mosquito exclusionary methods could limit mosquito access. Ten vaults were modified to block mosquitoes from entering the vaults through the vents of the enclosures, while the other ten vaults were left unmodified. The exclusionary modification consisted in retrofitting the vault vents with sections of pond filter (Figure 1a). Temperature data loggers were placed into each modified vault to determine if the exclusion modification would overheat the vaults, resulting in dangerous conditions for PG&E equipment. Additionally, emergence traps made of ½ inch PVC pipes, weed barrier cloth and adhesive panels (Figure 1b) were placed over both sets of vaults (modified and unmodified), and monitored weekly for four weeks.

Data analysis

All statistical analysis used R v4.2.1 (R Core Team 2022). To analyze the effect of the exclusionary modification in weekly *Ae. aegypti* abundance per vault, we fitted a generalized linear mixed effect model (GLMM) using the lme4 package to examine the relationship between mosquito abundance, vault modification and water level (Bates 2015). We fitted several models that included or excluded different parameters and interactions and selected the model that had the lowest AIC value. To compare the average temperature between modified and unmodified vaults we used an unpaired two sample t test.

Results and Discussion

Overall, we found a significant effect of vault modification (Estimate = 2.07, SE = 1.04, z = 1.97, p = 0.047) as well as the interaction between vault modification and water level (Estimate = −0.024, SE = 0.006, z = −3.70, $p < 0.001$) on mosquito abundance. In general, unmodified vaults were more likely to have mosquitoes than modified ones (Figure 2a). Lower water levels correlated to lower mosquito abundance, but only after adjusting for vault modification, water levels alone did not predict mosquito presence or absence (Figure 2b). The most adults trapped in one week were 628 *Ae. aegypti* in an unmodified vault. Finally, there was no significant effect of modification on internal vault temperature ($t_{(18)} = -1.88$, p = 0.07, modified temperature mean = 90.9 F ± 2 F, unmodified temperature mean = 96.0 F ± 1.7 F).

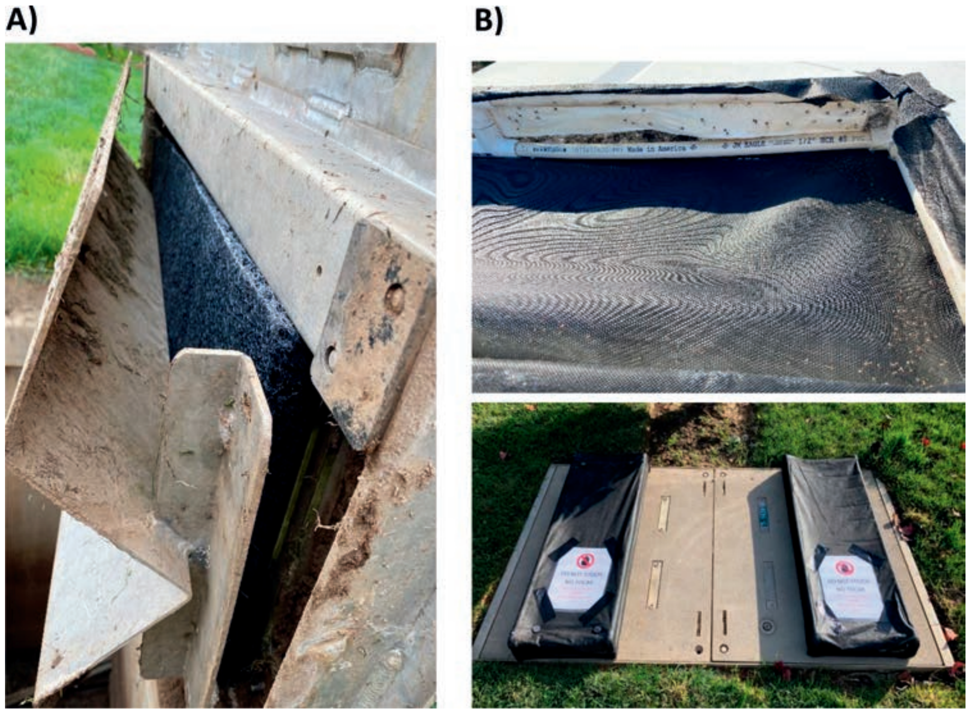

Figure 1.—Mosquito exclusionary modifications and emergence traps. A) Mosquito exclusionary modifications consisted of retrofitting the public utility vaults vents with pond filter and B) Emergence traps made of ½ inch PVC pipes, weed barrier cloth and adhesive panels place over the vault vents.

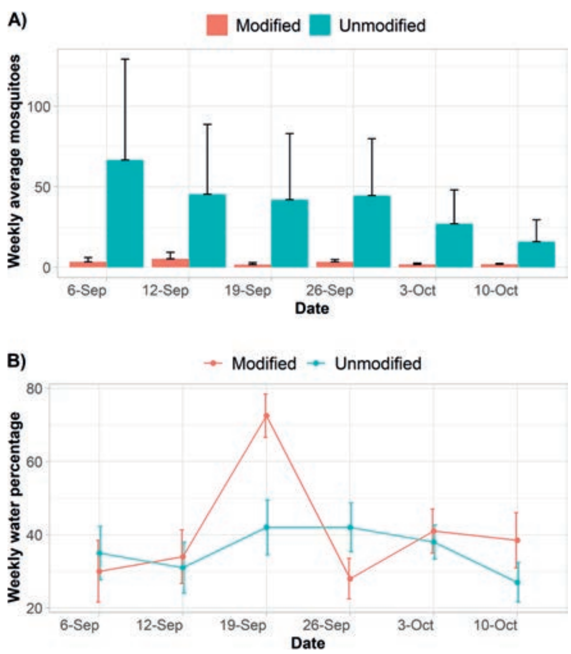

Figure 2.—Weekly mosquito abundance and water level across modified and unmodified vaults from September 6 to October 10, 2022. A) Weekly mean number of mosquitoes collected per week in modified and unmodified vaults and B) Average water level per date between modified and unmodified vaults. Black and color bars represent the SEM.

Conclusions

Public utility vaults served as important sources for the yellow fever mosquito*, Ae. aegypti*, in Madera County, California. Our findings showed that retrofitting the utility vault vents with pond filter material was an effective exclusionary method for limiting mosquito access to these sources without causing dangerous overheating conditions. The significant reduction in mosquito abundance in the modified vaults, compared to unmodified ones, highlighted the potential usefulness of this approach. Additionally, our study underscored the importance of continued efforts to identify and monitor potential mosquito sources, particularly those associated with human-made water sources. The MCMVCD is currently working with PG&E to expand the exclusion modifications to more utility vaults around the Madera County. Finally, the results of our study may inform future efforts to control mosquito populations and limit the spread of arboviruses of public health concern.

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What leads to pyrethroid resistance in *Culex tarsalis*?

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Abstract

Culex tarsalis is one of the most abundant vectors of encephalitis viruses in California. Pyrethroid insecticides are crucial in reducing populations and therefore the transmission of vector-borne pathogens. Over the last several years, there have been increased reports of insecticide resistance in *Cx. tarsalis*. Several graduate students in the Thiemann Laboratory have worked to characterize the mechanisms of this resistance at several sites in Northern California. Data analysis is ongoing, but it appears that both a target-site genetic mutation and increased enzyme levels are contributing to *Cx. tarsalis* pyrethroid resistance. Here, we examined which resistance mechanisms seems to be most important.

Knockdown resistance mutations in California: monitoring insecticide resistance in *Culex tarsalis*

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Introduction

Culex tarsalis is the primary vector of West Nile, Western equine encephalitis, and St. Louis encephalitis viruses in the western United States (Goddard et al., 2002). During the summer months, female *Cx. tarsalis* exhibit opportunistic feeding behavior and are potential candidates for spreading vector-borne diseases in agricultural and residential areas (Reisen and Reeves, 1990). Historically insecticides such as pyrethroids have effectively controlled *Cx. tarsalis* populations across California (Elliot et al., 1978). Pyrethroids target and prevent the closure of voltage-gated sodium channels (VGSC) within the peripheral and central nervous systems, inducing the depletion of energy stores within individuals.

A singular polymorphic mutation, knockdown resistance (*kdr*), was first identified at codon 1014 of the VGSC site in *Anopheles gambiaee*. *Culex tarsalis* exhibit the same mutation, with the wildtype (leucine) replaced by either phenylalanine (F) or, less commonly, serine (S), rendering pyrethroids less or ineffective at controlling populations (Plapp et al., 1968). Factors such as repeated insecticide usage, climate change, and even the expansion of human settlements may all contribute to resistance (Georghiou, 1990). Increasing concerns about *kdr* prevalence in local populations make surveillance efforts crucial.

Materials and Methods

Culex tarsalis females were collected by Kern MVCD (Mosquito and Vector Control District), Sacramento-Yolo MVCD, Sutter-Yuba MVCD, Lake County VCD, and Coachella Valley MVCD. We separated mosquito samples into head/thorax and abdomen, with the head/thorax used for DNA extraction with the GeneJET DNA genomic purification kit. Following extraction, SYBR Green qPCR fluorescence assays were implemented to identify the *kdr* L1014 site genotype. For each sample, qPCR derivative results (melt temperatures) were compared to designed primer melt temperatures to determine the *kdr* genotype of each individual. Samples that exhibited inconclusive qPCR results underwent Sanger Sequencing at Quintara Biosciences in Hayward, CA. We recorded allelic and genotypic frequencies from each site and time point.

Results and Discussion

Wildtype alleles have decreased over time, with samples from 2007-2009 having an allele frequency >75% of wildtype (leucine) compared to recent samples from 2021-2022 that contained <15% wild-type alleles. Recent samples from Kern and Sutter-Yuba contained no wildtype alleles. With the decrease in wildtype alleles, phenylalanine and serine mutants have increased over time. In the 2007-2009 samples, <25% of alleles included phenylalanine mutants, whereas recent samples had >80% were phenylalanine. Of 2021-2022 samples, about 5% of observed alleles were serine. Phenylalanine mutants were prominent first in Southern California, but recent data displayed an even distribution of mutant alleles across the state.

Our data indicates that the *kdr* mutation, L1014F, is not only present in California *Culex tarsalis* populations but predominated in our study areas. There was an exception to this trend at our Coachella Valley site. Although the limitation of one site per county may have affected that region's data, our data showed that recent *Cx. tarsalis* populations have not increased the prevalence of the L1014F mutation.

Our next step is correlating changes in allelic frequencies over time with the data we obtained from the California Department of Pesticide Regulation. Across the state, pyrethroids have been continuously applied by several constituents, perhaps placing continuous selective pressure on mosquito populations. We plan on testing linear regressions between the amounts of pesticide sprayed in these regions against the changes in allele frequencies over time, using data from two previous studies from the Thiemann laboratory to fill in data from 2016-2018, as well as further historic (1990) samples from Coachella Valley. With this information, we will better understand the selective pressure that *Cx. tarsalis* populations are under. When we know how selection pressures affect population evolution, we can make more informed decisions on the selection of adulticides to control these populations of concern.

Conclusion

Knockdown resistance mutations have become the predominant genotypes in California populations of *Cx. tarsalis*. The wildtype, leucine, has decreased at all sampled sites while one mutation, phenylalanine, has increased at all locations. The prevalence of these mutations urges the need for the development of novel forms of vector control and the implementation of Integrated Vector Management.

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Assessing permethrin resistance for *Culex tarsalis* in Southern California

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Abstract

In the western United States, *Culex tarsalis* is the most important vector of West Nile virus. Due to the wide range of environments where *Cx. tarsalis* are found, this species is under insecticide pressure from both agricultural spraying and vector control. Resistant mosquitoes may display resistance mechanisms such as target site insensitivity. The most common target site insensitivity in *Cx. tarsalis* is a knockdown resistance (*kdr*) mutation. Here, *Cx. tarsalis* were collected from five southern California locations with varying environmental and habitat characteristics to determine prevalence of permethrin resistance. Permethrin resistance was evaluated through CDC bottle bioassays to assess a resistance phenotype and by qPCR and sequencing to detect the presence of a *kdr* mutation. Mosquitoes were placed within glass bottles coated with 43 *u*g of permethrin with a diagnostic survival time of 20 minutes indicating resistance. Phenotypic permethrin resistance was evident in *Cx. tarsalis* populations collected from the southern California desert communities (Coachella Valley), whereas *Cx. tarsalis* from the southern California inland valley region (western Riverside County) were all susceptible. Individual *Cx. tarsalis* from the Coachella Valley were found to have homozygous or heterozygous *kdr* mutation genotypes. In contrast, individual *Cx. tarsalis* collected from western Riverside County were found to have a mix of homozygous or heterozygous wild type alleles.

Low dose effects of larval bifenthrin exposure on *Culex quinquefasciatus* permethrin resistance

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Introduction

The increased prevalence of pyrethroid resistance in mosquitoes warrants investigation into potential contributing factors (Yoshimizu et al. 2020). Due to the tendency for surface water runoff to transport pesticides and other contaminants to downstream waters, it is hypothesized that residues of pyrethroids may be selecting for resistance in larval populations developing in urban storm drains (Weston et al. 2009). The primary objective of the current study was to evaluate the effects of bifenthrin, a pyrethroid often detected in urban runoff in California, on resistance metrics of *Culex quinquefasciatus*, a species commonly found in California storm drains.

Methods

Two field collected *Cx. quinquefasciatus* strains (LA from Los Angeles and CV from Coachella Valley) from southern California and a susceptible laboratory strain (CQ1) were split into control and treatment lineages; bifenthrin was spiked into treatment larval pans at an environmentally relevant concentration (580 ng/L). Adults from each population were used in bioassays to determine permethrin susceptibility, *kdr* genotype, and enzyme activity (Chen et al. 2010, Burgess et al. 2022, "CONUS Manual for Evaluating Insecticide Resistance in Mosquitoes Using the CDC Bottle Bioassay Kit" 2022). Populations were reared for 3 consecutive generations, and comparisons were made between populations to determine the effect of strain, treatment, and generation number.

Results and Discussion

The KC_{50} (1-hour knockdown) and LC_{50} (24-hour mortality) of each strain were determined prior to treatment. KC_{50} (μg/bottle) values for CQ1, LA, and CV were 0.97, 2.98, and 34.42, respectively. LC_{50} (μg/bottle) values for CQ1, LA, and CV were 1.00, 3.31, and 39.58, respectively. At the time of publication, sample processing and data analysis was still ongoing.

Conclusions

The findings from this work will provide insight into whether residual pyrethroids, such as bifenthrin, in storm drains contribute to the emergence or maintenance of pyrethroid resistance in urban mosquitoes. This project also will assess how specific metrics, such as *kdr* allele frequency and enzyme-mediated resistance, may be affected over time because of exposure.

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A milligram of sugar alcohol helps the toxicant go down

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Abstract

Effective control of mosquitoes involves a multicomponent approach known as integrated mosquito management (IMM). In most places a large component of the control of mosquitoes in an IMM framework involves the use of insecticides, which is the most controversial aspect of IMM. One approach to defeat this obstacle is to use a targeted application of insecticides that is perceived to be less damaging to the environment. One emerging control strategy for mosquitoes that fits this profile is the us use of attractive toxic sugar baits (ATSB). The use of toxic baits has been in use for many decades in pest control and recently has been examined and evaluated in a number of environments for mosquitoes. Sugar alcohols are naturally occurring molecules, many of which are safe for human consumption and are found in foods that are regularly consumed. We screened a number of sugar alcohols and found one of them, erythritol, to be toxic to adult mosquitoes in an ATSB formulation. Later work was done to look at different concentrations to sugar and erythritol to see if low amounts of erythitol could be effective. Additional toxicants including Spinosad, boric acid and Bti were included in erythritol ATSB formulations and showed additive toxicity in some combinations.

What adulticides should the District use? An analytical method for adulticide resistance bottle bioassays to direct field product rotation

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Abstract

The Coachella Valley Mosquito and Vector Control District (District) conducts adulticide bottle bioassays annually by comparing local wild mosquito populations with their respective susceptible strains. The bottle bioassay results from previous years demonstrated that *Culex quinquefasciatus* were resistant or possibly resistant to all adulticide products tested. This makes it challenging to determine which products would be appropriate for District use. Semi-field assays demonstrated products were effective despite the bottle bioassay evidence of resistance. Through these assays, the District could confidently keep select active ingredients in rotation. Unfortunately, there are limitations on the number of assays that can be accomplished. The bottle bioassay results are sometimes the only information for deciding what adulticides can be used for the integrated vector management program. Fortunately, bottle bioassay data can be additionally analyzed using a probit method that estimates lethal times from successive observations at a single concentration. Our paper will discuss the results of using the software, the limitations of the program, and the results from the analysis.

Introduction

The Coachella Valley Mosquito and Vector Control District monitors adulticide resistance annually by bottle bioassays and semi-field assays. Bottle bioassays are conducted by comparing local, field-collected mosquito populations with their respective susceptible strains (CDC). The accepted practice for analyzing these results is to record the percentage knockdown of the field strain at the diagnostic time. Although this is an easy strategy, often the results have demonstrated that populations of *Culex quinquefasciatus* were resistant or possibly resistant to all adulticide products tested (Hung et al. 2021a, Hung et al. 2022). Furthermore, this evidence of adulticide resistance in bottle bioassays may not translate to a product being ineffective in a field application. With that, it was a challenge to determine which products would be appropriate for District use if relying on only these bottle bioassay results. Checking these results with semi-field applications ensures that the products are effective in the field despite evidence of resistance. Unfortunately, the limited resources and ability to collect enough field mosquitoes in a timely manner are constraints on the number of semi-field assays that can be accomplished. As such, the bottle bioassay results are often the main evidence on hand for deciding what adulticides can be used for the District's integrated vector management program.

Throne et al. (1995) describes a probit analysis for time-mortality data from successive observations at a single concentration, and this analysis may serve as an alternative method to analyzing single dose-time bottle bioassay results. If the bottle bioassays were conducted with the same product at multiple doses, an alternative probit analysis would be appropriate (Robertson et al. 2007). The objective of our paper is to compare the Throne probit analysis with the traditional CDC analysis and determine whether it may predict field efficacy.

Methods

Mosquito collections

Mosquito collections were completed as described in Hung et al. (2022). Briefly, *Cx. quinquefasciatus* were collected by placing gravid water bait outside overnight and collecting eggs the next morning. Larvae were fed daily and kept in rearing chambers at 50% relative humidity, 28°C, and a 16:8 L:D h cycle. During the pupal stage, first pupae were removed to increase the female:male ratio, and subsequent pupae were placed in a cage for emergence. Adults were 3-5 days old and fed 10% sugar water daily. Mosquitoes were collected from Cathedral City, Indio, La Quinta, and Palm Springs, California. The susceptible CQ1 strain (originally collected as adults from Merced, CA in the 1950s) was used as a susceptible control and was kept in colony under similar rearing conditions.

Table 1.—Ranking system for assay results.

	Bottle bioassay traditional analysis	Bottle bioassay probit analysis	Semi-field results
Good	>98% mortality, no resistance	RR < 5 susceptible	More than 80% mortality at 48 hr
OK	Between 80-98% mortality, possible resistance	5 < RR < 10 moderately resistant	Between 50-80% mortality at 48 hr
Bad	<80% resistance evident	RR > 10 highly resistant	Less than 50% mortality at 48 hr

Bottle bioassays

The bottle bioassays were conducted according to CDC manual (CDC) except adulticide product formulations were used rather than technical grade material. Field strains from Palm Springs and Cathedral City were tested against Aqua-Reslin (permethrin, PBO), DeltaGard (deltamethrin), and Fyfanon ULV (malathion). An average of 28 mosquitoes ranging from 18 to 48 mixed sex adults (ca. 63% females) were aspirated into each bottle. Four replicate bottles were used for each population for each adulticide tested. Mosquito mortality was assessed at 15 min after initial exposure and every 15 min thereafter until all the mosquitoes in a bottle were dead or the 2 h count was completed. Mosquitoes were observed as knocked down or moribund if they could no longer have controlled flight or stand. Results of mosquito mortality were analyzed using CDC guidelines for mosquito mortality for bottle bioassays. Mortality of the field strain was noted at the time when the susceptible strain reached 100% mortality (diagnostic time). If the percent knockdown of the field strain was less than 98% at the diagnostic time, the population was considered showing signs of possible resistance. Resistance was evident when fewer than 80% of the mosquitoes died at the diagnostic time (Table 1).

Probit analysis

A probit analysis was used to analyze the bottle bioassay time data from above (Throne et al. 1995). This required the use of Wolfram Mathematica software (https://www.wolfram.com, Champaign, IL) and a program package available from the author (https://www.ars.usda.gov/plains-area/mhk/cgahr/spieru/docs/probit-download-throne-draft/). The number of mosquitoes knocked down at each time interval out of the total from the bottle bioassay was used as the input values. The program output included the estimated time when 50 to 90% of mosquitoes killed, known as the lethal time (LT). The estimated LT50 and LT90 of the wild strains and the susceptible strain were used to calculate the resistance ratio (RR) as defined by the WHO guidelines (2006). WHO guidelines for resistance ratios were used to interpret the results (WHO 2006) where $RR < 5$ was susceptible, $RR = 5\text{-}10$ was moderately resistant, and $RR > 10$ was highly resistant (Table 1).

Semi-field assays

Field and susceptible mosquitoes for semi-field assays were collected as described above and the cages were prepared as described previously (Hung et al. 2021b). The mosquitoes from Palm Springs and Cathedral City were used together and treated with Aqua Reslin. The field strain from Indio and La Quinta were treated in separate cages with DeltaGard. An average of 24 mosquitoes ranging from 19 to 29 mixed sex adults (ca. 99% females) were aspirated into cylindrical cages with mesh screening on both sides.

For the study, 4 cages each of field strain and susceptible mosquitoes were placed on a stand 5 ft high and 25 ft downwind from the spray origin. Each product was applied at the maximum label rate using a Colt-4 handheld sprayer

Table 2.—Bottle bioassay results with percent mosquito knockdown at 15-minute intervals. Included in the colored cells are the rankings from Table 1.

Time (min):	15	30	45	60	75	90	105	120
Aqua-Reslin								
Susceptible	95	100	100	100	100	100	100	100
Cathedral City	0	5.3	14.2	44.7	66.8	90.2	92.6	93.8
Palm Springs	3.25	6.3	19.4	53.6	76.5	84.2	89.8	89.3
DeltaGard								
Susceptible	55.0	98.8	100	100	100	100	100	100
Cathedral City	13.4	45.7	55.5	86.0	88.2	94.8	91.9	92.0
Palm Springs	33.7	48.0	87.9	95.7	97.0	98.3	97.5	98.8
Fyfanon ULV								
Susceptible	1.0	52.0	100	100	100	100	100	100
Cathedral City	0	1.2	38.9	91.2	97.7	100	100	100
Palm Springs	0.9	30.1	73.2	90.1	98.4	98.4	99.1	97.6

Table 3.—Probit analysis results with LT50 and LT90 for each strain. Confidence intervals were included if available. The RR50 and RR90 include the color ranking system from Table 1.

Palm Springs	LT50	CI_{lower}	CI_{upper}	LT90	CI_{lower}	CI_{upper}	RR50	RR90
Aqua-Reslin	64.8	0	218.9	139.4	80.5	1230.4	7.0	10.1
DeltaGard	16.7	Undef	Undef	79.9	Undef	Undef	1.2	3.5
Fyfanon	38.1	8.5	64.2	65.0	45.5	141.2	1.3	1.7
Cathedral City								
Aqua-Reslin	65.0	45.4	84.5	106.4	86.5	144.1	7.1	7.7
DeltaGard	37.0	Undef	Undef	112.7	Undef	Undef	2.6	4.9
Fyfanon	47.5	41.1	53.9	61.7	55.1	73.8	1.6	1.6
Susceptible Colony								
Aqua-Reslin	9.2	Undef	Undef	13.7	Undef	Undef		
DeltaGard	14.1	Undef	Undef	23.0	Undef	Undef		
Fyfanon	30.2	Undef	Undef	38.7	Undef	Undef		

(London Foggers, Long Lake, MN). Temperature, wind speed, and humidity at the time of applications were recorded to ensure applications were conducted within appropriate conditions. We conducted 2 passes per product with new cages placed per pass. For Aqua-Reslin, one pass was excluded because the susceptible strain did not reach 100% mortality, suggesting the mosquitoes were not fully treated. Ten minutes after each pass, the cages were removed from the stand, counted, and stored in a cooler at room temperature. Cotton balls with 10% sugar water were placed on each cage and a damp towel was used to maintain humidity. The cotton balls were replenished daily by soaking in 10% sugar water. Mosquito mortality was assessed immediately before treatment and at 10 min, 6 hr, 24 hr and 48 hr after treatment. Mosquitoes were observed to be knocked down or "dead" if they could no longer have controlled flight or stand. Untreated control mosquitoes had 0-4.3% mortality at the end of the evaluation period and are not included in this report. Untreated control cages were placed at least 50 ft upwind of the spray origin to avoid treatment.

Results were interpreted using a ranking system (Table 1).

Results and Discussion

Bottle bioassay results with traditional analysis

Susceptible colony, Cathedral City, and Palm Springs were the mosquito populations examined against Aqua-Reslin, DeltaGard, and Fyfanon ULV. Results at diagnostic times are underlined with the color ranking system included from Table 1. The population from Cathedral City was considered to be resistant to Aqua-Reslin, DeltaGard, and Fyfanon ULV (Table 2). The population from Palm Springs was resistant to Aqua-Reslin and Fyfanon ULV and was possibly resistant to DeltaGard.

Table 4.—Semi-field assay percent knockdown of susceptible and field (Palm Springs and Cathedral City) mosquitoes exposed to Aqua-Reslin treatments.

Aqua-Reslin	Susceptible	Palm Springs and Cathedral City
0 min	0	1.0
10 min	22.0	4.9
8 hr	100	73.1
24 hr	100	65.2
48 hr	100	45.3

Bottle bioassay results with probit analysis

With the probit method, the populations from Palm Springs and Cathedral City were considered to be susceptible to DeltaGard, and Fyfanon. They were moderately or highly resistant to Aqua-Reslin.

Semi-field assay results

Semi-field assays demonstrated that DeltaGard was effective against the field populations despite the evidence of resistance from the CDC bottle bioassay. For Palm Springs and Cathedral City populations, at 8 hr after exposure, the population reached 73% knockdown, which would have been interpreted as yellow or "OK" at that time. Then the mosquitoes recovered, resulting in less than 50% of the mosquitoes knocked down at 48 hr.

The semi-field outcomes better reflected the results from the Throne probit analysis than the traditional bottle bioassay analysis. Although the traditional bottle bioassay analysis indicated that there was resistance present, the probit analysis more accurately designated which products may be acceptable to use in the field. This isn't to say that the traditional analysis should be disregarded. Both analysis methods were valid with an understanding that each has their limitations. Because the probit analysis showed that the field populations exposed to Fyfanon ULV were susceptible, Fyfanon ULV was expected to have a high knockdown rate against field populations. The District plans to verify this assumption with a field application in the future.

The Throne probit analysis was used by Sharabyani et al. (2020) to examine permethrin resistance from different habitat types. Su et al. (2019) used this probit analysis for mosquitoes from Utah to examine permethrin resistance. Although both papers used this method to demonstrate evidence of resistance, neither field or semi-field comparisons were conducted to provide an operational perspective.

There are limitations to using the Throne analysis for bottle bioassays. Although not reported here, the output

Table 5.—Semi-field assay percent knockdown of susceptible and field (Indio and La Quinta) mosquitoes exposed to DeltaGard treatments.

DeltaGard	Susceptible	Indio	La Quinta
0 hr	0.5	0.5	1.0
15 min	86.6	44.5	18.4
8 hr	100	90.4	81.9
24 hr	100	99.0	88.5
48 hr	100	100	91.4

could be undefined or unusual if the mosquitoes had a knockdown rate that was too fast or too slow. The outputs also would not make sense if there was recovery in the bottles, resulting in the estimated mortality declining rather than increasing over time. Some of the results reported here did not provide a confidence interval for the lethal times. Although the results of the probit analysis may not meet the statistical rigors for a scientific manuscript, the analysis may be useful for the District to better make use of the adulticide products available.

Conclusion

The probit method is potentially useful for providing information that the traditional CDC analysis cannot. More bottle bioassay data paired with semi-field assays are needed to determine how well these results agree. The District plans to continue using this analysis method alongside the semi-field assay when possible.

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Evaluation of a novel long-term treatment station in sensitive species tidal zones

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Introduction

Aedes taeniorhynchus or the "black salt marsh mosquito" is found along coastal salt marshes and lagoons in San Diego County. They typically occur between April – October, when tidal flood waters cause eggs previously laid on moist soil or vegetation to hatch. Development from hatch to emeregence may take as little as 5 days. Mostly considered as a pest species, *Ae. taeniorhynchus* has a long flight range and is an aggressive daytime biter causing discomfort to people living in San Diego's coastal communities. The larval habitat of *Ae. taeniorhynchus* often coincides with sensitive species such as Belding's savannah sparrow, Ridgway's rail and Western snowy plover. These species are ground nesting and are often concealed within coastal marsh vegetation. Due to the nesting habits of these sensitive species, it is discouraged and often prohibited for vector control staff to do "on foot" assessments of coastal areas without the supervision of a registered avian biologist. With access restrictions and quick maturation, the ability to control *Ae. taeniorhynchus* has become problematic. There is need for a product that would provide long term larval control so that each tidal influx does not require treatment. The current project evaluated two long term residual treatment products, the FourStar Briquet BTI 150 Day and Natular XRT, dispersed using an inexpensive "treatment station" that required minimal servicing.

Methods

A site was selected in the San Dieguito lagoon at a known *Ae. taeniorhynchus* larval habitat that consistently produces large numbers of *Aedes* mosquitoes after tidal events greater than 6.0 feet. At the site, 7 treatment stations were installed, each 10 feet apart and placed in the center of a 10 sq ft grid. Each treatment station was comprised of a 30-inch earth anchor (tent stake) with spinnable corkscrew base, a 2″ × 1″ piece of FOAMULAR® insulation, a universal aluminum leaf strainer, treatment product, station ID card, larvicide ID tag, and zip ties. During the 2020 season, BTI 150 day FourStar Briquets were used in all 7 stations. During 2021, Natular XRT was used in all the stations. The tent stakes were placed in April and twisted into the ground until only 18″ remained above ground to reduce the likelihood of predatory birds would use the stations as a perch. The treatment product was placed inside the aluminum leaf strainer and FOAMULAR® insulation piece. The FOAMULAR® insulation piece was provided buoyancy to the strainer so the product would stay in contact with the water throughout tidal cycles, and thereby resisted becoming coated with mud as it would if it was in a fixed position at the base of the stake. The strainer was pinched shut at the open end and secured closed with a zip tie. The strainer then was attached to the stake with a zip tie, pinched side up to avoid the strainer getting buried in the mud. The zip tie left approx. an inch space allowing for the strainer to move up and down in the water column. Lastly the station ID card and larvicide ID tag were attached to the top of the stake. At each tidal cycle, staff visited the site pre, peak and post tide. Staff would visit pre tide to ensure no larvae were present from the previous tidal cycle. Each visit at peak and post tide, staff would do larval counts via traditional dipping 2-3 feet out from each station (4 dips in a square pattern) and additionally 5 feet out from each station (4 dips in a square pattern). This was to verify product was sufficiently moving through the 10′ × 10′ grid. Additionally, two untreated control sites were selected and sampled for larvae at similar intervals. One untreated control site was adjacent to the study and shared the same environmental conditions. The second site was 220 yards away but had 50% less vegetation. After the first season using FourStar, the stations were collected in October and redeployed in April using the same methods, except that Natular XRT replaced the FourStar product in the stations.

Results and Discussion

The first summer using the FourStar Briquet BTI 150 Day, the average larval count across all stations and distances was 14 larvae per dip in the treatment areas and 8 larvae per dip in the control areas. All treatment stations withstood tidal flows, salt water, and other environmental conditions and could be used the following year. In year two, Natular XRT was evaluated using the same methods and using the same station materials from the previous season. The average larval count across all stations and distances for Natular was less than 1 larvae per dip in the treatment and untreated control areas. Once again the stations withstood tidal flows, salt water, and environmental conditions and will be deployed for a third season.

Conclusion

Moving forward, additional study sites with varying vegetation, tidal pressures and environmental conditions will be selected. Natular XRT seemed to be best at controlling mosquitoes in the one site for one season; however, additional trials with Natular as well as other products will be conducted. Modifications will also be made to improve the durability of the treatment stations such as metal clips instead of zip ties and possible 3D printed product cages to optimize water flow around the product.

Acknowledgements

Chris Conlan, Garret Tice, Steve Rivera, Ryan Salamat and Sophia Bejarano were instrumental in collection of data, and Nikos Gurfield and Chris Conlan reviewed on the manuscript. Big thank you County of San Diego Vector Control Program and California Department of Fish and Wildlife for the support in this project.

Evaluating the effectiveness of adulticiding to reduce West Nile virus transmission risk in California, 2009-2018

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Abstract

West Nile virus (WNV), a flavivirus transmitted by mosquitoes in the genus *Culex*, causes human disease that can range from febrile to neuroinvasive and can even cause death. There is no vaccine or target treatment for WNV disease; the only effective tools are prevention by public education and vector control, including, but not limited to, chemical control via the application of pesticides. Although larvicide use by California mosquito and vector control agencies is relatively ubiquitous, not all agencies use adulticides, particularly distributed on a large scale, such as by truck-mounted or aerial application. In this study, we quantified how the use of adulticides might reduce human WNV disease by comparing WNV incidence in ZIP codes and time periods that used adulticides to those that did not. Records of WNV environmental surveillance, pesticide applications, and human disease cases were analyzed for six high burden counties (Butte, Kern, Los Angeles, Orange, Sacramento, and Yolo) for 2009-2018. The use of adulticides within a ZIP code was associated with a decrease in WNV disease burden. Treating large areas and/or using more product further decreased human disease. Large-scale application of adulticides should be considered when indicated in integrated vector control programs for the control of WNV.

Rat Race: Changes in Norway rat distribution in Alameda County, California (2018-2022)

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Introduction

Research on the ecology and behavior of urban wildlife is severely lacking, even though information on this topic could inform IPM strategies. For example, Alameda County Vector Control Services District (District) performs regular rodenticide baiting of sanitary sewers in the city of Oakland to control Norway rat populations. During the five-year period between 2013-2017, the number of sewers inspected and baited remained consistent (Why et al. 2018). Knowledge of Norway rat population dynamics over time would help focus these efforts and lead to a decrease in the overall amount of rodenticides used by our District. The aim of the current study was to analyze the population movements of Norway rats in Alameda County over the years 2018-2022.

Methods

The location data extracted from Requests for Service (RFS) from the District's database was used to map

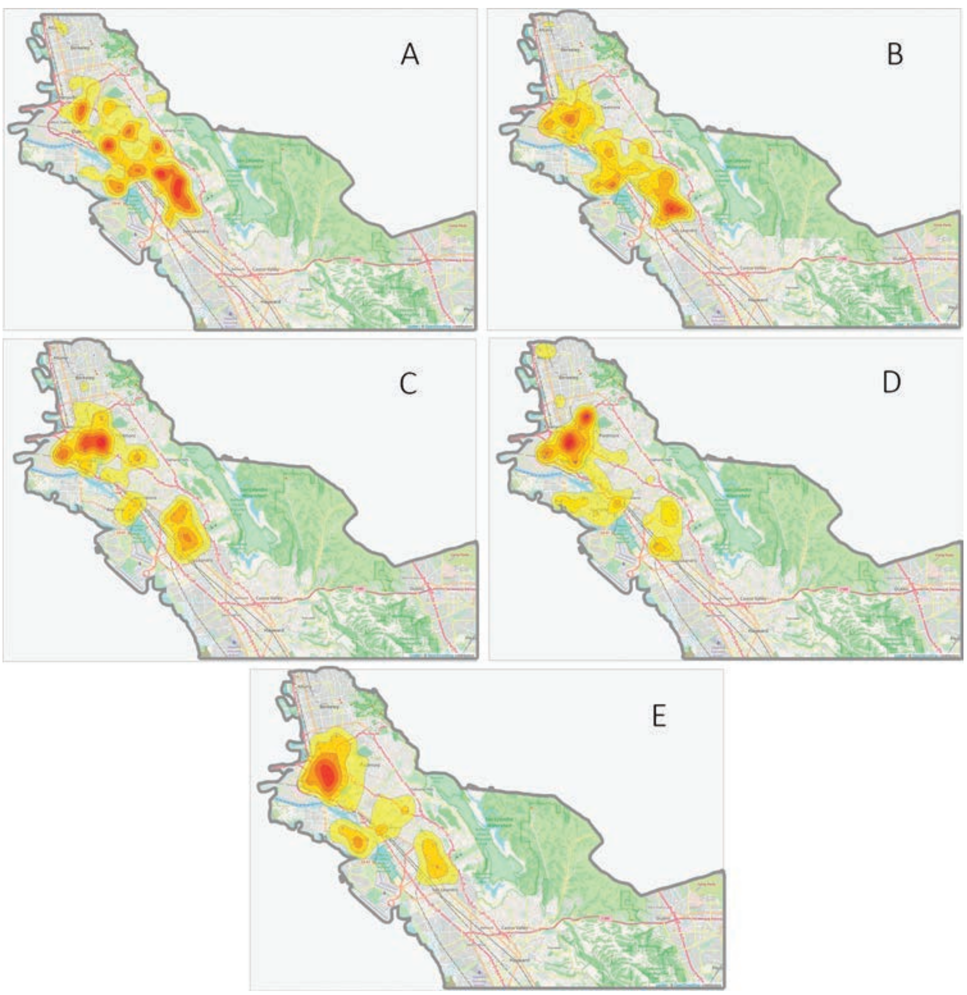

Figure 1.—Heatmaps of Norway rat RFS in Alameda County, CA. (A) 2018, (B) 2019, (C) 2020, (D) 2021, (E) 2022. Heatmaps have been focussed to only show the relevant parts of the county.

individual calls for Norway rats throughout Alameda County using geographic information system (GIS) tools. Longitude and latitude data from each RFS was collected and analyzed using Heatmapper, a free-to-use online heatmapping tool developed by the Wishart Research Group at the University of Alberta (Babcki et al, 2016). Individual heat maps were created for Norway rat RFS's for each year from 2018 to 2022.

Results and Discussion

The heatmaps of 2018 and 2019 showed similar hot spots of Norway rat activity (Fig. 1A, 1B). However, during the years of 2020 and 2021, the hotspots notably shifted, moving northward and westward (Fig. 1C, 1D). During these years, previous hot spots in East Oakland virtually disappeared. These years corresponded with the most strict COVID-19 lockdown protocols in Alameda County. In the year 2022, when most pandemic response measures had been lifted, the hotspot of Norway rat activity in East Oakland returned (Fig. 1E). This change in Norway rat distribution has been noted in other studies, including a 2021 study by Parsons et al. (2021) in New York City, where certain areas that had hotspots of Norway rat activity did not express the same special relationship during the month of April 2020, the first full month of COVID lockdown restrictions.

Conclusions

As commensal synanthropes, Norway rats change their behavior, in part, based on changes in human activity. This makes a case for increased monitoring of Norway rat populations and focusing IPM efforts in areas where human activity is the most attractive to Norway rats. Future studies to identify what factors are influencing the differences between 'hot' and 'cold' spots of Norway rat activity could assist in focusing IPM practices.

Acknowledgements

I would like to acknowledge all my coworkers at Alameda County Vector Control Services District for generating the data used in this study. I would also like to particularly thank Stephanie Kurniawan for helping me extract the data from our online database and Dr. Adena Why for all her help in reviewing my research and presentation.

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Paw-sible knowledge: Differentiating common vertebrate vector tracks

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Introduction

In Alameda County, the Alameda County Vector Control Services District assists residents with their requests for service regarding a variety of vertebrate species that vector disease or are hosts to invertebrate species that vector diseases. Some of the most common urban wildlife species encountered include raccoons, skunks, and opossums. They are often found on residents' properties, for example, in crawl spaces, around sheds or under decks. While investigating these requests for service, animal tracks are found that can provide evidence of the species present. This is important because the species of wildlife must be identified so that methods can be targeted to properly evict or control the animal present. The residents may have seen the wildlife present on the property, and their observations may be helpful with identification, but there are many scenarios when they are unsure of the species of wildlife present. While the application of motion-activated trail cameras will provide the proof needed, it will require multiple nights of monitoring. By learning how to analyze local wildlife tracks, one could identify the species and begin the eviction process within a matter of minutes rather than days.

Methods

To differentiate among the tracks of our urban wildlife species, foot anatomy and track morphology are examined as well as the possible effects of substrate and environmental conditions on how a track looks. Information regarding our most common urban wildlife species will be collected. This will include the shape, size, and general morphology of the typical tracks left behind, which can then be compared and contrasted to differentiate among species in the field.

Results and Discussion

Tracks will be more difficult to identify in certain types of substrates, including hard or dry environments, or in extremely loose substrates such as sand. In deep substrates such as wet mud, the toes may splay more to create more stability for the animal, however, in drier mud, the tracks will be cleaner and easily recognizable (Elbroch et al. 2012). Environmental conditions such as wind or rain can wash away, distort, or cover tracks and make it more difficult to identify them (Elbroch et al. 2012). Foot anatomy should be analyzed to help with species identification, focusing on the most important features such as claws, toe pads, palm pads, heel pads, and negative space. When discussing the anatomy and morphology of tracks, toes are numbered. Toe 1 is the medial toe, or the one placed closest to the midline of the body, whereas toe 5 is the most lateral toe. The directionality and spacing of the toes within the track, the shape of the palm pads, and how much the nails register is helpful in differentiating the tracks of different species.

Raccoons are very dexterous animals and are able to climb, dig and use their front paws as if they are hands. Raccoon paws have a tapered claw which may or may not register. If they do register, they will be relatively close to the toe pads. They have symmetrical front tracks and asymmetrical hind tracks, although the palm pad registers on both. The heel pad also typically registers, although it will be more defined on the hind track. The anterior portion of the palm pad will appear as a C-shaped edge, and the toes are often connected to the palm pad, leaving no negative space (Elbroch and McFarland 2019). Raccoon tracks can be easily identified by these features, which often cause them to resemble a gloved hand.

Another animal commonly encountered is the striped skunk. Although they have tracks that can be similar to raccoons, there are several key features that can be used to identify them. Skunks are not strong climbers, although they are very successful diggers, which can be seen in their paw morphology. Skunks have asymmetrical front and hind tracks, that largely resemble each other. Toe 1 is the smallest and may not register on the hind track. The heel pad may register on both but is more common on the hind track and is not as defined as a raccoon's heel pad. The claws are long and reliably register on the front foot, and they may be the only part of the track visible in hard substrates. These claws are very large, so they register much higher above the palm pad than the claws of raccoons. The toes do not splay, and between the toes, palm pad, and heel pad, there is a slight crease or line instead of a large negative space (Elbroch and McFarland 2019).

Opossums have the most distinctive prints. They are very able climbers with opposable thumbs on their hind paws that allow them to grasp and hold branches. Their front and hind tracks do not resemble each other at all, which makes identification more obvious. The front track is symmetrical with the 5 toes splaying out like in the shape of a rising sun so that toes 1 and 5 are pointing in opposite directions. Each toe has bulbous metacarpal pads attached with a negative space posterior to it. However, the hind track is very asymmetrical and looks very hand-like. Toe 1 is an opposable thumb and sits opposite from the track. Toes 2-4 sit evenly together, and toe 5 is slightly lower. Toe 1 lacks a claw, the other claws may or may not register in the substrate. The heel pad registers on the hind track with no negative space (Elbroch and McFarland 2019).

Conclusion

It is important to differentiate among the common urban wildlife to use the correct control techniques and maintain proper records. Between a raccoon, skunk and an opossum, the most distinctive features are the shape of the track, the way the toes splay, and the negative spaces. If tracks are the only visible sign in the area, knowing these features will allow the determination of the species present at the location. Once the species has been identified, the proper control methods can be initiated.

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Use of insecticide baits in water meter boxes for control of Turkestan Cockroaches in Alameda County

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Introduction

Turkestan cockroaches, *Blatta (Shelfordella) lateralis* (Walker & F. 1868), also known colloquially as the "red runner" cockroach in the pet food trade, are an increasingly common peridomestic species of cockroach in Alameda County. Like the Oriental cockroach, *Blatta orientalis* (Linnaeus, 1758), the Turkestan cockroach is a peridomestic pest and an accidental indoor invader. In Alameda County, Turkestan cockroaches typically are found in ground level utility boxes, cracks in pavement and soil, and near sources of water and vegetation. They are native to central and southwest Asia and were introduced to California in the 1970s (Kim and Rust 2013). Like most peridomestic cockroach species, they are considered outdoor pests that can sometimes wander indoors in search of water sources. However, they cannot thrive indoors and are often found dead (UC IPM 2023). Turkestan cockroaches have a similar niche and habitat preference as Oriental cockroaches but have a shorter development time and produce more offspring. Kim and Rust (2013) reported that Turkestan cockroaches are outcompeting Oriental cockroaches in many regions of California. In Alameda County, Oriental cockroaches are still the most dominant peridomestic species for which we receive complaint calls from the public. Verified Turkestan cockroach Requests For Service (RFS) comprise less than one-third of the calls received compared to those for Oriental cockroaches (Figure 1).

Alameda County Vector Control Services District (ACVCSD) first reported Turkestan cockroaches in 2013 in South Fremont, and as of 2022, Turkestan cockroaches have been positively identified as far north as the city of Oakland (Figure 2). Reports from the public have both increased in number and range over this time, starting in the south of the county and then spreading northwards (Figure 2). Increases in RFS typically occur after hot temperature events in late spring, when large populations of cockroaches come out at night in search of water sources, often startling residents (Figure 3). Although peridomestic cockroaches are not considered an important vector, they cause distress in many residents, especially when high numbers are seen in close proximity to homes.

As part of ACVCSD's mission, residential water meter boxes are treated with insecticidal gel baits in response to residential complaints. ACVCSD biologists perform an investigation of the complaint, identify the cockroach species present, and provide tailored recommendations. If there is cockroach activity in the water meter box, an insecticide gel bait treatment may be applied (with permission from the local water district). The current study evaluated the effectiveness of one application of gel bait insecticide in residential water meter boxes for control of Turkestan cockroaches.

Methods

Pesticide selection

At ACVCSD, Maxforce FC Select (0.01% Fipronil, Bayer Environmental Science, Raleigh, NC) was historically used. However, the active ingredient fipronil may not be safe for long term use in water meter boxes (Tingle et al. 2003) due to risk of non-target effects and groundwater contamination. For our study, we selected Maxforce Impact (Clothianidin 1.00%) as our gel bait insecticide. It is registered as a reduced risk pesticide by the EPA, which is a status given to pesticides that have a lower impact on human health, non-target organisms, and lower potential for groundwater contamination compared to conventional alternatives (EPA 2022).

Selection of Test Sites

Test locations were selected from ACVCSD's RFS database using the following criteria: (1) Turkestan cockroach activity was confirmed within the last 10 years; (2) No insecticide treatment was made in water meter box by ACVCSD within the past 1-2 years; (3) "Easy to access" water meter boxes were not covered by vegetation/obstructions, etc.; and (4) Sites were within 10 miles from other test locations with similar landscape and land-use (suburban front yard in this study). At each potential test site, we placed a single Victor Roach and Trap Monitor (Model BX M327, Woodstream, Lancaster, PA) inside all accessible water meter boxes at the location. Victor Roach and Trap Monitors are paper and glue insect monitoring boards commonly used for monitoring cockroach populations. We placed a small amount (approximately 3 mm sphere) of Maxforce Impact gel bait as an attractant on each board. Monitoring boards were left in the water meter box overnight and the number and life stage of Turkestan cockroaches were recorded the following day.

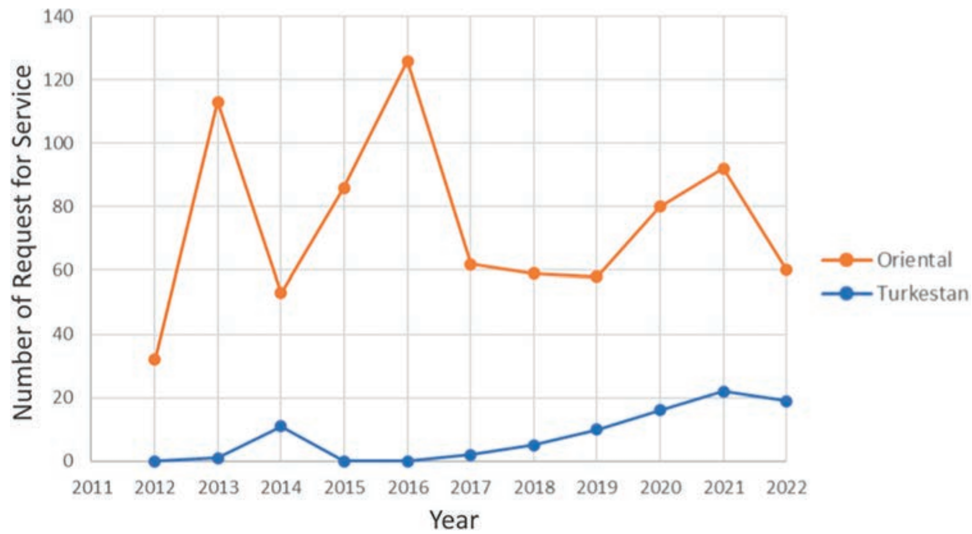

Figure 1.—Yearly number of requests for service from the public and local water district regarding Turkestan and Oriental cockroaches. Alameda County Water District stopped submitting cockroach abatement requests around 2017 due to time constraints on their technicians.

We ranked the populations as "high" (over 10 cockroaches found), "low" (less than 10) or "no activity". Locations with multiple water meter boxes within street block, and with confirmed populations of Turkestan cockroaches, were selected for the study. Four locations were selected, with one location serving as a control.

Bait receptacle creation

Pre-measured 1.5 g Maxforce Impact were applied to aluminum weight boats (5.7 cm diameter, 1.6 cm depth, Thomas Scientific, Swedesboro, NJ) according the recommend label rate of 1.5 g application per square yard for "low" populations. The weight boats were then folded in

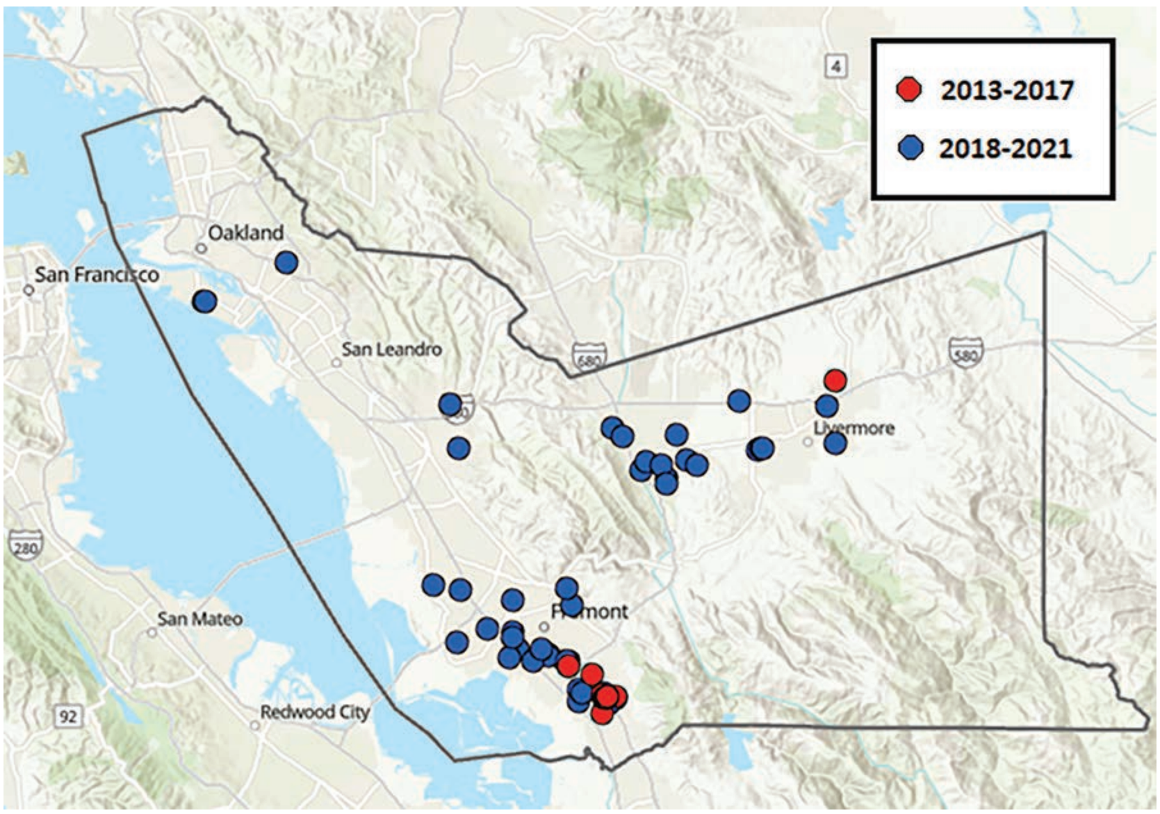

Figure 2.—Distribution of request of services involving Turkestan cockroaches submitted to Alameda County Vector Control Services District from 2013-2017 (red circles) and 2018-2022 (blue circles).

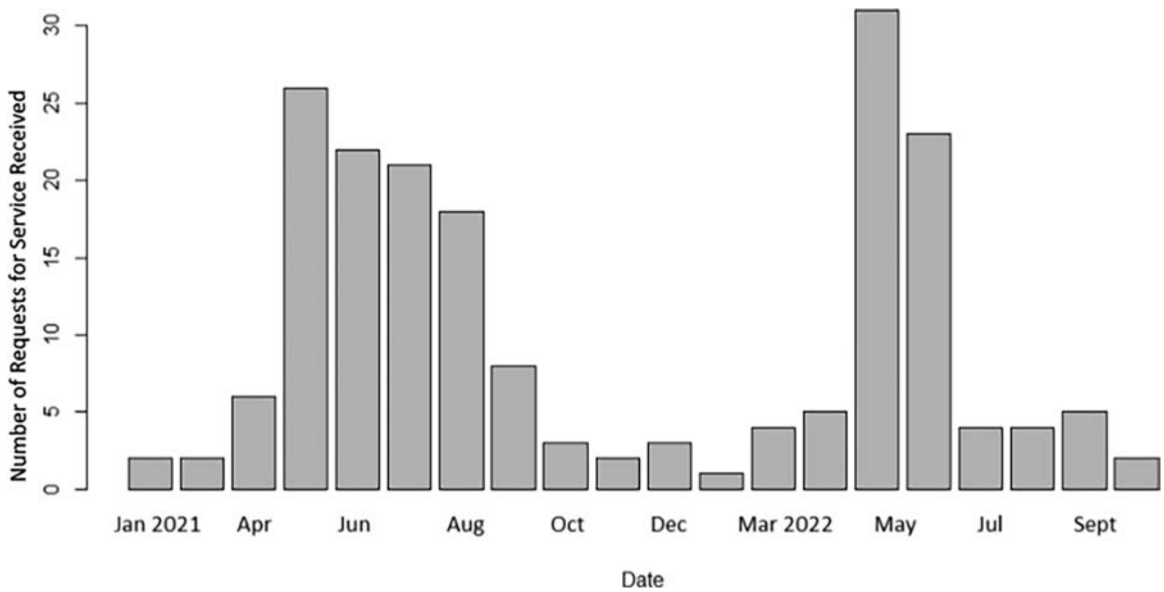

Figure 3.—Monthly total Requests For Service (RFS) submitted to ACVCSD for Turkestan cockroaches in 2021-2022.

half creating a crescent shape that protected the bait during transport and allowed quick and easy deployment of the bait into water meter boxes.

Bait deployment

Each weight boat was unfolded and placed into the water meter boxes in the treatment locations. Locations with low populations (1-10 cockroaches) were treated with one weigh boat. Locations with "high" populations (more than 10 cockroaches) received two weigh boats. Water meter boxes in the control location did not have any gel bait treatment. Water meter boxes with no activity were not included in the study.

Data collection

Data collection started at the end of May 2021, before peak of activity of Turkestan cockroaches in Alameda County (S. Kurniawan and K. Daum personal observations) (Figure 3). The initial population(s), determined during location selection, are included in the data set. Temperature, relative humidity, general environmental conditions (presence of live or dead cockroaches, ootheca, general landscape and hardscape surrounding and inside the water meter box) were recorded. Monitoring boards were left in the water meter boxes overnight and the number and life stage of Turkestan cockroaches were recorded the following day.

The following week, insecticide bait receptacles were placed inside the water meter boxes. A week after bait deployment, baited monitoring boards were placed inside the water meter boxes and the number and life stages of Turkestan cockroaches, as well as environmental factors were recorded the following day. We also noted if there were any live or dead cockroaches, or cockroach ootheca, in the water meter boxes that were not on the monitoring boards. Data collection was done once a month for 6 months from June - November 2021. The steps are summarized in Table 1.

Analysis

Results were graphed using Microsoft Excel and Rstudio (Version 2022.12.0+353) using package ggplot2.

Results and Discussion

Within 1 week of the initial bait treatment, dead Turkestan cockroaches were observed on the monitoring boards and in the water meter boxes. Turkestan cockroaches were observed consuming bait in the receptacles (S.Kurniawan and K.Daum, personal observation), so therefore there was no apparent issue of the cockroaches accessing the bait. We observed a decrease in the number of live Turkestan cockroaches captured on the monitoring boards after bait deployment in all treatments (Figure 4), and by six months, there was only 1 live adult Turkestan cockroach captured in the treatment locations (Figure 4, 6). The control water meter boxes had, on average, an increase in the average number of cockroaches per box. However, the number of cockroaches varied in each box in the

Table 1.—Data collection and bait deployment schedule.

Day	Procedure
0	Test site selection and monitoring boards picked up (Initial population)
6	Monitoring boards placed
7	Monitoring boards picked up
20	**Bait receptacle deployed**
27	Monitoring boards placed
28	Monitoring boards picked up
...62, 63, 90, 91, 125, 126...	Traps were placed and retrieved monthly from Day 28

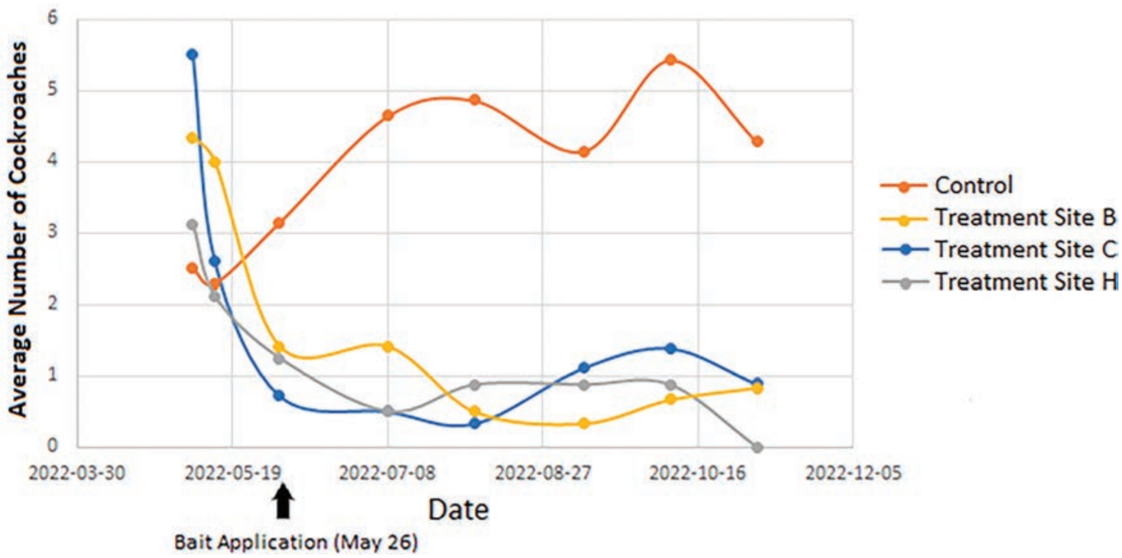

Figure 4.—Average number of Turkestan cockroaches collected on monitoring boards per water meter box at each location. Bait deployment was performed on May 26th.

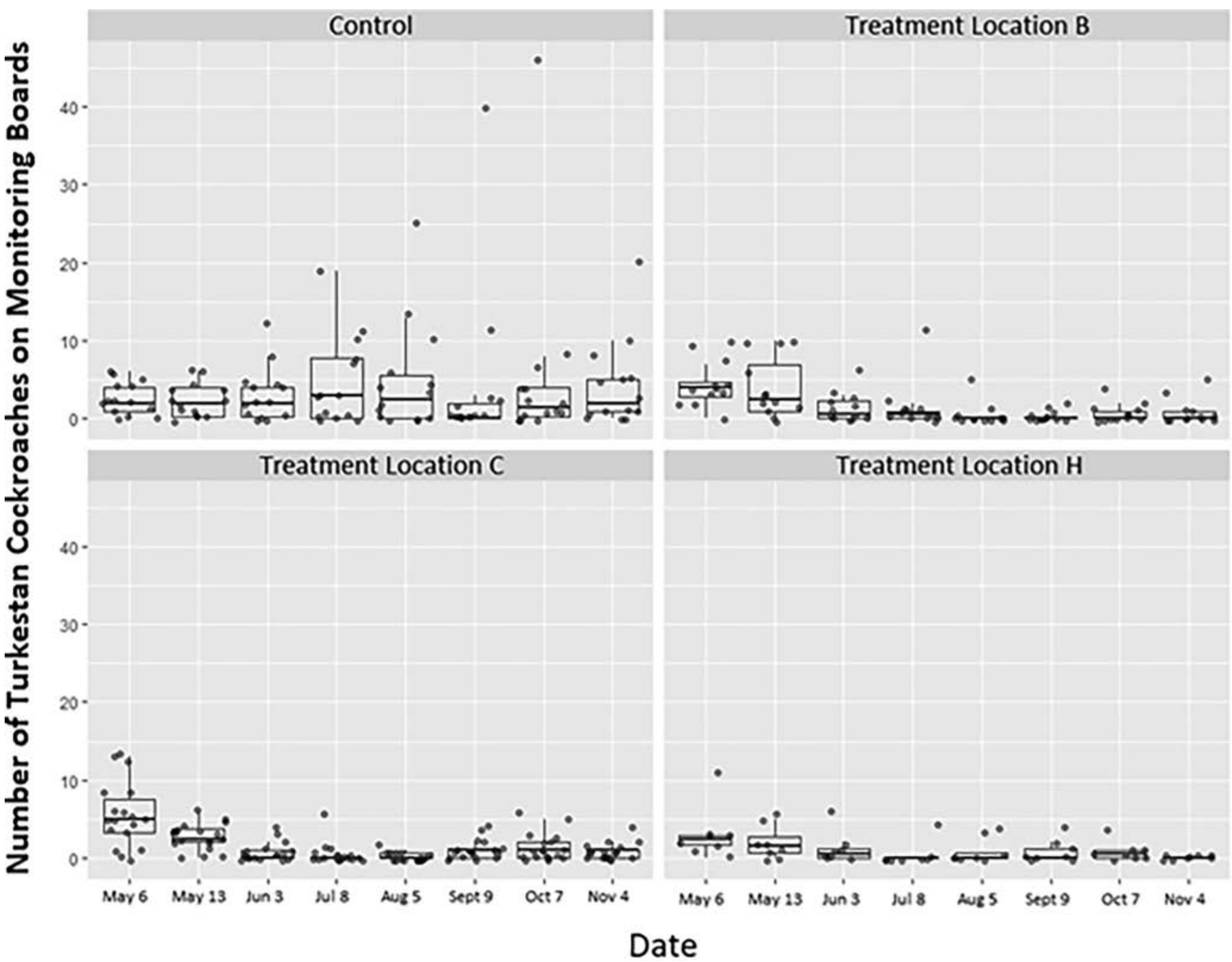

Figure 5.—Boxplot of distribution of median, and upper and lower quartiles of Turkestan cockroaches collected on monitoring boards in each water meter box by location and date. Points represent total number of cockroaches collected in a single water meter box. Bait deployment was on May 26th.

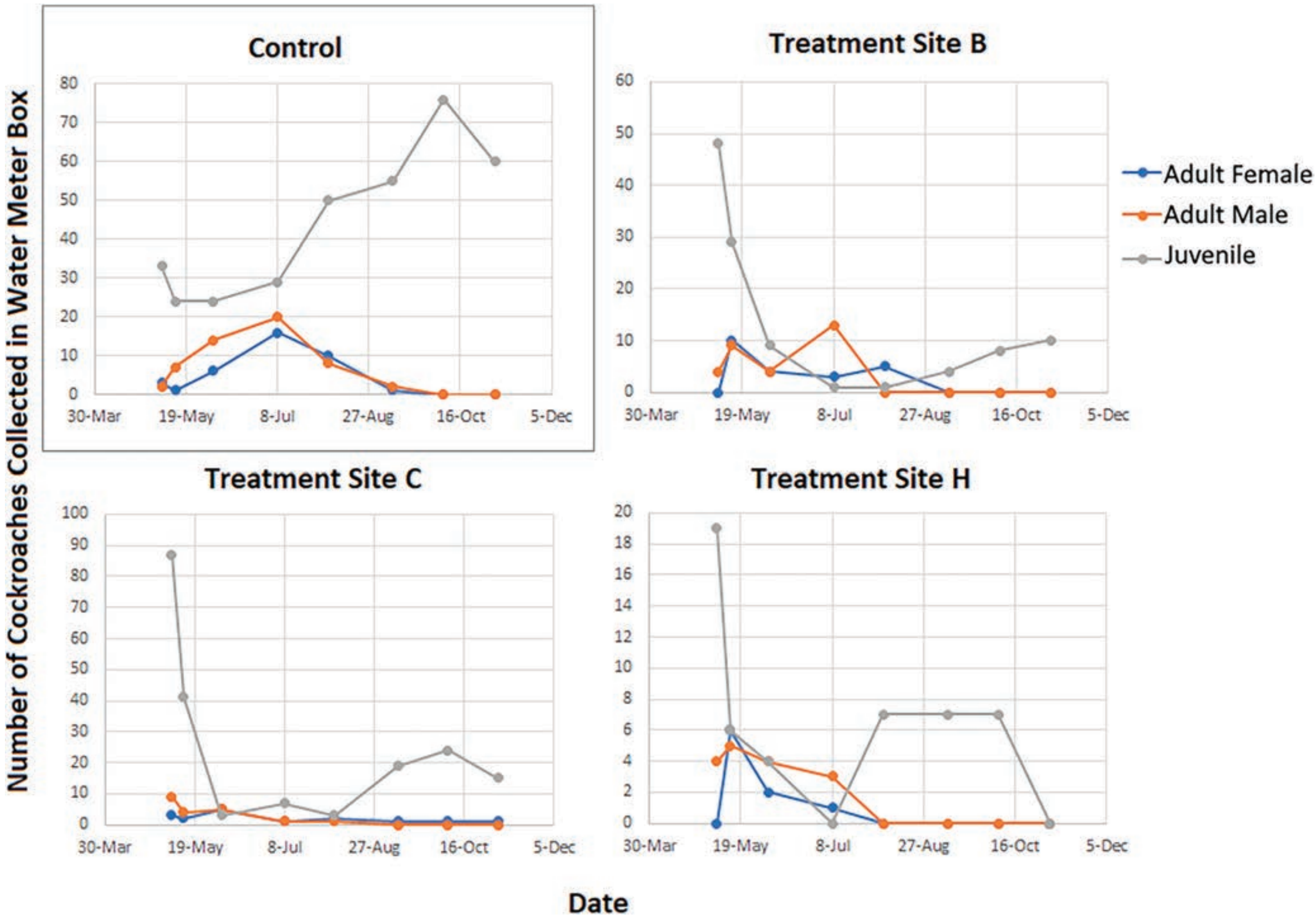

Figure 6.—Life stage composition of Turkestan cockroaches collected on monitoring boards inside water meter boxes. Sites B, C and H received a gel bait treatment.

controls, with some boxes have over 30-40 individual cockroaches and others with 0 (Figure 5).

Increases in the cockroach population in treatment locations were investigated further by breaking down the population data into three categories: adult male, adult female, and juvenile cockroaches. Two of the categories had increases during the study. At Site B, July 8th, the population increased and consisted of 64% adult male, with one meter box containing 11 adult males (Figure 6), with the population of adult males decreasing immediately. Increases in adult males may have been the result of mating flights of male Turkestan cockroaches from surrounding areas. At Site B (September 9), there was an increase in juvenile cockroaches, with the population of consisting of 19 nymphs (Figure 6) and at Site C (September 9) with the population consisting of 93-94% juveniles (1 adult female, 19 nymphs) (Figure 6). Increases in juveniles could have been caused by juveniles emerging from oothecas, suggesting that our gel bait application was too late in the season for optimal population reduction. According to research conducted by the University of California Cooperative Extension, bait should be applied when the population is high, but before any ootheca can be produced or before mating flights. An increase in juvenile cockroaches in the water meter boxes indicates that ootheca that were laid before the insecticide affected the gravid females and disrupted ootheca production. Turkestan cockroaches overwinter as juveniles, not in the ootheca stages, therefore reproduction can be interrupted by killing individuals before they reach sexual maturity (A. Sutherland, SF Bay Area IPM Advisor). In the future, we intend to bait earlier in the year, before the overwintering juveniles reach maturity.

We plan to re-run the experiment in the future, taking into account the population cycles of Turkestan cockroaches in water meter boxes throughout the entire year. Unfortunately, the monitoring boards used in this study were rendered useless during rainy weather and data collection had to be limited to dry times of the year. Water meter boxes were often found to be flooded during rainy weather and there were no waterproof solutions available. Flooding may also impact effectiveness of bait deployment by washing away the bait away from the location. There was also a concern about the waste of aluminum weight boats and future experiments will use biodegradable weight boats made of paper. Additionally, it is unknown if a treated water box affects the population of neighboring water meter boxes. If the treatment provides control beyond a single water meter box, pesticide treatment may not be necessary in adjacent boxes, reducing the overall pesticide use.

Conclusions

Despite not eliminating the Turkestan cockroach population in the treated water meter boxes, there was a reduction of adult cockroaches to only one individual in the time period after applying the gel bait as compared to the control water meter boxes. Placing monitoring boards 6 months after treatment can help detect any population rebounds and identify boxes that need to be re-treated. Re-treating the water meter boxes after 6 months may lead to complete control of the population by removing juvenile cockroaches that have emerged from oothecas after the initial treatment.

Acknowledgements

Joe Barile of Bayer for his knowledge of the different types of cockroach gel baits and appropriate dosage and selection. Andrew Sutherland and Casey Hubble of University of California Agriculture & Natural Resources for their input and resources on Turkestan cockroaches.

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Discovery of the black fly *Simulium ustulatum* (Diptera: Simuliidae) in San Joaquin County, California, USA[1]

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Abstract

The black fly *Simulium ustulatum* was described in 2022 from San Joaquin County, California, as a member of the *Simulium annulus* group. Females are morphologically indistinguishable from other members in the group but are ecologically distinct as the only western North American member living near sea level. Males can be separated from all other black fly species by the genitalia, which include a broad, flat ventral plate with finger-like lobes and multiple stout parameral spines. Larvae can be distinguished from all other black fly species by the brown triangular area on the ventral side of the head capsule. DNA barcoding strongly supports *S. ustulatum* as a member of the *S. annulus* group and its distinction from all other species in the BOLD (Barcode of Life Data System) database. Chromosomal barcoding shows that *S. ustulatum* has the most rearranged polytene chromosomes, compared with the eight other North American members of the *S. annulus* group. Additionally, it has a large pale-staining chromocenter where the three pairs of chromosomes are attached. *Simulium ustulatum* differs chromosomally in far more respects from the *S. annulus* species group standard than do any of the other Nearctic group members.

Introduction

Black flies are vectors of the parasitic filarial worm *Onchocerca volvulus*, the causative agent of human onchocerciasis or "river blindness" and other *Onchocerca* pathogens that can infect humans and other animals (Crosskey 1990). Zoonotic *Onchocerca* infections are rare in the United States, but *Onchocerca lupi* is an emerging zoonotic parasites, and several human cases have occurred in Arizona, New Mexico, and Texas (Cantey et al. 2016). Although human cases have not been documented in California, infections in dogs and black flies have been reported in southern California (Hassan et al. 2015). Deer-associated *Onchocerca cervipedis* (Weinmann 1973) and other *Onchocerca* species are also present in California (Kulpa 2021). Various avian protozoans, such as *Leucocytozoon* spp. and *Trypanosoma* spp. can be transmitted by black flies throughout North America (Adler et al. 2004). Black flies also can be severe nuisances due to their biting behavior.

San Joaquin County is centrally located in the Californian Central Valley, with black fly habitat primarily provided by the four major rivers flowing through the county and eventually merging into the Bay Delta. These rivers include the Mokelumne River, Calaveras River, San Joaquin River, and Stanislaus River, which all originate in the Sierra Nevada range (Fig. 1A). Furthermore, many small streams, creeks, and sloughs in the county provide additional habitat. Black fly populations are typically minimal and have not been of public health concern in San Joaquin County for the last several decades. In 2018, many black flies were caught in CO_2-baited encephalitis virus surveillance (EVS) traps set for mosquito vectors and West Nile virus surveillance. Sudden increases in black fly populations can be problematic and can affect public service request responses. Therefore, specimens were saved for identification to determine whether these black flies are species that bite humans. As a result, a new black fly species was discovered, supported by traditional morphology, chromosomal barcoding, and DNA barcoding (Adler and Huang 2022).

Methods

Specimen Collection and Habitat Characteristics

Initial collection of black flies was made in CO_2-baited EVS traps set for West Nile virus surveillance in San Joaquin County. Weekly trapping started in mid-March and was completed in early November. Black flies were stored at -80 °C until morphological identification. The type series of *S. ustulatum* was collected in March 2022 from the Lower Mokelumne River in San Joaquin County, California (38°12′36″ N 121°22′32″ W), at an elevation of 4.5 m above sea level (asl) (Adler and Huang 2022) (Fig. 1A). The riverbed consists mostly of sand with fine silt and mud along the banks. The predominant submerged aquatic vegetation includes Canadian waterweed (*Elodea canadensis*) along the banks and dense masses of the moss *Fontinalis hypnoides* on submerged logs (Fig. 1B, 1C). The larvae and pupae attached to both of these plants.

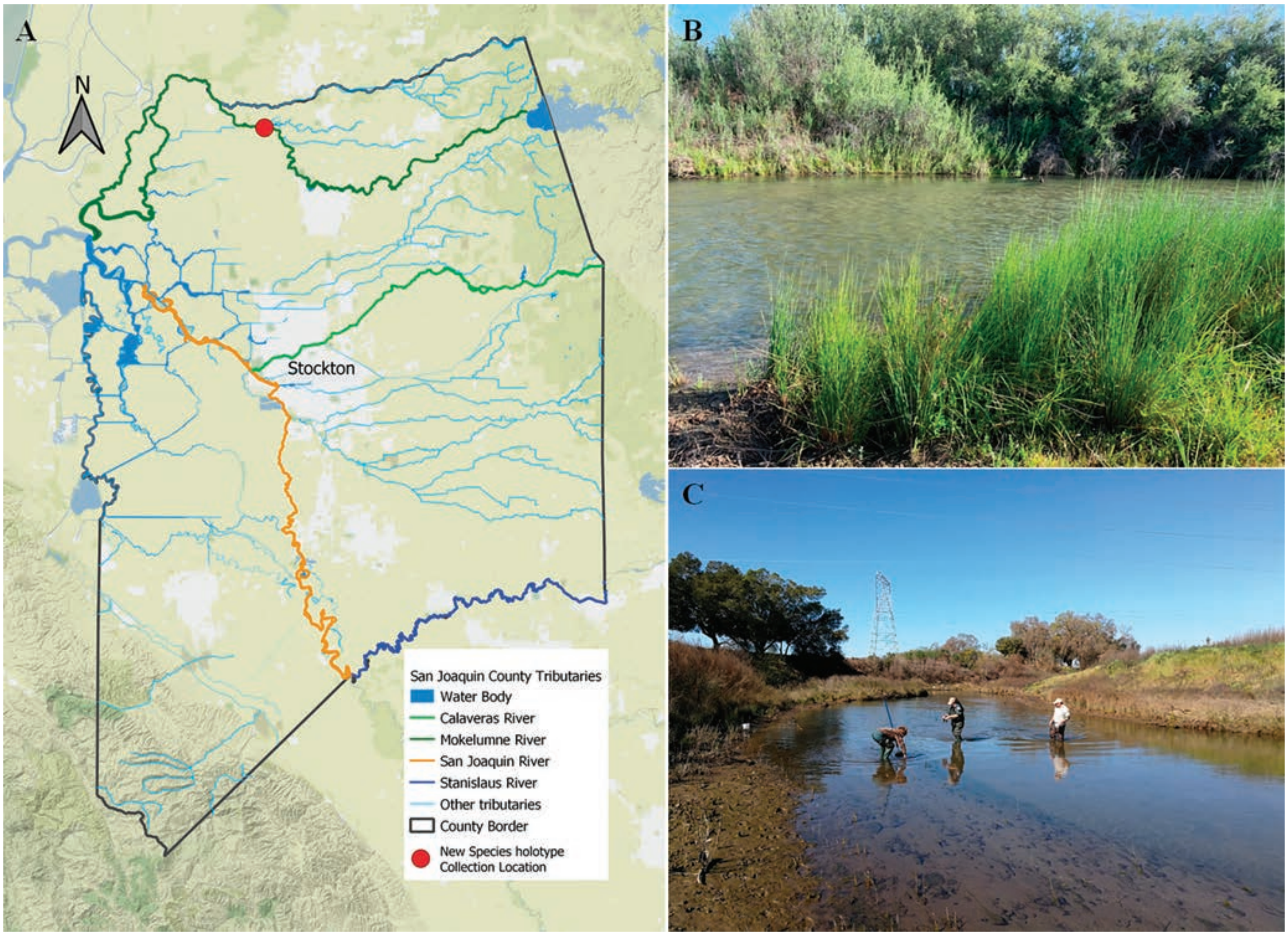

Figure 1.—Tributaries in San Joaquin County, California (A), and type locality of *Simulium ustulatum* (A, B and C).

DNA Barcoding

DNA barcoding was performed using the standard LCO1490 and HCO2198 primer pair for the Cytochrome C Oxidase I (*COI*) gene (Folmer et al. 1994). *COI* fragment amplification, sequencing, and analysis were described by Adler and Huang (2022). Sequence identity analysis for the *COI* sequences were conducted by searching the BOLD database, using the ANIMAL IDENTIFICATION [COI] module.

Chromosomal Barcoding

Chromosomal barcoding analysis was performed by examination of the giant polytene chromosomes in the salivary gland cells that were prepared from larvae according to the procedures described by Adler et al. (2004). The banding patterns of all six chromosome arms were compared with the standard reference map for the *S. annulus* group to evaluate differences (Golini and Rothfels 1984).

Results and Discussion

Collections of Adult Black Flies

Morphological identification of adults showed that the collections made from spring to early summer were dominated by the black form of *Simulium clarum,* followed by its orange form (Fig. 2). Both forms of *S. clarum* feed on a variety of hosts and are pests of horses, cattle, and occasionally humans (Moulton 1998). The second most frequently collected species was a taxon whose females initially were identified as a member of the *S. annulus* species group but could not be identified to species. Further study using characters of the polytene chromosomes, male genitalia, and larvae suggested that this unknown taxon was a new species (Adler and Huang 2022). The collections also included a human biting species, *Simulium vittatum,* but its abundance was minimal. Collection data showed that black fly populations continued to increase in 2019. Geographically, most of the adult black flies were collected along the rivers, especially in the lower Stanislaus River (Fig. 3). Fewer black flies were collected near small streams, creeks, and sloughs throughout the county.

Morphological Characteristics of Simulium ustulatum

The female has an overall grayish body covered with silvery hair, although the hair on the scutum is pale golden (Fig. 4A). The female resembles those of all members of the *S. annulus* group in the western United States (*Simulium balteatum, Simulium joculator, Simulium quadratum*, and *Simulium zephyrus*) but is ecologically distinct

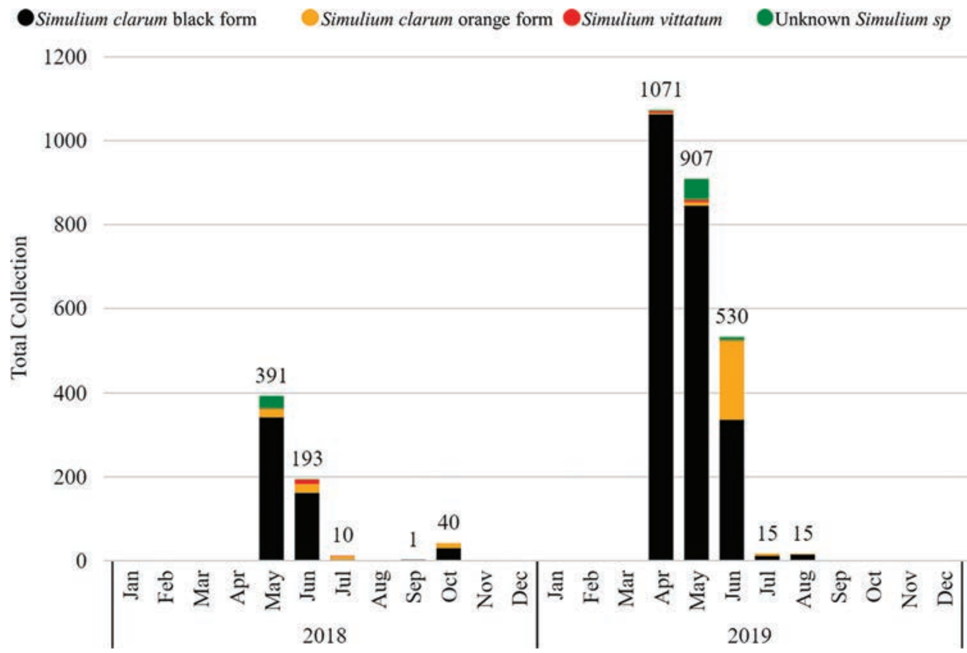

Figure 2.—Monthly collection of black flies in EVS traps from 2018 to 2019 in San Joaquin County, California.

in that it is the only member collected near sea level, whereas these four other group members inhabit mountain ranges. The female has bifid claws, each with a thumblike basal lobe, suggesting that *S. ustulatum* is ornithophilic.

The male is uniformly blackish brown and velvety with pale golden brown hair except on the stem vein and abdomen, where it is dark brown and brassy. The male genitalia have a broad, flat ventral plate with a slight finger-like lobe posterolaterally on each side. The parameres bear one stout spine near the midlength and three additional stout spines, often with smaller spines. These combined genitalic characters are diagnostic and separate *S. ustulatum* from all other species of black flies.

The larva has well-defined reddish brown banding on the body, and the head capsule is whitish-yellow with well-defined head spots (Fig. 4C). The ventral side of the head capsule has a brown area in the shape of an isosceles triangle that extends toward the hypostomal groove (Fig. 3D). This character renders the larva distinct from those of all other black fly species and was the inspiration for the name of the species, *ustulatum*, from Latin meaning singed or browned.

DNA barcoding

Eight *COI* sequences of 658-bp length for *S. ustulatum* are in GenBank (accession numbers: OP256416−OP256423). A sequence search of the BOLD database revealed that *COI* sequences of *S. ustulatum* share higher sequence identity with several members in the *S. annulus* group than with any other known species in the database (Table 1). An earlier study found that the maximum intraspecific divergence of *COI* sequences of analyzed Nearctic nominal species of black flies was below 3.84% (Rivera and Currie, 2009). Although not all members of the *S. annulus* group have been DNA barcoded, the results support the inclusion of *S. ustulatum* in the *S. annulus* species group and that its *COI* sequences are unique among those currently in the BOLD database. The tree constructed using the Neighbor-Joining method and Kimura 2-parameter model also strongly supports an evolutionary relationship between *S. ustulatum* and other members of the *S. annulus* species group.

Chromosomal barcoding

Chromosomal barcoding shows that *S. ustulatum* has the most rearranged polytene chromosomes compared to the eight other North American members of the *S. annulus* group. It has 12 fixed rearrangements whereas other members have no more than one fixed rearrangement. The long arm of the second chromosome pair (IIL) is highly scrambled and contains seven of the 12 fixed rearrangements. The most unique and striking chromosomal feature of *S. ustulatum* is a large, pale-staining chromocenter where the three pairs of chromosomes connect. In summary, *S. ustulatum* is chromosomally far more different from the *S. annulus* species group standard than any of the other Nearctic group members.

Black flies are notorious for their complicated taxonomy due, in part, to a high degree of morphological homogeneity and the existence of cryptic species. Recognizing and describing a new species of black fly typically requires an integrated approach using chromosomes, morphology, and DNA sequences. Chromosomal barcoding, loosely known as cytotaxonomy, has been used as a powerful taxonomic tool for the Simuliidae, as well as other families of Diptera (Michailova 1989, Coluzzi et al. 2002, Tosi et al. 2007, Adler et al. 2016). The dark and light banding patterns (barcodes) of the giant polytene chromosomes are typically

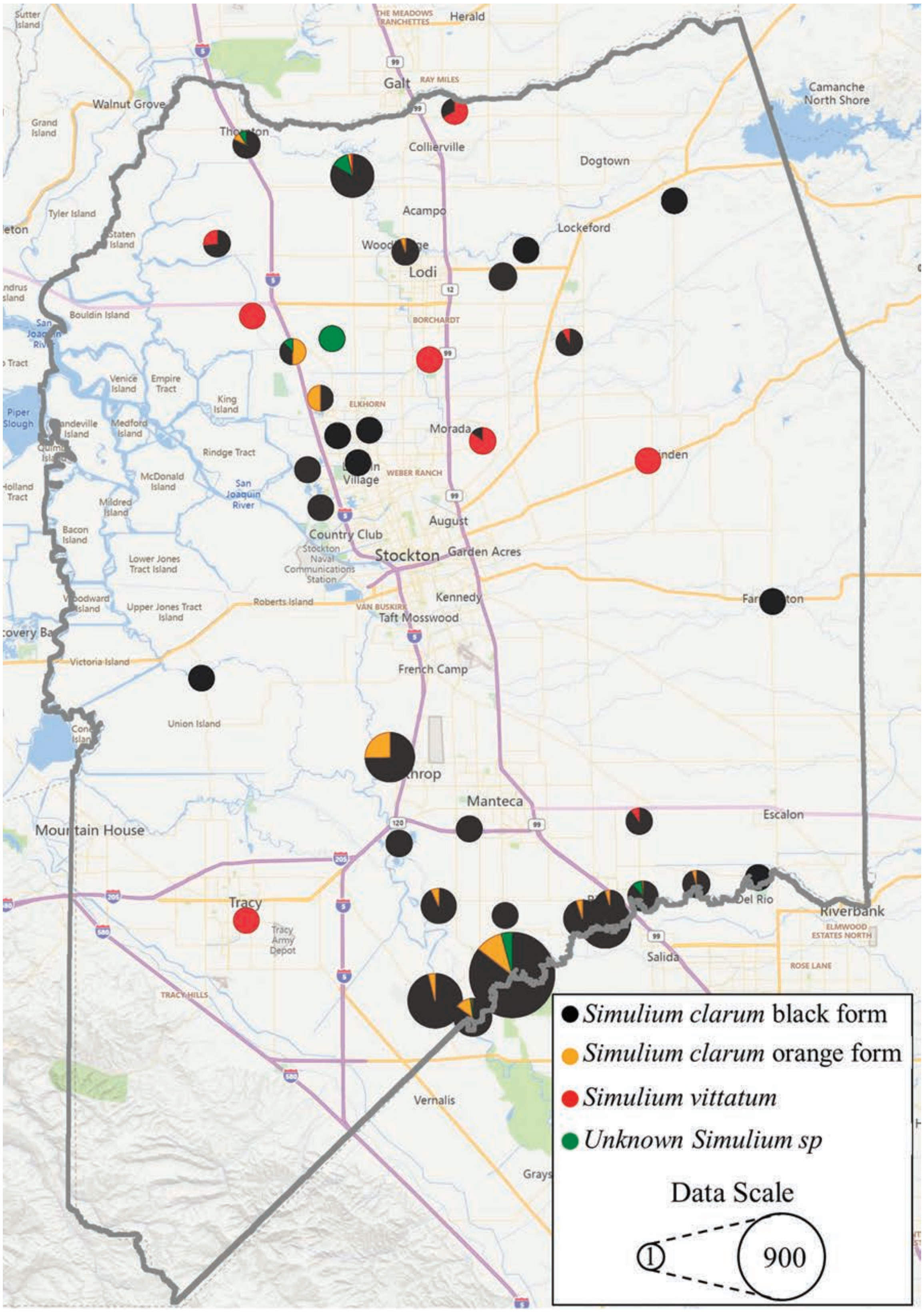

Figure 3.—Geographical distribution of black flies collected from 2018 to 2019 in San Joaquin County, California.

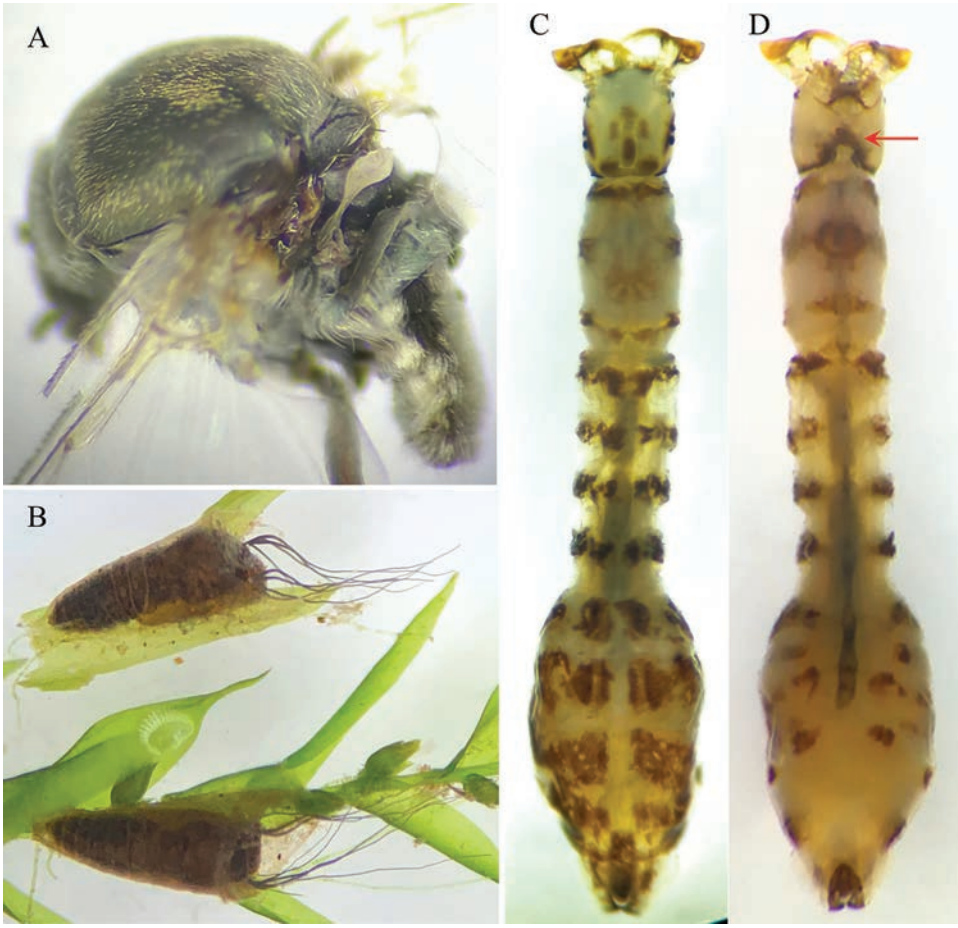

Figure 4.—Life stages of *Simulium ustulatum*. A. Female. B. Pupa. C. Larva, dorsal view. D. Larva, ventral view. Red arrow: larval character for which *Simulium ustulatum* is named.

species-specific. Different barcodes mostly result from chromosomal rearrangements, such as inversions, which are abundant throughout the Simuliidae. Consequently, chromosomal barcodes can provide resolution for delimiting closely related taxa that are indistinguishable morphologically. For example, polytene chromosome analyses revealed that the African *Simulium damnosum* complex, which includes the vectors of the causal agent of river blindness, consists of more than 50 cryptic species and genetically distinct forms (Post et al. 2007). The study of the *S. damnosum* complex demonstrates the power of chromosomal barcoding for providing accurate means of species identification.

Table 1.—Species with *COI* sequences most similar to *Simulium ustulatum*.

Species	Number of Hits	Percent Identity
Simulium annulus group	20	90.2-92.4
Simulium balteatum	36	91.6-92.2
Simulium emarginatum	36	90.8-92.2
Simulium annulus	26	91.1-91.9
Simulium johannseni	16	90.8-91.2
Simulium angustitarse	3	90.1-90.4
Simulium lundstromi	28	90.0-90.4
Simulium ibleum	1	90.0-90.0

Ecological Implications

Simulium ustulatum was the second species of black fly described from North America in the past 18 years. It was not previously discovered possibly due to its low abundance, early seasonal occurrence, and apparently restricted distribution. After one of the most severe drought periods from 2012 to 2016, California received the greatest precipitation on record in early 2017 that resulted in major flooding in all the major rivers in San Joaquin County. The floods probably expanded the habitat availability for *S. ustulatum*, and might also have brought larvae or eggs downstream from higher elevations. Future studies to investigate the presence of *S. ustulatum* at higher elevations could help understand the impact of flooding events in its primary habitats. Like other members in the group, *S. ustulatum* requires the flow of cold water in the spring. If this species is confined to low-elevation rivers, it could become vulnerable to its habitat being frequently disturbed by flow diversions for irrigation and municipal use and global climate changes resulting in fluctuations in temperature and snowpack.

Conclusion

The inadvertent collection of black flies in EVS traps for mosquito vectors and West Nile virus surveillance led to the surprising discovery of a new black fly species, *S. ustulatum*, originally described by Adler and Huang (2022). Although the females are morphologically inseparable from those of other species in the *S. annulus* group, the males and larvae bear unique characters that justify species status, supported by chromosomal and DNA barcoding. An integrated taxonomic approach using different methods provides promising outcomes for identifying species of black flies and understanding their evolutionary relationships.

Acknowledgements

The authors thank Sumiko De La Vega of San Joaquin County Mosquito & Vector Control District for field and laboratory support for specimen collection, identification, DNA barcoding, and habitat investigation; Mary Iverson and former employee Andrew Provencio for helping in specimen collection; and the District's manager Omar H. Khweis for approval of all the resources needed for conducting this study.

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District program overview for culturing *Gambusia affinis* and overall earthen pond health

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Introduction

The Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) has a robust *Gambusia affinis* (mosquitofish) rearing program that consists of 22 earthen ponds and 6 indoor rearing tanks. On average, SYMVCD produces 3,600 pounds of mosquitofish that are used to control immature mosquitoes in rice fields, wetlands, and agriculture sites including ditches, sumps, and irrigation ponds. There are over 50,000 acres of rice fields and wetlands within Sacramento and Yolo County where mosquitofish can be used to control mosquito larvae. Currently, 80% of the total mosquitofish produced are planted into rice fields adjacent to dense residential housing areas. Increased mosquitofish production within the current system of ponds and tanks is paramount to keeping up with demand for this biocontrol agent. Several improvements were made to mosquitofish production procedures to optimize fish yield. These procedural steps and resultant outcomes are described below.

Methods

The first improvement made to increase mosquitofish yield was to add predatory bird exclusion to 12 ponds (Fig. 1a). This exclusion method included 2 in chain link fencing around each pond and 2 in top netting. The netting is custom made to size (Christensen Net Works; Everson, Washington) to prevent birds from accessing the ponds. The second improvement was the addition of solar-powered aeration systems (Fig. 1b) to raise dissolved oxygen (DO) levels. The solar-powered aerator with battery backup system (Keeton Solaer SB-1.2; Wellington, Colorado), included 10″ diffuser discs that produced high oxygen transfer micro bubbles. These aerator units were capable of running for 20 hours on a fully charged battery. The aerators were programmed to operate from before dusk to mid-morning, to prevent nighty decreases in dissolved oxygen levels due to the lack of photosynthesis. These improvements were quantified through water quality monitoring including turbidity, temperature, DO, and mosquitofish yield. Weekly water quality monitoring began in January, and during the study all pond readings were taken twice daily, once in the early morning (0700h) and again in the afternoon (1400h). Turbidity was measured using both a portable turbidity meter (LaMotte; Chestertown, Maryland) and the Secchi disc method (Bowers et al. 2020). Temperature and DO readings were collected with a Milwaukee MW600 dissolved oxygen meter. During 2019 four unaerated ponds and three aerated ponds were monitored for differences in morning DO levels.

All statistical analysis were conducted using R v4.2.1 (R Core Team 2022). A Wilcoxon-signed rank test was used to compare dissolved oxygen concentrations between aerated and non-aerated ponds. To analyze the effect of aeration on total fish yield, we fitted a generalized linear model (GLM) using the lme4 package, model parameters included aeration status, pond, and year. Fish yields from 2016−2022 were evaluated, ponds were coded by year as

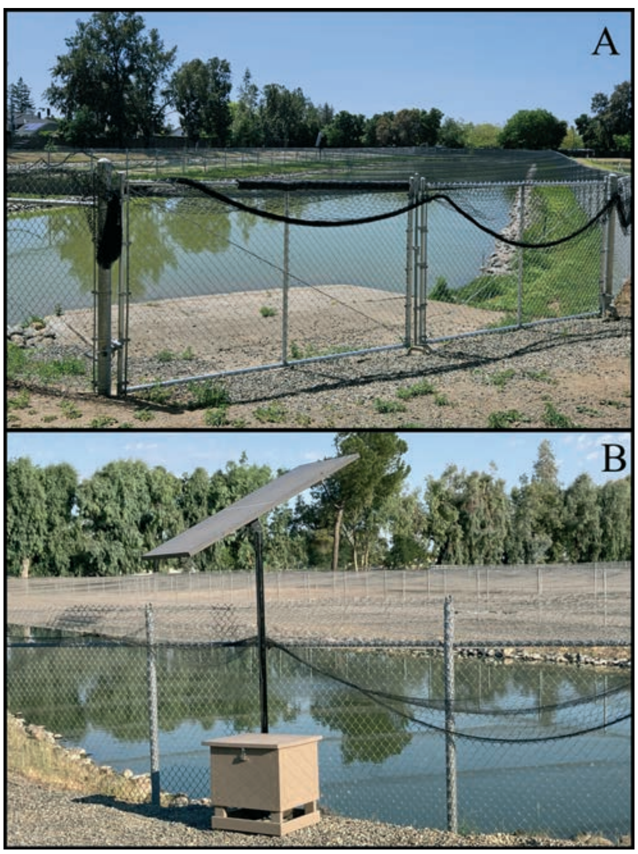

Figure 1.—A: Predatory bird exclusion including 2 in chain link fencing and 2 in top netting; B: solar-powered aeration system.

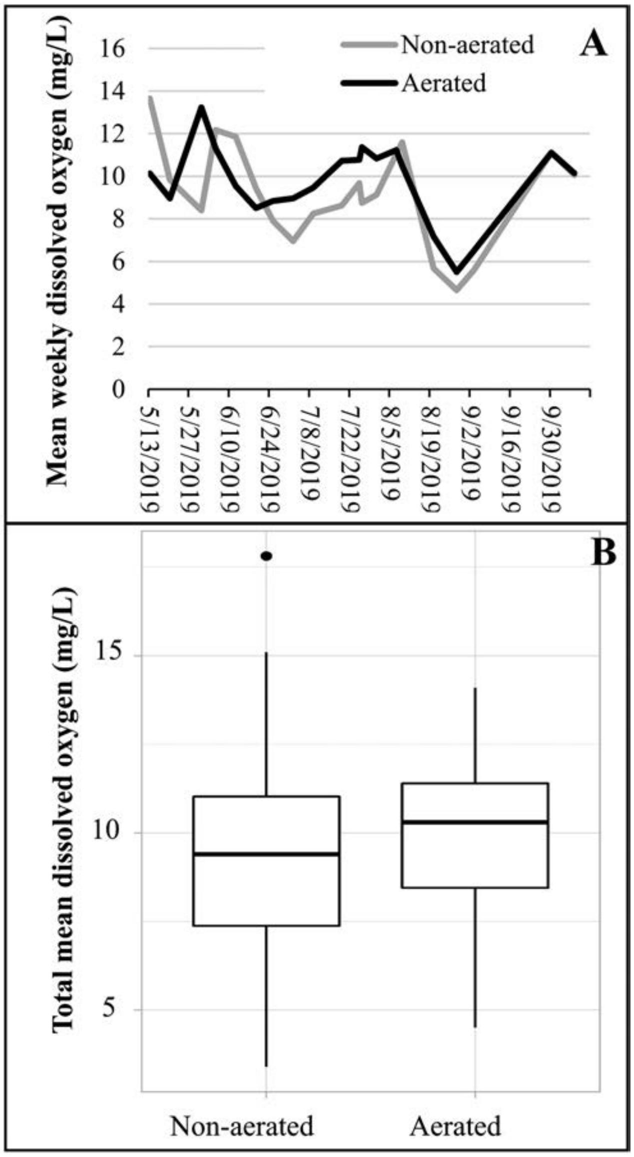

Figure 2.—A: Mean weekly dissolved oxygen levels in aerated and non-aerated ponds during 2019. B: Total dissolved oxygen levels in aerated and non-aerated ponds. Bars represent the SE.

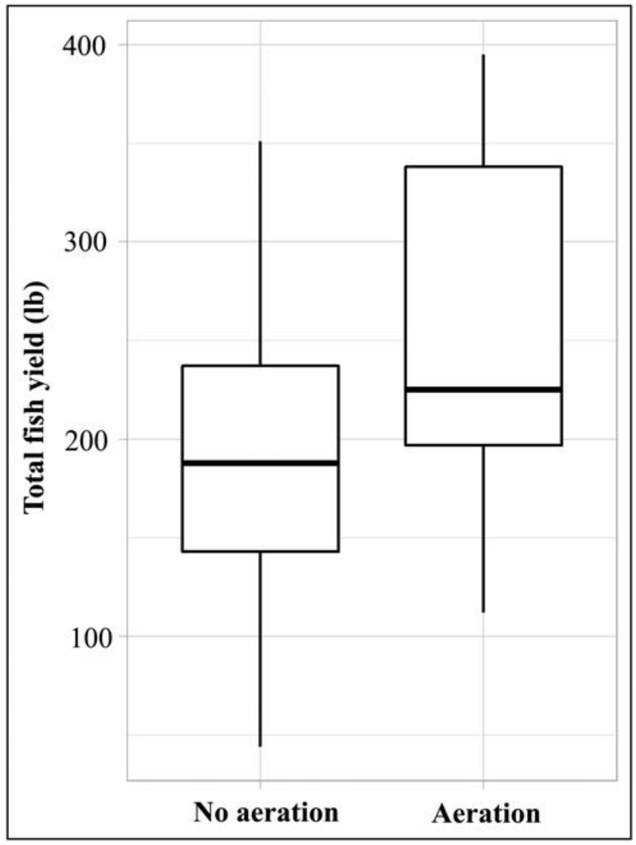

Figure 3.—Mean total mosquitofish yield (lb) for aerated and non-aerated ponds across all study years (2016−2022). Bars represent the SE.

either aerated or non-aerated, and the best fit model was selected by lowest Akaike information criterion (AIC).

Results and Discussion

Over the course of the mosquito control season, each earthen pond was repeatedly seined until we failed to collect fish in the nets. Several additional ponds were maintained as nursery ponds that were used to replant ponds in the spring. Each year, in March each earthen pond was stocked with 50 pounds of mosquitofish. The production of mosquitofish was monitored following the addition of supplemental aeration. Additionally, changes in pond health, as measured by turbidity, temperature and most importantly dissolved oxygen were recorded before and after the addition of the aerators (data not shown).

Solar aerators raised the weekly levels of DO (Fig. 2A), but differences in total DO between aerated and not aerated ponds was not significant (Fig. 2B) ($Z = 2692$, $P = 0.15$). Fish harvesting began in June and continued through late October. When evaluating fish yield using GLM, the best fit model ($AIC = 494$) contained all parameters (aerations status, pond, and year). There was a significant effect of aeration (Estimate = 1, SE = 4.4, $t = -2.28$, $p = 0.03$), year (Estimate = 1.1, SE = 5.1, $t = 2.2$, $p = 0.02$) and pond (Estimate = 1.8, SE = 7.6, $t = 2.3$, $p = 0.02$) on total fish yield. Aerated ponds had a significantly higher fish yield (total fish yield mean = 261 ± 25 lb.) compared to non-aerated (total fish yield mean = 193 ± 14 lb.) and the magnitude of that difference varied between years and ponds.

Conclusion

Mosquitofish are an important part of the SYMVCD integrated pest management program. There currently are more areas suitable for mosquitofish planting in Sacramento and Yolo Counties than our current fish production can support. Therefore, SYMVCD is continually striving to improve fish yield; the addition of predatory bird exclusion and solar aerators have both contributed towards meeting this goal.

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Dog heartworm (*Dirofilaria immitis*) in mosquitoes of Orange County, California

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Introduction

Mosquitoes are the vectors of *Dirofilaria immitis*, a filarial nematode that causes heartworm infection in dogs as well as other mammalian hosts, including humans (Dantas-Torres and Otranto 2020). The number of heartworm cases has increased nationally in the last decade both in areas historically considered to be endemic for dog heartworm as well as in areas of low endemicity such as in Orange County, California (Couper and Mordecai 2022, AHS, 2019). The mosquito fauna of Orange County now includes the genus *Aedes* of which several species are known vectors of *D. immitis* (Ledesma and Harrington 2011). The Orange County Mosquito and Vector Control District (District) first detected the presence of the invasive *Ae. aegypti* (L.) and *Ae. albopictus* (Skuse) in 2015 and *Ae. notoscriptus* (Skuse) in 2018, but it is unknown if *D. immitis* is present in these mosquito populations. Therefore, our study was designed to test for the prevalence of *D. immitis* in the introduced populations of *Ae. aegypti*, *Ae. albopictus* and *Ae. notoscriptus* as well as in native *Culiseta incidens* (Thompson). Evidence for *Ae. aegypti* contributing to the transmission of dog heartworm (Scavo et al. 2022) and the discovery by Chaban et al. (2021) in detecting *D. immitis* in *Ae. aegypti*, *Ae. albopictus*, and *Cs. incidens* mosquitoes collected from nearby Los Angeles County, further compelled us to investigate the potential infection rates in the mosquitoes collected in our District.

Methods

As part of our ongoing disease surveillance program, mosquitoes are collected using different surveillance traps: CO_2-baited, BG-Sentinel with human lure (BioGents, Regensburg, Germany), in-house constructed EVS traps (Rohe and Fall 1979), and the Reiter-Cummings CDC gravid trap (Cummings 1992). *Aedes* and *Culiseta* mosquitoes are routinely tested for the prevalence of West Nile, St. Louis encephalitis and Western equine encephalitis viruses as well as Zika, chikungunya and dengue viruses. Mosquitoes collected during the 2020-2022 seasons were included in this study. DNA from whole mosquito homogenates was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Cat # 69506, Germantown, MD) following manufacturer recommendations. A two-step PCR protocol was implemented by first using a duplex real-time PCR protocol that was described by Spinks and Buettner (2019). The real-time duplex utilizes two sets of primers, the first set detects a wide range of filarial worms using the relatively highly conserved *5s* ribosomal RNA gene (*5s*), whereas the second set is species specific for *D. immitis* and targets the relatively highly variable mitochondrial cytochrome c oxidase I (*COI*) gene. Primers for the *5s* gene include the following forward and reverse primers: 5′- TACCACGTTGAAAGCACGAC and 5′- CCAAGTACTAACCAGGCCCA respectively, with the probe, VIC- CGTCCGATCTGTCAAGTTAAGCAACGT-QSY. Forward and reverse primers for *COI* are 5′- TTACTTTTGTTGCGTTGTTG and 5′- TGACCCTCTA-CACTCAAAGG, respectively, with the probe, FAM-GGGGGTCCTGGGAGTAGTTGA-QSY. The duplex PCR reactions were carried out using the Applied Biosystems™ 7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA). Ambion® Path-ID™ qPCR Master Mix (ThermoFisher Scientific, part # 388644, Waltham, MA) was used in the PCR reactions and performed in 25 µl volumes including 12.5 µl of Ambion® Path-ID™ qPCR Master Mix, 4 µl with Nuclease-free Water (Applied Biosystems, part # 4388514), 3.5 µl of primer and probe working stock and 5 µl

Table 1.—Prevalence of dog heartworm throughout Orange County, California, in *Aedes aegypti*, *Aedes notoscriptus*, and *Culiseta incidens* collected in 2020-2022.

Ae. aegypti	*Ae. notoscriptus*	*Cs. incidens*
	2020	
(2/531, 0.38%)	(1/9, 11.1%)	(0/0, 0%)
	Total: (3/540, 0.55%)	
	2021	
(7/693, 1.01%)	(0/1, 0%)	(2/10, 20%)
	Total: (9/704, 1.28%)	
	2022	
(14/512, 2.73%)	(0/3, 0%)	(3/356, 0.84%)
	Total: (17/871, 1.95%)	

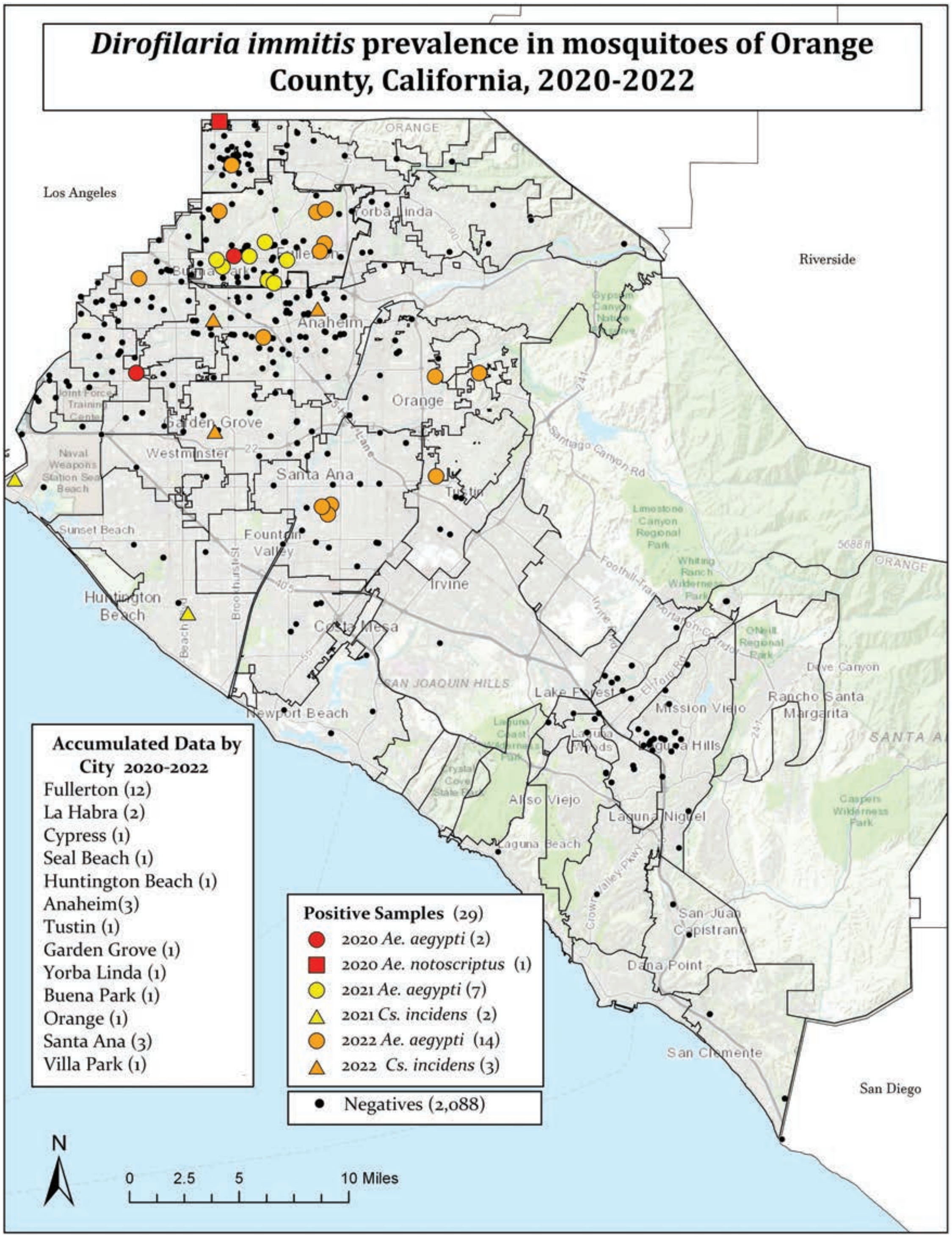

Figure 1.—Distribution of dog heartworm (*Dirofilaria immitis*) positive mosquito pools collected in Orange County between 2020-2022. Positive mosquito pool samples are denoted in color by their respective collection year and all negative mosquito pools collected between 2020-2022 are denoted by a smaller black circle.

of extracted DNA. The primer and probe working stock consisted of 0.3 μM of each *CO1* primer and 0.2 μM of *CO1* probe, along with 0.5 μM of each *5s* primer and 0.4 μM of *5s* probe. PCR cycling conditions using the 7500 Fast Real-Time PCR System were as follows: one cycle at 50° C for 2 min, one cycle at 95° C for 10 min, 45 cycles at 95° C for 15 sec and 60° C for 1 min. Previously extracted mosquitoes positive for dog heartworm were used as positive controls, as well as L3 *D. immitis* larvae. Previously confirmed negative mosquito samples, master mix alone, and nuclease-free water alone were used as no-template negative controls. To help confirm our results, samples that amplified using our duplex real time

PCR protocol were subsequently tested following the approach of Huang et al. (2013). Amplicon products produced from the second PCR screening were run on a 2% agarose gel where the band was excised and the PCR product purified using the GeneJET Gel Extraction Kit (ThermoFisher Scientific, Cat # K0692, Waltham, MA) according to manufacturer recommendations. The purified PCR product was then sent to Genewiz, (Azenta Life Sciences, La Jolla, CA) for sequencing. Sequences were analyzed and compared to accessioned *D. immitis* sequences using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) site to confirm the *D. immitis* identification.

Results and Discussion

Dirofilaira immitis was detected in three species, including the introduced species, *Ae. aegypti,* and *Ae. notoscriptus* and native *Cs.incidens* (Table 1). Dog heartworm infection rates in the tested mosquito samples increased every year from 0.55% (2020) to 1.28% (2021) to 1.95% (2022). *Aedes aegypti* mosquitoes accounted for the most samples collected and tested and had the highest infection rates. Samples that were confirmed *D. immitis* positive had a cycle threshold (Ct) value of 33 or less from the initial duplex real-time PCR screening. Although this would suggest that a cut-off Ct value of 33 would confirm a true *D. immitis* positive, some samples with Ct values between 30-33 were not confirmed positive using the second PCR test, demonstrating the need for the double screening process. Efficiency testing will be performed on the initial duplex assay to determine if conditions can be improved to reduce non-specific amplification. For every year of surveillance, *D. immitis* positive mosquito pools were caught between June and November. Of note, *D. immitis*-infected *Ae. aegypti* were captured by both the BG-Sentinel and gravid traps, whereas mosquitoes in the *Ae. notoscriptus* positive pool sample were captured using a gravid trap. Mosquitoes collected in the *D. immitis*-infected *Cs. incidens* positive pools were from both CO_2 and gravid traps. All positive samples were collected in the northern part of Orange County (Fig. 1), with most of the positives occurring near the border with Los Angeles County. Interestingly, in 2022, two sites had multiple positive samples recovered several weeks or months apart. The first site was in Fullerton, where a *D. immitis*-positive *Ae. aegypti* pool was first detected in June and then again in September. The second multi-positive site was in Santa Ana, which had a confirmed *D. immitis*-positive *Ae. aegypti* pool detected in the first week of August and then again, during the last week of the same month.

Conclusion

Although *D. immitis* infection rates were low in the mosquitoes sampled, the increasing trend in dirofilarial-positive infection rates indicated the importance of continued surveillance for this vector borne pathogen. In addition, positive mosquito samples caught by gravid traps in a relatively short period of time, in similar or nearby locations, implies local *D. immitis* transmission occurring within Orange County; however, further investigation is required to understand the extent of mosquito contribution to transmission. Unfortunately, dog heartworm veterinary cases are not reportable in Orange County and the lack of published data limits our knowledge of the cases that are affecting our communities. With the abundance of invasive *Aedes* likely to increase in the future, our results highlight the need to inform and educate veterinarians, animal care agencies, and the affected communities about the risk of dog heartworm infections in Orange County.

Acknowledgements

We are grateful to all Orange County Mosquito and Vector Control District staff, especially those involved in setting mosquito traps, collecting samples, and identifying mosquitoes to genus and species. We also thank District staff for helpful discussions regarding this project, especially Tim Morgan, and a special thank you to Robert Cummings for reviewing this manuscript.

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Updates and improvements to the caged mosquito field trial

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Abstract

Using small mesh cages to evaluate mosquito adulticide products at field-relevant doses has been a tool employed by mosquito control districts and industry alike for several years. However, much of the equipment used for these trials is homemade, and there is wide variability in the tools and protocols used. Recently, new research has allowed for streamlining the process of conducting these trials, and advances in 3D printing have made creating the necessary equipment precise and user-friendly. By making these trials easier to conduct and creating equipment that is more readily available and cost effective, these evaluations can become more standardized and easier to compare. This session shared field testimony of the impacts of innovations resulting from years of collaboration between industry and mosquito control districts.

Mind the gap: lessons learned from an expanded mosquito surveillance program

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Introduction

Mosquito surveillance is the foundation of mosquito control programs. An effective surveillance program provides timely data to make and evaluate control decisions that protect the public health while justifying funding requirements (AMCA 2021, Fournet et al. 2018, NAACHO 2021). Starting in 2016, Delta Mosquito and Vector Control District (DMVCD) expanded its mosquito surveillance program with the goals of establishing a database of mosquito and arbovirus activity at a finer geographic scale than previous years and using that data to improve decision-making for control efforts (Tushar et al. 2017, Tushar and Erandio 2018, Bear-Johnson et al. 2019). DMVCD re-evaluates the surveillance program yearly to identify gaps in coverage, surveillance information needed to improve decision-making, and goals for the next mosquito season.

2016-2017 Surveillance Strategy Overview

During the 2016 to 2017 mosquito seasons, 4 gravid mosquito traps were placed per square mile of populated area in urban and suburban settings per week (Figure 1A). Each gravid trap was placed at a fixed location within each quarter-mile section. Since *Aedes aegypti* (L.) were initially discovered in the District in 2014, BG Sentinel traps were placed in areas with suspected invasive mosquito presence from service requests. In rural areas, dry ice-baited Encephalitis Virus Surveillance (EVS) traps were placed at historical high abundance sites and by technician request, with a goal of deploying 20 traps per week.

Results

The average number of traps set per week ranged from 11.4 to 21.8 for BG Sentinel traps, 139.4 to 153.2 for gravid traps, and 14.0 to 16.7 for EVS traps during the 2016 to 2017 mosquito seasons (Figure 2). *Aedes aegypti* were rediscovered in the District in 2017, resulting in a redistribution of District resources to address this issue. Additionally, the BG Sentinel traps did well at catching female *Culex* species that could transmit West Nile virus (WNV), leading to a surplus of mosquito pools that could not all be tested at that time.

2018-2021 Surveillance Strategy Overview

With the rediscovery of *Ae. aegypti*, the District needed to build a baseline database of *Ae. aegypti* distribution and abundance as well as establish a surveillance strategy with a finer spatial scale to take into account the mosquito's shorter flight range. To address these surveillance gaps during the 2018 to 2021 mosquito seasons, 5 adult mosquito traps were placed per square mile of populated area in urban and suburban settings per week (Figure 1B). This included 1 fixed BG Sentinel site and 4 fixed gravid trap sites, one in each quarter-mile section. Additional BG Sentinel traps were placed at service request locations where *Ae. aegypti* were suspected. In rural areas, EVS trap sites with a history of WNV activity had a higher priority than other sites, with the goal of setting 20 traps per week.

Results

The average number of traps set ranged from 54.9 to 70.2 for BG Sentinel traps, 162.3 to 170.5 for gravid traps, and 14.5 to 19.4 for EVS traps during the 2018 to 2021 mosquito seasons (Figure 2). Additionally, EVS traps were set only one time at 35.5% of rural sites in 2021.Although the addition of a weekly fixed location BG Sentinel trap improved surveillance results, the reliance on BG Sentinel traps placed at service request locations proved to be ineffective for decision-making as *Ae. aegypti* spread and the number of service requests increased. By 2020, the average delay from inspecting a residence to setting a strategic BG Sentinel trap was over 20 days during the peak season. A similar delay was seen with EVS sites where a lack of uniform trapping led to over or under sampling of mosquitoes in rural areas.

2022 Surveillance Strategy Overview

Surveillance program goals for 2022 included increasing routine surveillance for host-seeking mosquitoes, including *Ae. aegypti* (L.), and improving surveillance in rural areas with high WNV risk. During the 2022 mosquito season, 7 adult mosquito traps were placed per square mile of populated area in urban and suburban settings per week (Figure 1C). This included 1 fixed location BG Sentinel site, 2 rotating BG Sentinel sites, and the 4 fixed location gravid trap sites. In rural areas, the District set a goal of setting

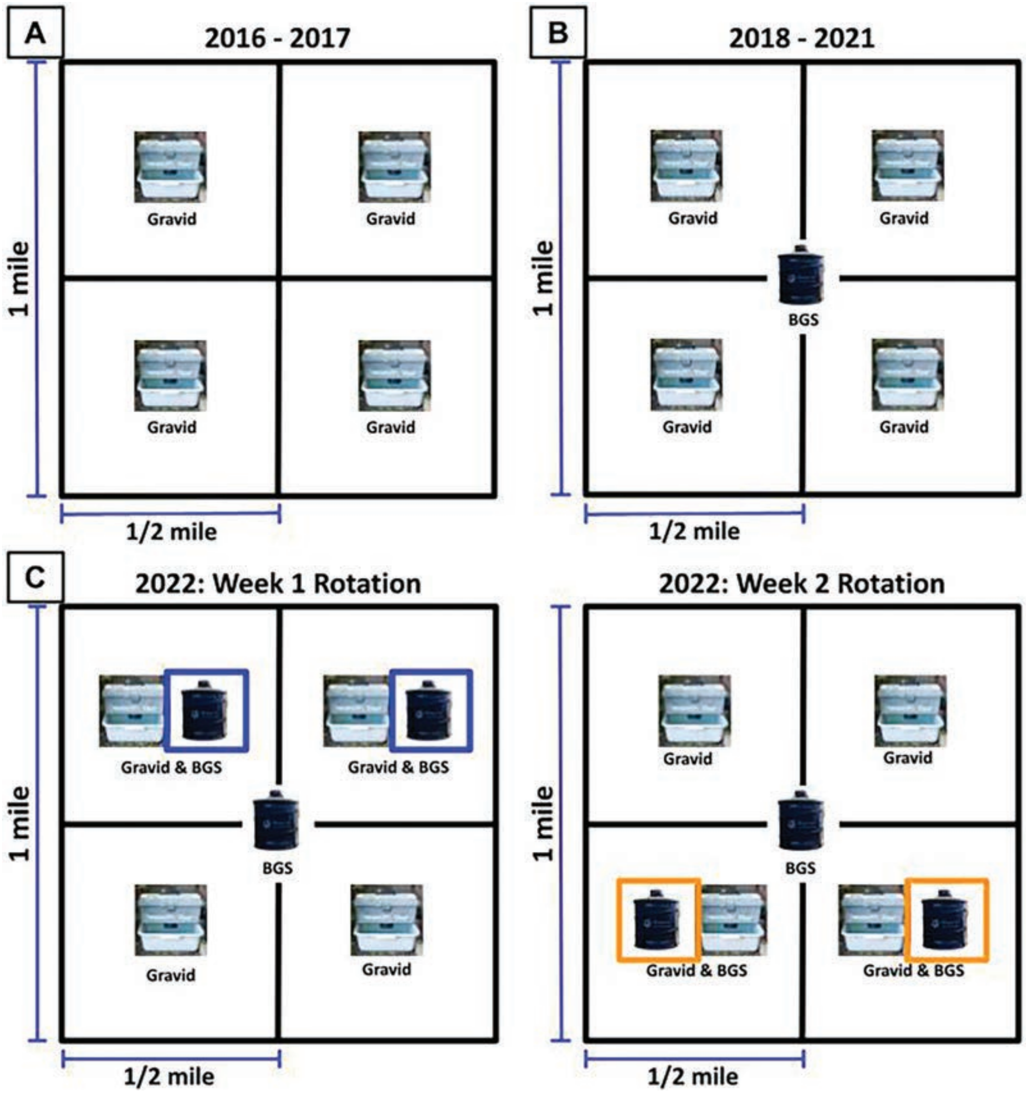

Figure 1.—Adult mosquito surveillance placement during (A) 2016 to 2017, (B) 2018 to 2021, and (C) 2022 mosquito seasons.

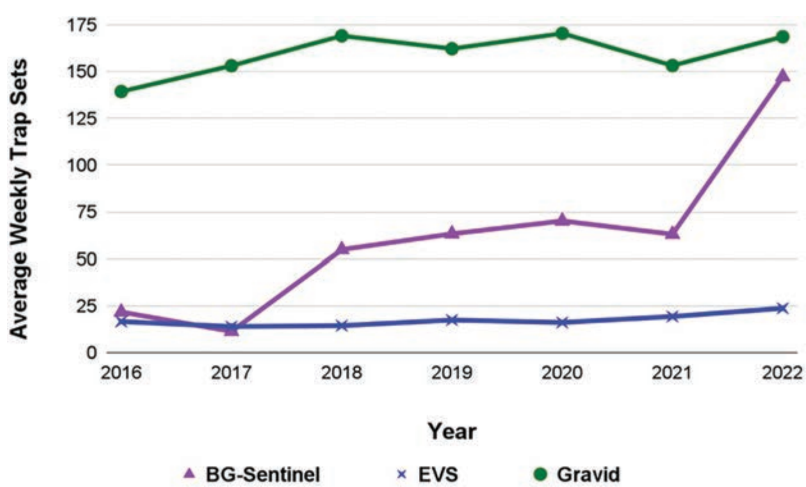

Figure 2.—Average number of weekly trap collections made from 2016 to 2022 by trap type.

28 EVS traps per week, with a 3-4 week rotation period for each site.

Results

During the 2022 mosquito season, an average of 147.3 BG Sentinel traps, 168.7 gravid traps, and 23.8 EVS traps were set per week (Figure 2). EVS trap sets increased to 6 to 7 times at 48.4% of rural sites.

Conclusion

The annual review of the expanded surveillance program demonstrated that consistent trapping, at a fine scale, is more likely to reflect true changes in mosquito abundance and arbovirus risk to the public, providing better data for control and funding decisions.

Acknowledgements

We thank the District Manager, Mustapha Debboun, and the District Board of Trustees for their continued support.

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Are you growing invasive mosquitoes in your backyard? : First detection of *Aedes aegypti* in Contra Costa County

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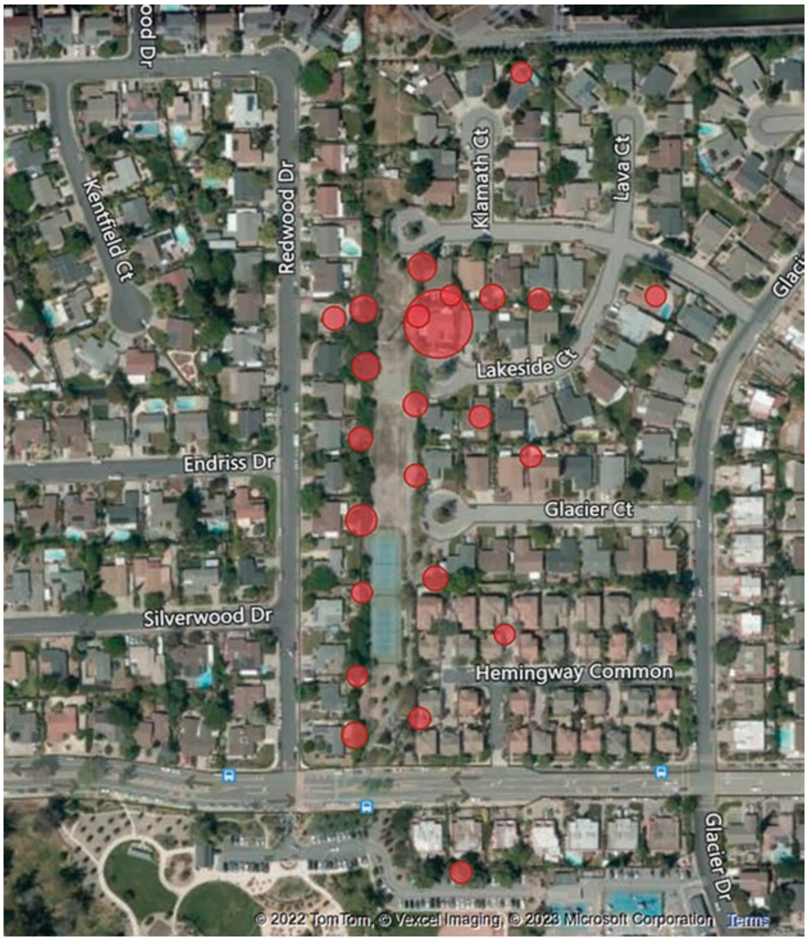
Introduction

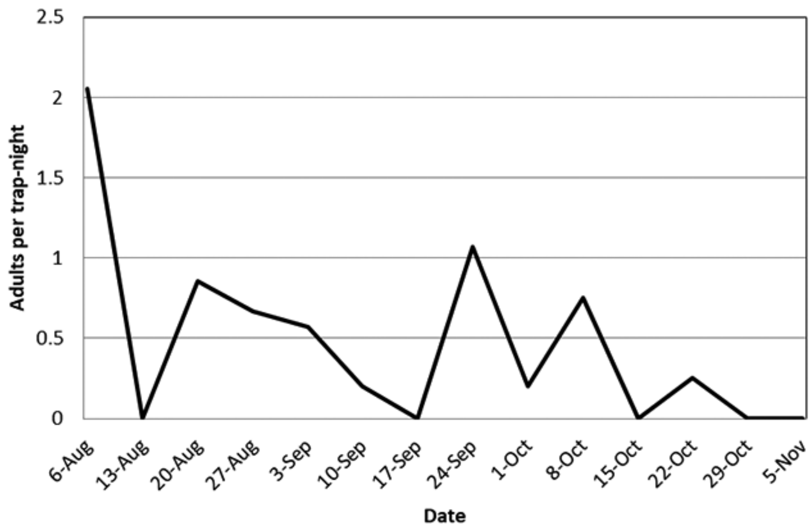
On August 2, 2022 one of our inspectors collected a single adult female *Aedes aegypti* while responding to a public service request from a resident of Martinez, Contra Costa County, California who complained of daytime-biting mosquitoes in her back yard. Laboratory staff accompanied the inspector back to the property and was able to collect additional adult specimens. This was the first detection of *Ae. aegypti* in Contra Costa County, although there had been previous detections in other nearby counties including San Mateo, San Joaquin and Sacramento (VectorSurv Maps 2022).

Materials and Methods

No larval sources were found on the property, although inspection of a neighboring residential property



Figures 1.—Locations in Martinez, CA where adult *Ae. aegypti* were collected in BG Sentinel-2 traps. Note: locations shown are approximate and do not indicate specific addresses. Size of circles is proportionate to numbers collected.



Figures 2.—Numbers per trap-night of adult *Ae. aegypti* collected in BG Sentinel-2 traps.

on the following day revealed numerous small water-filled plant saucers and other containers in the back yard, as well as an un-maintained hot tub, which contained larvae of *Ae. aegypti* in addition to local *Culex* mosquitoes. The resident of this property reported being bitten indoors, and our inspector recovered a small Mason jar used to root plant cuttings inside the house, which contained eggs and larvae of *Ae. aegypti*. A BG Sentinel-2® trap baited with BG-Lure and dry ice placed in the backyard overnight collected several male and female adults.

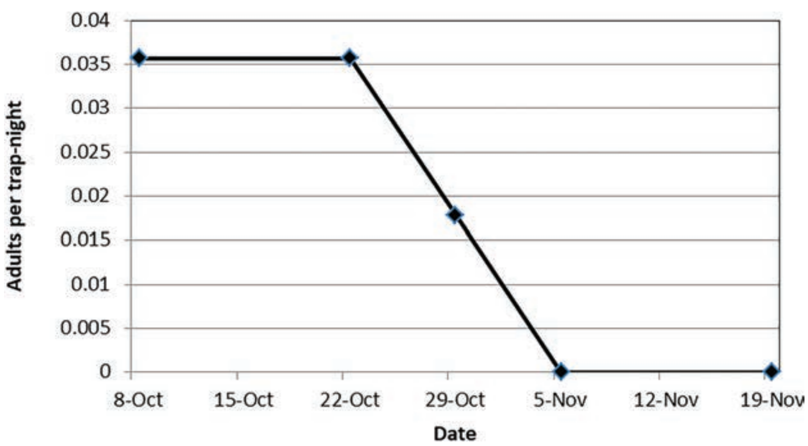
Following identification of the first *Ae. aegypti* specimens, we notified County and State Departments of Public Health and neighboring mosquito and vector control districts. On August 5, a media release was sent to local news agencies, and a mass-mailing was sent to 980 addresses within ½ mile of the initial detection to inform residents that invasive mosquitoes had been found in their area and that the District would be conducting door-to-door inspections. Over the next two months, approximately 1,600 inspections were conducted within the ½ mile radius, which was later narrowed to ¼ mile, because since the infestation appeared to be localized. All District employees, including Laboratory, Office and Public Affairs staff were asked to assist with these inspections. because many residents were not home on weekdays, rotating teams were used to conduct additional inspections on Saturdays. Containers found to be holding water were sampled for larvae and dumped or drained when possible. Larger containers such as decorative water features and unmaintained hot tubs and swimming pools that could not be drained were treated with a variety of larvicides. At locations where adult *Ae. aegypti* were found, surface treatments were done with Suspend® SC. BG-Sentinel-2® traps baited with BG Lure and dry ice (and later GAT-2 traps baited with water and powdered rabbit pellets) were placed weekly at locations with obvious harborages for adult mosquitoes. Sentinel traps were set for one night and GAT-2 traps for seven nights. Trapping and inspections continued until early November, when we were no longer collecting any adults or larvae.

Table 1.—Adult mosquitoes collected in BG Sentinel-2 traps.

Species	male	female	total
Ae. aegypti	55	96	151
Ae. dorsalis	0	12	12
An. franciscanus	0	1	1
An. punctipennis	0	1	1
Cx. pipiens	12	269	281
Cx. tarsalis	7	340	347
Cs. incidens	2	29	31
Cs. inornata	0	12	12
Cs. particeps	0	2	2

Results and Discussion

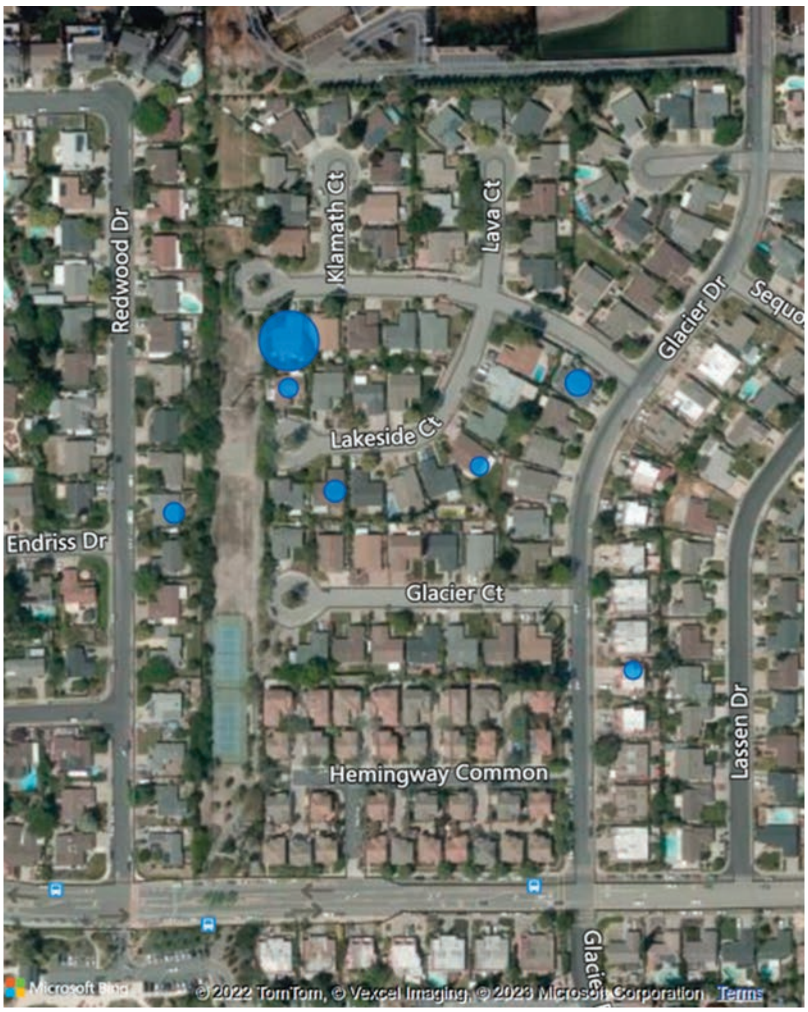
A total of 151 adult *Ae. aegypti* (55 males, 96 females) were collected in BG Sentinel® traps at 24 locations (Fig. 1) over a total of 183 trap-nights, between August 6 and October 22 (Fig. 2). All 24 locations were within less than ¼ mile of the initial detection. Because traps were baited with dry ice in addition to BG-Lure, we also collected individuals of other local mosquito species, with *Culex tarsalis* and *Culex pipiens* the most abundant (Table 1). A small number of additional *Ae. aegypt*i adults were collected in GAT-2 traps deployed in the same area, although these traps were not deployed until quite late in the season (Fig. 3). Trapping was suspended after several weeks of zero collections. Additionally, a total of 101 larvae were collected at seven locations (Fig. 4), with a range of 1-31 larvae per collection. At one site we recovered larvae on five occasions, indicating that the resident was not complying with our request to dump and drain containers. Sources included variety of artificial containers, including plant saucers, trays,



Figures 3.—Adult *Ae. aegypti* collected in GAT-2 traps.

buckets, water troughs, bird baths, and unmaintained hot tubs and swimming pools (Fig. 5).

A majority of residents were receptive to, and appreciative of, our efforts to prevent the spread of *Ae. aegypti* in their community, although some required more explanation and convincing than others, and were initially suspicious that we were 'selling something' or trying to blame them for causing a mosquito problem. Having laboratory or public affairs staff accompany our inspectors was helpful, because one could engage with



Figures 4.—Locations where *Aedes aegypti* larvae were collected. Note: locations are approximate and do not indicate specific addresses.

Figures 5.—Typical backyard artificial containers where *Ae. aegypti* larvae were collected.

the resident and answer questions while the other conducted the inspection. It also helped to have an 'extra pair of eyes', because sources were often cryptic and easy to miss. Some residents were either reluctant to allow 'strangers' onto their property, or outright hostile to what they perceived as government intrusion into their privacy. Notices were posted on properties to which we were unable to gain access, asking the resident to call and schedule an appointment. Those who failed to respond to the first notice received a second notice explaining that if they failed to contact us we would be obtaining inspection warrants. Only seven residents failed to respond after the second notice, and inspection warrants were obtained for these properties on November 7. Ultimately, only one warrant needed to be served, with a law enforcement escort.

Based on our inspection and trapping results, we appear to have caught this infestation early and found no evidence that it had spread outside of a 1/8 to 1/4 mile radius of the initial find. A probable source of introduction could not be identified. We are not confident that the population has been eradicated and will be conducting additional enhanced surveillance in the area in 2023.

Acknowledgements

The authors wish to acknowledge the entire staff and Board of Trustees of the Contra Costa Mosquito and Vector Control District for their assistance and support, and the residents of the affected neighborhood in Martinez, California for their cooperation.

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The third time is not a charm: The third time with *Aedes aegypti* in Moab, Utah

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Moab is a small, rural town in southeastern Utah known for the surrounding national parks and outdoor recreational opportunities. Moab receives substantial tourist traffic with nearly three million visitors annually, yet there are only 5,500 permanent residents. *Aedes aegypti* (L.) was collected in Moab for the first time in 2019, but an aggressive vector control campaign eliminated the species prior to the end of the season. Although *Ae. aegypti* was not collected during 2020, it was detected again in 2021 and 2022. Moab is outside of the original predicted northern range of *Ae. aegypti* estimated by the CDC. The Moab Mosquito Abatement District has a small team of employees, making it a challenge to handle routine mosquito concerns in addition to those imposed by an invasive mosquito.

Population genetic analysis was conducted on *Ae. aegypti* specimens collected from the 2019 and 2021 infestations. Specimens from 2019 were found to have likely originated from Tucson, AZ (Gloria-Soria et al. 2022). What was unique about the 2019 infestation was that it was initiated by a single female mosquito that 'hitchhiked', possibly within a vehicle. Often mosquito eggs are what is primarily thought to travel from one location to another and the 2019 infestation is a reminder that adult mosquitoes can travel just as effectively. The 2021 adult specimens were unrelated to the 2019 specimens, but instead came from a source related to those that invaded York, Nebraska in 2019, possibly from a southcentral or southeastern U.S. population (Gloria-Soria et al. 2022). Specimens collected from 2022 are currently being analyzed.

As a response to *Ae. aegypti* in Moab, an outreach project was initiated and partially funded through the Western Integrated Pest Management Center and USDA in 2022. This *Ae. aegypti* project focused on implementing an educational outreach campaign and a citizen science project geared towards reducing *Ae. aegypti* in Moab. Local students and residents participated in the monitoring of container breeding mosquitoes using ovitraps. Moab *Ae. aegypti* originating from eggs collected from ovitrap surveillance sites in 2022 will be investigated for insecticide resistance.

The citizen science project involved residents and students of Moab to engage them in monitoring activities of *Ae. aegypti*. Participants were supplied all materials for the project and attended an hour-long workshop which included training and a presentation on basic mosquito biology, arboviruses, Integrated Mosquito Management, the operations of the abatement district, and *Ae. aegypti* biology. The outreach project during 2022 laid the building blocks for future seasons to continue monitoring invasive, container breeding mosquitoes and engage the community of Moab.

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Thermal preferences of *Aedes aegypti* in California's Central Valley alters relative transmission risk

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Introduction

Temperature drives the transmission of mosquito-borne pathogens by altering traits of the ectothermic mosquito vector and interactions between the mosquito vector and the pathogen (Paaijmans et al. 2012, Mordecai et al. 2019). Current mosquito-borne pathogen transmission models primarily use air or land-surface temperatures from weather stations or thermal imagery as a proxy for the temperatures mosquitoes experience (Reiner et al. 2013). However, temperatures from these sources are an inadequate representation of the temperatures of local environments or microclimates available to adult mosquitoes (Murdock et al. 2017). A small number of studies have examined mosquito thermal preferences in the laboratory (Muirhead Thomson 1938, Blanford et al. 2009, Verhulst et al. 2020, Reinhold et al. 2022). This is the first study to examine *Ae. aegypti* thermal preferences in the field, and how those preferences may affect transmission risk.

Methods

Thirty resting boxes adapted from Edman et al. (1997) were placed in the front or back yards of 10 homes in Madera, California, for six weeks in August and September 2021 during the late-summer period of peak adult *Ae. aegypti* abundance. Temperature sensors (iButton, Maxim Integrated, San Jose, California) were placed in each box, and mosquitoes were collected by aspiration two times per day. Mosquitoes were identified morphologically. Hourly temperature data were obtained from NOAA National Centers for Environmental Information Local Climatological Data at Madera Municipal Airport, CA (Station ID: GHCND:USW00093242) for the time period studied. Analyses was performed using R software version 4.1.3 (R Core Team 2022).

Results and Discussion

Aedes aegypti chose relatively cooler microhabitats in the field as ambient temperature increased. When *Ae. aegypti* thermal preferences were accommodated, transmission risk for *Ae. aegypti*-borne viruses on representative days during peak season in Madera was relatively lower than when calculated using weather station data as a proxy for mosquito temperature.

Conclusions

Using weather station data to calculate transmission risk in California's Central Valley during peak *Ae. aegypti* season may overestimate transmission risk compared to using mosquito resting temperatures. This may explain why we haven't seen local transmission of *Aedes aegypti*-borne viruses in California.

Acknowledgements

The authors thank Istvan Menyhay and Teresa Hamilton of Madera Mosquito and Vector Control for their help organizing the study and providing laboratory space during the study period, and the residents in Madera that allowed us to place resting boxes in their yards.

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Plague, Politics and Race: The San Francisco bubonic plague epidemics of 1900-1909

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Abstract

This presentation examines the politics of vector control in suppressing bubonic plague in San Francisco in 1900-1904 and later in 1907-1909. Analyzed were the plague suppressive measures undertaken by local and federal authorities and how physicians, politicians and members of the Chinese community contested the diagnosis of plague in San Francisco and the vilification of the Chinese community. Examined were the evolution of public health bubonic plague suppressive measures in San Francisco from quarantine of the Chinatown district and forcible inoculation with an experimental vaccine of Chinese and Japanese residents to travel prevention in 1900, to fumigation, house to house inspection and rat eradication in subsequent years. Examined were how Chinese merchants and diplomats protested through the federal courts and the riots that erupted among poorer Chinese workers who were trapped by public health regulations. The public health policy of vilifying and persecuting Chinese residents during the initial epidemic in1900/01 was replaced by widespread sanitary management in 1903. When bubonic plague erupted again after the San Francisco earthquake and fire, public health authorities recognized the misguided dangers of targeting residents by race and instead implemented widespread sanitary cleaning and rat eradication.

Black Death in the City of the Future: Plague in L.A., 1924

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Abstract

In the late fall of 1924, disturbing indicators suggested that one or more varieties of plague had appeared in the densely-packed neighborhoods flanking downtown Los Angeles. Numerous deaths had been reported, and public health physicians scrambled to identify victims, name the disease(s), and institute quarantine and other procedures. This presentation examined that era and the several months of frenzied efforts made by health and other officials to eradicate plague and the vector contributors to its spread. The discussion is historical, and it takes up the various social, cultural, and racial dimensions to the region's response. An interpretive thread running through this brief presentation is that a century is not necessarily "a long time ago," in that perhaps we have not moved as far beyond the attitudes and assumptions of our forebears as we might think.

The archaeology of Macy Street and the 1924-1925 Los Angeles plague outbreak

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Abstract

Major events such as the 1924-1925 Los Angeles plague outbreak alter human activity and material culture. Archaeology provides theoretical approaches that supplement history. The current paper will use historical and archaeological data sets to analyze the conditions which led to this plague outbreak and the human response as reflected in material culture. It will focus on the Macy Street District as reflected in archaeological finds, including structural debris and refuse deposits, associated with residences along historic Clara Street. Some archaeological deposits were associated with 700 Clara Street, which was the home of Jesus Lejun and his daughter Francisca who were the first to contract the plague during the outbreak. The remains and historical data shed light on the sanitation of the district which led to the outbreak and efforts to improve that sanitation, but also households' attempts to combat the disease before it was known to be plague.

Introduction

Between October 1924 and January 1925, Los Angeles experienced the last major outbreak of human plague in the United States. The first known victim, Jesus Lejun, contracted the disease at his home at 700 Clara Street in the heart of the Macy Street District, an urban working class area northeast of downtown. Roughly ninety years later, construction of a new bus maintenance and operations facility north of today's Union Station uncovered archaeological remains of Clara Street residences. Among the archaeological features were ruins of Lejun's house and trash deposits from about the time of the plague outbreak. Together with historic documents, the remains helped tell the story of a transient immigrant neighborhood struggling with overcrowding and poor sanitation. They also told of these people's response to illness and agencies' efforts to eradicate both the disease and the conditions that led to the outbreak.

Historic Background

The Third Pandemic

By 1924, California had been fighting plague for nearly a quarter of a century. The Third Pandemic—the third time the plague swept the known world—began in Asia about 1855 and killed ten to fifteen million people worldwide before ending about 1960 (Gregg 1985; Link 1955). It reached San Francisco in 1900, sparking a human outbreak that lasted until 1904. A second outbreak in San Francisco broke out in 1907 and continued into 1908. In Oakland in 1919, a squirrel hunter contracted plague from his kill, and that infection led to the deaths of 13 others, most of them healthcare workers (Gregg 1985; Link 1955). In Los Angeles County, a boy contracted plague after contact with a squirrel (*Long Beach Daily Telegram* 1908). The possibility that plague would jump from squirrels to the urban Los Angeles rat population was a fear that was realized in 1924.

The pandemic was less deadly in the United States than in much of the rest of the world, in part, due to America's response. It began in China during an extended civil war and caused the most deaths in areas of political instability. When plague reached California, Governor Henry Gage and other political leaders initially denied its existence, leading to human deaths and Gage's political demise. But in the Los Angeles American outbreak, well-funded federal and state officials fought against the disease and won.

In late October 1924, City and County health officials encountered a strange disease in the Macy Street District of Los Angeles and unincorporated Belvedere Gardens. Later it was discovered that a mass rat die-off in the early fall preceded the human outbreak. The disease was not identified as plague until late October, when plague struck ten individuals at a boarding house at 742 Clara Street and then spread to their close contacts (Dickie 1926). Early victims were misdiagnosed with a variety of maladies, including venereal disease, flu and pneumonia. The United States Public Health Service and the Department of Public Health of California closely monitored plague in animals and people, especially in northern California. But despite occasional singular cases such as the 1908 infection, plague had never had a major outbreak in southern California.

The City of Los Angeles Department of Health published the names and addresses of known victims in a table in their annual report (McGonigle 1925). Two names that do not appear in the City's table, Jesus Lejun and Francisca Lejun, were nevertheless thoroughly discussed in

Table 1.—Victims of the 1924-1925 plague outbreak (After McGonigle 1925: 20). Variant spellings of family names in the original.

Address	Quarantine District	Name	Age	Date admitted to County Hospital	Date of Death	Pneumonic or Bubonic
1217 S. Hill	South Hill	Christionson, Josefa	58	10/31/1924	11/1/1924	P
		Hurtado, Urbana	23	10/31/1924	11/2/1924	P
1716 Marengo	Marengo	Enriqueez, Remedio	48	11/11/1924	-	B
		Moreno, Juana	26	-	11/1/1924	P
		Ruiz, Refugio	24	11/5/1924	11/6/1924	P
1804 Pomeroy	Pomeroy	Hernandez, Peter	33	11/2/1924	11/3/1924	P
2039 E. 7th	-	Dominguez, Marie	11	1/4/1925	-	B
234 N. Anderson	-	Rodriguez, Marie	1	-	11/8/1924	B
		Rodriguez, Merced	9	-	11/8/1924	B
249 N. Mariana	Belvedere	Valenzuela, Guadalupe	52	11/1/1924	11/3/1924	P
		Samarano, Mary	18	10/31/1924	11/1/1924	P
332 Bauchet	Macy Street	Herrera, Efren	51	10/30/1924	11/1/1924	P
342 Carmelita	Belvedere	Guilland, Juliana	63	10/30/1924	11/6/1924	P
343 Carmelita	Belvedere	Samareno, Maria	80	10/29/1924	10/30/1924	P
		Samarano, Victor	35	10/29/1924	11/1/1924	P
438 Bauchet	Macy	Peralta, Eulogio	22	11/4/1924	11/5/1924	P
441 S. Flower	-	McGlaughlin, Emmett R.	48	-	11/1/1924	P
507 S. Sichel	-	Costello, Mary	32	10/31/1924	-	P
5725 Brooklyn Ave	-	Hernandez, Martin (Abelganio)	15	11/23/1924	11/24/1924	B
695 Imperial	-	Perez, Jose	14	1/11/1925	1/15/1925	B
700 Clara	Macy Street	Jesus Lejun			10/11/1924	B
		Francisca Lejun	15		10/5/1924	P
712 Clara	Macy Street	Vera, Tony	23	11/6/1924	11/7/1924	P
730 Date	Macy Street	Jiminez, Jose	25	11/4/1924	11/7/1924	P
		Jiminez, Mike	27	10/31/1924	11/2/1924	P
741 Clara	Macy Street	Florez, Jessie	21	-	10/26/1924	P
		San Romano, Ruth	23	10/31/1924	10/31/1924	P
742 Clara	Macy Street	Baganola, Jose	58	10/30/1924	11/1/1924	P
		Burnett, Alfredo	11	10/29/1924	11/11/1924	P
		Gutierrez, Arthur	16	10/30/1924	10/31/1924	P
		Gutierrez, Horace	25	10/30/1924	10/30/1924	P
		Ortega, Fred	26	10/30/1924	10/31/1924	P
		Samarano, Gilbert	7	10/29/1924	11/1/1924	P
		Samarano, Guadalupe	37	-	10/26/1924	P
		Samarano, Raul	8	10/29/1924	-	P
		Samarano, Robert	10	10/29/1924	10/30/1924	P
		Samareno, Luciana	39	-	10/19/1924	P
750 Yale	-	Perinlo, Frank	40	11/1/1924	11/3/1924	P
Plaza Church	-	Brualla, Father Medrano, CMF	48	-	11/2/1924	P

City documents and were added to the Table. One additional individual, Carolina Moreno, age 14 months, appears as a pneumonic plague victim alongside Juana Moreno in the county records (Cremation Number 1440). A total of 40 victims were identified, 38 of whom died, including a Spanish missionary priest who tended the afflicted, two Irish-American healthcare workers, one individual identified as Italian-American, and 36 Mexicans and Mexican-Americans, primarily laborers.

The data suggests that the bacillus spread from fleas to humans causing five separate outbreaks (Figure 1):

- Macy Street, Belvedere Gardens, Hill Street, and outliers – approximately 31 victims, October 5, 1924, through November 11, 1924.
- Marengo and Pomeroy Streets – 4 victims, November 2, 1924, through November 11, 1924.
- Anderson Street – 2 victims, November 8, 1924.
- Brooklyn Avenue – 1 victim, November 24, 1924.

Table 2.—Archaeological sites associated with the plague in the Macy Street District.

California State Primary Number	Associated Archaeological Features	Historic-Period Address
19-004649	Feature 8 (concrete basement)	722 Clara Street
19-004650	Feature 1 (refuse deposit)	718 Clara Street
19-004651	Feature 2 (refuse deposit)	710 Clara Street
19-004652	Feature 6 (concrete basement); Feature 7 (brick wall and concrete foundation)	700 Clara Street
19-004657	Feature 3 (refuse deposit)	735 Lyon Street
19-004659	Feature 15 (brick manholes); Feature 16 (sewer pipe)	Lyon Street

Table 3.—Victims of pneumonic plague listed in Los Angeles County Department of Charities Cemetery Division Register of Cremations, October 1, 1924 through January 31, 1925.

Cremation Number	Name	Age	Sex	Race	Date of Death	Date of Cremation	Date of Permit
1428	Joe Bagnola "Begnola"	30	Male	Mexican	11/01	11/01	11/03
1429	Efren Hanard "Herrara"	51	Male	Mexican	11/01	11/01	11/03
1430	Roberto Samarano	10	Male	Mexican	10/30	11/01	11/03
1431	Mary Samarano	80	Female	Mexican	10/30	11/01	11/03
1432	Horace Guiterez	25	Male	Mexican	10/30	11/01	11/03
1433	Arthur Guiterez	16	Male	Mexican	10/31	11/01	11/03
1434	Ruth San Roman	23	Female	Mexican	10/31	11/01	11/03
1435	Fred Ortego	26	Male	Mexican	10/31	11/01	11/03
1436	Gilbert Samorano	7	Male	Mexican	11/01	11/01	11/03
1439	Juana Moreno	26	Female	Mexican	11/01	11/02	11/03
1440	Carolina Moreno (Baby)	14 months	Female	Mexican	11/01	11/02	11/03
1441	Mike Jose Jiminez	27	Male	Mexican	11/02	11/02	11/03
1442	Urbano Hurtado	23	Male	Mexican	11/02	11/02	11/03
1443	Joseph Christianson	58	Female	White	11/01	11/02	11/03
1444	Victor Samorino	35	Male	Mexican	11/01	11/02	11/03
1445	Maria Zamorino	18	Female	Mexican	11/01	11/02	11/03
1446	Frank Perinlo	40	Male	White	11/03	11/03	11/05
1147	Peter Hernandez	33	Male	Mexican	11/03	11/03	11/05
1449	Guadalupe Valenzuella	52	Female	Mexican	11/03	11/04	11/05
1454	Eulogio Peralta	30	Male	Mexican	11/05	11/06	11/06
1456	Juliana Giulland	63	Female	White	11/06	11/06	11/07
1458	Tony Vera	22	Male	Mexican	11/07	11/07	11/08
1459	Refugia Ruiz	24	Female	White	11/06	11/07	11/08
1460	Jose Jiminez	25	Male	Mexican	11/07	11/07	11/08
1461	Merced Rodriguez	12	Male	Mexican	11/08	11/10	11/12
1462	Baby Marie Rodriguez	1	Female	Mexican	11/08	11/10	11/12
1470	Alfredo Burnett	11	Male	Mexican	11/10	11/11	11/12

- Seventh Street at Imperial – 2 victims, January 4, 1925-January 15, 1925.

In each instance, one or more persons contracted the bubonic form of the disease from flea bite, but sometimes later the bacillus spread in its pneumonic form. In the first and most deadly outbreak, Lejun contracted the bubonic form of the disease. It transitioned to its pneumonic form and spread first to his

Figure 1.—Map: Distribution of victims of the 1924-1925 plague outbreak, as shown on the 1926 Alhambra and 1928 Los Angeles 1:24,000 scale United States Geological Survey topographic quadrangles (map by Jonathan Perez, Michael Baker International).

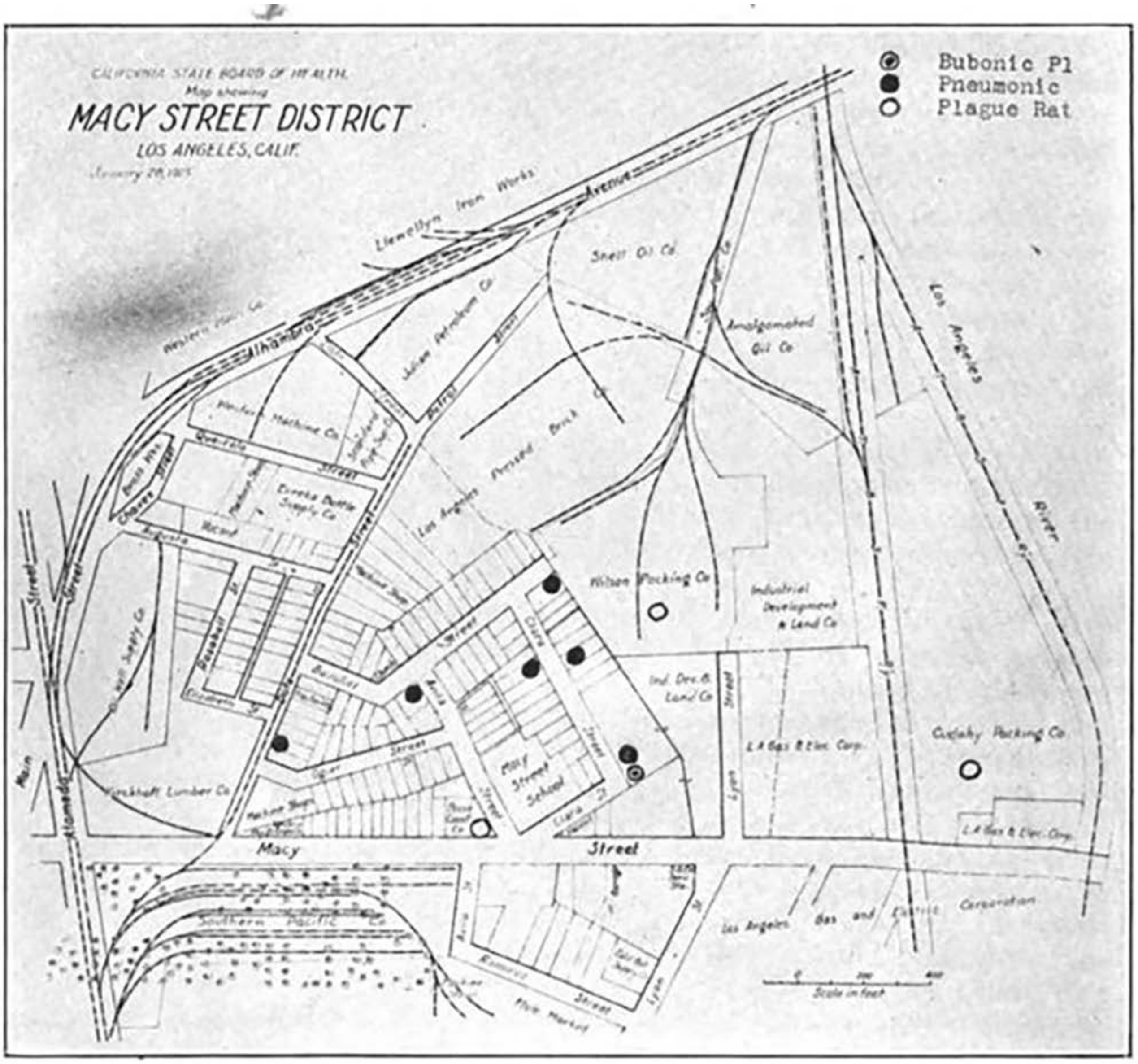

Figure 2.—Map: Cases of plague in the Macy Street District (Dickie 1926).

daughter, then to neighbors. It spread to Belvedere Gardens and elsewhere, primarily along family lines. Those who attended victims' funerals were, in turn, exposed. The later outbreaks were controlled as soon as they emerged. In each case the disease was identified before it could spread and died out with its initial human hosts.

As the origin of the human plague outbreak, it is necessary to look at life in the Macy Street District to understand the social conditions that led to the plague outbreak and its response.

Life in the Macy Street District

The Macy Street District, named for the major east-west boulevard that is today called Cesar Chavez Avenue, was an encapsulated area northeast of downtown (Figure 2). It proved easy to isolate from the world. "The district is situated at the north-east corner of the business portion of the city, in the heart of an industrial section. Its boundaries are logical: the river to the east, the Southern Pacific railroad yards to the north, and three streets to the south and the west which carry heavy traffic—Main, Arcadia and Aliso—all preclude easy transit. Together they enclose about one fifth of a square mile" (Sterry 1924).

The district was unhealthy for humans, but a haven for rats. Period maps illustrate the vast Wilson & Company Packing House bordering homes on the north side of the district, and the Cudahy Packing Company located between the residential district and the Los Angeles River. Industries occupied much of the northern part of the district (Baist 1921). In the 1920s horses were still an important

Figure 3.—Backyard of 741 Clara Street, the home where Jessie Florez and Ruth San Romano contracted plague (Photographic Documentation of Pneumonic Plague Outbreak Sites and Rats in Los Angeles, UC Berkeley, Bancroft Library).

form of locomotion, and horse waste was brought into the district for disposal.

The Commission of Immigration and Housing of California described the scene: "On the outer edge of Chinatown and near the Macy School standing on a siding are a number of cars into which manure is dumped every day, collected from stables throughout the city. Much of the manure which falls to the ground is left rotting in the sun, breeding countless flies. The adjacent packinghouses, with their corrals for sheep and hogs, and the smell and smoke from the gas plant, combine to make the place an unfortunate residence district" (Commission of Immigration and Housing of California 1916). Photographic evidence from the time of the plague confirms the unhealthy nature of the neighborhood. A photograph of the backyard of 741 Clara Street, taken after the plague deaths of Jessie Florez and Ruth San Romano, shows a yard filled with piles of wood debris: ideal rat harborage (Figure 3).

In 1923, Macy Street School Principal Nora Sterry conducted a census of the district's population. She counted 2,878 residents. Sixty percent were what she called Mexican. Approximately 19.5% were Chinese, mostly living in Chinatown, located where Union Station is today. Eleven percent were Italian, 8% were Syrian, and 2.5% were "distributed among the remaining races" (Sterry 1924). The population was predominantly male, adult and transitory. Sterry found 1.75 men to every woman, and three adults to every two children. In one year, from 1921 to 1922, a 71% "change in district personnel" occurred (Sterry 1924). Although the district had a resident rooming house and shop owners, generally people did not come to Macy Street to stay, and few of its inhabitants had reason to improve living conditions.

The district was notoriously overcrowded. The Commission of Immigration and Housing discovered up to seven persons living without beds in a single room. On Clara Street, up to five persons were crowded into a single bed (Commission of Immigration and Housing of California 1916). Sterry reported that many of her pupils had never slept in a bed in their lives (Sterry 1924). The district was primed for the rat and flea infestations that would bring the plague, and overcrowding led to swift spread of the highly contagious pneumonic form.

Plague Response

When the outbreak was first identified, it wasn't clear where the disease originated, whether plague had become endemic in rats, or if the already known host, squirrels, were responsible for the outbreak. The primary means of controlling infectious disease in the 1920s was quarantine.

Over the course of the plague outbreak, the city quarantined 16 homes (McGonigle 1925). But at the beginning, when the cause of the outbreak was unclear and the number of victims unknown, health officials decided that more than household quarantine was needed. On October 31, 1924, the City and County of Los Angeles took the unusual, but not unprecedented, action of creating what was known as a military quarantine around the Macy Street District and Belvedere Gardens. In the Macy Street District, "about eight city blocks, and which housed about 1800 Mexicans, was placed in quarantine" (Dickie 1926). Chinatown was left out of the quarantined area because it had no cases of plague. Headcounts were taken, and food was delivered to all the homes. Guards were stationed around the quarantined areas to ensure no one entered or left except on department business. However, a lot of people entered and left on a daily basis, including trappers and hunters, and charity and health workers. Residents were required to stay put.

Local, state, and federal agencies joined to combat the plague. The cooperation was not always easy, and the response was fraught with inter-agency competition (Viseltear 1974). At minimum, those agencies involved in the plague response included: United States Public Health Service, Department of Public Health of California, California State Board of Horticulture, Los Angeles County Department of Public Health, Los Angeles County Horticulture Commission, City of Los Angeles Department of Health, and City of Vernon. Civil society also contributed. The Macy Street School, Los Angeles County Charities, and Catholic Board of Charities fed the quarantined, and Spanish-speaking Catholic priests and social workers kept the calm and facilitated the work. The Baptist mission assisted and even gave the Bauchet Baptist Church to serve as a temporary laboratory (Dickie 1926; *Los Angeles Times* 1924b). Most famously, Sterry opened her school, but she also risked spreading the disease by defying rules against holding gatherings.

On November 3, city, county, state, and federal agencies met to coordinate and plan the ongoing response. State Health Secretary Walter M. Dickie and the Department of Public Health of California led the effort. An eleven-point plan was established (Dickie 1926) and included:

1. Strict quarantine of all areas where plague cases had occurred or might occur.
2. Segregation of the inhabitants of these districts and prevention of the gathering of groups of people.
3. A daily house to house inspection within the quarantined areas.
4. Hospitalization of all known contacts with pneumonic cases and hospitalization of all cases of illness found within the quarantined areas.
5. The examination of all dead bodies by physicians, and autopsies by the pathologist at the Los Angeles General Hospital, on all cases dying of unknown causes or in which a diagnosis had not been definitely established.
6. The establishment of a bacteriological laboratory for exclusive examination for rodent and human plague.
7. A special force of men for widespread trapping, poisoning, and rat-proofing of buildings.
8. The disinfection of premises by petroleum spray.
9. The tagging of all rats, mice and squirrels collected, so the locations of any infected rodent would be known and the progress of infection mapped out; and the tagging and special marking of all rodents from the harbor district.
10. The proper disposal of garbage and the separation of the rat from his food supply.
11. Eradication of ground squirrels, under the direction of the county horticultural commissioner, who had an efficient and well-organized department of rodent control.

Dickie advised the press, "More men experienced in fighting plague have been placed in the local field than were ever before gathered together in any previous epidemic" (*Los Angeles Times* 1924c). Dickie and bacteriologist Walter Kellogg served during the San Francisco outbreaks. Frank L. Kelly served as epidemiologist during the 1919 Oakland outbreak. Epidemiologist Charles H. Halliday who fought plague in Manila, and chief sanitary inspector Edward T. Ross, led rat and squirrel eradication in San Francisco and New Orleans (California State Board of Health 1924:158). They were experienced, and their plan was based on precedent.

Modern myths about the plague response can be disregarded. One is that a conspiracy of silence surrounded the plague. The Los Angeles city newspapers avoided the phrases "pneumonic plague" and "bubonic plague" for the first week, but they accurately reported the outbreak. Government officials and newspapers outside the city freely used the phrase "pneumonic plague". During the military quarantine health officials issued a daily bulletin documenting who was hospitalized and where they lived. There was no attempt on the part of government officials to pretend that there was no outbreak (Viseltear 1974). Bodies were examined with the intent to identify all cases of plague. There was no attempt to cover-up or ignore the plague.

Another myth is that healthy people were shut up in quarantine with the sick and left to die. The quarantined were provided with food and care. House inspections and hospitalizations of suspected cases and close contacts were designed to prevent unnecessary death. Eighty-two suspected cases and 114 close contacts were taken to the newly-constructed General Hospital Communicable Disease Building (Dickie 1926; Martin 1979). Most were found not to have plague. Although the treatments, which included a Mercurochrome drip and rarely plague serum, were not effective, health officials sought to care for the sick, thoroughly document the outbreak, and share that information with the public.

It is sometimes alleged that neighborhoods were quarantined simply because they were predominantly Mexican. This too was untrue. Mexican and Mexican-American laborers disproportionally suffered as a result of the plague (Deverell 2004; Feldinger 2008). But they were

Figure 4.—"Negro shack in business section," near the outbreak at 2039 East Seventh Street, prior to demolition (Photographic Documentation of Pneumonic Plague Outbreak Sites and Rats in Los Angeles, UC Berkeley, Bancroft Library).

not specifically targeted by agencies. In the first two weeks of the outbreak, when plague was identified outside the Macy Street District and Belvedere Gardens, additional locations were quarantined. They included an apartment building on South Hill Street and city blocks on Pomeroy Street and Marengo Street. In total, five locations were quarantined, but only after each saw human death. Rat deaths did not lead to quarantine, nor was any ethnicity targeted. Vast Mexican-American neighborhoods were never quarantined. The military quarantine was known to be unpopular, and it was lifted on November 13, 1924, as soon as the outbreak appeared to be controlled. No neighborhood-wide quarantines were imposed after that date. In the last outbreaks—Anderson Street, Brooklyn Avenue, and at the intersection of Seventh Street and Imperial Avenue, all of which occurred after the military quarantine was lifted, individual homes were quarantined, but neighborhoods were not.

Federal, state, and local agencies destroyed rodent hosts. The City Department of Health created a new Rodent Control Division, which eradicated 154,879 rats, 29,189 mice and 39,175 squirrels. Mice were unintended victims; so many rats were killed that mice were caught in rat traps (Peterson 1925). The California State Board of Horticulture and the Los Angeles County Horticulture Commission similarly destroyed rats and squirrels in their jurisdictions, and the United States Public Health Service controlled rodents at the Port of Los Angeles, where an average of 115.3 men worked every day, killing 203,570 rodents (Draper 1925).

The agencies also sought to eradicate rat harborage. Trash was buried, removed, or burned. A total of 2,699 premises were cleaned (Peterson 1925:68). Another myth is that the disease was used as an excuse to raze entire neighborhoods. Enforcing health and safety codes included what was known as "wrecking", which sometimes extended to building demolitions. These demolitions were done by hand by teams of men (Figures 4−5). In the summer of 1924, voters passed a new City Charter that gave the Department of Health more power to condemn and demolish homes, and so increased demolitions were anticipated. In the year 1924-1925, a total of 2,473 homes were demolished in the city (Peterson 1925). The Chief Inspector estimated that roughly 1,000 of these, less than one-half of the total, were demolished due to plague (Sweger 1925).

More often, "wrecking" consisted "of the removal of rat harborages only, that is to say, wood floors, double walls, and ceilings" (Draper 1926). In 1925 the federal Public Health Service took over plague abatement in Los Angeles (Viseltear 1974). Then "Wrecking was confined to immediate premises and adjoining properties from which

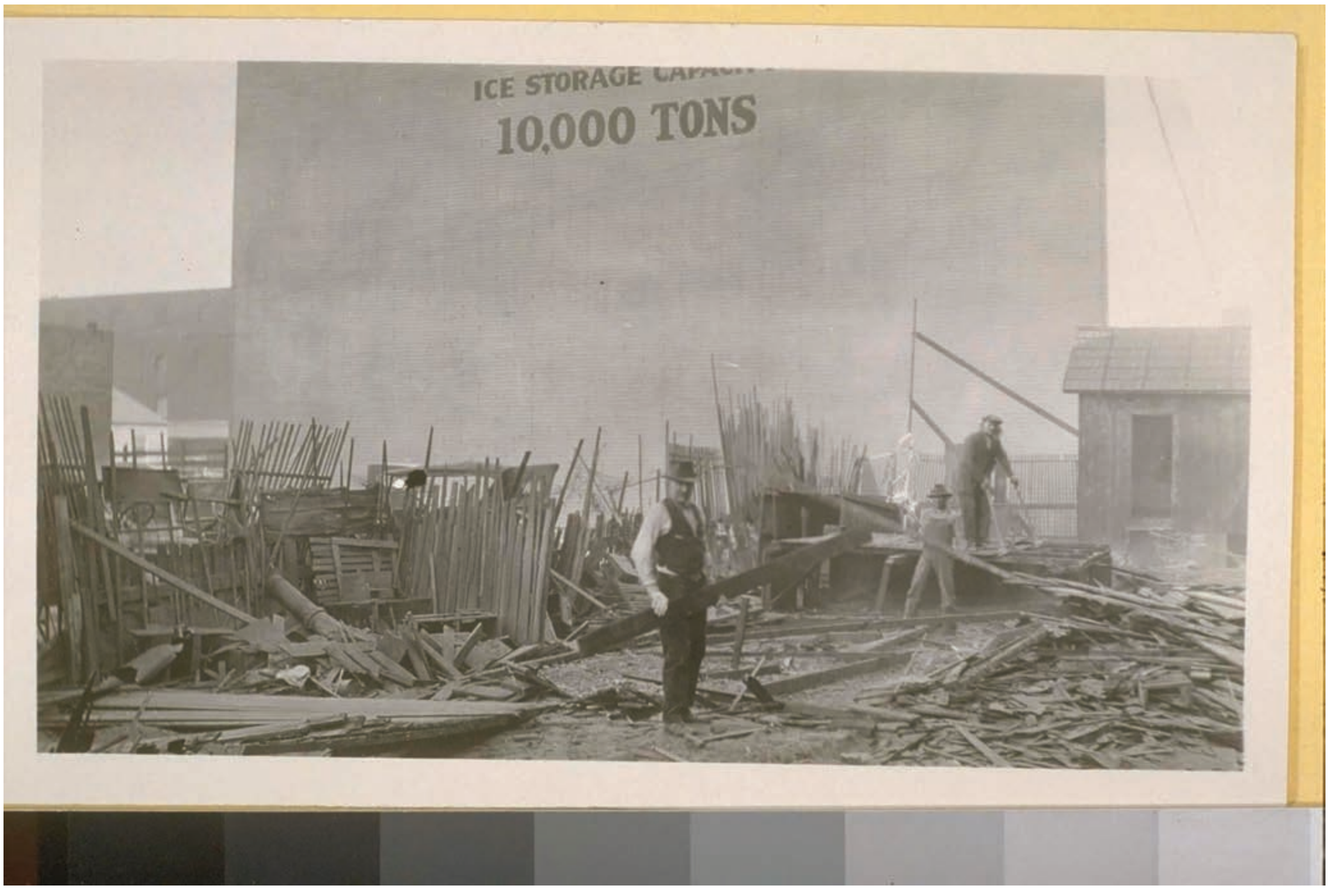

Figure 5.—"Negro shack in business section," near the outbreak at 2039 East Seventh Street, being "wrecked" (Photographic Documentation of Pneumonic Plague Outbreak Sites and Rats in Los Angeles, UC Berkeley, Bancroft Library).

infected or suspected rodents were taken. . . . It has been found that the demolition of buildings in plague work is not a good policy. It is expensive, time consuming, and devoid of any great benefits" (Draper 1926).

None of the homes along Clara Street were razed. There life quickly went on. Building records are incomplete from the period, but one shows that Joe Palroz, a landlord living in Chavez Ravine, filed for a permit to reroof 741 Clara Street just a month after two people took ill there (Department of Building and Safety 1924). The effort was to improve Clara Street, not tear it down.

An analysis of the Los Angeles City Directories dating from 1909 to 1938 showed that the neighborhood was quickly reinhabited after the plague, by the same demography as before: Mexican and Mexican-American laborers. In the 1926 city directory, a laborer named Pedro Aragon owned the house where the Lujons died. He lived there with at least two other people, a laborer named Francisco de la Cruz, and Ignes Hernandez, a woman who was not listed as either man's wife, (AECOM 2015:29). The house was quickly reinhabited by multiple and apparently unrelated adults. It was not until 1937, when Vignes Street was lengthened to serve Union Station, that the houses along Clara Street were finally vacated. Some were demolished, but others were moved to new locations (Bolger 1937; Department of Building and Safety 1937).

In the 1920s, it was far more cost-efficient to improve homes than demolish them. In the year 1924-1925, the City of Los Angeles rat-proofed 4,080 homes by lifting the baseboards up off the ground and 5,107 by adding concrete floors (Peterson 1925:68). Other jurisdictions did the same. The demolition of entire neighborhoods such as Bunker Hill and Chavez Ravine, which required heavy machinery, was still nearly two decades in the future.

Archaeology

Archaeology has the potential to gather information about the conditions that led to the outbreak of disease, the societal response to disease, and changes in beliefs and worldview caused by disease (Antoine 2008). Historic accounts and archaeological data allow us to study these themes in our not-so-distant past.

Plague Response

Material evidence of how regulators and the medical community responded to the plague is unlikely to be found. Rat traps and carcasses, petroleum sprays, empty bottles of mercurochrome and plague serum, and the cages that held

Figure 6.—"Attire worn by attendants while caring for pneumonic plague patients." A hazardous material suit including a hood constructed of a pillow case suspended on an armature

inoculated guinea pigs werer biological hazards and medical waste and therefore tended to be incinerated and thoroughly disposed of. The General Hospital Communicable Disease Building was demolished in the 1980s using modern methods and the debris removed, which left no trace of the building where scores were treated and dozens died. No archaeological sites have been reported at the hospital or laboratory locations.

Historic documents describe the changing material culture of health care. The doctor who initially identified the plague began wearing gloves during autopsies for the first time (Martin 1979). Nurses developed a hazardous material suit to protect themselves from aerosolized contamination (Figure 6). A mask was constructed of a pillowcase stretched over an armature constructed from wire coat hangers. A window cut in the mask's face was covered with celluloid. Tape sealed suit openings (Link 1955). The fact that no healthcare workers contracted the plague after it was diagnosed indicated that the suit was effective.

The Archaeology of the Macy Street District

More data is available at the household level. From 2012 to 2015 excavations for the Division 13 Bus Maintenance and Operations Facility, just north of today's Union Station, unearthed remains associated with the plague outbreak, including the 700 Clara Street home of the Lejuns. Complete details of the monitoring effort are documented in a technical report (AECOM 2015), and I have previously discussed the finds in technical detail (Beherec 2018).

A total of 18 archaeological features were encountered, some of which dated to the period of the plague. Features 1 through 3 were refuse deposits. They contained short-lived artifacts like food bottles that date to the middle-1920s, and no artifacts that postdate that time. These accumulations of household trash, found in what would have been the yards of the Clara Street homes and dating to the middle 1920s, were most likely buried during the clean-up efforts in 1924 and 1925. Their contents show evidence of cleaning and that the residents were treating symptoms that could result from plague.

Antiseptics included chemicals meant to clean people and homes. A Lavoris Chemical Company bottle held an antiseptic marketed as a mouthwash and deodorant (Fike 2006). An embossed bottle once held Florida water that was used as a cologne, but also to wash floors or in baths. Pain remedies included Hick's Capudine Liquid (for headaches and muscle aches), Dr. J.H. McLean's Volcanic Oil (a liniment for body aches), and two bottles of Foley's Honey and Tar Compound expectorant and demulcent for coughs, colds, hoarseness and children's cough. These treatments targeted the symptoms caused by pneumonic plague. Despite the fact that Prohibition was in full force in 1924 and alcohol was illegal, some of the plague victims were drunk when health officials arrived. Bottles of high-quality, imported alcohol were recovered from the trash pits. This alcohol was probably being saved for a special occasion, but instead was used to dull the extreme pain of the plague.

Some of the artifacts suggest an attempt to nurse the sick back to health. Several bottles of Horlick's Malted Milk and Mellin's Food were recovered. Horlick's was marketed as "health food for infants and invalids." Lightweight, nonperishable, and high in calories, it was often stored as emergency rations (Wisconsin Historical Society n.d.). It may have been delivered by charities to the quarantined on Clara Street, and may be the remains of attempts to nurse the early plague victims before the disease was properly diagnosed. These attempts by neighbors and relatives proved futile, but instead may have spread the disease.

Structural remains including 8-inch vitrified clay sewer pipes may not have been adequate for the overcrowded conditions that existed at the time. Two small windowless concrete basements indicate efforts to improve sanitation after the plague. They were encountered in what had been the yards of the homes at 700 and 722 Clara Street. Each measured approximately 10 feet by 15 feet and was constructed of poured-in-place rebar-reinforced concrete walls, six inches thick, and surviving down to 10 feet in depth. The tops of the walls and the roofs were torn off when the basements backfilled and the associated houses

Figure 7.—The ruins of Jesus Lejun's home at 700 Clara Street.

removed. What appeared to be metal drain openings were observed in the base of each. These structures appear to be toilets constructed after the original houses were built. The poured-in-place, rebar-reinforced concrete construction resembles nearby constructions from the 1920s and appear later than the original construction of the frame homes. Broken toilet fragments found in the Feature 2 refuse deposit also testify to the renovations at the time.

Low brick wall stubs and a partial concrete floor of Lejun's house were also uncovered (Figure 7). The floor was apparently installed as a response to the plague. No photographs of 700 Clara Street have been located, but all the available information indicates that it was a small frame cottage constructed in 1905 (Baist 1921; *Los Angeles Herald* 1905; Sanborn 1906).

The concrete pad appeared to be a deviation from these descriptions, but was likely a rat-proofing measure implemented in response to the plague (Peterson 1925:68).

Final Disposition of the Deceased

Analysis of the treatment of the deceased is one of the strongest tools of archaeology to understand the status of the dead and the beliefs of the living.

The people of the Macy Street District lived in a predominantly Roman Catholic community. The authorities counted on the fact that the Mexican-American community would reach out to the Church for help in their distress, and enlisted the aid of Catholic Charities and priests. The plague victims were at least nominal members of the Catholic Church. Catholic treatment of the dead before the reforms of Second Vatican Council in the 1960s was bound up with a belief in bodily resurrection. Cremation increased in popularity during the twentieth century, but the Code of Canon Law was unequivocal: "The bodies of the faithful departed shall be buried, their cremation being reprobated" (Canon 1203, Section 1; Peters 2001). The families would normally have desired Christian burial.

A study of Los Angeles burial records may identify the locations of some of the plague victims. Those who died before November 1, 1924, were most likely buried. The plague spread at Lucina Samarano's funeral. A search for their final resting places, located only one grave associated with the plague—that of the Spanish missionary priest Father Medardo Brualla. Father Brualla's order, the Claritins, obtained a permit to bury him in a sealed casket (Feldinger 2008:86), and did so in the old cemetery along the east wall of Mission San Gabriel. Today it is the burial ground of the Claritin Order. In 1924 these efforts were a sign of his high status.

Completed early in 1924, the Los Angeles County Crematory is the only building intimately associated with the outbreak that still stands. Cremation records indicate that 27 individuals listed with the cause of death "pneumonic plague" were cremated between November 1 to 11, 1924 (Department of Charities 1923-1937:22-29). With one possible exception, all were cremated before a permit was issued. By contrast, those who died of any other cause during this period were invariably cremated after the issuance of a permit. The haste with which the 27 were cremated without permits suggest urgency on the part of the Department of Public Health and a lack of control on behalf of next of kin. The ashes of those cremated were usually buried on the crematory grounds (Gust et al. 2007). After the military quarantine was lifted and the emergency seemed over, no more cremations of plague victims took place. Bodies were often the subjects of study. Nine were autopsied at the General Hospital (Martin 1979). The first, Guadalupe Samarano, was such an object of interest that his autopsy's findings were published in the General Hospital's official history (Martin 1979).

Some of the early victims were likely exhumed, studied and disposed. It is unclear from the records how some of those who died in October were diagnosed with plague, and a strange collection of news articles in the first week of November 1924 report that Jesus Lejun was mysteriously alive. "Loujon is alive and will recover, it is believed," *The San Pedro Daily Pilot* reported on November 6 of the man who had died on October 11 (*San Pedro Daily Pilot* 1924); and this claim appears in other newspapers (e.g., *Los Angeles Times* 1924a; *Pomona Progress* 1924). It is a surprising inaccuracy in reporting at a time when the State Board of Health worked very hard to control the flow of information. Dickie stated that Lejun "was located on October 31, at which date a culture was made from the sinus at the site of the old bulbo" (Dickie 1926). Somehow, 20 days after his death, doctors had access to Lejun's body.

The most likely explanation for the strange press stories and the doctors' ability to probe his bulbo is that his body was exhumed. Knowing the exhumation would be unpopular, I speculate that Dickie deceived the press. What happened to the body after October 31 remains unclear. In the search for early plague victims, others whose cause of death resembled plague may have been exhumed and studied.

Such treatment reflects both fear of the disease and the decedents' roles in society. Brualla's superiors had the influence and resources to bury him. However, those whom he labored to serve lacked the power to save their loved ones' bodies from summary cremation.

Conclusion

In 1924 and into 1925, at least 39 people died in five separate outbreaks, and Los Angeles was only saved from a more deadly outcome by decisive public health action based on experience drawn fighting earlier outbreaks.

The plague first spread to humans in the Macy Street District. Blending history and archaeology, one can witness that poor sanitation led to the plague, responses to plague symptoms, and efforts expended to clean up and prevent such an outbreak occurring again.

I hope this review will honor those who perished in the plague and those who fought it, and that it may serve as some small aid to the Mosquito and Vector Control Association of California, which prevents such misery and death from returning to Los Angeles one century later.

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A forty year (1983-2022) review of environmental surveillance and ecology of plague in California

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Abstract

Plague, a flea-borne disease caused by the bacterium *Yersinia pestis*, was introduced into California in 1900. It soon moved from commensal rodents at port cities to sylvatic rodent species, and subsequently spread throughout the state. The California Department of Public Health (CDPH) and partner agencies have long been responsible for monitoring and responding to periodic increases in plague activity in wild mammals to help reduce the occurrence of human cases. Plague ecology in California has evolved since its introduction, with a changing understanding of the species and geographic regions important to the maintenance and potential transmission of plague to humans. Here, the past forty years (1983-2022) of environmental surveillance data was reviewed with an emphasis on providing a contemporary view of plague ecology in California. Described was the recent spatiotemporal distribution of plague and the roles of primary rodent hosts and their fleas. Briefly reviewed was a recent analyses conducted by CDPH and collaborators that evaluated sampling biases and the use of rodent and carnivore surveillance data to predict the distribution and climatic relationships of plague in California.

The history of plague surveillance in Alameda County, California

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Abstract

Plague (*Yersinia pestis*) became established in Alameda County in 1909 after, it is assumed, infected rats and fleas crossed the bay from San Francisco to the City of Oakland. The bacillus then spread to ground squirrels county-wide and over the next two decades a ground squirrel erradication program was established and run by the U.S. Public Health Service to try and stop the continued spread of plague in the county. The last documented human case of plague in Alameda County was in 1970. Alameda County Health Care Services Agency conducted plague surveillance in 1981 and found two coyotes and one ground squirrel positive for plague from two different areas of the county. A wide scale plague surveillance program has not been conducted in Alameda County since the 1980s, and Alameda County Vector Control Services District plans to resume plague surveillance in the summer of 2023.

Introduction

The following is a history of how the plague bacillus (*Yersinia pestis*) became established in Alameda County, California, in the early 1900s and the human cases and disease surveillance that followed its introduction. Plague was brought to San Francisco, California, in 1900, when it was introduced via rats and fleas that dispersed from a ship docked in the harbor (Creel 1941). Creel (1941) hypothesized that a ship, which had docked in Honolulu the year before and had introduced plague to Hawaii, was the same vessel that later docked in San Francisco and introduced plague to California.

It is well documented that over the next 2-3 years, 121 human cases of plague were reported in San Francisco, with 113 deaths by 1904 (Kellogg 1920); almost all of these deaths were confined to the Chinese quarter of the city (Creel 1941). "The infection was pronounced eradicated in 1904, based solely upon the cessation of human cases. However, no rodent survey was maintained and from subsequent developments, we know that the rodent infection was merely latent, to be spread throughout San Francisco after the catastrophe of 1906" (Creel 1941). After the devastating San Francisco earthquake in 1906 leveled a large portion of the city, the most likely scenario is that the infected rats and associated fleas scattered to other areas of the city and beyond. In May of 1907 new cases of plague began to appear outside of San Francisco, with 13 cases documented by the end of August 1907 (Kellogg 1920). Twelve of those cases were reported from the city of Oakland and one case was reported from Berkeley (Alameda County HCSA 1981). During the period of 1907-1908, 126 rats infected with plague were collected in the city of Oakland, with the last infected rat detected in October 1908 (Kellogg 1920).

The first documented human case of plague being contracted in Alameda County occurred in 1909. A boy contracted bubonic plague while hunting ground squirrels in the city of Sunol (Creel 1941). While no direct evidence from that time exists, it seems very plausible that the rats carrying infected fleas made their way across the estuary that separates San Francisco from Alameda County and the City of Oakland, and that these rats had direct contact with uninfected rats along the waterfront areas of the city. An eradication program was started by the U.S. Public Health Service in 1908 upon the discovery of infected ground squirrels in Contra Costa County (Creel 1941).

In 1909, 5,000 ground squirrels were trapped and examined for plague in Alameda and Contra Costa Counties and 42 were found to be infected with plague (Creel 1941). Alameda County shares its entire northern border with Contra Costa County. In 1910 an additional 122,000 ground squirrels were shot and examined with 386 found to be infected with plague (Creel 1941). By this time, plague had spread to eight California counties (Contra Costa, Santa Clara, Alameda, San Benito, Santa Cruz, Stanislaus, San Joaquin and Merced), with the greatest infection rates being detected in animals trapped in Alameda and Contra Costa counties (Creel 1941). In 1912, 56,000 ground squirrels were trapped and examined and 613 were found to be infected. This was an infection rate of roughly 11 per 1,000 animals, with the highest numbers of infected animals once again coming from Alameda and Contra Costa counties (Creel 1941). In 1915, plague was again detected in Alameda, Contra Costa and San Benito Counties when 30,000 ground squirrels were trapped. The rate of infection was 1.3 per 1000 animals, with 39 animals

Table 1.—Rodents trapped and tested for plague antibodies in Alameda County, 1981.

Species Trapped	Common Name	Location	N	Positive
Spermophilus beecheyi	Beechey's Ground Squirrel	Camp Parks Military Base	24	1
		Sunol Regional Wilderness	11	0
		Sycamore Grove Park	19	0
		Calaveras Reservoir	12	0
		Del Valle Regional Park	25	0
Peromyscus truei	Pinon Mouse	Sunol Regional Wilderness	1	0
		Sycamore Grove Park	1	0
Neotoma fuscipes	Dusky-footed Woodrat	Sycamore Grove Park	2	0
		Total	95	1

infected with plague. More infected ground squirrels were found in Alameda County, and six additional California counties, in 1916 (Creel 1941). In 1917, an additional 124 infected squirrels were examined from Alameda, Contra Costa and San Mateo Counties, with an infection rate of 2 per 1000 animals (Creel 1941).

A series of thirteen pneumonic cases of plague occurred in 1919 in the City of Oakland. The first confirmed case being a boy exposed to plague-infected ground squirrels (Kellogg 1920, Creel 1941). In 1924, rats were found to be infected with plague at the Oakland city dump. "Circumstantial evidence pointed to infection from the ground squirrels on the outskirts of Oakland and the possibility of an infected rodent being picked up dead and deposited in the household garbage and from there transported to the city dump where infected fleas lighted up an epizootic among Norway rats." (Creel 1941). From 1909, when the plague surveillance program began by the U.S. Public Health Service, until 1919, 431 infected ground squirrels were captured in Alameda County (Creel 1941). An additional 2000 infected ground squirrels were collected in Contra Costa County during the same time period (Kellogg 1920, Creel 1941). Plague was reintroduced into Oakland in 1924 by infected ground squirrels (Creel 1941).

From 1920 – 1934, the plague control program statewide began to wane and in 1927 control of ground squirrels and rodents was turned over to the State Department of Agriculture (Creel 1941). "This served the double purpose of utilizing a force already engaged in squirrel suppressive measures for economic reasons and at the same time did not unduly publicize the continued prevalence of the infection in California." (Creel 1941). "A survey by the U.S. Public Health Service forces starting in 1935 showed in progressive years that the infection had spread into Nevada, Idaho, Montana, and Utah, gradually infiltrating into Arizona, New Mexico and Wyoming. In addition to ground squirrels, prairie dogs, wood rats, and various other wild rodents were found to be infected." (Creel 1941).

Another human case was not detected in Alameda County until 1970, when an 8 year-old boy who contracted plague in Shasta County from ground squirrels at a recreational area was diagnosed in Alameda County, where he recovered. (Nelson 1980). From January 1, 1981 – December 31, 1981, the Alameda County Health Care Services Agency conducted a plague surveillance and suppression program in the county. The historical presence of human plague cases and the spread of plague throughout the county had resulted in the establishment of a permanent natural foci among the wild rodent population. (Alameda County HCSA 1982). Alameda County Health Care Service Agency (HCSA) oversees the Environmental Health Department and "is responsible for the administration and coordination of plague surveillance and suppression activities." (Alameda County HCSA 1982).

Methods

The following section summarizes plague surveillance conducted by Alameda County (HCSA) in 1981, the full study can be found online and is open access. Seven areas of the county were identified as important to survey based on their epizootic plague history. These specific areas support a chaparral habitat, along with an increased suburban/rural interface and recreational usage.

Ninety-five blood samples from commensal rodents and an additional twenty-five blood samples from large carnivores, i.e. coyotes and bobcats were sent to the Centers for Disease Control laboratory in Fort Collins, CO, for serologic testing. For the full Materials and Methods, please see the Alameda County Health Care Services Agency Plague Surveillance and Suppression Program Report of Activities January 1, 1981 – December 31, 1981. In addition, all specimens were examined for ectoparasites.

Results

Samples were collected from five of the seven designated areas from August 20 – September 23, 1981. The 95 rodent blood samples comprised: 91 Beechey ground squirrels (formerly *Spermophilus beecheyi* now *Otospermophilus beecheyi* Richardson, 1829), 2 Pinon mice (*Peromyscus truei* Shufedlt, 1885) and 2 Dusky-footed wood rats (*Neotoma fuscipes* (Baird, 1858) (Table 1). One Beechey ground squirrel tested positive for antibodies against plague with a titer level of 1/128. The animal was trapped at Camp Parks Military Base in the city of Dublin. This was the first campestral rodent to test positive for plague in Alameda County since 1942. Six Norway rats collected in Oakland also tested positive for plague in 1943 (Alameda County HCSA 1982).

Table 2.—Large carnivores tested for antibodies against plague in Alameda County, 1981.

Carnivore Species	Location	Number of Samples	Sex	Positive for Plague
Coyote	Sunol	9	7F/2M	2
	Livermore	15	7F/8M	0
Bobcat	Pleasanton	1	1F	0

Twenty-five large carnivore sera were collected in Alameda County, including 24 coyotes (*Canis latrans* Say 1819) and one bobcat (*Felis rufus* Schreber 1777). Blood was collected via Nobuto strips and tested by the CDC laboratory in Fort Collins, CO. Two coyotes tested positive for plague antibodies with titer of 1/64 (Table 2). Both coyotes were captured in the area near the city of Sunol. These were the first large carnivores testing positive for plague in the county since 1943 (Alameda HCSA 1982).

The second objective of this study was to collect ectoparasites from the rodents to determine the diversity and abundance of flea species. Overall, 106 rodents were combed for ectoparasites: including 102 *S. beecheyi*, 1 *N. fuscipes* and 2 *P. truei* (Tables 3, 4). No fleas were recovered from the dusky-footed wood rat or the Pinyon mice. A total of 3,714 fleas comprising three flea species were collected from 102 Beechey's ground squirrels: *Hoplopsyllus anomalus, Oropyslla montana* and *Echidnophaga gallinacea*. The most common flea collected was *H. anomalus,* with 1,530 fleas collected from 42 animals from Sycamore Grove Park in the city of Livermore, for a flea index = 36.4. An additional 917 fleas were collected from 10 animals at Camp Parks Military Base in Dublin for a flea index = 91.7. A total of 1,020 *O. montana* were collected, with the highest total numbers being from Sycamore Grove Park (N = 353, flea index = 8.4) and Del Valle Regional Park in Livermore (N = 369, flea index = 23.1). A total of 315 *E. gallinacea* were collected, with 153 from 16 Beechy ground squirrels from Del Valle Regional Park in Livermore (flea index = 9.6). Of the three flea species collected, *O. montana* is considered the most efficient at transmitting plague, with *H. anomalus* second (CDC 2023). A possible transmission cycle occurred at Camp Parks Military Base, which had the highest flea index = 91.7 of *H. anomalus,* and where one Beechy ground squirrel tested positive for plague antibodies.

The other ectoparasites collected included lice, mites and ticks. Ticks were collected off rodents from Calaveras Reservoir (N = 2) and Sycamore Grove Park (N = 2). Mites were collected from rodents collected at Calaveras Reservoir, Sycamore Grove Park and Sunol Regional Wilderness. The highest numbers of mites (N = 280) were collected from animals trapped in Sunol Regional Wilderness. Lice were collected from all five locations: Calaveras Reservoir, Camp Parks, Del Valle Regional Park, Sunol Regional Wilderness and Sycamore Grove Park. The areas that had the highest lice abundance were Sunol Regional Wilderness (N = 108) and Sycamore Grove Park (N = 96) (Table 4).

Ninety-five rodent samples were submitted to the CDC laboratory in Fort Collins, CO, for serological testing and one sample from a Beechey's ground squirrel (from the City of Dublin) came back positive for plague. Twenty-five large carnivore samples were also submitted for testing and two samples, taken from coyotes in the Sunol area, came back positive for plague antibodies. Since this study was completed in 1981, Nobuto strips samples sent to the California Department of Public Health Laboratory has been the only plague surveillance conducted in Alameda County. No additional rodent surveys have been completed. However, the same animal species are still present in the, so the possibility of sylvatic plague still being present in the more rural areas is possible. Currently our District routinely traps commensal rodents county-wide for pathogen testing and surveillance, and we intend to start a plague surveillance program within the next year. Plague surveillance will consist of obtaining blood samples from rodents and wildlife in the County for antibody testing and testing the associated fleas for the presence of the plague bacillus.

Table 3.—Fleas collected from rodents trapped for plague surveillance in Alameda County, 1981.

	Total # of Animals Surveyed	*H. anomalus*			*O. montana*			*E. gallinacea*		
Location	N	# of Fleas	Average # per animal	% Spp by Site	# of Fleas	Average # per animal	% Spp by Site	# of Fleas	Average # per animal	% Spp by Site
Calaveras Reservoir	10	436	43.6	75.7	93	9.3	16.1	47	4.7	8.25
Camp Parks	10	917	91.7	91.1	78	7.8	7.8	11	1.1	1.1
Del Valle Regional Park	16	474	29.6	47.6	369	23.1	37.1	153	9.6	15.4
Sunol Regional Wilderness	28	357	12.7	73.6	127	4.5	27.2	1	0.3	0.2
Sycamore Grove Park	42	1530	36.4	77	353	8.4	17.8	103	2.5	5.2
Total	**N=106**	**3714**	**35**	**73.6**	**1020**	**9.6**	**20.2**	**315**	**3**	**6.2**

Table 4.—Ectoparasites collected from rodents as part of the plague study in Alameda County, 1981.

Location	Total # Animals Surveyed	Lice (Total #)	Average # per Animal	Mites (Total #)	Average # per Animal	Ticks (Total #)	Average # per Animal	Total Fleas	Average # per Animal	Total Ectoparasites
Calaveras Reservoir	10	45	4.5	16	1.6	2	0.2	576	57.6	639
Camp Parks	10	43	4.3	-	-	-	-	1006	100.6	1049
Del Valle Regional Park	16	96	6.0	38	2.4	-	-	996	62.3	1130
Sunol Regional Wilderness	28	108	309	280	10.0	4	0.1	485	17.3	877
Sycamore Grove Park	42	77	1.8	199	4.7	4	0.1	1986	47.3	2266

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LA County Agricultural Commissioner Weights and Measures Department's response to plague, past and present

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Abstract

In 1917, an amendment to the Political Code added animal pests to the pests which came under the jurisdiction of the Horticultural Commissioner (now the Agricultural Commissioner). This gave the Horticultural Commissioner authority to abate ground squirrels by service of notice (public nuisance). This authority was sought to boost agricultural production due to the worldwide food shortage caused by World War I. The first squirrel drive was organized in 1919 under the newly appointed Superintendent of Rodent Control, Mr. L.S. Neville. During 1924-25, following the bubonic plague outbreak in Los Angeles, the ground squirrel eradication work in plague areas was placed in the hands of the Horticultural Commissioner. Ten Plague Districts were created in the county between 1924 and 1942 with a total acreage of 572,600. Over the years several different rodenticides were used, including strychnine, thallium, carbon bisulphide, zinc phosphide, sodium fluoroacetate (1080), tetrachloroethane and diphacinone. Many agencies cooperated in this project including the LA County Department of Health, California State Department of Public Health, US Forest Service, US Fish and Wildlife Service, Works Progress Administration (WPA) and the LA County Agricultural Commissioner Department. This presentation provides a short overview of this history, where the program is now and where it might be in the future.

A recent history of plague activity at South Lake Tahoe

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Abstract

From 2012 to 2022 plague (*Yersinia pestis*) activity has been detected annually in South Lake Tahoe. The California Department of Public Health Vector-Borne Disease Section has tested over 600 rodent blood samples, 78 rodent carcasses, and over 500 flea pools from this area to detect locations of increased plague activity and human risk. Detections of increased plague transmission led to the closure and insecticide treatments in several recreational areas on United States Forest Service lands. A recreational site closure and treatment in 2020 was the result of a reported human plague case and the subsequent environmental investigation, which led to the testing of over 200 rodents and 150 flea pools. This presentation included a review of plague history and ecology in South Lake Tahoe, including the testing, treatments, and case investigations conducted over the previous 11 years.

Sylvatic plague outside California: Lessons from plague in prairie dogs from the Great Plains

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Introduction

Plague is believed to have invaded the Rocky Mountains and the Great Plains regions by the 1940s, presumably through infections of ground-dwelling squirrels or woodrats. The most conspicuous current epizootics were in prairie dogs (genus *Cynomys*), disease patterns that, on the surface, differed from those seen in California rodents.

Methods

Herein, I discuss findings from nearly a decade of plague research and its ecological consequences in black-tailed prairie dog (*C. ludovicianus*) colonies in the shortgrass prairie of eastern Colorado. Epizootics spread rapidly in these social, burrowing rodents, usually resulting in mortality of more than 90% of individuals. Key questions were then: 1) how the non-native pathogen, *Yersinia pestis*, persists when it causes such high mortality of its primary host, and 2) how disease spreads from one spatially isolated colony to another. These problems have major conservation implications because of the importance of prairie dogs as prey for the endangered black-footed ferret (*Mustela nigripes*) and as an ecological engineer that increases landscape-scale diversity in western grasslands.

Results

Plague outbreaks were influenced by weather conditions during El Niño events that likely improved survival and population growth of prairie dogs and their fleas. During these periods, some 50-82% of colonies were extirpated by plague, creating temporal synchrony in colony extinctions. Only 11% of extirpated colonies had suffered a plague event during the previous 5 years, suggesting that colonies were not persistent foci. Large colonies were nearly as likely as the smallest ones to be extirpated during epizootic events, and proximity to a large colony suffering plague increased the odds that a neighboring colony also was extirpated. Prior to the appearance of plague, these large colonies probably persisted indefinitely and, through dispersal, could have rescued nearby small colonies that would have been more prone to extinction through natural causes (flooding, predation). In these ways, the plague invasion has greatly modified the metapopulation structure and dynamics of black-tailed prairie dogs, with important consequences for rangeland natural resources management.

A major focus of our research effort was investigating the potential role of alternate rodent hosts in the epizootic spread and maintenance of plague. Through intensive field studies of rodent and flea populations in and out of colonies and laboratory studies of plague exposure and host feeding relationships, our results implicated the northern grasshopper mouse (*Onychomys leucogaster*) as potentially involved in epizootic spread. Unlike other rodents, these carnivorous mice were abundant within colonies, especially those colonies that eventually suffered plague, were seropositive for *Y. pestis*, and suffered modest population declines during epizootics. Grasshopper mice were infested by and fed upon by prairie-dog fleas, with very high loads during periods of prairie dog mortality. They also could be infected with *Y. pestis* by consuming carcasses. Grasshopper mice are wide-ranging and visit multiple prairie dog burrows each night in search of prey. We speculate that, at moderate to high densities, grasshopper mice carrying infected fleas between burrows could increase the functional connectivity of a colony across prairie dog territorial boundaries and thereby spark an epizootic. That said, we found no evidence of plague exposure in grasshopper mice or any other rodents or their fleas outside of epizootic events or off prairie dog colonies, suggesting that neither rodents nor fleas were a long-term reservoir for *Y. pestis*. This suggested the involvement of another environmental reservoir, e.g., soil or buried carcasses, cryptic enzootic persistence within prairie dogs themselves (perhaps due to resistance to mortality), or the transport of plague at the landscape scale through prairie dog dispersal or movements of mammalian carnivores that spread infected fleas between colonies.

Conclusions

Our understanding of plague in prairie dogs has benefited from several aspects of the prairie-dog system that seemingly make it different from sylvatic plague in California. Prairie dogs live in discrete, spatially isolated colonies whose size and plague status can be readily mapped and monitored on the landscape. Prairie dogs themselves are conspicuous, making population crashes obvious, and burrows can be monitored easily for signs of activity and fleas. Vegetation of the grasslands inhabited by prairie dogs is structurally simple compared to many areas where plague occurs in California, supporting much lower densities and lower diversity of alternate rodent hosts, i.e., no chipmunks or woodrats. The reliance of species of conservation concern on prairie dogs and their important ecological role has attracted interest and funding for basic research, whereas studies of plague in California have tended to focus on public health surveillance. Although there is greater support for the involvement of alternate rodent hosts as enzootic reservoirs and for predictable plague foci in California, more intensive ecological studies are needed to elucidate the dynamics of sylvatic plague across the state and the extent to which the two systems are truly different.

Bubonic plague disease ecology in urban-wildland interfaces in Orange County, California

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Abstract

Orange County is home to 3.2 million residents who reside in urban and suburban neighborhoods traversed by flood control channels, green belts, and open spaces that interface with the Cleveland National Forest, Chino Hills State Park and Puente Hills Habitat Preservation Authority. Several bubonic plague-competent flea species and mammalian hosts reside within these ecotone communities, and plague-positive rodents have been detected at three separate wildland interfaces in the county. Prior to a national Boy Scout Jamboree in 1953 in Newport Beach, several plague-positive rodents were found at the proposed meeting location. Following detection, the site and surrounding area were subjected to intense rodent control and monitored by state and county health departments to ensure the safety of participants for the gathering. In 1975 the Orange County Mosquito and Vector Control District (District) assumed responsibilities for bubonic plague surveillance from the Orange County Health Department. The District's program has consisted primarily of trapping and sampling wild and commensal rodents in ruderal and natural habitats bordering residential developments and performing in-house testing of mammalian sera using a CDC-supplied antibody assay. Serologic detections of plague-positive California ground squirrels (*Otospermophilus beecheyi*) occurred at a golf course in 1982 and in a roof rat (*Rattus rattus*) captured in 1998 at a home located next to a riparian corridor. Although no human cases of bubonic plague have been recorded in Orange County, plague bacillus-infected fleas, animals, and humans have been reported in neighboring counties over many years. These detections illustrate low-level enzootic transmission and persistence of *Yersinia pestis* in wildlife, the complexity of sylvatic plague ecology within urban-wildland ecotones, and the potential for epizootic spillover to peridomestic mammals and humans in southern California.

Panel discussion of inspection and enforcement of unmaintained swimming pools

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Abstract

Mosquito-borne diseases, such as West Nile virus (WNV), are an important public health issue in California where three species of mosquito are effective vectors of the virus for humans and reservoir birds. The spread of WNV is closely linked to the co-occurrence of birds and mosquitoes, the latter of which can reproduce effectively in stagnant water. Unmaintained swimming pools are a prime example of such an environment, especially in urban and suburban areas with high densities of people. Timely detection and remediation of unmaintained swimming pools is crucial for effective mosquito control and disease prevention. Traditional methods for locating unmaintained swimming pools have relied upon manual "boots-on-the-ground" field surveys, which are labor intensive and highly constrained by legal rights that prevent physical access to private properties. Aerial imagery obtained using aircraft or satellites that is analyzed using artificial intelligence algorithms to automate workflows offer a potential solution to these challenges. This symposium highlighted the approaches used by several vector control districts to capture aerial imagery, automate its analysis, and the legal enforcement approaches used to abate unmaintained swimming pools. The presentations were followed by a moderated panel discussion of successes, lessons learned and where we are going from here.

Introduction

MVCAC held its first panel discussion on the inspection and enforcement of unmaintained swimming pools during the 2023 Annual Conference in Anaheim, California. As aerial, inspection, and notification technologies continue to improve, so do the mosquito control programs that are needed to access and control mosquitoes in unmaintained swimming pools. Remote sensing, which involves aircraft or satellites to capture images of the earth, has been used by vector control agencies in California since at least 2006 to identify unmaintained swimming pools that may produce mosquitoes (Alameda County Mosquito Abatement District 2008). Early approaches relied upon color photographs that were manually inspected by district staff to locate unmaintained pools. The success of this and similar programs motivated the use of increasingly advanced approaches that utilized multispectral imagery that distinguished unmaintained green from functional blue pools (Minho et al. 2011, McFeeters 2013) and artificial intelligence (AI) algorithms that automate identification (Cunha et al. 2021). This symposium explored several defined and critical aspects of unmaintained pool programs across California including: visual and aerial imagery acquisition, notification, inspection, enforcement, abatement and warrant processes. A moderated panel discussion followed with information technology and enforcement experts from the Mosquito and Vector Control Association (MVCAC) membership.

To start the symposium, the audience was asked to raise hands if they used mailers in their program to notify swimming pool owners of unacceptable water conditions that can produce mosquitoes (three quarters indicated they do), and who uses text messaging to communicate with the owners (a third raised hands). With the knowledge that most in the audience had good familiarity with unmaintained swimming pool programs, the speakers tailored their pace and focus on the more technical aspects of their presentations.

Panelist presentations

Mark Daniel, the Director of Operations with Greater Los Angeles County VCD (GLACVCD), presented the abatement process used by their district, and their thought process on whether an abatement should be pursued. The abatement process at GLACVCD starts with their Vector Control Specialists (VCS) who work directly with the resident or owner to resolve problems with vectors on the property. In the absence of sufficient progress, the VCS sends official notices to the property and notifies their Supervisor of the issue. Once the Supervisor verifies non-compliance andthe Director of Operations decides that an abatement should be pursued, they proceed with collecting physical evidence, verifying the chain of custody, and present a report to the General Manager for review with legal counsel. Upon deciding to move forward, an abatement notice is sent to responsible parties that accounts the reasoning, potential fines, and other consequences of an abatement in a manner similar to that of a civil hearing. The GLACVCD Board of Trustees has empowered the General Manager to assign a hearing officer to preside over the proceedings where the property owner and GLACVCD staff present their cases. If the hearing officer determines that the evidence merits an abatement, an order to abate is issued, which can be contested by the property owner with a Judicial Challenge to the county court. In the absence of a successful Judicial Challenge, a penalty of up to $1,000 per day can be assessed along with all costs to GLACVCD for the abatement, which can be charged as a lien on the property that is levied once the property is sold. Advantages for pursuing an abatement include highlighting a legal path that may motivate property owners to comply with vector control workers and a means to recover extraordinary costs for vector control on non-compliant properties.

How Alameda County MAD (ACMAD) has shifted the workload from the field to office staff in their response to unmaintained swimming pools was discussed by Robert Ferdan, the Information Technology Director, and Eric Haas-Stapleton, the Laboratory Director. Before automation was incorporated into their unmaintained swimming pool program, field staff reviewed paper prints of all aerial swimming pool imagery, identified those that needed inspection, located each property on paper maps, and visited each to help the resident bring the pool into compliance. Consequently, all of the service requests that were related to swimming pools required the attention of field staff, who were responsible for resolving each, and closing the service request. Obtaining georeferenced data from the County of Alameda Office of Assessor to determine location and ownership of property parcels was the first step in automating the pool process. Next, they combined the aerial imagery with AI to identify unmaintained pools that may need attention. Regardless of the vendor used to obtain and annotate the aerial images, ACMAD found some pools that were incorrectly identified as needing the attention of vector control. Misannotations were most common for "PebbleTech" surfaces at the bottom of swimming pools that made the water appear green or swimming pools that had been converted to ornamental ponds that contained green water but did not produce mosquitoes because fish were present. To reduce the number of notices sent to homeowners with pools that were clean, but appeared green in the images, ACMAD staff examined each image of a pool that was flagged by the AI algorithm as 'unmaintained' before a notice of violation was sent to the owner of the parcel. Similar to the process that was developed by San Gabriel Valley MVCD, ACMAD staff utilized software that automatically filled the violation notice with the owner name, address, and an image of the unmaintained pool. Like Sacramento-Yolo MVCD, ACMAD utilized a text messaging and email workflow that enabled owners to send a photo of a pool that had been cleaned or emptied. Of note, if an ACMAD field worker had inspected or treated a pool within the prior 90 days, it was excluded from further follow-up by field or office staff. Once a resident provided evidence that a pool was clean or empty, the property was removed from needing to have field staff inspect the site. For 2022, all of the unmaintained pools that were found in the district were returned to service or treated with larvicide or mosquito fish. The new automations that were fully in place by 2019 challenged office staff with long or multiple telephone calls with owners to resolve questions or errors. However, the end result was office staff resolving 74% of the service requests during 2022, with field staff responding to one-quarter of the unmaintained swimming pools with a site visit to help the resident. This shift in work effort from the field staff to the office enabled the former to focus upon mosquito control, and has more broadly fortified all programs at ACMAD for responding to invasive *Aedes* mosquitoes, such as *Aedes aegypti*, when they eventually arrive to Alameda County. Challenges remain, which include a lack of integrated software that would further streamline the process, aerial imagery that has sufficient resolution and geographic coverage to identify all of the unmaintained swimming pools in the county, and AI algorithms that accurately identify target pools without the need for human eyes on each image to confirm that it is indeed unmaintained and not a shadowed or pigmented pool bottom.

The approach used by Orange County MVCD (OCMVCD) to find unmaintained swimming pools using aerial imagery and AI was highlighted by Steve Shephard, their Director of Operations. The potential for large numbers of unmaintained swimming pools in the county is daunting, as Orange County have over 113,000 swimming pools, which translates to a swimming pool for every 28 people in the county or 142 pools per square mile. They currently have a little over 9,000 swimming pools in the database at OCMVCD, with 1,195 of those being observed or treated to control mosquitoes (approximately 1 % of the total). They are aiming to fill the gap between the number of known swimming pools that are reported by the county and those that are recorded in the OCMVCD database using high resolution aerial imagery that is coupled to AI that distinguishes among unmaintained (green, murky, not uniform in color), "Pebbletech" bottoms (green), empty (usually white), partially filled and likely producing mosquitoes (white with brown or green), covered pools (variable color), clean pools with shadows (grey to green), and clean (blue). The importance of imagery that is regularly updated was highlighted using an example of a neighborhood fire in 2022 that quickly transformed all of the functioning pools to ones that produced mosquitoes in a matter of weeks. Fortunately, aerial imagery was captured just prior to the fire, and again after a few weeks, allowing OCMVCD staff to identify the geographic extent of the response that was needed to limit mosquito production.

The role of information technology for locating, recording and managing the data that is needed for the field response to unmaintained swimming pools at Sacramento-Yolo MVCD (SYMVCD) was described by Dan Fisher, their Information Technology Administrator. During prior years, aerial imaging services were contracted to fly small regions of the district each year (~ 50 sq. mi.) so that office staff had sufficient resources to process the data and field staff had sufficient time for a prompt response to the unmaintained pools that were identified. Recently, in collaboration with other districts, SYMVCD contracted with NearMap to obtain high resolution aerial imagery of the entire county, with a much faster stream for data production and analysis. They were also able to bring NearMap imagery into the field where technicians used it in their day-to-day workflows. Early iterations of the NearMap data process allowed SYMVCD to remove nearly 15 % of the pools (more than 200) from their list of historically problematic pool sites, allowing them to focus efforts on those that remained. Ongoing collaborations with NearMap engineers has produced an AI system that automates identifying unmaintained pools, but it needs further refinement to improve accuracy and throughput. The public response to unmaintained swimming pool notices has shifted from 100% phone calls that need extensive office resources to responses via text messages (45%) and their website (35%), with the remainder called in via the telephone (20%). For unmaintained pools that needed a site visit from SYMVCD staff, scheduling was automated using Acuity Scheduling which offered residents multiple time slots to select from, Zapier to automate staff workflows, and Google Sheets to record the field response. Plans for the next seasons include reducing the time for managing responses to notices using chat AI bots and a custom web site, and fine tuning the imagery AI to discover both unmaintained pools and clean blue pools. However, increasing the number of pools that need attention by SYMVCD staff may require changes in policy, staffing levels, and workflow so they can be addressed in timely manner.

The collective bargaining that was needed to bring multiple districts together under a unified aerial image acquisition contract with NearMap was highlighted by Luan Ngo, the Information Technology Manager with Orange County MVCD. A tiered pricing structure was agreed upon that binned imagery fees charged to each district using their total annual budget, with higher resourced districts contributing more funds. Other factors, such as the number of flights and access to AI systems affected the final cost for each district. Lastly, Luan described the post image processing workflow whereby they automated offline delivery of the image data processing of orthomosaic maps to extract parcels with unmaintained swimming pools, and custom MS Excel macro scripts that enabled viewing and condition status marking for each image.

The final presentation of the symposium was from Marty Scholl, the Program Coordinator for Sacramento-Yolo MVCD. Marty outlined the District's pool mailer and door posting program. Mailers are sent to each known unmaintained pool location at the beginning of each season or after a recent aerial survey identified new pools. The First Notice requested a picture of their maintained or dry pool to be sent to the District. If they are unable to fix or drain their pool, they can schedule a site inspection. After a period of a couple weeks. a Second Notice mailer is sent to the owner of record as well as to the physical address. Door postings use a carbon copy-based notice, where the first notice information is transferred to the Second Notice. A First notice is placed on a property with a suspected unmaintained pool requesting that the occupant call the District to discuss their unmaintained pool. If there is no response after one week, a second notice is then posted at the address. If no response has been received from either the mailer or door posting program, a Final Notice is sent to the Owner of Record requesting them to contact the District about the status of their pool. The notice also outlines the enforcement process that includes obtaining an Inspection and Abatement Warrant as well as possible abatement by the Board of Trustees if they fail to communicate with the District.

Marty then briefly outlined the Warrant process to include posting of the property 24 hours in advance, and scheduling to serve each warrant with local law enforcement. Some of the current issues that Marty detailed was the length of time it takes to go through the entire process as well as the life span of an aerial image.

Moderated panel discussion

Once the presentations were finished, the symposium transitioned to a moderated discussion amongst the panel and audience. The first question to kick off the discussion was to see how many pools each panelists agency managed in a year, so that everyone could appreciate the magnitude of the issue. Most districts that were represented by panelists had vector control staff inspect in person or virtually via email or text message from 500 - 2,000 unmaintained swimming pools each year. For many, that represented approximately 10% of the mosquito production sites in their service area. Therefore, although there can be 10's of thousands of pools in a district, only a small number of them require service from vector control workers. Fortunately, a much smaller proportion of the pools needing service went to final notices to the resident that had the potential to initiate abatement hearings (panelists indicated that their district initiate zero to 30 abatement proceedings each year). Districts in cooler climates often limit their pool response to begin during the late spring season, while those in warmer climates investigate unmaintained pools throughout the year. As districts gain access to aerial imagery for the entire service area that is captured more than once a year, all of the panelists expected that the number of pools that require attention by vector control is anticipated to increase substantially. This led to a discussion of how such increases will be acted upon, and most recognized that increases in staff numbers, changes in policies for pool conditions that require a response, advances in technologies such as machine learning that automate the workflow, or a combination of them all will likely be needed.

The next question to the panel asked if their district captured aerial imagery for the entire or smaller region of their service area. No district had imagery for the entire district as rural regions or those that were geographically unsupportive of a swimming pool were excluded (e.g., regions with steep hillsides). Most districts focused on acquiring aerial imagery in regions with known high densities of swimming pools, primarily as a means to conserve costs as tasking manned aircraft with outboard cameras have to date been most effective. Others intentionally constrain the size of the regions that are imaged each year to what current staffing capacities can accommodate, rotating those regions over several years until most of the service area is covered.

Satellites can capture images over very large geographic regions (10s − 100s of sq miles in each image). However, those that evaluated the suitability of satellite imagery from NASA or Google found the resolution too low to determine if a pool is unmaintained. The entrance of NearMap into the market with very high-resolution aerial imagery that covers broad geographic regions, often multiple times each year, has improved the capacity of districts to locate unmaintained pools. Once district noted that their vector control workers in the field now have access to that imagery on their mobile tablets. However, it remains to be seen if it is economically or technologically feasible to develop an automated system that uses the images to distinguish among the variety of conditions that distinguish maintained from unmaintained pools.

The next panelist question was to understand bottlenecks that are limiting the success of the pool programs. All noted that long periods of time between image acquisition and delivery to the district (often more than a month) can substantially reduce the utility of the imagery. A two week turnaround time would be ideal. Another problem is the time of year that the images are collected. Those that are collected before Memorial Day (the last Monday in May) are more likely to show unmaintained pools that have been cleaned for use that holiday weekend, which is typically before mosquitoes of public health consequence are produced. Concerns over resident fatigue with receiving multiple violation notices in a single or over multiple years is an issue for some. Lastly, budgets and staffing are of substantial concern for all. That constrain alone likely makes it infeasible for a district to mount a response for all unmaintained swimming pools, and an administrative policy or procedure will needed to establish priorities.

Concluding discussion with the symposium attendees

The symposium next transitioned to a robust discussion with the audience that continued well into the scheduled break time. Topics of interest included: new laws that limit the ability to broadly broadcast unsolicited text messages, the use of supplemental drone imagery, pools that are miscategorized by AI algorithms, challenges with repeated visits, motivating residents to clean pools without needing to start a warrant process, inconsistent perspectives in the judiciary when seeking a warrant, and a suggestion that districts connect residents local non-profit organizations that help those without fiscal resources to permanently remediate pools that they find are unmaintained each year.

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CalSurv Adventure: a review of bulk data entry methods and data extraction tools

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Introduction

The CalSurv Gateway (VectorSurv Development Team 2023a) is the web-based platform used for management and analysis of data on mosquito surveillance and control throughout California. As the original member of the VectorSurv family of websites that now serves many U.S. states, the CalSurv Gateway has been in continuous use since its launch in 2006 and now serves > 80 vector-control and public-health agencies in California, with > 1,000 registered users. In recent years as the user base of the system has expanded, along with increasing sophistication of software available for data visualization and analysis, there has been a need to import and export data in new ways to meet the needs of various user groups. The CalSurv Gateway provides multiple ways to enter new data records or to retrieve data already stored within the system.

CalSurv Web Forms

The simplest way to enter or view data from within the CalSurv Gateway is through the use of the CalSurv Gateway's web forms. One example of a web form is the "New Collection" form that can be found by visiting Arthropod → Abundance → New Collection from within the CalSurv Gateway. Additional examples of web forms include forms for the entry of surveillance sites, arthropod pools, test results, sentinel chickens, dead bird carcasses, and pesticide applications, among other data types. Web forms provide a simple and formatted structure that allows users to easily enter individual observations or to retrieve and edit previously entered data that are stored within the CalSurv Gateway.

While useful in many situations, the CalSurv web forms do not easily allow users to enter or retrieve large numbers of surveillance or control records in one action. Therefore, the CalSurv Gateway also provides users with separate tools that allow for both bulk data entry and bulk data extraction from the CalSurv Gateway.

CalSurv Web Services

The CalSurv web services are tools that provide local agencies with the ability to import new data records into the CalSurv Gateway or to retrieve existing records from the CalSurv Gateway in bulk. The web services are application programming interfaces (APIs) that can connect local software or data sets used by mosquito control agencies to the CalSurv database. The web services are used by local agencies through either in-house software applications that can connect to the web services or by programming scripts that send the data request to the CalSurv database from the agency.

To assist with the use of the CalSurv web services, documentation pages have been created for both web service data importation (VectorSurv Development Team 2023b) and data export (VectorSurv Development Team 2023c). These documentation pages outline how to structure data imports or exports, both of which utilize JavaScript Object Notation (JSON) data formatting. These documentation pages also outline where to send the data request via specific URL paths and how to submit the data request to the CalSurv database via a POST request.

In order to utilize the CalSurv web services, users must first create an agency token that verifies that the individual submitting the data request via the web services has the proper credentials to do so for that specific agency. Agency tokens can be generated by each agency's designated agency manager(s) within the CalSurv Gateway by visiting Settings → My Agency → Agency Tokens → New Tokens → Create New Token. This token then will be included in the URL and POST request that is submitted to the CalSurv database as outlined within the web service documentation pages.

CalSurv Data Tools (Excel Import / Export)

As an alternative solution for bulk data imports and exports without the need for writing computer programming code, CalSurv also has modules for uploading and extracting data using Excel spreadsheets and CSV files.

To extract data, users can navigate to the Export Data page (Tools → Data Tools → Export Data), and choose their preferred export type. They then will be presented with a choice of filters and given the option to receive the file as a Microsoft Excel spreadsheet or CSV file.

Importing data is a similar process. Users can navigate to the Import Data page (Tools → Data Tools → Data Import) and choose their preferred import type. After selecting a file for upload, the system will walk the user through a series of validation steps before finally committing the data to the system.

At the bottom of the import page, links are provided for downloading import specification files and example spreadsheets. These files serve as guides to help users to format their data and make it compatible with the CalSurv system.

Recent developments in the import and export modules include the following:

- CalSurv is now accepting .XLSX file types in addition to the .XLS and .CSV types.
- Users can import tick data into the system.
- Capacity of the data import and export modules has been expanded to handle larger file sizes.
- A new "Arthro Collection - Formatted" import style has been added. It was designed to mimic laboratory forms that are commonly used by many agencies. The layout is intended as a template that can be used as a weekly worksheet for entering trap collection data by surveillance staff, and edits can be made to the provided templates to accommodate an agency's specific needs.

CalSurv Aggregate Reports

When a broad overview of an agency's data is preferred, the Aggregate Reports tool can be useful. To see aggregate reports for both Sentinel and Arthropod test results CalSurv Gateway users can navigate to the Aggregate Reports page (Tools → Data Tools → Aggregate Reports), choose a date range and report type, and then generate the needed report. Data will be organized into a set of tables and provide information about positive sample details, the number of pools tested by test target, pools tested by agency, and more.

Need more help?

For more information on the issues above as well as other CalSurv Gateway capabilities, please visit our documentation page (VectorSurv Development Team 2023d). We also post new training videos as they become available on our YouTube Channel (VectorSurv Development Team 2023e). If your question is not answered in the documentation above, feel free to send an email to the development team anytime at help@calsurv.org.

Acknowledgements

We thank our partners, especially the Mosquito and Vector Control Association of California and the California Department of Public Health, for their many invaluable 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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Be Our Guest: Open access to CalSurv data for research

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Introduction

Vector control agencies throughout California use the CalSurv Gateway to enter, store, process, and analyze vector abundance and arbovirus infection data. Users belonging to each local agency have access to their agency's data through various mechanisms, and any agency who utilizes CalSurv's services is free to export and share their data with any collaborator they choose.

CalSurv also has a process by which externbal researchers may request statewide data for use in research by providing basic information about the requestor(s) and intended uses of the data. Requests then are shared by email with the primary contacts for all California agencies involved in CalSurv, and comments are received from the agencies over the next two weeks. All comments then are shared with the CalSurv Steering Committee, which includes voting members from the Mosquito and Vector Control Association of California, California Department of Public Health, and UC Davis. The steering committee then votes to approve or decline the request. The CalSurv data request process typically requires approximately three to four weeks from the request to the provision of the data to the requestor.

To accelerate research and reduce barriers to data acquisition, the CalSurv development team has worked with the CalSurv Steering Committee to develop a new open data portal that will allow immediate downloads of aggregated CalSurv data for all agencies who have opted to provide their data via the portal.

Methods

Visitors to the open data portal are required to enter personal and organizational information before defining the type of data and any filters (e.g., particular years, counties, mosquito species, or trap types) for the data they seek. Before receiving data, visitors must also verify their email address to ensure authenticity. Currently, the open data portal serves data on mosquito abundance and mosquito pool testing, but in the future the CalSurv Steering Committee will consider adding other data types in consultation with the MVCAC.

Citation of CalSurv and acknowledgement of contributing agencies are required when using the data for publication, and a suggested citation and list of contributing agencies is provided with each data download.

Open data are aggregated to a relatively fine spatio-temporal scale that is useful for modeling and other ecological and epidemiological studies. Data are aggregated by collection date within two-week intervals to accommodate variation in local trapping routines because some agencies repeat trapping routes on alternate weeks. Data are also aggregated spatially by census county subdivision to avoid any privacy issues associated with traps at individual households or neighborhoods (e.g., during follow-up for human disease cases). Open data are available beginning 18 months after initial collection of the data to allow for routine edits to the data and use of the data by MVCAC agencies for internal research purposes prior to public release.

The spatio-temporal scale and lagged availability ensure that accuracy and research opportunities are maximized for CalSurv's partner agencies while still providing a rich selection of data that researchers can use to investigate the relationships between vector activity, geographic location, and collection dates on a standardized scale.

Results and Discussion

MVCAC agencies who opt to participate in the CalSurv open data portal will encourage and accelerate the work of researchers by enabling them to use real-world surveillance datasets more easily. We expect that this will encourage more predictive modeling and increase awareness and data use among researchers who are often on tight timelines for class projects or grant proposals.

Open data will also allow vector control district personnel to perform multi-agency analyses that were possible previously only through data requests. This will allow for more comparative analyses to understand regional patterns in mosquito and arbovirus activity and inform local decision-making. Information provided by users when downloading data will help to justify state and federal financial support for CalSurv and MVCAC agencies' contributions of data to the program. Fig. 1 shows an example that comparea the abundance of *Cx. pipiens* females per trap-night between Sacramento County and Shasta County for the year 2020 compared to the previous ten-year average.

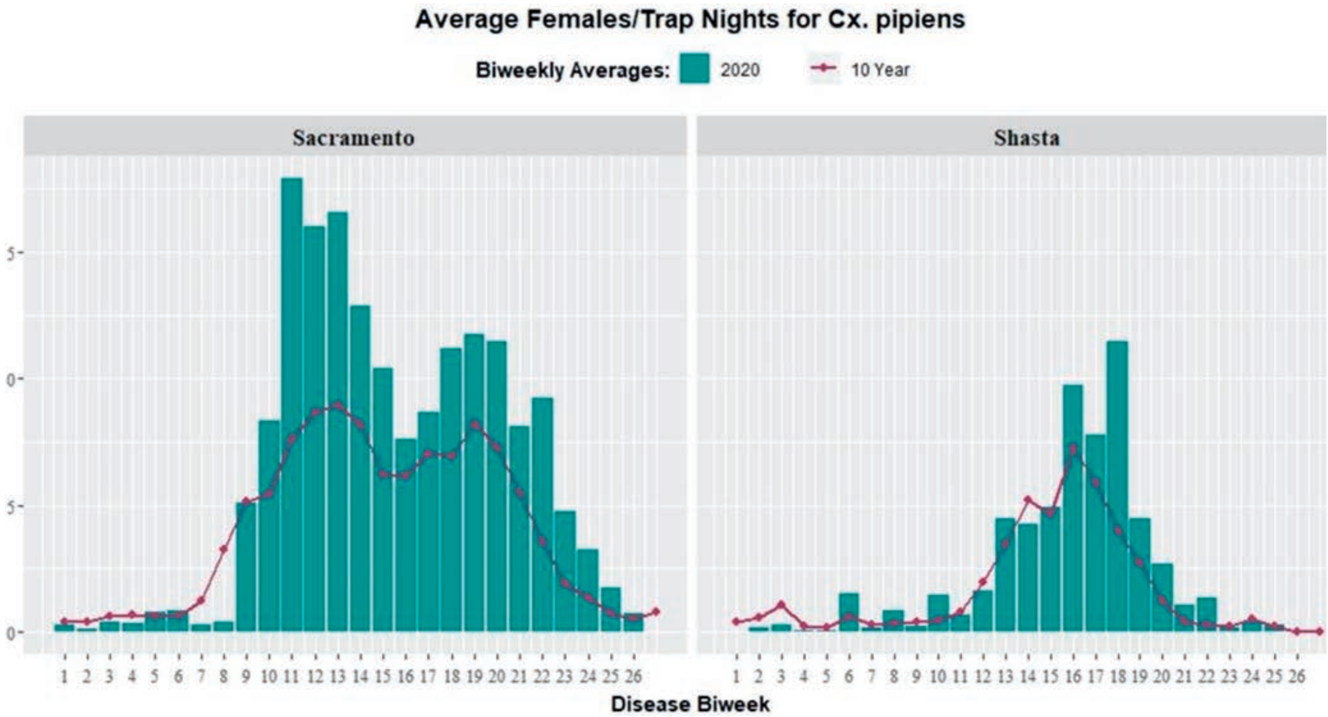

Figure 1.—Example comparing the biweekly temporal resolution of CalSurv open data for *Cx. pipiens* females per trap-night between Sacramento County and Shasta County for the year 2020 compared to the ten-year average.

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.

A whole new world: The present and future of CalSurv

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Introduction

The California Vectorborne Disease Surveillance System, known as CalSurv, is part of the broader VectorSurv family of web services and is used by vector control agencies throughout California to manage, analyze, and report surveillance and control data (VectorSurv Development Team 2023a). Beginning in 2022, the CalSurv team worked with the Mosquito and Vector Control Association of California, California Department of Public Health, CDC, and individual agencies throughout California on strategic planning to identify features and functionality which will improve the ability of CalSurv users to monitor and control vector-borne diseases. The current presentation describes some of the changes to CalSurv that are expected in 2023.

Methods

The CalSurv team communicates with stakeholders to identify development projects that will equip state and local agencies with the tools they need to monitor and control vector-borne disease in their district. The projects are prioritized based on the balance between anticipated value to users and development effort required. Projects scheduled to be completed in 2023 focus on data entry and management, analytical tools, tick surveillance, service records, and user communication.

Results and Discussion

CalSurv users can expect several improvements to the ways data are entered and managed in CalSurv. A new user interface will be available for managing mosquito collections and pools. The new interface will embrace modern design principles, making it easier for users to manage data using a wider variety of devices including desktop or laptop computers as well as mobile tablets and phones. Users will be able to import data from spreadsheets with a print-friendly format similar to laboratory worksheets commonly used by many agencies. This will improve consistency and reduce data input errors. Finally, a new RESTful API will be available that will provide a simple interface for third-party software to interface with CalSurv.

CalSurv provides many tools to analyze surveillance data. Users will soon be able to save settings for calculators. This will allow for anyone to easily save and retrieve commonly used calculations, including filters and other criteria. The technology to generate charts in CalSurv will be upgraded. This will allow users to download charts to use in reports created in other software, such as Microsoft Word (Microsoft, Redmond, WA, USA). Charts showing the results of saved calculations will be accessible directly via a unique web address, meaning it will be possible to link directly to the charts from compatible word processor software. VectorSurv Maps (VectorSurv Development Team 2023b) also will be updated to make enhance the visualization surveillance data.

Surveillance of ticks is a growing element of vector-borne disease programs in California and elsewhere. CalSurv will empower users to manage tick surveillance data by updating the current tick data module to align with the needs of California-based vector control agencies. Tick data collected in CalSurv will soon be available on a new tick surveillance map displaying information about species and pathogen distributions. CalSurv also will provide calculators to enable users to make data-based decisions utilizing tick surveillance data and to understand seasonal and spatial patterns in tick activity in their districts.

Growth in the user base and the number of system features has made onboarding and training users a high priority for the CalSurv team. Beginning in 2023, we will provide instruction and training to users at two live training sessions each year. The training sessions will include instructional content, and question and answer sessions. Training sessions will be recorded and posted as a series of online videos (https://www.youtube.com/@vectorsurv). To empower users to find answers anytime, a Frequently Asked Questions section will be developed for the CalSurv website.

Beyond 2023, longer-term development goals will include the addition of county-level human and veterinary case data for use in risk calculations, a user dashboard highlighting key local surveillance trends, and a webpage featuring predictive models and other web applications built with CalSurv data. We anticipate that the development of these features will enhance the ability of agencies who use CalSurv to monitor and control vector-borne disease within their agency.

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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Implementation of an automated mosquito identification and sorting system at the SYMVCD (District)

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Introduction

Mosquito control agencies rely on precise enumeration and identification of mosquito species as part of their surveillance programs, which ultimately drives targeted and effective vector control decisions. Adult mosquito surveillance requires extensive trapping, sorting, identifying, and collection of specimens for viral testing (pooling) by district technicians, activities that are both labor and time intensive. The automation of this process can save time and reduce the labor typically associated with these activities. Additionally, the use of artificial intelligence and image processing can improve the accuracy and precision of mosquito identification, reducing the likelihood of misidentifying important vector species. The development of these technologies is particularly important for public health organizations such as the Sacramento-Yolo Mosquito and Vector Control District (District). In 2022, the District acquired an automated identification and pooling robot (Senecio Robotics; Kfar Saba, Israel) to help streamline the surveillance process, and ultimately allow for timely, efficient, and effectively control measures to protect public health from mosquito-borne disease outbreaks.

Methods

Since acquisition, most of the work with the automated identification and pooling machine has focused on training the machine learning algorithms, with the goal of attaining >95% species identification accuracy for the most common mosquito species collected in Sacramento and Yolo Counties. This activity was limited by species availability based on seasonality. The training process involved collecting and identifying each target species of mosquito, then running the collections through the machine in training mode so that image data could be collected and added to image libraries. Each specimen was run through the machine a maximum of three times. The goal was to acquire a minimum of 10,000 images for each species of interest, which according to Senecio, is the minimum number of images required to obtain accurate identification. Training is currently ongoing both at the District and the Senecio laboratory.

Results

Over a period of nine months more than 200,000 images from 16 species were collected, and for six of those species, more than 10,000 specimens were imaged (Table 1). The District is currently collecting training data to strengthen mosquito identification algorithms so that the most common mosquito species present in the Sacramento and Yolo counties are identified with >95% accuracy. Subsequent plans include expanding its capabilities to identify mosquito species from different geographic areas,

Table 1.—Mosquito species of interest for the SYMVCD (District) and total number of images in the library.

Species	Senecio Lab	SYMVCD (District)	Sum
Aedes melanimon	11,703	1,091	12,794
Culex tarsalis	47,593	16,857	64,450
Anopheles freeborni	51,108	4,714	55,822
Culex pipiens	45,470	5,132	50,602
Culiseta incidens	6,868	7,646	14,514
Aedes vexans	13,806	66	13,872
Aedes nigromaculis	6,776	0	6,776
Culex erythrothorax	4,622	368	4,990
Aedes stiticus	2,094	0	2,094
Anopheles franciscanus	40	1,566	1,606
Culex stigmatosoma	1,240	1	1,241
Culiseta inornata	1,022	123	1,145
Aedes sirrensis	168	0	168
Culiseta particeps	64	0	64
Anopheles punctipennis	40	2	42
Aedes washinoi	20	0	20
Total	**192,634**	**37,566**	**230,200**

with the ultimate goal of supporting and optimizing wide-ranging vector surveillance and control efforts.

Conclusion

The successful implementation of automated mosquito identification technologies has important implications for public health organizations worldwide. This technology can streamline the mosquito surveillance process and enhance the efficiency and effectiveness of mosquito control efforts. However, despite the potential benefits of automated mosquito identification technologies, there are some important caveats to consider. These technologies are still in the developmental stage and require ongoing testing and refinement; additionally, these systems may not be accessible or affordable for all public health organizations, particularly those with limited resources.

Creating and Sharing Data using Google My Maps

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Abstract

The ability to visualize data has become critically important to mosquito control districts to conduct effective and targeted efforts. Understanding the spatial components of mosquito control can be beneficial and lead to better management and decision making. The Sacramento-Yolo Mosquito and Vector Control District (District) has implemented mapping strategies to develop data driven mosquito control efforts and has integrated location data with descriptive data to manage and respond to events. By utilizing Google My Maps (https://www.google.com/maps/about/mymaps/), the District can create interactive maps with minimal efforts. Maps can be shared amongst colleagues as well as the public making it easy to disseminate information quickly and effectively. With Google My Maps, users can conduct useful mapping functions such as creating data, conducting measurements, mapping coordinates and the ability to import map files that have been created by county agencies. The District also uses Google My Maps to generate spray routes for wide area larvicide sprays making it a diverse tool for operation planning (For example, Fig. 1). Google My Maps has provided the District an additional mapping platform that is free and easy to use. By utilizing custom maps, staff and management can communicate effectively and plan accordingly for projects, trials, and pesticide applications.

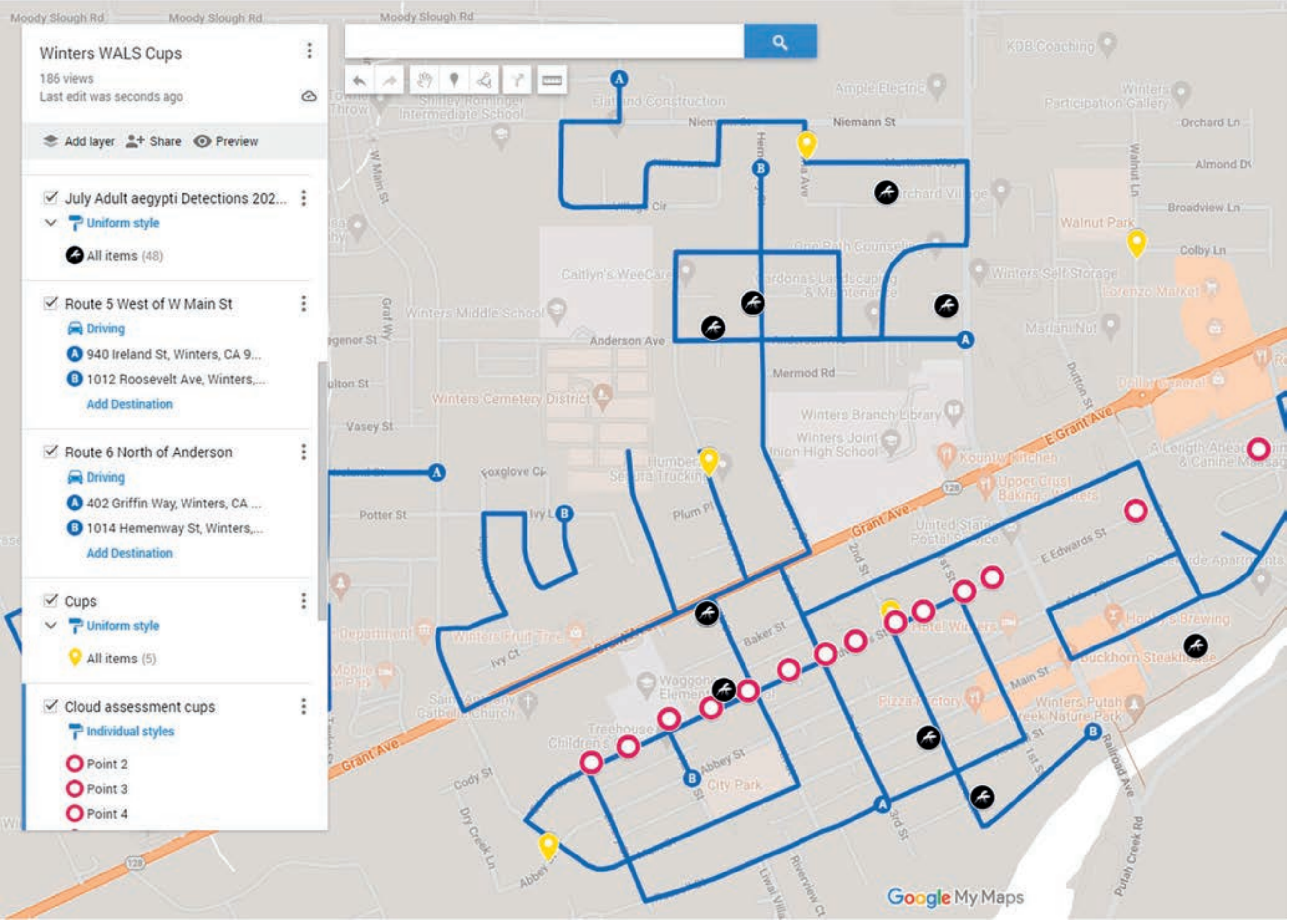

Figure 1.—Google My Map illustrating *Aedes aegypti* detections, spray routes and cup placements for post application analysis.

Web GIS – How a single source of information benefits your organization

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Abstract

Leveraging the Esri ArcGIS Online platform as the single source of information for your organization has many benefits. Coupled with the Frontier FieldSeeker GIS mosquito control software, the user is provided with simple, intuitive tools for finding, collecting, and reporting data related to control activities. Data from other sources (ATV/truck spraying systems, aerial data from drones, planes, or helicopters systems, asset management systems, public notification systems, etc.) can be stored in or integrated within the GIS. Field and office users benefit from seeing a common up-to-date picture of their operations. The more data an organization can share, the better able it is to make plans to move forward with its goals while minimizing time spent reconciling data sets for required reporting. Described were recent product updates to FieldSeeker Core software (workflows for Larviciding with storm drain treatments, Surveillance, and Service Request) and the FieldSeeker ULV Adulticiding system.

Internal Production Mapping for Mosquito Disease

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Introduction

In 2022, the Consolidated Mosquito Abatement District modified mapping strategies to meet surveillance and operational needs. The District transitioned from generating single quarter-mile sections targeting control and surveillance activities following the detection of an arbovirus to a district-wide quarter-mile grid system. A quarter-mile grid layer was developed by subdividing the township range section (TRS) layer into four quarter-square mile sections (Fig. 1). Disease and virus indicators in these sections would influence future sampling for arboviruses as well as operational responses to sources in the area.

Prior to 2022, static maps with a single highlighted quarter-mile section were provided to staff (Fig. 2). These maps displayed positive mosquito pool data and targeted ultra-low volume (ULV) treatment areas. An ArcGIS Online dashboard was subsequently developed to replace the static maps. This tool provided the same information but as an interactive time-enabled map (Fig. 3).

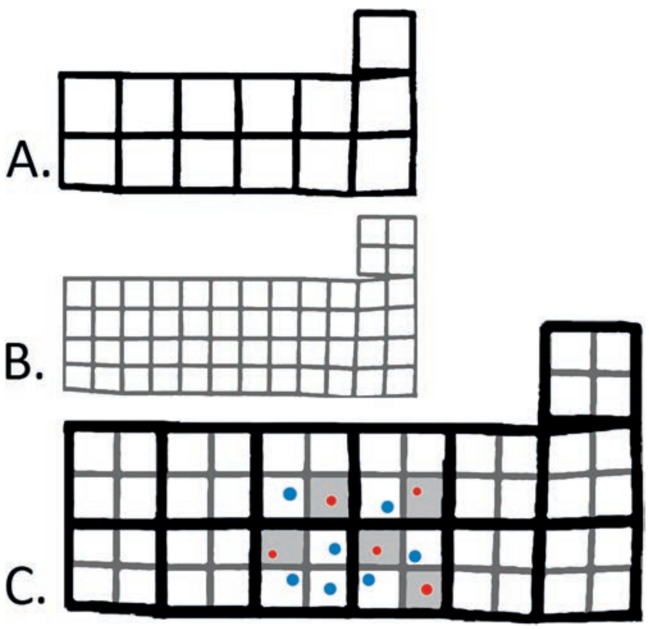

Figure 1.—A. TRS grid layer, B. TRS layer subdivided into quarter mile sections, C. quarter mile section grid with sampling points, and areas greyed out to discontinue sample submissions.

The dashboard became the primary method for laboratory staff to identify areas of arbovirus activity and to delineate ULV treatment areas. A decision was made to adapt the 2021 static maps for operational use. When a confirmed arbovirus sample was detected, maps would be generated to provide additional information on known mosquito sources within a quarter mile of the location. Maps were distributed to supervisory staff to respond accordingly. Weekly tabular source reports have historically been provided to staff but do not contain information on arbovirus activity. The newly designed operational maps would need to display detailed site information in a clear and concise manner. The symbology and labeling scheme would enable staff to assess and prioritize mosquito sources that should be re-inspected (Fig. 4).

Methods

2.1 Labeling and Symbology

Labels were formatted to provide critical source details without requiring a large area to display. Arcade syntax labels were formatted to provide the following information: site ID suffix, last activity, and date visited (Fig. 5). Labeling was applied to storm drain locations to show the last date of treatment and current status as depicted by the symbology. The software platform FieldSeeker (field data system) developed a revolving set of fields that correspond with the most recently submitted inspection and treatment records. Details of a source's informational page can be updated every 24 hours.

District symbology classes (Fig. 6) were predefined as follows:

Green symbology: a source with *'No Action Required'*.
Blue symbology: a source with *'Action Required'*.
Grey symbology: a source that is *'Inactive'*.
Red Catch Basin symbology: a source *'Needs Treatment'*.

A new symbology class (yellow) was introduced, depicting sites visited in the last 10 days to allow for rapid identification of sources recently inspected.

2.2 Dynamic Text

Both the 2021 and 2022 map approaches utilized ArcGIS Pro's 'Map Series' feature. Map series allows

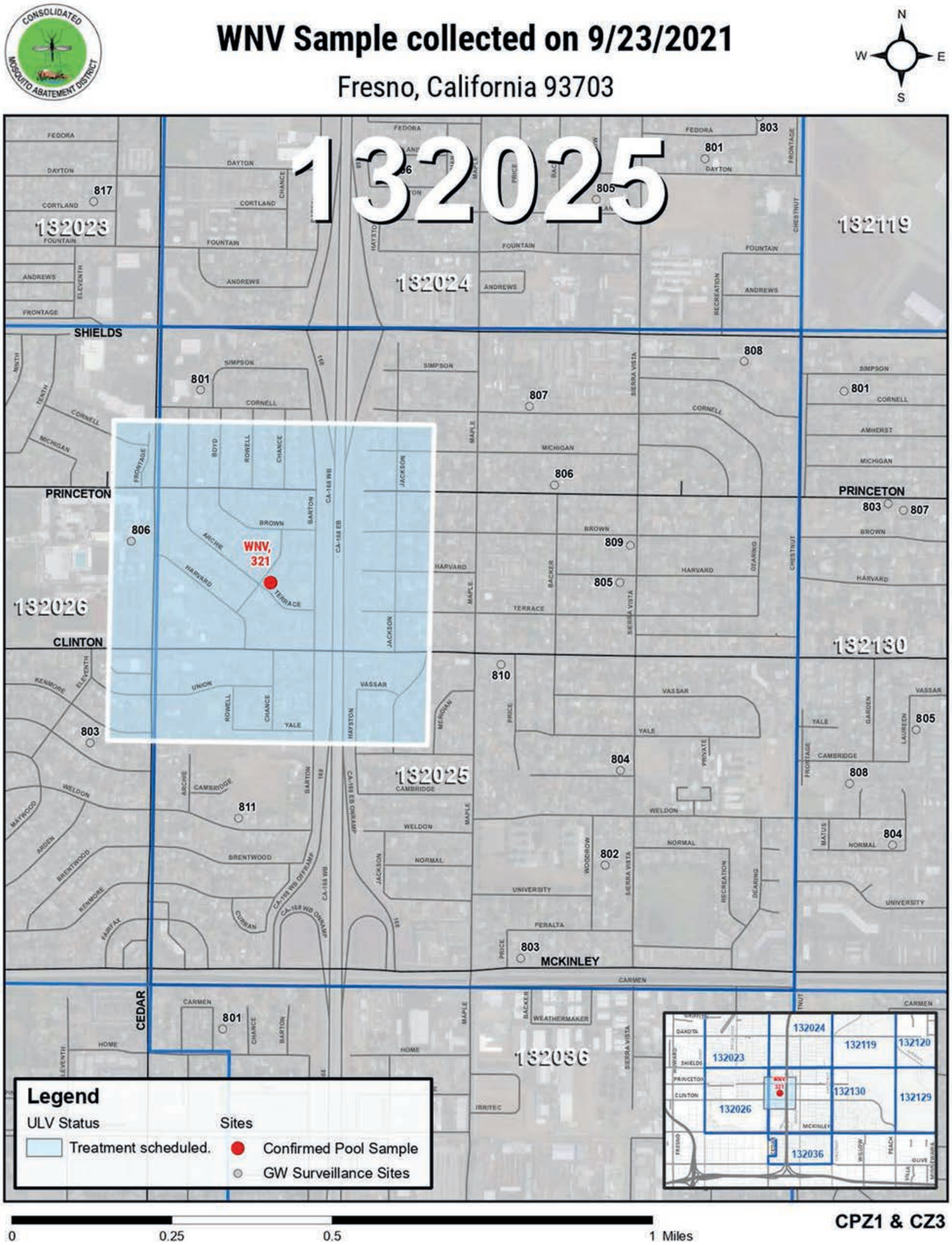

Figure 2.—Sample of static surveillance map displaying information for ULV and positive mosquito pool locations.

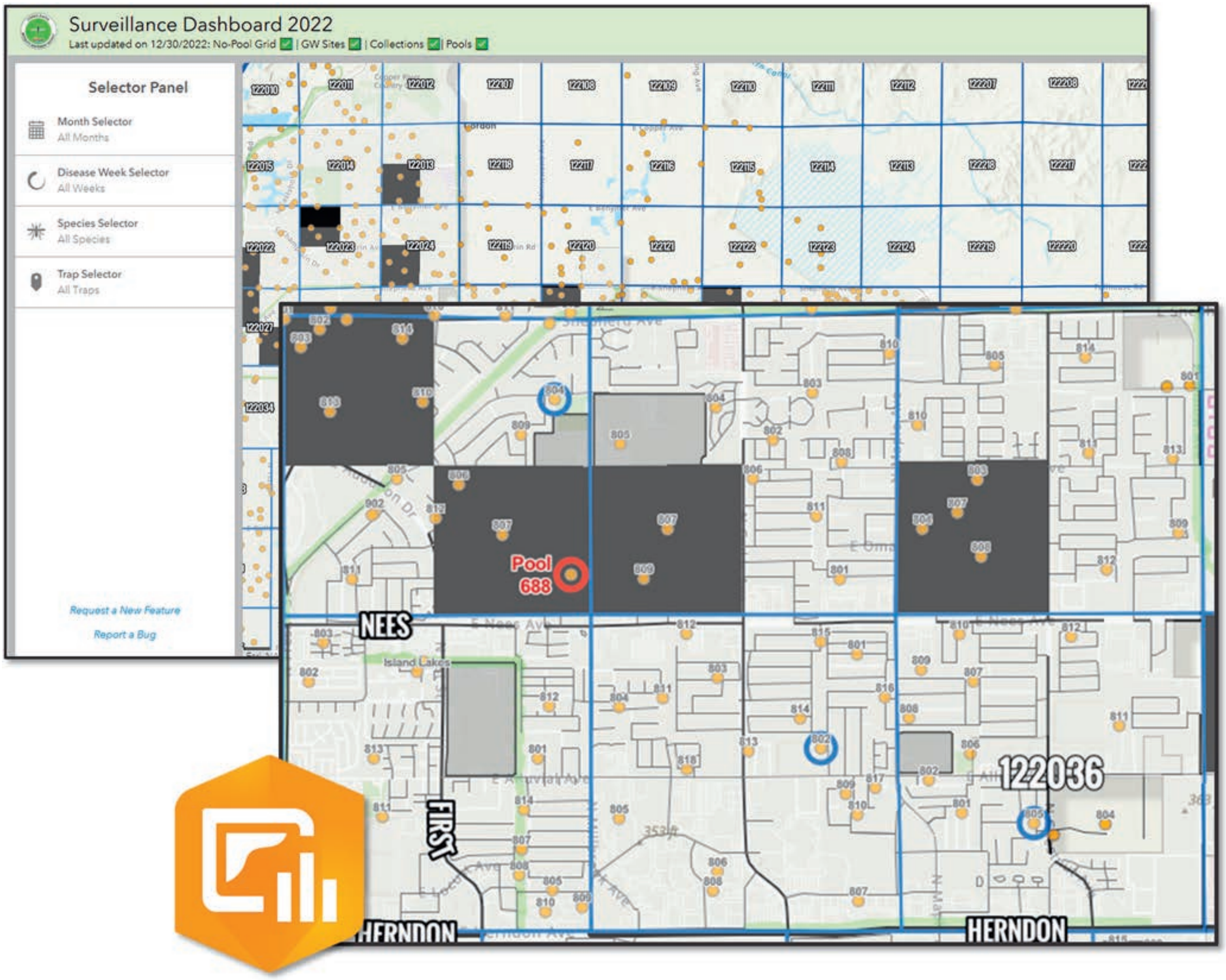

Figure 3.—Screenshot of interactive ArcGIS surveillance dashboard.

cartographers to incorporate dynamically contextual layout items, tailored to a specific geographic area. Each record from a sourced shapefile or feature layer dictates a single page in the total report. This allowed for additional content to be displayed at the top of each map, providing additional context (Fig. 7) without having to configure each map manually.

- ▶ The geographic location of the current extent.
- ▶ The subzone work assignment for that extent.
- ▶ The status and date of Adult Control measures carried out in the area.

The legend of each map also was configured to dynamically display information current to the geographic extent set by the page on the map series. The legend (Fig. 8) allowed viewers to have a clear understanding of sources and their status.

Conclusion

By utilizing advancements in GIS techniques and vendor features, the Consolidated Mosquito Abatement District improved overall awareness of existing sources adjacent to confirmed arbovirus detections. Operational response maps provided rapid insight into sources that were targeted for intervention without having to go site by site and view individual source information. Advances in digital tools and technology must continue to be leveraged in public health to ensure a timely response to disease threats and accountability to disease response programs.

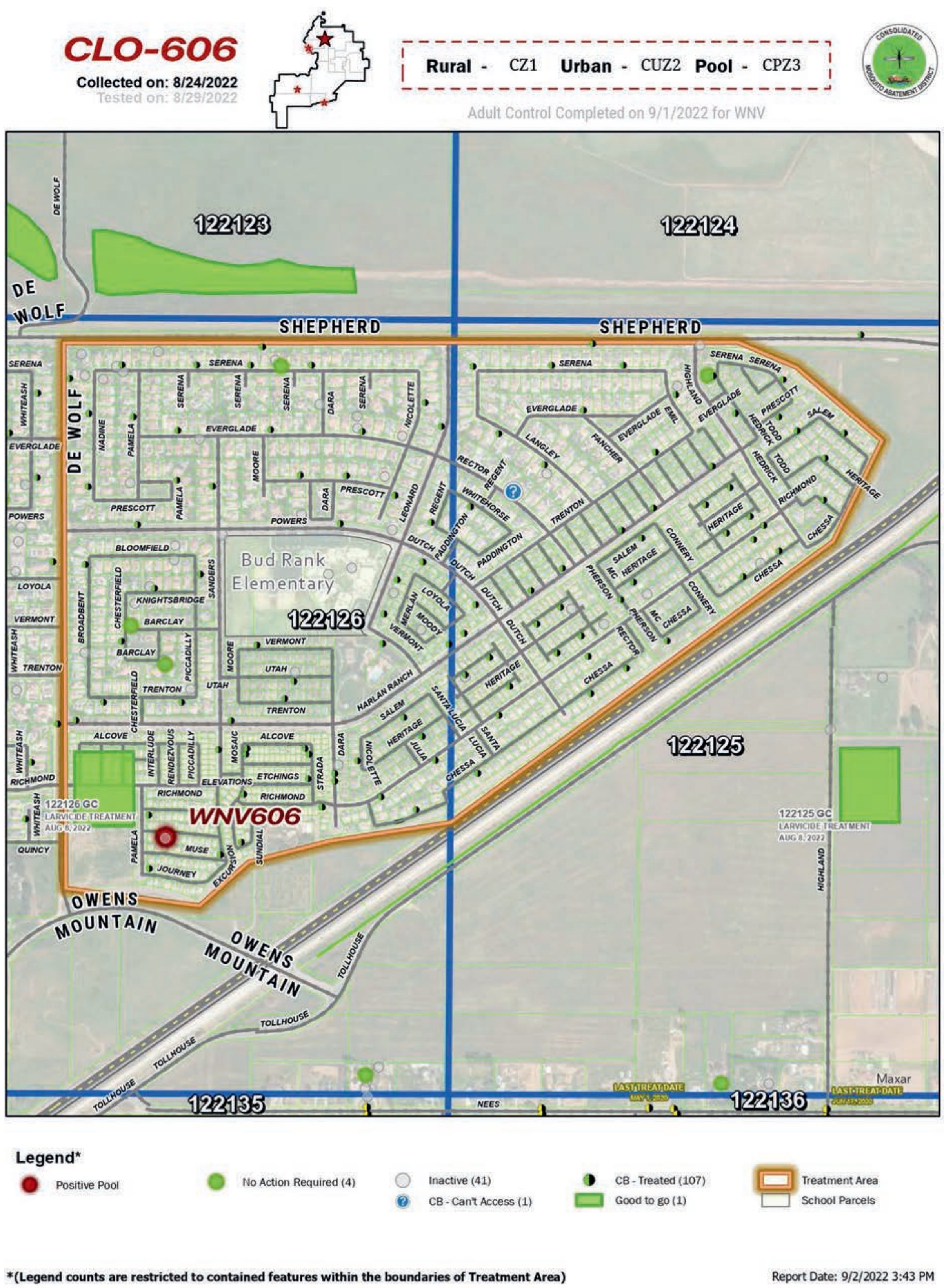

Figure 4.—Sample of static operational map showing confirmed arbovirus mosquito sample and sources along with source details in the area.

```
Expression
var siteName= split($feature.NAME, "-")
var lastinsp = $feature.LASTINSPECTACTIVITY
var lastdate = $feature.LASTINSPECTDATE

return "<BOL>"+siteName[1]+"</
  BOL>"+TextFormatting.NewLine + "<FNT size =
  '6'>"+lastinsp+"</FNT>" +TextFormatting.Newline
  +"<FNT size = '6'>"+Text(lastdate, 'MMM D, Y')
  +"</FNT>"
```

Figure 5.—An expression excerpt using Arcade syntax to enforce multi-value formatting for labels of mosquito sources.

Figure 6.—Primary symbology classes for the map's known sources.

Figure 7.—Enriched information of the focused area was provided by using data-driven dynamic text elements.

Figure 8.—Enriched information of the focused area was provided by using data-driven dynamic text elements.

Utilizing data analysis tools to maximize mosquito control efforts in underground storm drain systems

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Abstract

The expansive network of underground storm drain systems (USDS) beneath Los Angeles and surrounding communities account for a large portion of the overall mosquito abundance observed in Los Angeles County. For the past two decades, the Greater Los Angeles County Vector Control District (District) has had resources in place dedicated to mitigating the impact of USDS on mosquito populations within its jurisdictional boundaries. Initially founded in response to the threat of West Nile virus, the USDS program focuses control efforts primarily on *Culex* species. Recent in-house development and implementation of in-the-field digital record-keeping software and an internal SQL server database have allowed the Dsitrict to capture more accurate USDS treatment and inspection data. However, with the District's mosquito collection data stored on the Vectorborne Disease Surveillance Gateway (VectorSurv) database, analyzing the datasets together meant downloading data from each database. Without a centralized database, the District couldn't visualize data in real-time, nor could it easily evaluate its treatment strategies. To do this, the VectorSurv database was merged with the District's internal SQL server database, with the SQL server set up to receive daily updates from VectorSurv. With the data in one centralized database, the District used visualization software, Microsoft Power BI Desktop (Power BI), to generate reports for the USDS program. Calculations were created using Power BI's built-in syntax, DAX, to depict the total number of USDS treatments, the average number of female *Culex quinquefasciatus* mosquitoes in gravid traps per trap-night (F/TN), and the 5-year average for F/TN over time (Fig. 1). Visualization of these pooled data revealed that peak treatments occurred from July to October. In contrast, peak *Cx. quinquefasciatus* abundance occurred from April to July. Early season treatments may lead to lower abundance throughout the year. In March 2022, treatments increased more than 26-fold from previous years, and 2022 saw lower *Cx. quinquefasciatus* abundance compared to the 5-year average. Higher numbers of early-season treatments in subsequent years must be conducted to confirm this result. Utilizing technological tools such as an SQL server database and Microsoft Power BI Desktop allowed GLACVCD to monitor treatment strategies and mosquito abundance trends in real-time to maximize control efforts and help control the spread of West Nile virus.

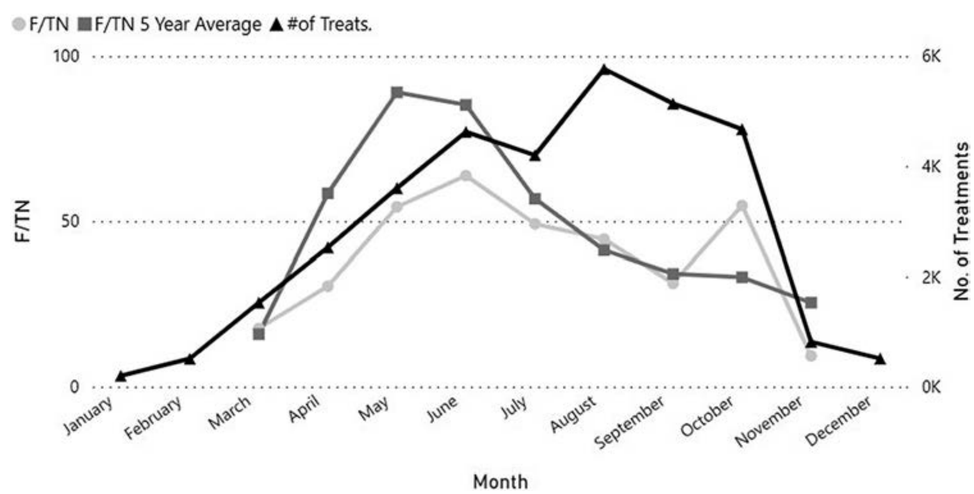

Figure 1.—The total number of USDS treatments, *Cx. quinquefasciatus* F/TN, and the 5-year average for *Cx. quinquefasciatus* F/TN over time for 2022.

BacDrop™ - A cloud based tool to characterize larvicide droplet parameters

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Abstract

Accurate droplet characterization is essential for optimizing product efficacy, cost, and ensurance that applications are within label requirements. More specifically for WALS applications, larvicide droplets must be in the extremely fine to fine range (30−235 μm) to effectively drift and move through the environment to reach cryptic habitats in urban and suburban environments. Valent BioSciences recently has developed an easy-to-use larvicide droplet analysis cloud application that is freely available on the Valent BioSciences webpage. This user-friendly interactive app can be used for liquid larvicides and allows applicators to conduct droplet and swath analysis by uploading a single document of droplet data collected from kromekote cards analyzed using ImageJ (http://imagej.nih.gov), a free image processing and analysis program developed by the National Institute of Health (NIH). After uploading the raw droplet data to BacDrop™, downloadable graphs are generated plotting the calculated volume median diameter (VMD) and number median diameter (NMD) with respect to swath distance. Additionally, a downloadable excel file is available detailing droplet density and individual VMD with respect to swath distance.

Efficacy and safety of the Takeda teravalent dengue vaccine

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Introduction

An ongoing long-term efficacy trial in eight dengue-endemic countries is evaluating a recombinant tetravalent dengue vaccine based on a DENV-2 backbone (TAK-003). Here we present an additional 18 months of follow-up data for a total of 4.5 years of evaluation.

Methods

From September 2016 to March 2017, healthy 4−16-year-old children (n=20,099) were randomized 2:1 to receive two doses of TAK-003 or a placebo three months apart and were under active febrile illness surveillance to detect symptomatic dengue (both outpatient and hospitalized) using a serotype-specific RT-PCR. Serious adverse events (SAEs) were collected throughout the trial.

Table 1.—Cumulative vaccine efficacy (VE) from dose 1 to 4.5 years post-Dose 2

	VE %	95% CI
Cumulative VE from Dose 1 to 4.5 years post-Dose 2		
Against VCD	61.2	56.0−65.8
Against hospitalized VCD	84.1	77.8−88.6
VE in baseline seronegative participants		
Against VCD	53.5	41.6−62.9
Against hospitalized VCD	79.3	63.5−88.2
VE in baseline seropositive participants		
Against VCD	64.2	58.4−69.2
Against hospitalized VCD	85.9	78.7−90.7

VCD = virologically-confirmed dengue infection.

Results and Discussion

Overall, 20,071 children received ≥1 dose of TAK-003 or the placebo; 27.6% (5,547/20,063) were seronegative at baseline. A total of 18,260 (91.0%) completed up to 4.5 years post vaccination follow-up and 27,684 febrile illnesses were reported. These led to detection of 1,007 RT-PCR confirmed dengue cases, 188 of which required hospitalization. The cumulative vaccine efficacy (VE) from first dose until 4.5 years after the second dose is summarized in Table 1. Efficacy continued beyond 3 years of vaccination regardless of baseline serostatus, with sustained robust protection against hospitalized virologically-confirmed dengue (VCD). Rates of SAEs were similar between the vaccine and placebo groups and no important safety risks were identified.

Conclusions

Two doses of TAK-003 three months apart were well tolerated and protected against symptomatic dengue through 4.5 years after vaccination in both dengue-naïve and pre-exposed children in dengue endemic countries. Efficacy was higher and sustained against dengue, preventing hospitalization.

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No code, no problem: A year with a homemade mobile app

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Introduction

The daunting task of information management at special districts and other government agencies lead many to think that creating their own self-sufficient system is not feasible given available resources. In 2022, the Catch Basin Program at the Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) began full implementation of a "no-code" mobile application developed in-house over the course of the previous year. The new in-house application system proved to be an easily adaptable solution for departments with numerous data points and no existing geolocation data. Previous efforts to collect catch basin location data involved methods ranging from hand-drawn parcel maps to overlaying satellite images on scanned paper maps, and required tedious annual updating to ensure accuracy against new construction. The program's new application allows for ongoing modification and can facilitate report generation, record archives and instant data accessibility. The current overview describes the development process, the manifold benefits to the application's implementation, and how the concept is now being applied interdepartmentally.

Methods

The management of hundreds of thousands of catch basin data points - including the maintenance of a growing related historical database - was not a sustainable endeavor using paper records. Seeking outside developer options for a software solution proved prohibitive with respect to cost and the feasibility of progressive modification. Taking advantage of a nascent software concept - the "no-code" development platform - allowed for the complete abrogation of paper records, accommodated the cost needs of SYMVCD, and created an adaptable system for managing all data related to catch basin treatments, inspections, and sampling. The SYMVCD mobile app was built using AppSheet (Google, Mountain View, CA), that utilized a spreadsheet format that could be configured to create a mobile application. Using language akin to spreadsheet formulas, all actions and relationships within the application could be defined and tailored to suit the high-volume field activity of the Catch Basin Program as well as the incorporation of a scheduling feature, automated notifications, and a messaging system. Although a basic understanding of spreadsheet formulas was required, agencies similar to SYMVCD are typically engaged in managing information using similar methods, and ample resources are readily available online at no cost. To anticipate scaling issues and maintain the dexterity of the application, the program undertook a migration of all collected data from spreadsheets to a cloud SQL database - BigQuery (Google, Mountain View, CA) - which allowed for a massive amount of information with no needed change to the configuration of the application.

Results and Discussion

Upon the creation of a custom mobile application, the use of paper maps was eliminated and larvicide treatment scheduling, pesticide use reporting and catch basin sampling data were all moved to the mobile app. Data were analyzed in real-time within the application, and a host of previously disparate processes was achieved within one platform in a readily visible way. Changes to data in the application, such as catch basin larvicide applications, can be seen by other users in the field without the hindrance of prolonged load times. Searches across all column types – timestamps, user name and location, and much more – can likewise be performed without the need to load or otherwise refresh the application, allowing for instant recall of date ranges and all other forms of recorded activity.

Conclusion

The transition from paper maps to a custom mobile application has increased productivity both in the field and in the reporting process. Numerous tasks have been automated, while the execution and analysis of other tasks is now centralized in one digital platform.

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Did we change behavior? Find out by using strategic planning for PR measurement.

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Introduction

Public relations (PR) professionals at mosquito and vector control districts across California report they conduct outreach and provide information to the residents in their districts' jurisdictions, most often by passing out brochures, posting messages on social media, sharing videos, giving presentations, participating in events and school programs, and conducting advertising. Surveys show these public information options have been successful in raising awareness about vectors and vector-borne disease, but simply sharing information has not necessarily prompted residents to take action to reduce a vector habitat or food supply. Implementing a strategic plan prior to passing out brochures, or conducting other forms of outreach, may provide district personnel with information that allows for a targeted outreach plan that is more likely to be successful in changing behavior.

Materials and Methods

A strategic plan includes background research, strategy, objectives (goals), tactics, and measurement. Research provides insight into stakeholders' priorities, level of understanding and trusted sources. Research methods include using a jurisdiction-wide survey, creating a do-it-yourself (DIY) telephone survey, sending direct mail questionnaires, asking community influencers to hand out questionnaires at meetings and gatherings, taking a social media poll, or by reviewing existing data from state or county demographics. These research options are diverse and allow both PR Professionals and districts that lack dedicated outreach staff to conduct useful research toward a comprehensive strategic plan.

A strategy, that is informed by research, is designed to determine how to share information with stakeholders that prompts behavior change. Studies show that most often people are moved to behavior change by a successful triggering event that allows PR professionals to build relationships with stakeholders. Goals and objectives are designed to support the strategy and the district's larger goals of protecting public health by minimizing the risk of vector-borne disease for the community. SMART goals are most effective because by definition they are specific, measurable, attainable, relevant, and time bound. Tactics are how PR professionals reach the goals and objectives. Tactics provide information to stakeholders in the most effective ways possible— from the source residents say they receive trustworthy information. To determine if the strategic efforts have been successful, PR professionals create a baseline measurement using surveys, website analytics, media monitoring, or the information field employees report after providing a district service; options to determine what residents know related to the planned outreach. During the outreach campaign, PR professionals continue to monitor success using the same means as used during the baseline measurement and thereby gauge how the campaign is progressing. Once the outreach plan has been completed, a final survey reveals whether the outreach effort was successful.

Conclusions

Raising awareness among constituents is not enough to reduce the risk of vectors or vector-borne disease. Stakeholders must change their behavior. Strategic planning for PR measurement is how Public Relations, Public Affairs, and Public Education professionals determine if efforts to change stakeholders' behavior were successful. Strategic planning involves conducting research, planning strategy, creating goals and objectives, designing tactics to achieve the goals and objectives, and conducting measurements before, during and after the outreach effort. Strategic Planning by a district's Public Relations, Public Affairs, or Public Education department staff results in data that can benefit the entire agency, because it reveals how residents are responding to public health messaging and if they are making the changes necessary to reduce the risk of vectors of disease or vector-borne disease in the community.

Design the most effective risk communication message and get the most action

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Abstract

Risk communication studies and theories in public health messaging were explored and applied to a public outreach campaign in Southern California over the summer of 2022. This paper examines factors affecting behavior change, including awareness, acceptance, and how persuasive messaging affects preparedness. The case study in the Coachella Valley MVCD used targeting and then tailoring messages to test which messages had the greatest influence in persuading the population to complete the 'calls to action' from the campaign. The findings showed tailoring messages, coupled with specific actions stressing community consciousness, had a positive influence in persuading residents to complete actions that help prevent a human outbreak of mosquito-borne disease.

Introduction

As a public relations expert, the communications department has a multitude of charges to help fulfill the agency's mission. Beyond being the spokesperson and media manager, department staff are also expected to be fluent in the area of persuasion. Messaging campaigns are not only meant to create awareness, but also shape attitudes, alter practices and influence behavior in a meaningful way. The purpose of this paper is to explore risk communication studies and theories, and apply these concepts to a campaign by the Coachella Valley Mosquito and Vector Control District (CVMVCD) in the summer of 2022.

It might be easier to understand how public relations influence people by examining one of the first public relations experts, George Creel. Creel began a massive campaign during World War I with the goal of getting the U.S. public to support the war, in part, by hating the enemy (Maxwell, 2015). Creel was the ultimate persuasive communicator. He theorized people are more easily swayed in small and personal groups, so he trained and deployed a series of spokespersons to spread his messaging in places people gather such as movie theaters. We use many of his techniques today. His campaigns were wildly successful and directed social scientists on a decades-long study of propaganda.

Up until WWI, the word "propaganda" did not have a negative connotation. Propaganda only referred to persuasion of the masses by communicating one side of an idea or point of view with the hope of the public accepting that suggestion (Black, 2001). Creel's campaigns were effective in changing public opinion and behavior on a massive scale.

The current paper navigates different aspects of persuasive speech through risk communication; that is, preventative or preparedness messaging to create the most effective campaigns preventing poor behavioral practices – or influence good behaviors – in public health. The challenge for public relations in effectively conveying risk depends on the variety of environmental influences the receiver of the message has surrounding them. How you convey messages is equally important to what is said, which is equally as important as how the receiver receives the message. Even if the receiver receives your message, they still need to accept the message – then act on it – for the message to be successful.

This paper is divided into two sections. The first section will explore research on influential messaging campaigns including awareness, acceptance and persuasion, and how those factors influence messages in risk communication campaigns for public health messaging. The second section will identify how to best utilize other researchers' findings to influence a messaging campaign in Southern California. Finally, a discussion of the findings of the 2022 summer campaign and the lessons learned are presented.

Awareness

Gaining audience awareness is one of the first concepts learned in communication but may be misunderstood in relation to risk communication. In disciplines such as marketing awareness is the first step to purchasing a product (Neimand and Christiano, 2017), or, as Michaelson and Stacks (2011) suggest, exposing your audience to a key message is the most basic concept. Additionally, Grunig's situational theory of publics (Moffitt, 1999) explains an audience's level of awareness and categorizes them into likely areas of action. The general idea behind awareness is if people are exposed to a message (being made aware), they will make the decisions we, as risk communicators, want them to make. However, researchers (Winneg et al., 2018; Michaelson & Stacks, 2011) have found knowledge (awareness) does not have a direct impact on behavior. In fact, one human health related study from Noar et al. (2007) explored the awareness of healthy lifestyles and found even though U.S. adults were aware of healthy lifestyles, only 3% completed all four of the criteria set for healthy living. Awareness of healthy habits was not enough.

One of the most compelling arguments against awareness leading to behavior change is what is commonly termed "going viral". Communicators want messages to go viral and are secretly (or openly) jealous of those getting there. Because going viral measures a post by a tangible number (likes, shares, views, etc.), the numbers become a measure of success. However, in the article "Stop Raising Awareness Already" Neimand and Christiano (2017) examined a CDC campaign called Preparedness 101: Zombie Apocalypse. The campaign's goal was getting people to create a preparedness kit by telling them: if you can prepare for a zombie attack, you will also be prepared for a disaster. The campaign was both funny and clever. But the study found that was even though the campaign reached hundreds of thousands of people (went viral), this awareness did not translate into more people actually packing a preparedness kit, and in many cases, had the opposite effect called 'reactance', which is discussed later in this paper.

Behavior

So, if awareness doesn't drive behavior change, what does? Here again, it's complicated. Theories vary from asserting that perceived threats will motivate change (Winneg et al., 2018) to beseeching change based on moral exigency and shared responsibility (Nan et al., 2022). One theory presented in the book *Change or Die* (2008), suggests that intensive intervention can change behavior. The author Alan Deutschman describes a group of people who have a heart condition and unless lifestyle habits change, they will need major heart surgery – or die. In the study, after months of intensive support including dieticians, personal trainers, mental health experts, 77% of the patients were able to avoid surgery at a cost savings of almost $40,000 each.

Unfortunately, government communicators rarely have those kinds of intensive programs available for preparedness campaigns. When the public faces an emerging or impending disaster, there is little or no reaction, because the threat is not considered important enough (Smith, 2002) or their perceived susceptibility of getting sick is low. Risk communication doesn't get much attention because people just don't care or don't believe that disaster will strike them.

Risk Communication

Some researchers suggest the level at which you believe your health is at risk will influence your investment in prevention. Noar et al. (2007) stated "a prerequisite for behavior change is an individual recognizing that he or she is at risk and that the seriousness of the disease or outcome is severe enough to motivate protective action". Winneg et al. (2018) tested this theory by studying the Zika outbreak in Florida. The Zika virus is transmitted by either mosquito bites or less commonly sexual transmission and may cause birth defects which should be concerning to both males and females of childbearing age. When first reported on the island of Yap, over 73% of the population contracted the virus making it a significant public health concern. Winneg et al. (2018) theorized that if the behavior change messaging focused on the risk of getting the disease (awareness plus threat), they could effectively stop the outbreak. After a large-scale campaign educating Floridians, the study found Floridians did indeed have more knowledge about Zika. However, of those at higher risk needing to take preventative measures, only about 37% took any action to prevent either mosquito bites or mosquito larval habitats. Even households with a pregnant woman did not take significant steps to prevent mosquitoes or mosquito bites compared with other Florida households. Those not living in Florida felt a kind of optimism bias, believing they would not be affected by the disease and therefore took no action to prevent it.

Too much emphasis on threat in risk communication may be, in part, responsible for this low level response. Steindl et al. (2015) explained that "reactance" is a human response to the loss or threat of loss of personal freedom and the motivation to regain lost freedom. They used the example of a health class at a fitness club to explain this concept. Participants that were told by their instructor "you have to do this", experienced more reactance and did not do the specific exercise, unlike when instructors said, "consider doing this". Similarly, if you tell an adult they are at risk or their lifestyle choices are unhealthy, they often have a similar reaction of immediately wanting to do the opposite (Gerrard et al., 1999). Another example of reactance is studying the smoking habits of teens. Teens know the risks and negative consequences of smoking, but some, even today, still do it (Berger, 2017). Being overbearing about risk is not an effective way to influence behavior.

Other documented feelings have been studied, using positive versus negative emotions with conflicting opinions. "[P]ositive emotions can be more productive" (Nan et al., 2022) versus "fear appeals can be effective" (Smith, 2002). They agree, however, that it's a delicate balance easily tipped either way. Fear, according to Smith (2002), can evoke changing health behavior by appealing to a person's guilt or shame sentiments. Anxiety, according to Basch et al. (2020), helps "[t]he body become energized to cope with threat, and attention becomes narrowly focused on threat-related information".

From Awareness to Acceptance

All these ideas culminate in the ability to persuade. Christiano and Neimand (2017) compared two health campaigns targeting children, the "Just Say No" campaign and the "Let's Move" campaign in their infancy stages. Just Say No explained the negative consequences of drugs and smoking, but studies showed that these children were more likely to use drugs (reactance) after being exposed to this messaging. In contrast, Let's Move in which the main objective was to reduce childhood obesity, gave suggestions instead of language like "don't eat that". Suggesting that children consume less fat and sodium was positively correlated to reducing the number of obese children.

Acceptance of a message when it comes to persuasion is heavily influenced by culture. For example, reducing drinking and driving has been a national campaign for decades and although the language is often compelling and emotional which should influence the acceptance of the message, Wakefield et al. (2010) found these campaigns have limited success because drinking is often perceived as a social norm rather than a dangerous practice.

Berger (2017) also found cultural influence when studying acceptance leading to behavior. The cartoon character Popeye ate spinach to become stronger, and the cultural desire, or acceptance of a latent message like "eat spinach and you'll be strong like Popeye" is thought to be the reason spinach consumption increased by one third at the time. If a salty old sailor who smoked a pipe could be the voice of healthy food choices, health messages should be able to elevate their messages to be persuasive.

Acceptance to Action

To examine the causation of turning acceptance into action, the concepts of targeting versus tailoring should be considered. The term population means every single person in your campaign. The idea of targeting is to identify groups within the population you want to affect. Tailoring takes targeting one step further. Noar et al. (2007) describes tailoring as "any combination of strategies and information intended to reach one specific person, based on characteristics that are unique to that person, related to the outcome of interest, and derived from an individual assessment". Tailoring may be one of the most influential components of persuasion, and the most difficult to achieve. Although mass media campaigns can be clever and funny like the Zombie Apocalypse showed, spending those kinds of resources does not seem to achieve the desired action. Coupling tailored messages to a positive cultural desire to reduce risk was studied in the current project.

Public Health Messaging

Vector control, specifically mosquito abatement, has been a government service for over a hundred years in many areas of the world, including the United States. No matter where you live in the world, you have likely been bitten by a mosquito. But the annoyance of being bitten is not classified as a public health concern. The mosquito is the deadliest animal in the world. "Each year, more than one billion people are infected and more than one million die from a mosquito-borne disease" (Potter et al., 2016). When studying the effectiveness of mosquito control messaging over the last few decades, findings are disheartening. Communicating science in mass campaigns is complex and difficult to both explain and understand which leads to the receiver of the message distrusting the source (Hulcr et al., 2019; Basch et al. 2020). Furthermore, knowledge of mosquito-borne disease varies between demographics (Potter et al., 2016; Ahluwalia et al. 2021), which means any message communicated needs to take demographics into consideration. In the case of the mosquito-borne disease Zika, between 2015 and 2016 there were over 4,400 confirmed cases in the high-risk demographic in the United States alone (Samuel et al., 2018). Even in Winneg et al. (2018) there were disappointing findings when studying messages to a high-risk demographic during a Zika outbreak in Florida. They found "less than half of the public had done anything personally to prevent the spread of the virus". There was awareness within the high-risk demographic, they accepted the risk as truth, but messaging was still not effective enough to influence action. The world's deadliest animal was actively transmitting disease, and that was not enough to influence action.

Methodology

In the fall of 2021, the Coachella Valley Mosquito and Vector Control District (CVMVCD) conducted a series of information gathering events from the residents of the Coachella Valley using qualitative and quantitative methods. The project goals were to determine knowledge, attitudes, and practices of the residents in the CVMVCD jurisdiction and to gain insights that might identify the most persuasive messaging technique for future prevention campaigns (risk communication).

Information was gathered in two ways. First, two focus groups were used to find knowledge, attitudes, and practices of people within the population being researched. The intent was to use qualitative means to discover how people are talking about an issue, the language they used, and what language was most effective. As part of the focus group project, participants were also required to fill out an activities log − a behavior audit − daily for the 10 days leading up to the scheduled focus group. The second part of project was an area-wide survey. A total of 351 surveys were completed by people residing in the valley at least part of the year.

Hypothesis and Research Question

With background information from the project of 2021 and the supporting literature review, the research team

Table 1.—Campaign effort, surveys sent and results from mosquito surveys in three communitiues within the Coachella Valley.

	Community Unity (Indio)	Control (La Quinta)	Self Preservation (Rancho Mirage)	TOTAL
Postcards sent	936	1,003	860	2,799
Emails sent	125	127	76	328
Surveys	16	53	35	220 starts, 113 completed
Inspections	7	20	12	39
Breeding mosquitoes	1	3	0	4

hypothesized awareness messaging was not enough to influence behavior. However, if messages were directed to a specific person in the household (tailoring), coupled with specific actions, and those actions are linked to an emotion − those messages should influence source reduction activities. The importance placed on community effects or personal responsibility should influence source reduction activities. But which message would be most effective?

Summer 2022 Campaign Implementation

The survey portion of the research yielded findings that influenced the implementation of the following summer's campaign. For example, the project found households with higher incomes were more willing to participate (36% Very Likely or Somewhat Likely) in a program similar to neighborhood watch indicating persons of higher socioeconomic status might be more willing to complete actions requested of them by CVMVCD for the good of the community. This part of the research also found 76% of respondents look for and remove standing water on their own property.

Part two of this research was the campaign that emerged from the market research findings (District-wide Market Research Project. https://www.cvmosquito.org/sites/g/files/vyhlif4551/f/uploads/2022.05.10_may_board_agenda_packet.pdf). In the summer of 2022, three cities in the Coachella Valley were targeted with the intent of reducing the public health risk mosquitoes create by persuading residents within those areas to complete the most effective mosquito control − source reduction.

To remove the variable of attaining awareness, only residents having interacted with the CVMVCD by requesting service were messaged. Messaging pieces suggesting action were sent to tailored persons within the targeted group separated by city (Table 1). All things being relatively equal and randomized, groups were asked to do something or not, then the researcher observed how each group responded. This method is generally described as A/B Testing. Residents received a different postcard containing a message based on their grouping. Group one, Indio, received persuasion messaging based on suggesting community commitment (community unity). Group two, Rancho Mirage, received messaging focused on personal responsibility (self-preservation). Group three − the control group − La Quinta received messaging stressing neither community nor personal responsibility. Their messaging included information about the problem, but actions were

Table 2.—Survey results for three communities within the Coachella Valley.

Percent of respondents to say YES				
	Indio	La Quinta	Rancho Mirage	AVERAGE
Recognize the postcard?	30.7	50.9	51.4	49.1
Self inspect?	30.7	52.8	45.7	44.6
Know how often it's necessary?	100	79.2	91.4	87.5
Share with friends?	57.1	43.3	65.7	43.8

1. 47% of respondents did not inspect their property for standing water (the action item) after reading the postcard.
2. 13% didn't know inspections needed to be done weekly.
3. 43% did not share information with friends or family.
4. Within 2 weeks of receiving a postcard, Service Requests were: Indio 0, LQ 8 (3 with mosquitoes) and RM 3 (no mosquitoes).

not emphasized. A sample of each group received a follow up email with similar messaging along with a request to complete an attached survey. Each group was surveyed to try and capture which group was most persuaded to complete the action item. Lastly, vector control technicians were deployed to a sample from each area to substantiate the respondents claims that the action had in fact been completed.

Results

The first two weeks after the postcard was sent led to some interesting results. The control group, which received an informational postcard not stressing action called CVMVCD for service more often than the other two groups suggesting − as hypothesized − simply being aware of an issue did not lead to action (Table 1). The control group expected CVMVCD to resolve the problem for them. Further, technicians deployed to these sites found 63% of the properties positive for mosquitoes. The self-preservation group also called in for service rather than taking responsibility for their property and expected the CVMVCD to resolve their problems. However, inspections by technicians in this area did not find mosquitoes (Table 1). The community unity group did not call in for service. In fact, no requests for service came from the community unity group within two weeks of receiving a tailored postcard stressing the importance of community commitment.

Responses to the survey (Table 2) revealed interesting results. Again, the control group, although the messaging was tailored, seemed more confused as to the action requested and were unsure of basic mosquito life cycle concepts such as the frequency needed to remove larval sources. But in general, respondents knew landscaping should be self-inspected every week, although less than half of them (47%) did it. It was encouraging that the other half self-inspected as they have learned/been taught and may suggest over time that more people will buy into weekly self-inspecting.

To further test the effectiveness of this campaign, there cannot be sole reliance on survey responses. Even anonymous surveys are problematic because, to summarize social scientist Stephens-Davidowitz (2017) "everybody lies". Responses need to be verified and validated – as the survey suggests – at least half of the properties inspected will be dry and have no mosquito breeding.

Interestingly, or perhaps validating the key indicators of persuasion, tailoring key messages did seem to work in this case study. Ninety percent of the 39 completed inspections remained dry, although there were plenty of potential sources according to technician notes. Further, of the 10% not dry and actively having mosquito larvae (not completing the action), 90% of those were in the control group not receiving specific calls to action in messaging.

In answering the research question which group would be most persuaded, not using an emotional or cultural connection to tailored messages resulted in the least desirable outcome. Deploying technicians to individual homes took additional time and worse, the technicians found mosquito larvae present. According to this case study, tailoring messages to individuals at a specified address coupled with an emphasis on actions providing protection and safety for the community at large resulted in the most desirable outcomes.

Lessons Learned, Recommendations

The recommendation to risk communicators is to tailor your messaging as specifically as possible. This suggestion might seem small but can have great effect gaining focus and attention. Use language that is persuasive and not threatening. Must, need, and no are examples of harsh language that induce reactance rather than acceptance. Instead, use terms that suggest, not force. Further, pleading to an individual's moral responsibility does seem to promote a positive response as suggested by Nan et al. (2022). Their claim to incorporate this approach has the potential to significantly impact the community. The emotional connection to be a part of a community rather than preserving oneself was most effective in stimulating actions such as going outside and removing water sources.

The lessons learned, or limitations of this research, include the demographics of each group tested. Although steps were taken to randomize and reach residents sharing similar demographics, race, language, political affiliation and income varied greatly among groups. Future research is required to test whether similar results are observed when the messages are rotated among groups.

Summary

This paper that examined different parts of creating effective messaging campaigns was meant to utilize awareness to shape attitudes and alter practices related to risk communication. Risk communication was analyzed through the lens of public health and how emotions can influence one's sense of community insomuch a person is willing to complete an action for the good of the community. If the community's cultural desire is to reduce public health risk, this study suggested individuals will support the actions asked of them. From this case study, it appeared tailored messaging had a positive influence on residents in persuading them to complete actions to help prevent a human outbreak of mosquito-borne disease. Much like in Smith's (2002) recall of the healthy heart study, intensive intervention such as tailoring to a specific person coupled with specific actions stressing community consciousness reach the desired outcome.

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Building EXTRA-ordinary campaigns: Best Practices to enhance Fight the Bite messaging

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Introduction

A key element in the Sacramento-Yolo Mosquito and Vector Control District's Public Information and Outreach program is its extensive media relations, advertising and earned media campaign. The annual Fight the Bite campaign strives to raise awareness of mosquitoes and the prevention of mosquito transmitted diseases as well as District services and activities. This presentation provided an overview of the various public outreach strategies that have been used to communicate with residents including paid advertising, public affairs shows, enhanced news coverage, studio partnerships and participation in community events.

Methods

The District's Public Information and Education Department provides dynamic messaging that is purposely planned and placed to raise awareness of mosquitoes and how as the District works to prevent and abate the spread of disease and protect the public health. The District uses a wide array of advertising, media tools, events, and public outreach to accomplish our communication and outreach objectives. Our paid advertising which runs from April-October utilizes market, consumer and media data to help create a multiplatform campaign that includes a mix of elements such as paid television and radio commercials; targeted cable, outdoor billboard and bus advertisements; and extensive digital, streaming advertisements and social media. We purposely target homeowners, adults with children at home, outdoor enthusiasts and adults 55 years and older.

Aside from paid advertising, the District utilizes earned and enhanced media coverage which is free publicity and exposure gained from methods not included in paid advertising. The most common types of earned media are news coverage, participation in community events, non-paid news features, social media mentions or partnerships and collaborations. One of the most important strategies commonly used by the District is disseminating information through media coverage as a result of a news release or pitching of a story. Building relationships with the media and giving them the visuals and information they want is extremely important. Typically the District has had great success with stories where media crews follow District staff as they conduct their daily work such as conducting home inspections, making treatments or setting laboratory equipment in the field. These stories often provide an in depth overview of a specific problem and provide useful information for viewers.

Two other important components of enhanced media include live in-studio segments on lifestyle and entertainment programs and recorded public affairs shows on television or radio. Both of these elements are unique because they offer the opportunity to provide in depth information on a specific topic such as invasive mosquitoes, use of repellent, aerial spraying for West Nile disease, etc. Public affairs shows can be recorded and aired across various stations and repeated throughout the season.

Another element traditionally used by the District to disseminate information is through participation in community events. Typically District booths are set up at 30 or more events between April and October, the months of peak mosquito activity. These events could be health fairs, cultural events, movies in the park, county fairs, etc. where we talk to people about District services, pass out repellents, encourage everyone to look for mosquito breeding sources around their home, and to call the District concerning any mosquito related issues. Events provide an opportunity to come face to face with residents and offer services.

A key element of our outreach includes contacting local elected officials and keeping them informed of all District activities relevant to their constituents. For example, this can include increased WNV activity, invasive mosquitoes in their area and our District response plan. We encourage them to sign up for our treatment notifications and provide information they can use to help us disseminate content to their constituents. In addition we set up annual spring presentations with each city and county to provide an update on West Nile virus activity, invasive mosquitoes and other relevant information going into the mosquito season ahead.

Results and Discussion

The implementation of ongoing public outreach strategies is critical to ensure residents are informed of the District's Fight the Bite messages. Aside from utilizing a paid media campaign, it is important to implement earned media coverage because this builds confidence and a strong reputation as a trusted public health partner rooted in the community. By being present through advertising messages, participating in community events, and creating community partnerships the District sends the message that we are a valuable community resource striving to fulfill our mission of protecting the health of residents served by the District.

Conclusion

For more than 15 years the District has been implementing a comprehensive and multi-faceted advertising, media and public information campaign that successfully keeps residents informed about mosquitoes, West Nile virus and other mosquito transmitted diseases as part of our annual Fight the Bite campaign. Although messages may change depending on the needs and arising issues, the strategies utilized have proved to be very effective. Moving forward the District will continue implementing these tactics and evolving or changing messages as necessary. In addition we will use research and data to evaluate the campaign and ensure we are successful in reaching our intended target audience.

Acknowledgements

The Sacramento-Yolo Mosquito and Vector Control District staff for their assistance in helping to ensure public outreach to promote ongoing Fight the Bite messages.

Lightning in a bottle: When earned media truly pays off

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Introduction

The phrase, "lightning in a bottle" means a great, unlikely success, particularly entrepreneurial or in the media. This paper defines earned media, illustrates how our District (Contra Costa Mosquito and Vector Control District) attains earned media coverage, and the methods used to achieve media coverage in general. One particular story about mosquitoes turned into lightning in a bottle.

Methods: Defining and Achieving Earned Media

Earned media is free news coverage by print, television, radio, and social media outlets. It is a highly coveted concept, meaning that this type of earned news coverage is very much desired or sought after. Earned media is an important way to share a District's message in a timely fashion to a potentially wider audience. Our District seeks to achieve earned media by posting news releases on our website, emailing news releases to all Bay Area media contacts who have subscribed to our online marketing platform, posting our news releases to our social media channels, which include Twitter, Nextdoor, and Facebook, and posting articles relevant to news releases or vector-related issues via an online newsletter published in-house and available on our website and to subscribers.

Results and Discussion

On July 29th, a news release was published, informing the media and our residents of the first sample of mosquitoes testing positive for West Nile virus in the county. We provided this type of information to county residents routinely, and we post all news releases to our website. They were also sent to local media contacts and residents who subscribed to our email marketing platform. We used this initial news release as an education opportunity to remind residents about the significance of the virus, how weather influences it, what residents can do to avoid being bitten, and how they can contact the District to report mosquito issues. As a result of this news release, the District received media coverage by several Bay Area news stations. Our District also received coverage by way of several popular local news blog sites that cover the eastern and central portions of the county.

The news coverage we received regarding West Nile virus mosquitoes from July 29th resulted in a resident who viewed one of these news stories contacting the District and reporting a mosquito problem in their neighborhood. The resident explained they had just moved to this area of the county, and although they were being bitten by aggressive mosquitoes, they did not know who to contact until viewing the news story. A subsequent inspection resulted in the discovery of *Aedes aegypti* for the first time in Contra Costa county on August 3rd. As a result of that discovery, the District published a news release on August 5th that resulted in more media coverage. This time, it was about the newly discovered invasive species.

As a result of the news coverage regarding *Ae. aegypti*, the District published an online newsletter article on August 17th that further highlighted the discovery and educated residents about their significance, why we needed to eliminate them, and how they could do their part and help the District protect public health.

KRON, a Bay Area news station, subscribes to our notifications and as a result of receiving the online newsletter, contacted the District to do a story and live interview, which aired on August 18th. The live interview occurred at 9 a.m. and the story aired on the station throughout the day. The live interview focused on the importance of District technicians going door to door requesting resident's cooperation and allow us to inspect their properties for the possible presence of *Ae. aegypti*. The news story also resulted in residents of the affected neighborhood to contact the District and request an inspection of their property.

Conclusion

In conclusion, the concept of earned media, while highly desired and sought after, is actually easily achievable. The keys to success include: proper utilization and distribution of news releases by making sure the written content is appropriate for publication, distribution of news releases through district websites, social media channels, and perhaps an online marketing platform. Also, be prepared for immediate contact from local media outlets. Our District will often receive contact from news stations requesting to do a story just minutes after a news release is published.

You never know; your next earned media may lead to 'lightning in a bottle.'

Figure 1.—Public Information and Technology Officer Andrew Pierce interviewed by a reporter from Bay Area news station KRON.

Acknowledgements

The author would like to thank local media personnel for helping to disseminate important public health messages on behalf of the Contra Costa Mosquito and Vector Control District. Our public health agency truly relies on the media to help communicate timely and important information related to the insects and animals for which our District provides service.

All aboard the campaign train: Getting cities en route to effective mosquito control

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Introduction

The current analysis explores the latest campaigns implemented by Orange County Mosquito and Vector Control District (OCMVCD) and the foundation laid throughout the past five years to secure full city engagement in the County. Through collaboration, partnership, and relationship building, city members and organizations have assisted OCMVCD in building trust with the community and in reaching a new level of outreach and engagement.

Through planning, implementation and evaluation, OCMVCD has gained full support from all 34 cities that it serves to increase mosquito control awareness and education. The current presentation highlights the challenges of developing a county-wide campaign by exploring what worked and did not. The goal was to facilitate successful campaigns by sharing measurements and budgets with other agencies that can benefit from collaboration in outreach efforts.

Methods

An effective mosquito abatement campaign requires coordinated planning, preparation, execution, feedback, and review. Otherwise, the campaign cannot operate at optimum levels. To be successful OCMVCD must:

- Monitor all aspects of the campaign to adjust approaches where needed and incorporate lessons learned into future endeavors.
- Maintain close relationships with city officials to optimize and amplify messaging across communication channels and ensure that communication and outreach efforts are tailored to the needs, language and preferred media of the intended audiences.
- Listen to residents to understand and properly respond to their concerns and ensure that communication and outreach efforts are tailored to their needs and presented in language they can readily understand and digest.

Results and Discussion

In 2022, more than 1,000 truck magnets were delivered to 16 cities and public work agencies (Fig. 1). Each magnet can generate over 30,000 daily impressions. Having 1,000 truck magnets on vehicles travelling throughout Orange County resulted in an estimated 120 million impressions throughout the four-week campaign. However, magnets can be costly due to high demands for magnetic materials and a growing number of fleet truck bodies are made from aluminum which is not magnetic.

OCMVCD's campaign kit included a link on which cities could 'click' to access everything they needed to help

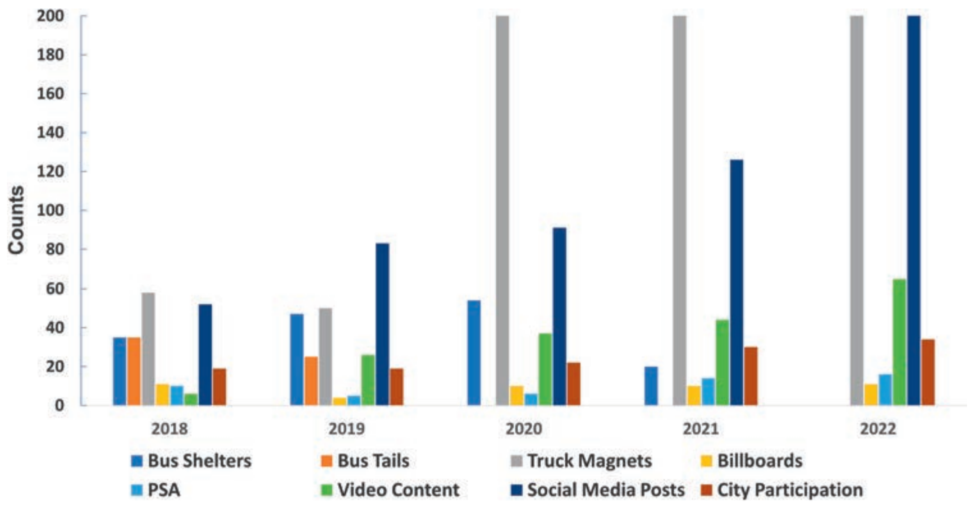

Figure 1.—Five-year measurement of annual mosquito campaign media presence from 2020-2022.

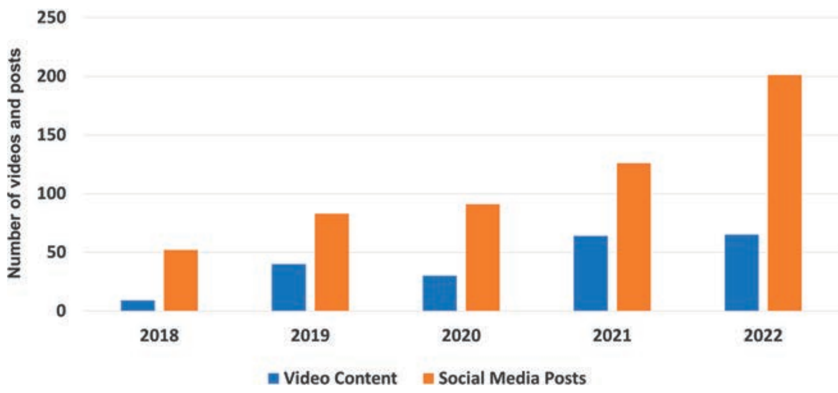

Figure 2.—Number of social media posts and video content per year increased from nine to 65 videos during 2018-2022.

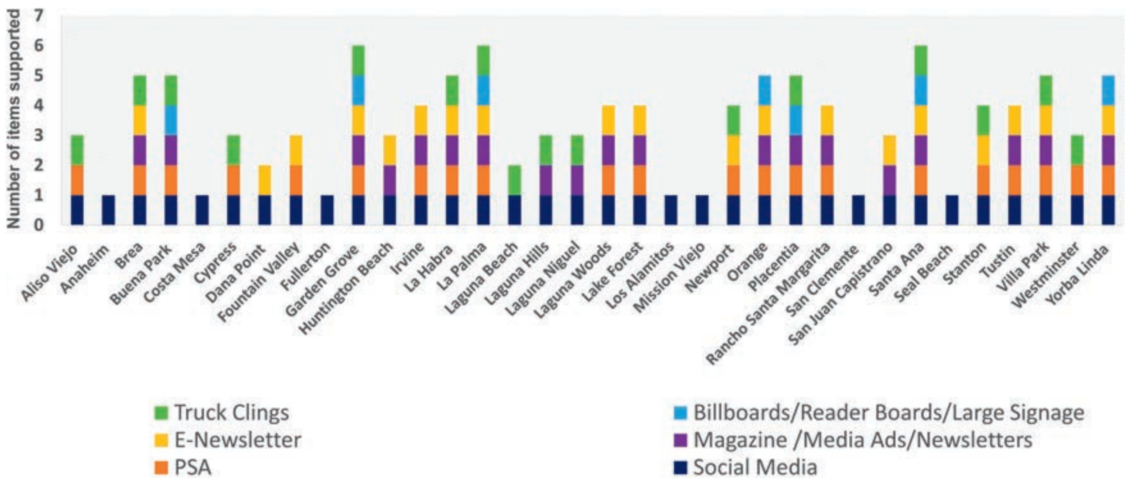

Figure 3.—Number of media items supported by each city during 2021.

support the campaign, including verbiage and hashtags, and accurate information that they could easily post to their social media accounts (Fig. 2). The United States boasts a staggering 302.35 million social media users as of 2023, with most of the public using social media as an information source (Djaziri 2023).

Conclusion

OCMVCD successfully gained the full support of all 34 cities it serves in increasing mosquito control awareness and education through planning, implementation, and evaluation of multiple approaches (Fig. 3). The implementation of a city-wide tracking system in 2021 introduced a healthy spirit of competition, as the tracker showcased which city would be awarded the coveted "Golden Mosquito" accolade for the most supportive municipality. By capabilities, information and techniques, other agencies may benefit from collaboration in outreach efforts and elevate campaigns for greater success. Based on input from cites, public agencies and the public itself, OCMVCD is developing a mobile app for the 2023 campaign. The app will contain current data input updated regularly by OCMVCD's GIS coordinator to map our West Nile virus 'hot spot' cities and areas highly infested with invasive *Aedes*. The mobile app can then reach out to residents in specific zip codes and locations with ads and notifications. We anticipate that this new mobile app will increase our impressions by the millions.

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The Proof is in the Pole Banners

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Introduction

The history of out-of-home advertising dates back to the 1850s and street railways. It has evolved from bill posters to street furniture, transit wraps, pole banners, digital billboards, and place-making experiences. Placer Mosquito and Vector Control District's (PMVCD) 2022 paid advertising campaign strategically used pole banners for out-of-home advertising to lower its cost per thousand and obtain a dramatic increase in its return on investment.

Methods

PMVCD's paid advertising campaign included consistent branding, digital billboards, pole banners, digital display advertisements, and analytic tracking and reporting. Pole banners were geographically situated in shopping centers where people visit frequently for daily necessities, entertainment and health with big box stores, supermarkets, pharmacies, and specialty retailers. To choose locations to advertise with pole banners in Placer County, we overlayed potential pole banner locations with the previous years' West Nile virus positive trap locations to see which four locations would reach people who experience higher risk levels of West Nile virus activity. The creative design and message on the advertisements encouraged EPA-registered repellent use and purchasing.

Results and Discussion

We chose four pole banner locations and deployed 24 total pole banners (48 faces – banners are double-sided) for 14 weeks, June through August. We received one complimentary week free of charge. We received 13,756,154 impressions from this campaign, with a total expenditure (not including production or installation fees) of $30,960. This was a 204% increase in impressions from our 2021 advertising campaign which was attributed to a longer run time and two additional locations as well as more community activity at stores as we entered the third year of the COVID-19 pandemic. The cost per thousand (advertising measurement for the advertisement being viewed by 1,000 people) of the pole banners was $1.72 and compared to other average advertising cost per thousand was less expensive than other media including poster billboards, digital ads, billboards, radio, TV, etc.

Conclusions

Overall, we found that pole banners were an effective use of our advertising budget to reach people in Placer County with important mosquito protection messages. We will continue to use this form of outdoor advertising to reach our county residents each summer.

Acknowledgements

Thank you to District Manager Joel Buettner for his support of our advertising program and our advertising vendor, Look Media.

Teaching in a virtual world: Measuring learning environments on Zoom

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Introduction

The challenge of teaching a virtual lesson is two-fold. The lack of immediate feedback from seeing and hearing the students makes program evaluation difficult. First, students are muted and often have their cameras off which makes it difficult for the presenter to gauge engagement and the lesson's success. Second, an intensive and interactive in-person lesson is ineffective in a virtual format. The approach to these problems was to restructure in-person lessons to virtual lessons by using teaching "best practices" and then survey teachers afterward to determine the effectiveness of the virtual lessons.

Methods

The Education Coordinator at Orange County Mosquito and Vector Control District (OC Vector) created a Google Forms survey to determine the effectiveness of the virtual education program. The goal was to receive maximum feedback from fourth grade teachers whose students had participated in the program. The survey was kept brief, with ten questions and a comment box to entice busy teachers to respond. Response options comprised a five-point scale ranging from "strongly agree" to "strongly disagree."

Results and Discussion

The survey response rate from the target audience was 49%, with 63 of 129 teachers responding to the survey questions and 40 providing comments. Wu et al. (2020) stated that the average survey response rate in the education-related field is 44%, which puts OC Vector's survey within an acceptable range for evaluating the collected data.

Appropriate pacing is crucial to the success of any virtual education program. Information needs to be chunked into manageable lessons and activities so that students pay attention and can retain the information. The original format of two 90-minute lessons was inappropriate for online learning. Ninety minutes was an unreasonable expectation of ten and eleven-year-olds' attention span for learning in front of a screen. Consequently, the content from the two in-person 90-minute lessons was reorganized into a week of virtual 45-minute blocks. *Virtual Mosquito Week* was structured to be five consecutive days of 40-minute lessons with about five minutes for questions at the end of each lesson. The survey indicated that 98% of the teachers felt this structure was appropriate (Fig. 1).

Developing clear objectives is an important aspect of any educational program. Students must understand their learning goals and teachers must ensure these objectives align with their curricula, activities, and assessments. In addition to explicitly stating the objective at the beginning of the lesson, the teacher should refer back to the objective throughout the lesson to keep student learning focused. All student activities and independent assignments should also align with the lesson's objective. Teachers can have students repeat the objective to take ownership of it. For instance, students can share the objective with a partner when zooming from a classroom. If zooming from home, students can write the objective down, visualize the objective, or whisper it to themselves or even an inanimate object, such as a stuffed animal. When surveyed, 100% of the teachers responded that the lesson objectives were clear (Fig. 2).

Using follow-up assignments is an excellent way to reinforce student learning. A best practice for virtual programs is to prepare assignments beforehand and have them ready for teachers to assign. Although the lessons can stand alone, many teachers appreciate assignments that are ready-to-use, standards-based, and connected to the lessons taught. With students zooming from home at the beginning

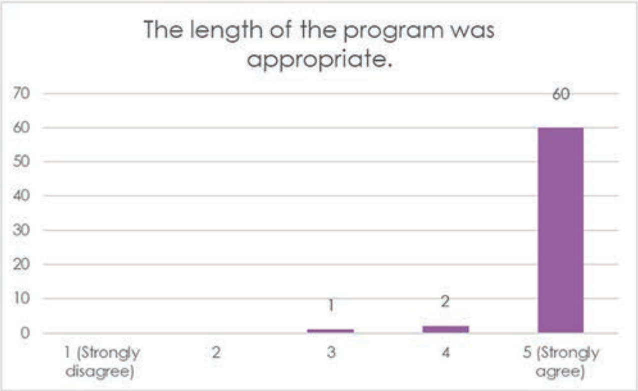

Figure 1.—Percentage of 63 teachers who felt that a 45 min length of session was appropriate.

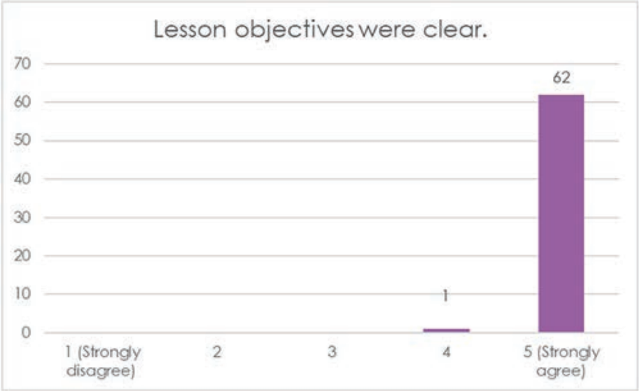

Figure 2.—Percentage of 63 of teachers who felt that the lesson objectives were clear.

of the COVID-19 pandemic, teachers were scrambling to develop independent work assignments online. The benefit of creating online assignments was two-fold: teachers had assignments they could upload with little effort to their online portal for students, and students were reinforcing their online learning with follow-up assignments. This reinforcement of learning increases the likelihood that the information will persist in students' long-term memory. This makes behavior change more likely, which is the ultimate goal in vector control outreach and education. When surveyed, 86% of teachers agreed that students did at least some of the follow-up assignments given. Only 3% of teachers did not do any follow-up work with students (Fig. 3).

Finally, it is important to incorporate opportunities for movement into the lesson. Physical activity in the classroom increases cognition, memory, and recall. There are positive associations between physical activity and mood, behavior and stress levels. For most children, dancing and singing is fun and children love to be goofy and have a good time while learning. If children are enjoying themselves while learning, they are more likely to pay attention and remember the lesson content. Also, most children are in a sitting position during virtual lessons, and

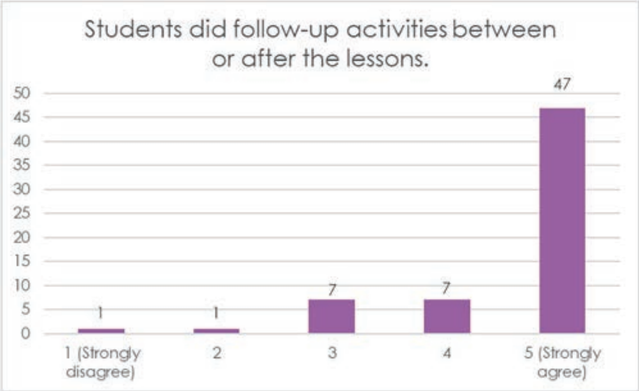

Figure 3.—Percentage of 63) teachers who agreed that students did some of the follow-up assignments.

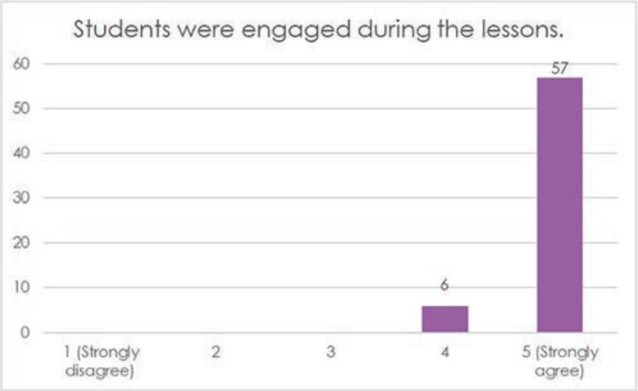

Figure 4.—Percentage of 63 teachers who felt that students were engaged in the lessons.

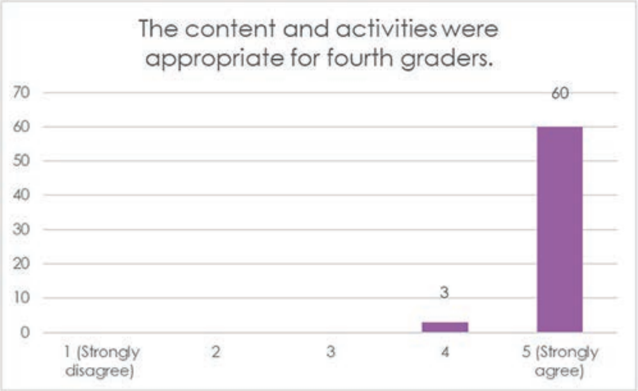

Figure 5.—Percentage of 63 teachers who felt that the lesson content and associated activities were appropriate.

they need breaks to exercise their brains and bodies. Incorporating planned movement breaks is fun and beneficial. For OC Vector's virtual program, each of the five days had at least one song and/or movement activity. When surveyed, 100% of teachers agreed that the students were engaged during the lessons (Fig. 4), and 100% of teachers felt the content and activities were appropriate (Fig. 5).

Conclusion

In conclusion, teachers provided overwhelmingly positive feedback about OC Vector's *Virtual Mosquito Week* education program. Of the teachers surveyed, 98% (all but one) indicated that they would book the program again. The strategies used to restructure the education program were effective and allowed mosquito education to continue, even during a global pandemic.

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Enriching practical learning resources for entomological, medical, and one-health curricula

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Introduction

The Pacific Southwest Center of Excellence in Vector-borne Disease (PacVec) is working to enhance public health entomology curricula for multiple audiences (vector control professionals, as well as undergraduate, graduate, and medical students) in our region. Currently, PacVec training opportunities include academic public-health entomology curricula at our collaborating campuses, training grants available for university students, and summer undergraduate internships, as well as publicly available resources on the PacVec website (pacvec.us), including larval mosquito resistance testing videos and information on ticks in our region.

Methods

PacVec's goals of enhancing public-health entomology curricula in the Pacific Southwest region focus on five main projects over the coming year:

(1) *Hands-on medical entomology laboratory*. Curriculum and materials will be developed initially at UC Davis in a format that can easily be transferred to other universities.
(2) *Visual guide to medically important arthropods*. This is intended to be used by vector control agencies and for use in public health curricula.
(3) *Training videos for vector control and public health programs*. Videos on field sampling methods and media outreach are being developed in collaboration with the MVCAC Laboratory Technologies and Public Relations Committees.
(4) *Asynchronous virtual vector-borne disease modules*. These are being developed for an online certificate in Global Health that will be available to medical students across the University of California system for training of future physicians.
(5) *Editable public health entomology slide decks*. These are intended to be used by professors in our region who may not be experts in public health entomology, but want to include it in their curriculum.

Results and Discussion

All projects are underway and are expected to be completed by the end of 2023. These projects will enrich practical learning resources both at the university and in public health and vector control agencies in the Pacific Southwest region.

Acknowledgements

The authors thank our project collaborators: Dr. Geoffrey Attardo of the Department of Entomology and Nematology at UC Davis, members of the MVCAC Laboratory Technologies and Public Relations committees, and Dr. Michael Wilkes of the UC Davis School of Medicine.

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Beyond the Booth: Increasing Engagement Through Interactive Activities

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Introduction

Public outreach is an extremely effective form of abatement. It is environmentally friendly, has a low cost and high rate of return, empowers residents to prevent mosquitoes, and the lessons people learn about preventing mosquito breeding can last a lifetime. Setting up outreach booths at events to connect with the public is certainly effective, but it is not the only way to reach the public.

Methods

To gauge the effectiveness of outreach booths at public events, Orange County Mosquito and Vector Control District (OCMVCD) surveyed attendees at 11 events from June through October 2022. An estimated 3,668 people visited OCMVCD booths at those events and 307 of them (8.37% of attendees) participated in the surveys.

Results

Of the 307 respondents: 43.60% had not previously heard of OCMVCD, 73.80% had experienced issues with mosquitoes, 89.20% learned new information about mosquitoes at the event, 79.30% participated in an OCMVCD outreach activity (i.e. Plinko, the Rat Hole beanbag toss game, or Fishin' for Larvae with magnets, and 81.57% lived or worked in Orange County.

The survey also indicated that school-age children were more likely to learn new information at outreach events than senior citizens: only 25.81% of respondents at the Seal Beach Leisure World Retirement Community Expo indicated they learned anything new about mosquitoes at the event, most likely because during more than 65 years of life they have been exposed to some messaging before the event, whereas 88.46% of school age children at the OC Discover Cube, Westminster Safety Day and OC Children's Book Fest events indicated they learned something new about mosquitoes. Finally, our experience conducting surveys at booths indicated that people were more likely to participate in the surveys if they could potentially earn a prize in a free drawing. It is important to remember that manning booths at events are not the only way to reach out to the public. For instance, more than 2,274 people participated in OCMVCD activities during the Discovery Cube Mosquito Awareness Week in June 2022, and 444 tasted OCMVCD Fire Ant Chili at the 2022 Tustin Chili Cook-Off.

Conclusions

OCMVCD has seen great success educating the public about mosquito control via its educational outreach program, public signage, children's events, and social media. A cohesive, comprehensive outreach program that takes all the aforementioned details into account and strives for continuous improvement is the most effective way to show the public that they too have an important stake in mosquito abatement.

Connecting with students using TikTok

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Introduction

The Greater Los Angeles County Vector Control District is the nation's largest vector control District. It serves over 1,000 square miles, 36 cities including the City of Los Angeles, and areas of Los Angeles County. The Education Program is part of the District Public Health education programs and offers a free 5th-grade curriculum to all elementary schools within the District boundaries.

With the continuous goal to educate the youth about vector-related public health concerns, the role of mosquitoes in the environment, and the importance of behavior change for vector management in everyday lives, the Education Program Coordinators (EPCs) continue their efforts to make a connection and lasting impression with students using social media. Social media platforms address diverse demographics with different audiences on every platform. The United States has the most TikTok users with over 135 million users. Generation Z is the leading group of users with ages ranging between 10-25 years old. Considering the demographics of the social media app and the GLAmosquito Education Program students, the EPCs use their @MosquitoSWATLab TikTok account to connect with their students.

Methods

The EPCs use TikTok to connect with their students, continue mosquito education beyond the in-classroom, and reach more people faster. It has been beneficial to use the @mosquitoSWATLab TikTok account as a learning tool because it is a free mobile app, easy to use, and has the largest music and sound library compared to any other social media platform allowing creative liberties. Lastly, it is fun and entertaining for both the user and the viewer. Content creation can be anything you want it to be and the TikTok app makes it easy to be creative. Before filming a TikTok video, the EPCs begin with the context of their TikTok video, deciding on what will be portrayed and its messaging. Perhaps the video will be about the mosquito anatomy, the invasive Aedes species in Los Angeles County, what to do about breeding sources after a rainstorm, or increasing reservations for the Education Program. The social media app gives you two options for recording, you can upload videos from your mobile phone or record through the TikTok app. Video edits can be done with the tools offered by the TikTok app; cut, move order, and add text and music/sound to your video directly from the app. Applying trendy music or sound to your video will increase your reach by the thousands. Create a short but sweet caption and always include relevant hashtags to your industry, demographics, or audience. Hashtags allow TikTok to categorize the content and expand the post reach. Staying active on social media is also key to increasing engagement. The EPCs post weekly on TikTok, encouraging and responding to students' mosquito questions and concerns in the comments section of their posts. The @mosquitoSWATLab branding has proven to be an effective way to gain followers. Presentations, print material, activity booklets, and giveaway items have the Education Program @mosquitoSWATLab username printed, which has helped boost its following by more than a third of the total followers in 2022.

Results and Discussion

Social media platforms are a great way to distribute information to the public. The demographics of social media vary by platform. The @mosquitoSWATLab TikTok account has been successful for the Education Program because the majority of TikTok users are made up of their target audience age group, Gen Z. It is a cost-free social media platform used to pursue its ultimate goal of raising mosquito awareness and changing the behavior of vector management.

Conclusion

In addition to the District's traditional outreach of events and presentations, social media has proven to be an effective way to communicate and continue the education surrounding the topic of mosquitoes and vector management. The continued outreach through the @mosquitoSWATLab TikTok account has increased its reach to a much larger audience at no cost.

Acknowledgements

Thank you to the Communications Department at GLACVCD, and a special thank you to Diana Garcia, because the success of the Education Program social media would not be possible without her contributions.

Increase your Instagram engagement 15 seconds at a time

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Introduction

There is no denying the dominance of social media in everyday life. In 2021, an estimated 48% of American adults said they get their news from social media (Walker & Matsa, 2021). The explosion of TikTok sent social media platforms such as Instagram into a frenzy to keep up with interactive short-form videos. Instagram released "Reels" on August 5, 2020, as a way to stay competitive against TikTok. According to Instagram (Bhatia et al., 2021), the platform currently has 1.4 billion active users worldwide, making it the fourth largest social media platform. With Reels, Instagram has increased its focus on short-form videos and agencies should be aware of effective engagement strategies. Reels are broadcast to a wide audience, allowing agencies to be a part of the larger narrative and reach audiences looking for educational content. Industries have already seen the benefits of Reels as a way to reach larger audiences and get their messages across. For example, professional dermatologists have turned to Instagram Reels to promote literacy and provide accurate information to health consumers and prospective patients. Vector control can do the same by using Reels as a tool for information and to educate the public on the platforms they currently utilize. The San Gabriel Valley Mosquito and Vector Control District (District) was an early adopter of Reels by posting its first Reel on August 18, 2020. Since then, the agency has created a strategy to amplify its reels by using trending audio, creative transitions, geo-tagging, an engaging two-second openings, and reusing content.

Methods

For creating Instagram Reels, the District's official verified account @SGVmosquito was used to post and upload content. The majority of the content was filmed on an iPhone XR. The video was edited using some of Instagram's Reels templates or Adobe Premier Pro. The analytics on how the Reels performed was obtained from Instagram's professional dashboard feature. Insight into the accounts following also was used to determine average age, general location, hours users are most active, and gender.

Results

The District activity started posting Reels in August of 2021. With no real strategy, in 2021 the District posted 63 reels that averaged 1,586 views with 26 post engagements. In 2022, with a refined strategy, the District posted 59 posts and averaged 3,504 views with 72 post engagements.

One of the biggest challenges with actively posting Reels, is the constantly changing trending audios. It was difficult to predict when the audio will become viral and if your Reel will best fit the trend. It also was difficult to maintain a video library that will always have what you are looking for when it comes to trends or creative transitions. To create an effective trend that hits the mark, original content may have to be created for that video or trend.

Discussion

By using five tips for Instagram Reels, agencies can amplify their audience, drive the narrative of their message, and provide educational resources:

1. Trending audio: This will give the content the 'eyes and ears' needed to get pushed into the algorithm. Instagram reel audios have two types of symbols near the name of the audio: arrows for trending audio and music icons for standard audio. Standard audios can be used, but trending audios are more likely to produce increased views.
2. Creative transitions: Creating fun or 'cool' transitions when shooting content will give it that extra 'wow' factor. Reels are very short, accounts only have a few seconds to get people's attention. Using zoom-in and zoom-out effects, reveal things in the video by removing objects from the camera, or chasing the never-ending loop that leaves the audience guessing where the video starts and ends.
3. Geo-tagging the post: Geo-tags help build a digital footprint on social media. It's also useful to strategically geo-tag the different cities in the district to get added to the city geo locations, allowing the post to expand its reach, and show more of the service area.
4. Engaging opening: The first 2-4 seconds of the video are critical to get the audience's attention. Think of something that will 'hook' people in right away that will make people want to watch the whole video.
5. Reuse content: Don't record something new every time. Social media evolves quickly. Space out content to prevent overuse of the same video clips.

Conclusion

Social media continues to dominate how we communicate and share ideas with large audiences. In order to stay relevant and engaged in the platforms where conversations and ideas are being shared, it's important for government agencies such as vector control to have an effective social media strategy. Instagram Reels continues to grow with trending audios going viral. The District has expanded its Reels consistency with #TipTossThursday and event recaps from community events. Reels continue to dominate Instagram and produce the highest level of engagement on the platform. As vector control agencies continue to post strategic and effective Reels, they can help amplify the industry and help eliminate misinformation about mosquitoes. Vector control agencies are the experts in this field and should be the ones leading the conversation on social media about this topic.

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Efficacy of bacterial larvicides in bromeliad phytotelmata for controlling invasive *Aedes* mosquitoes

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Introduction

The urban populations of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) in the USA have continued to expand despite a long history of control campaigns (Metzger 2017). The presence of these important vectors facilitates future disease introductions, especially as increased globalization and a changing climate create novel epidemiological patterns. Although phytotelmata, water-holding plant cavities, are well researched larval habitats for mosquitoes, ornamental bromeliads specifically may be playing a large and underappreciated role in the spread of *Aedes* (Frank 1983, Wilke 2018). In areas like southern California, many vector control efforts use *Bacillus thuringiensis* var. *israelensis* (*Bti*) based products to control larval mosquito populations, but their impact on these phytotelmal habitats is unknown (Lacey 2007). As a response to disease outbreaks or vector population rise, vector control intervention can use a combination of source reduction, adulticide spraying, and larvicides to suppress mosquito presence in a given area. Effective source reduction and larvicide treatment require specific targeting of cryptic sites, but agencies are often left to rely on large scale spraying like brigades of truck mounted ultra-low volume insecticide sprays for covering as much potential habitat as possible. Although this circumvents having technicians arduously locate and treat each small habitat by hand, the overall effect is subject to many factors that reduce control efficacy such as weather, application direction, foliage, and spray parameters (Mount 1996). Towards this aim, our study has two objectives: 1. Determine if the tank bromeliad phytotelmata habitat influences the effect and duration of *Bti* products, and 2. Evaluate current *Bti* spraying methods for their efficacy against mosquito larvae within these habitats.

Methods

To measure the efficacy of larvicidal treatments, in-situ emergence assays were conducted in both *Neoregalia medusa* plants and artificial containers subjected to varying treatments. Plants and cups were treated manually in the laboratory with Vectobac-WDG (Valent) and Duplex-G (Central Life Sciences) to understand if the plant habitat had a significant effect on the *Bti* products. Third instar larvae of *Ae. aegypti* and *Ae. albopictus* then were introduced biweekly along with food until no significant mortality as a result of treatment was observed. Fifiteen larvae of each species were added to each plant in individual leaf axils, with 4 plants per treatment repeated in 3 blocks. To test interactions with different field applications, plants and cups were placed outside during both truck and helicopter based (6 plants per treatment) ULV-WALS of Vectobac-WDG in the Coachella Valley administered by Coachella Valley MVCD. These were brought back to the laboratory greenhouse and subjected to similar emergence assays with 20 *Ae. aegypti* larvae each added directly after treatment, one month after, and two months after, to examine residual effect. Emergence rates as proportions were converted to individual binary outcomes, combined for all blocks, and analyzed for significant differences using probit based binary generalized linear regression models (GLM). Variance of these proportions was calculated per plant for illustrative purposes only as the model compared the total proportion emergence and mortality.

Results

Bromeliads were monitored for 24 weeks over 12 additions of larvae following initial treatment (Fig. 1). Emergence success in untreated bromeliad remained at around 75% for the duration of the study and there was no overall difference in survivorship between mosquito species. VectoBac treated plant emergence was not significantly different from control after twenty-one weeks, and Duplex-G treated plant emergence was not different from control after twenty-three weeks. Emergence success in the untreated cups generally declined from over 90% initially to under 70% after eleven weeks (Fig. 1). *Aedes albopictus* emergence was lower than *Ae. aegypti* in the control cups after eight weeks but not significantly so for the remainder of the product testing period. The VectoBac treated cups provided complete mortality for only the first introduction of larvae. Emergence increased in subsequent weeks until eleven weeks after treatment when there was no significant difference when compared to the untreated control. The treated cups also had lower average emergence each round of *Ae. albopictus* but never significantly so across the time points.

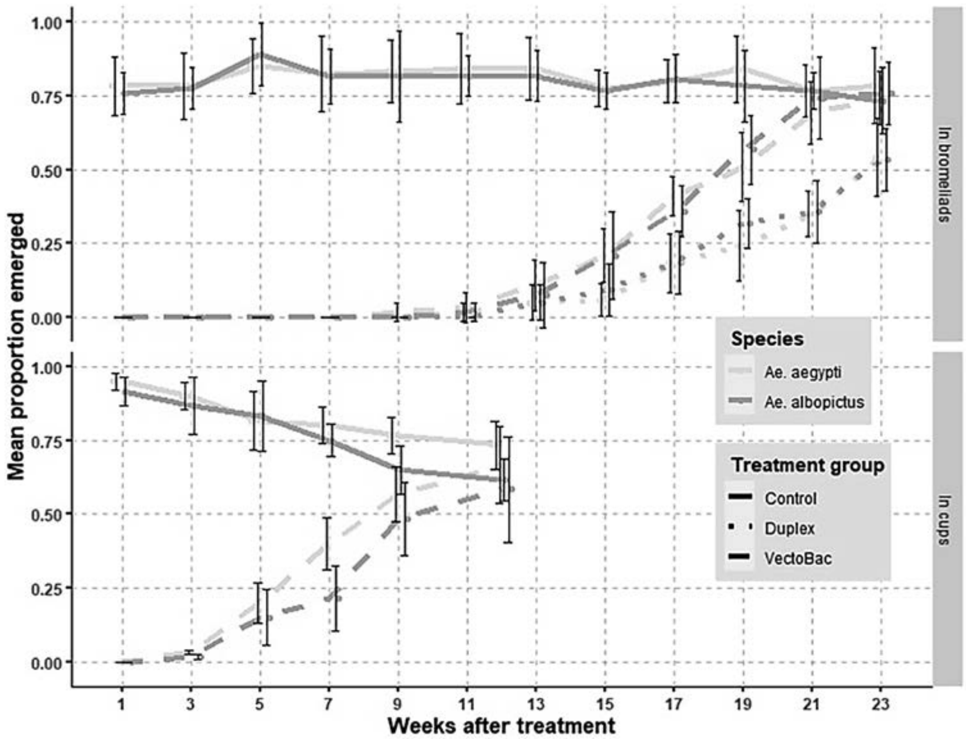

Figure 1.—Proportion of each *Aedes* cohort that emerged in plants or cups following manual application of *Bti* product (8mg/L A.I.). Error bars represent standard deviation across all sampling units and blocks.

Initial emergence assays in cups showed almost complete control of *Ae. aegypti* larvae for both truck and helicopter mounted treatments (Fig. 2a). Emergence from these habitats increased significantly in both cases to about 20% overall after one month, and after another month emergence was above 50%. Untreated controls did not differ in emergence at any of the time points. There were no differences in efficacy between the two treatment types in cups. Initial treatment and assays of the bromeliads showed significant effects of each treatment compared to the control, and the helicopter spray was significantly more effective than the truck spray, with emergence at around 10% compared to 32% (Fig. 2b). Emergence was higher after one and two months in the truck treatment but not significantly so. For the bromeliads treated by helicopter the time points showed significant effect overall, but the pairwise comparison was only significant between the first and third round.

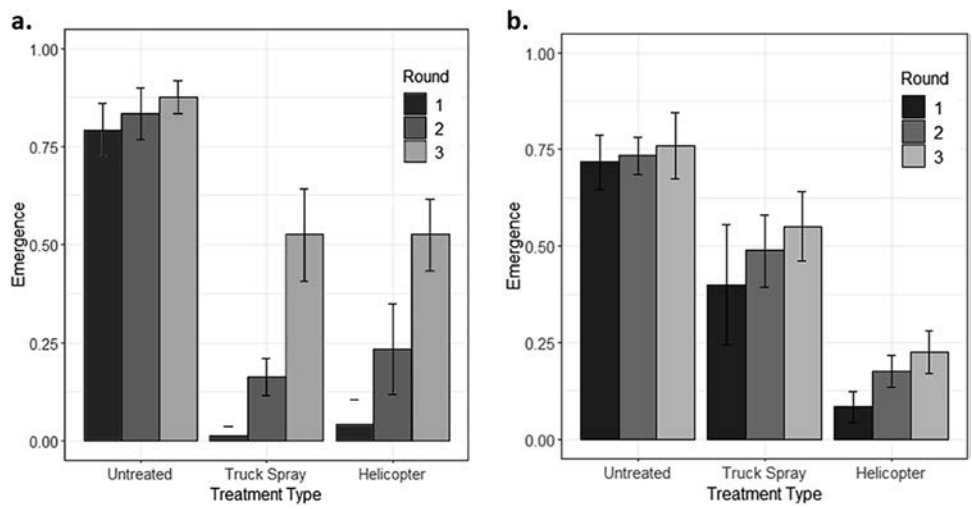

Figure 2.—Proportion of each *Aedes aegypti* cohort that emerged in **a.** Cups and **b.** Plants treated in the field (0.25 lbs/acre VB-WDG). Error bars represent standard deviation of emergence percentage. Round 1 is directly after treatment, round 2 is one month after, and round 3 is two months after.

Discussion

Overall our findings showed that *Bti* products were a promising candidate for targeting *Aedes* in bromeliad phytotelmata, but that application strategy was an important consideration. Duration of bacterial larvicide activity was extended in bromeliad phytotelmata compared to artificial containers in both experiments, but levels of control immediately after treatment were significantly lower in tests with field sprays on the plants. With our current understanding of interactions between the plants and the water they hold, it is not surprising that the products lasted much longer in the phytotelmata than in artificial containers. The factors that have been shown to reduce the duration or effectiveness of *Bti* were all mitigated in some way within the plant (Boisvert 2000). Nutrient uptake and decreased pH reduce the production of organic material and inhibit microbial life. Leaf structure and evaporative cooling lower the water temperature relative to artificial containers and provide more shade from damaging sunlight. In the laboratory experiments, every axil was ensured to get some amount of the larvicides, but in the field the ULV sprays created much more variation in the results. Uneven coverage of the phytotelmata due to overlapping leaf structure leads to overall lower application rates that were not as effective in reducing emergence. There was also a marked difference in the water between the plants and the cups, despite both undergoing the same additions of water, food, and larvae. While the cups quickly became murky and teeming with unpleasant growth, the plant water stayed relatively clear after even months of food additions with and without treatment. These conditions allowed the *Bti* to actively kill almost all larvae for more than twice as long in the treated groups.

Conclusions

Although unique in many ways, these plants may also serve as a model for many other cryptic and understudied larval habitats of mosquitoes. The native ranges of bromeliads include areas of the world most vulnerable to vector-borne diseases such as dengue and chikungunya, making their continued research paramount in reducing the public health burden created by *Aedes* mosquitoes. The interaction between different products, application methods, and target habitats is a field of knowledge that will continue to grow. Especially important as these habitats themselves are mediating interactions between mosquito species and dictating how they spread.

Acknowledgements

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ARGO trailer nurse tank for extended larvicide applications at remote sites

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Introduction

The Alameda County Mosquito Abatement District uses Argos to apply multiple batches of liquid larvicide via a mounted 50 gallon sprayer throughout the county in areas where access and permission is available. Access to a water source for refilling the sprayer is limited in many of these areas. Custom fabricated tanks and plumbing mounted to the trailer under the Argo provides technicians with an additional 100 gallons of water, reducing the need for another vehicle with a nurse tank or time spent traveling to an alternative site to obtain water.

Methods

Tanks were custom designed and fabricated with lightweight 5052 aluminum. The proposed tank volume was calculated using the formula (LxWxH)inches/231 = volume(gallons). The tanks were centered and spaced on the trailer platform for mounting, plumbing access, and Argo clearance (Fig. 1). Appropriate venting and modified valving and connectors were used for both filling and extracting the water from the tanks. The use of quick-disconnect fittings contributed to the time saving advantage. The truck and trailer GVWR (Gross Vehicle Weight Rating) were both within the limits of the new payload.

Results and Discussion

The trucks that tow the modified Argo trailers had a 50- or 100-gallon volume sprayer. With the addition of the trailer mounted nurse tanks, 4 to 5 50-gallon batches can be applied in the field from the Argo. The Field Technicians and Vector Biologists that use this equipment have found the nurse tank modifications to be an asset in their larvicidal treatments, saving time and resources.

Figure 1.—Two supplemental water aluminum tanks mounted onto an Argo trailer.

Taking Flight: Utilizing unmanned aircraft systems in the Delta Mosquito and Vector Control District

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Abstract

Delta Mosquito and Vector Control District (DMVCD) lies within an agricultural basin of the southern San Joaquin Valley in California. Corn, one of the main crops in the District, is densely grown through flood irrigation which is the perfect *Culex quinquefasciatus* Say larval habitat. The resulting abundant mosquito population creates an increase in West Nile virus (WNV) transmission. From 2017 to September 2022, DMVCD had an average of 457 positive WNV mosquito pools per year, with many found to be associated with corn production. This crop is difficult to treat, and traditional ground application methods have had minimal impact in reducing population abundance. To address this problem, DMVCD adopted new aerial application techniques utilizing Unmanned Aircraft Systems (UAS) to reach these inaccessible areas. The DMVCD's goal in establishing a UAS Program was to reduce WNV transmission by integrating safe and efficient aerial applications into our Integrated Mosquito Management Program.

Finding the right mix: A comparison of yeast fermentation solutions as an alternative carbon dioxide source for BG-Sentinel traps

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Introduction

The BG-Sentinel traps have been used worldwide as mosquito arbovirus surveillance tools since 2006 (Armed Forces Pest Management Board 2012, Geier et al. 2006). CO_2 is a known olfactory cue for host seeking mosquitoes and is often used in the form of compressed gas, dry ice, propane, or yeast fermentation (Gillies 1980, Kline 2002, Nakata et al. 2020, Saitoh et al. 2004). Yeast fermentation is recommended as a CO_2 source because of its low cost, being easily obtained and transportable, and relatively nonperishable compared to other forms of CO_2 (Nakata et al. 2020, Smallegange et al. 2010). Varying concentrations of sugar and temperature affect yeast fermentation through the utilization of both anaerobic and aerobic respiration to produce CO_2 (Koutsokali and Valahas 2020). Yeast fermentation is used by a small number of surveillance programs, which leads to only a few commercially available yeast products on the market. Our study evaluated the mosquito capture efficacy of Delta Mosquito and Vector Control District's (DMVCD) yeast solution and BG-CO_2 Powder as attractants.

Materials and Methods

For three weeks, one BG Sentinel-2 trap was set Monday through Thursday at five suburban locations with established *Aedes aegypti* (L.) populations. Each trap set consisted of a BG-Sentinel trap, 12v 22ah SLA battery, BG-Lure, Elitech RC-51H temperature and humidity data logger, and one CO_2 source (Figure 1).

The four CO_2 sources and containers included were: 1) DMVCD yeast solution containing 15g Red Star Active Dry Yeast, 250g sugar, 2.5L water; 2) DMVCD container consisting of an Uline EZ-pour F-style 1 gallon jug, Festo push-in bulkhead connector QBS-1/4T-U, 0.170in ID clear vinyl tubing, 1 inch aquarium airstone; 3) BG-CO_2 Powder containing 20g BG-CO_2 Powder, 500g of sugar, 2L of water; and 4) BG-Pro Bag consisting of a mixing bag with cap and vinyl hose, and insulated bag. The four CO_2 sources and containers were randomized among five traps; one negative control trap contained no CO_2 source. Traps were visited daily to collect mosquitoes, change the battery, and rotate the CO_2 source.

The CO_2 sources and containers were assigned to the five sites by randomization through GraphPad Prism. The temperature, humidity, yeast solution, and container were recorded for each site and the number of mosquitoes collected were enumerated by species and sex. Statistical significance ($P<0.05$) was calculated using one-way analysis of variance (ANOVA) and post-hoc Tukey Honest Significant Difference (HSD) test in R v4.2.2 statistical software.

Results

Objective 1: Verify that temperature, humidity, and relative mosquito abundance were similar among sites

The Elitech RC-51H data logger recorded temperature and humidity every 15 minutes. A daily average temperature and humidity were calculated based on a 24 hour time period from which the trap was set. The ANOVA indicated no significant difference in daily average temperature ($F=0.788$; $df=4,59$; $P=0.538$) or humidity ($F=1.317$; $df=4,59$; $P=0.274$) among sites. There was a significant difference in the average number of mosquitoes collected per trap set between sites ($F=33.01$; $df=4,59$; $P<0.001$; Figure 2). A post-hoc Tukey HSD test indicated that the catch of mosquitoes at site 1 was significantly greater than all other sites ($P<0.001$), but sites 2 through 5 were not significantly different from one another ($P>0.05$).

Objective 2: Determine if there is a difference in mosquito capture among yeast-container combinations

Total number of mosquitoes were totaled per trap day including all species and sexes for each treatment regardless of site. The ANOVA indicated a significant difference in average mosquitoes collected per trap set ($F=3.443$; $df=4,59$; $P=0.014$; Figure 3). A post-hoc Tukey HSD test showed that BG-CO_2 Powder & BG-Pro Bag caught significantly more mosquitoes per trap set than DMVCD yeast solution & BG-Pro Bag ($P=0.047$) and no CO_2 source ($P=0.021$). All other yeast solutions and

Figure 1.—12v 22ah SLA battery, Elitech RC-51H temperature and humidity data logger, BG-Sentinel 2 trap, DMVCD container with yeast solution, and BG-Pro Bag with BG-CO_2 Powder.

container combinations were not significantly different from each other ($P>0.05$).

Discussion

Unfortunately, no significant conclusions comparing yeast fermentation solutions could be made with the collected data due to the site bias of unequal mosquito abundance. The experiment also ended earlier than expected due to traps being tampered with. This resulted in certain combinations of yeast solution and containers being set more often at some sites and none at others. Although results were limited, every combination of yeast solution on average caught more total mosquitoes per trap set than no CO_2 source. This showed that the amount of CO_2 released was above ambient levels and played a role in improving mosquito capture.

From an efficiency and cost perspective, there were differences among yeast solutions and containers. The BG-Pro Bag consisted of a nonrigid, plastic mixing bag with a relatively small mouth and insulated bag. The structure of the mixing bag made it difficult to add the solution contents, clean it after use, and maneuver contents into the insulated bag. The approximate cost of the BG-Pro Bag was \$13.00, with the BG-CO_2 Powder solution costing \$2.93 per trap set. The DMVCD container solution consisted of a rigid container with a wide mouth opening making the container easier to prepare, clean, and dry. Yet, the DMVCD container was non-insulated which did not make a significant difference during summer months with average daily temperatures above 26.7°C. The approximate cost of the DMVCD container was \$8.74, with the DMVCD yeast solution costing \$0.51 per trap set.

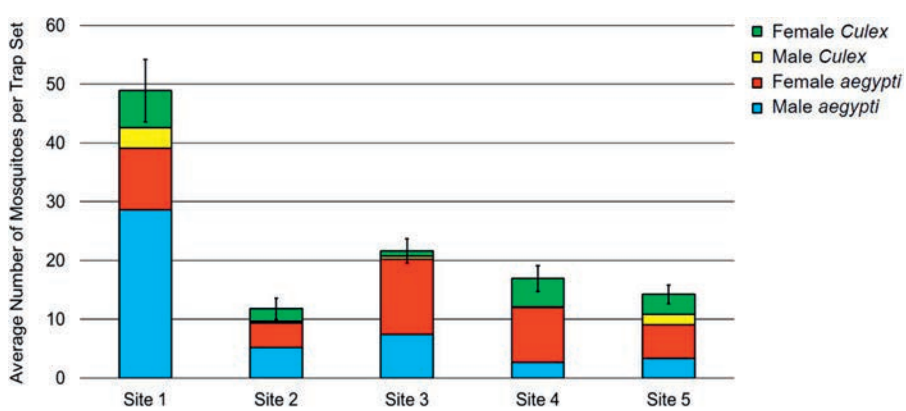

Figure 2.—Average number of mosquitoes collected per trap day at each site by species and sex. Data pooled over all treatments and days. Vertical bars show standard error.

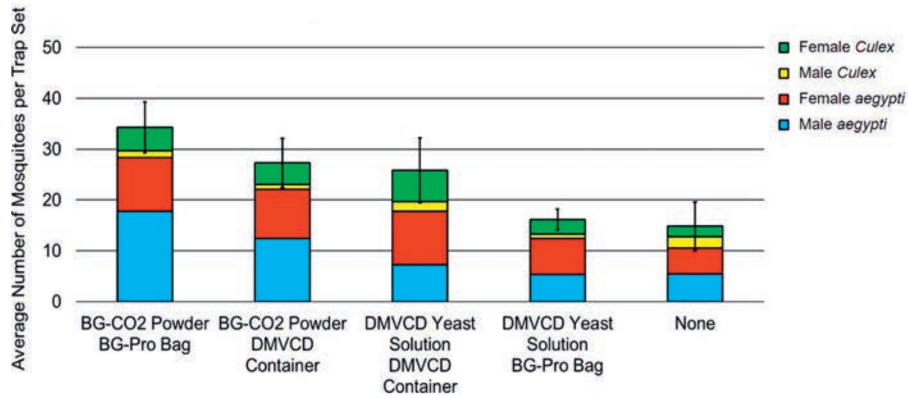

Figure 3.—Average number of mosquitoes collected per trap set for each yeast solution and container combination by species and sex. Data pooled over all sites and days. Vertical bars show standard error.

Conclusion

The DMVCD's Surveillance Program has been using yeast fermentation as the CO_2 source with BG-Sentinel traps since 2018. Future research includes maximizing CO_2 production with different yeasts and sugars. By advancing and developing cost effective trap attractants, surveillance data can be collected at a finer scale, leading to better control decisions in the field.

Acknowledgements

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Testing Artificial Intelligence accuracy in mosquito identification

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Introduction

Insect identification is a key component in vector control. Identification of mosquitoes is important because different species vary in their tendency to bite humans and their capacity for transmitting arboviruses, including dengue, chikungunya, and West Nile viruses (California Department of Public Health, 2022). Therefore, not all mosquitoes are important in vector control. Mosquito identification is a time and labor-intensive task that requires entomological knowledge and expertise. The purpose of the current project is to determine whether time spent on mosquito identification can be reduced by automating the process using artificial intelligence (AI). The creation of a smartphone application could reduce the need for expert staff and time spent on mosquito identification. This application could be useful in public health, especially vector control and surveillance in many underserved communities, where there is a lack of resources to hire expert staff. It also may be useful in understaffed communities that do not have access to expert staff or are in remote locations. If successful, such an application would be a useful tool both in laboratory settings as well as in field operations for quick identification.

Methods

A simple workflow with four steps was used for this project: (1) trap mosquitoes, (2) identify mosquitoes, (3) generate training data, and (4) test artificial intelligence for accuracy.

Trap Mosquitoes

BioQuip EVS traps and Biogents BG-Pro traps were used to collect mosquitoes in Alameda County, California during June to August 2022. Both traps are similar in structure with a fan (powered by USB rechargeable batteries), bait, and net at the bottom to hold the mosquitoes. All traps were baited with dry ice, a light and BG-Lure, a scent that mimics body odor to enhance trap efficacy (Hoel et al., 2007). The mosquito nets containing the mosquitoes were detached from the trap and placed on dry ice before being transported to the laboratory for identification.

Mosquito identification

A dichotomous key was used for morphological identification of mosquito specimens to species. The same mosquitoes were next photographed using a Google Pixel 6 smartphone camera. The phone was held the same height as the light source to take pictures, ensuring all the mosquitoes were within the frame and clearly visible. Images of the mosquitoes were taken on three different backgrounds – (A) a chill plate, (B) green cardstock paper and (C) blue cardstock paper (Fig. 1). The images were taken in a manner where the entire background and all the mosquitoes on it were clearly visible. Different backgrounds were used to ensure that the AI trained on the mosquitoes and not the

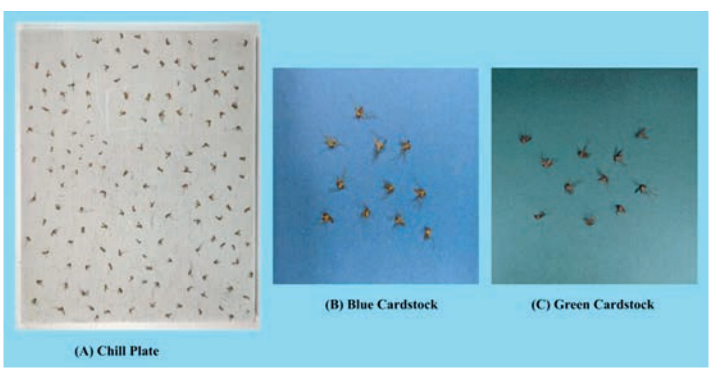

Figure 1.—Images of mosquitoes taken on different backgrounds: (A) Chill plate (B) Blue cardstock and (C) Green cardstock

Figure 2.—LabelMe annotation tool used to annotate mosquitoes.

backgrounds. These pictures were automatically uploaded to a Google Drive folder for storage and organized by trap ID.

Generate Training Data

To create a training data set for the AI algorithm, images of the mosquitoes from the traps were annotated. LabelMe, a free annotation tool from the Massachusetts Institute of Technology (Russell et al., 2008), was used to annotate the images. This was done by drawing rectangular polygons around each mosquito, and a label containing the genus and species as identified morphologically was attached (Fig. 2). Male mosquitoes were given an additional label. Any images where the mosquitoes appeared blurry or were not identifiable were not used. The annotated images were then saved as .JSON files in a separate Google Drive folder. The data set currently consists of 700+ images and will be used to train the AI.

Test the Artificial Intelligence

Machine learning was used to train the AI on the annotated images and test for accuracy. The ten species selected based on their relatively high abundance in Alameda County during the summer of 2022 were *Aedes dorsalis, Aedes vexans, Anopheles franciscanus, Anopheles freeborni, Anopheles punctipennis, Culex erythrothorax, Culex pipiens, Culex tarsalis, Culiseta incidens* and *Culiseta particeps*. There was a minimum threshold of 100 images for each species to train the AI that was easily surpassed with 700+ images. Although results are not final at the time of this publication, the goal for this project is 93% minimum accuracy in identification. Once results have been obtained, the next steps will be to finish developing the smartphone application (Fig. 3).

Challenges

Image Capture and Annotation Lighting

Correct placement of light sources was necessary to illuminate all mosquitoes within the frame. Two light bars were placed on either side of the background (chill plate or cardstock paper) and placed at approximately the same height for even lighting. Light sources closer to the background resulted in over-exposed mosquitoes and an image that is too bright, so that the distinct features of the mosquitoes could not be seen. However, a light source placed farther away from the background provided adequate illumination to clearly detect differences among species.

Image Quality

Overlapping or damaged mosquitoes were removed because they could potentially confuse the AI, particularly for training purposes. Damaged mosquitoes constituted any mosquitoes that had broken wings, abdomen and/or severely damaged legs. Mosquitoes missing scales on the legs, body or wings that were critical to differentiation of species were removed.

Background

To simplify training the AI, debris and non-mosquito insects were removed from the background. Debris such as

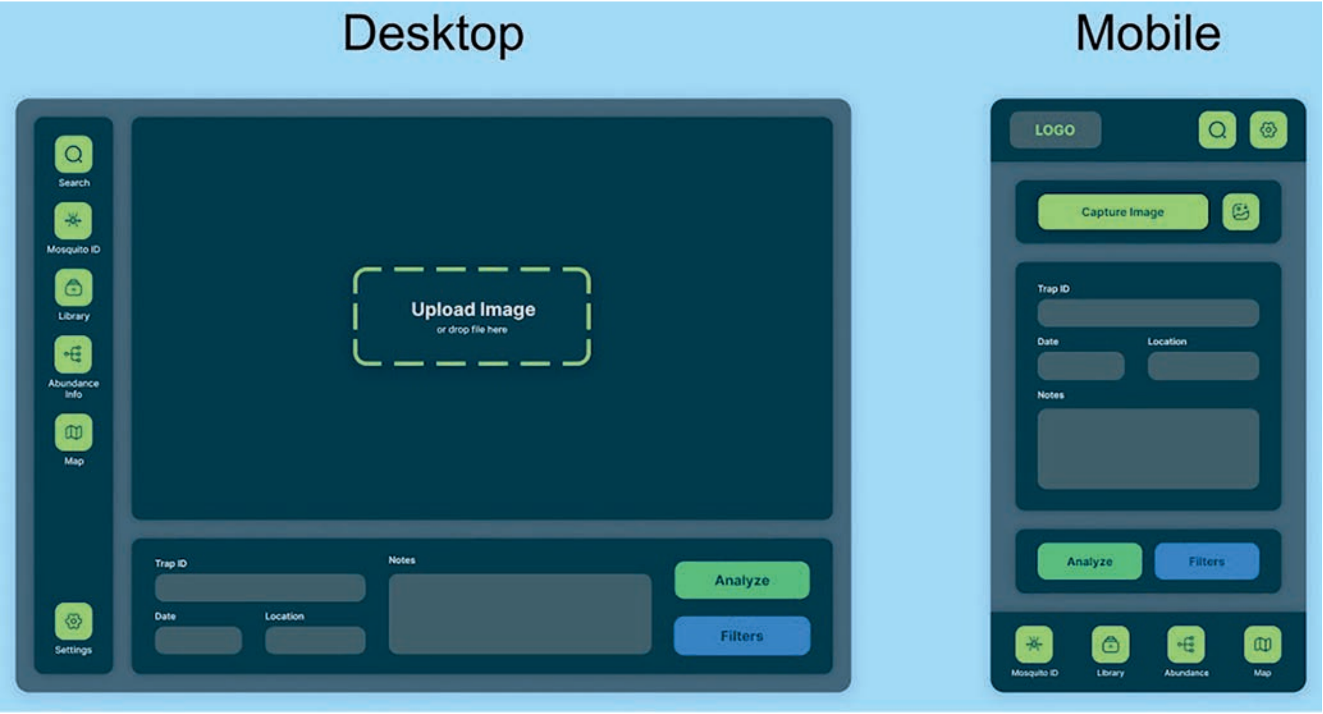

Figure 3.—Mock-up of desktop and mobile phone application.

broken legs or wings was removed to train the AI solely on whole mosquitoes. Although this was mainly done to generate training data, the goal for this application is for the AI to identify mosquitoes accurately, even in the presence of debris or other insects in the frame.

Image Format - RAW vs JPG

The original format choice for images was RAW because they contain purer data without manipulation or enhancement by the phone's operating system. Although these images were helpful for training and provided more detail, they are large files and require processing through software (such as Adobe Photoshop or Lightroom) before use. The final format chosen was JPG, which provided sufficient detail and did not require added processing, making the images immediately available for use.

Anticipated Challenges in Testing AI

Possible Species Confusion: There is potential for the AI to confuse species that are similar in appearance. Examples include *Culiseta incidens* and *Culiseta particeps* where the wing vein details are not always evident in each image as the magnification of the Google Pixel 6 camera lens is not as great as a microscope. Another example is *Culex pipiens* and *Culex erythrothorax*, which can have very similar coloring and features in images, making it hard to distinguish between the two species, especially when the dorsum of the abdomen is not in view.

Damaged Mosquitoes: The AI may have difficulty identifying mosquitoes with missing scales (damaged or lost with age) or broken appendages that were important for identifying key vector species.

Discussion

Applications to automate mosquito identification have the potential to make mosquito identification faster and more efficient, which could expand surveillance capacity in areas with limited resources. By decreasing the time spent identifying mosquitoes, the pooling of species that are important for research or vector control (such as testing for viruses) can be accelerated. This allows for time and resources to be redirected to other efforts, which can be useful in communities that lack the resources to hire expert staff. Our aim for this application is to be free or low-cost to make it more equitable and accessible. If successful, the application could be used by individuals or vector control organizations/agencies to monitor species abundance and for general surveillance. A feature of the application that makes it easy to use is that the AI can identify multiple species of mosquitoes at once. Pictures of each mosquito are not needed; they can all be identified at the same time within one picture. Once the trap has been emptied on a chill plate or background, the mosquitoes will only need to be lightly segregated before taking a picture. 'Light segregation' constitutes spreading out the mosquitoes to prevent specimen overlap. Our hope is that the light segregation is sufficient for accuracy in identification and need not require the manual labor of staging each mosquito. Expansion of the data set is also a future goal for this project. This allows the AI to be trained to identify mosquito species found in different geographic regions.

Acknowledgements

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Evaluation of a cordless heat gun for ovicidal treatment of *Aedes aegypti* eggs

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Introduction

Treatment protocols for *Aedes aegypti* tend to require more time and tedious labor than non-container breeding species. Eggs deposited in containers adhere to the surface even after the water level recedes, resist desiccation, remain viable, and hatch once water refills the container. Although a combination of scrubbing, bleaching, and disposal are the common methods for eliminating eggs on surfaces discovered in the field, this method is time-consuming and not always feasible for all container materials. Our study investigated an alternative method using a cordless heat gun for treatment of *Ae. aegypti* eggs.

Methods

Aged 2.54 cm square pieces of paper containing invasive *Ae. aegypti* eggs from the Gainesville lab strain or field-collection by Consolidated Mosquito Abatement District were pinned to a damp paper towel on a flat plywood surface. Each piece was assigned to either the negative control group or to a two, three or five second treatment of heat from a cordless 20 V heat gun from a distance of 2.54 cm. Treated papers then were submerged in a 10% gravid water infusion and vacuum-treated for 15 minutes to induce hatching. Larval hatch was recorded daily over the course of a two-week period.

Results and Discussion

The mean hatch rate for untreated *Ae. aegypti* eggs was 61.9% and 47.3% for the field and laboratory strains, respectively, and 12.7% and 0.57% for those treated for two seconds and 0% for both strains when treated for three or five seconds. Compared to the untreated control group, the two second heat gun treatment reduced the hatch rate by 79.5% and 98.8% in field and laboratory strains, respectively, and 100% in both strains subjected to three and five second treatments.

Conclusions

Heat treatment with a cordless heat gun at a distance of 2.54 cm for >3 seconds resulted in 100% control of *Ae. aegypti* egg hatch. Based on this laboratory evaluation, a cordless heat gun may be a useful method for faster treatment of *Ae. aegypti* eggs in the field. Heat-based methods of treatment warrant further investigation.

Acknowledgements

Consolidated MAD, Merced County MAD, and Sierra Research Laboratories (Modesto, CA) for providing *Ae. aegypti* eggs.

Truck-mounted wide area larviciding treatments for *Aedes aegypti* (L.) control in northern Tulare County

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Introduction

Aedes aegypti (L.) was first detected within Delta Mosquito and Vector Control District's (DMVCD) boundaries in 2014 (Lee 2019). In response to this detection, DMVCD created an Invasive *Aedes* Program that focused on surveillance, education, and physical control of mosquito sources through property inspections. Although effective, the process of conducting property inspections in neighborhoods of high *Ae. aegypti* abundance is time consuming, labor intensive, and largely dependent on homeowner participation. Truck-mounted Wide Area Larviciding Systems (WALS) was added to supplement the Invasive *Aedes* Program to treat high trap count (HTC) areas and control larvae in cryptic water sources.

Material and Methods

Eight sites were selected based on high *Ae. aegypti* abundance in neighborhoods in June 2022. Four were randomly selected as treatment sites and four were selected as untreated control sites. VectoBac® WDG (*Bacillus thuringiensis israelensis* (*Bti*) 37.44%) was applied at a rate of 0.5 kg per hectare (0.5 lbs/acre) to four of the neighborhoods using a truck-mounted A1 Super Duty Mist Sprayer (Azelis, Westport, CT; Valent BioSciences 2019). Seven WALS applications were made beginning in June, with one treatment per week for the first four applications and then one treatment every two weeks for the following three applications.

BG-Sentinel mosquito traps monitored *Ae. aegypti* abundance biweekly in each of the treatment and control areas. Mosquito abundance was calculated as *Ae. aegypti* per trap per night. BG-Sentinel 2 traps were powered with a Mighty Max 12-volt 22 ah sealed lead acid battery (Home Depot, Atlanta, GA). Each trap set was baited with a BG-Lure (BioGents, Regensburg, Germany) and CO_2 produced by yeast fermentation. The sugar yeast solution consisted of 15g Red Star Active Dry Yeast, 250g of table sugar, and 2.5L of tap water in a Uline EZ-Pour F-style 3.8L jug, Festo push-in bulkhead connector QBS-1/4T-U, 0.170in ID clear vinyl tubing, 1in aquarium airstone (Figure 1). Abundance per trap night in treatment and control sites were compared using Welch's Paired t-test in Graphpad Prism (v.21312.41.41.24124.124).

Results and Discussion

The mean *Ae. aegypti* collected per trap night was significantly higher in the control sites at 23.4 mosquitoes per trap compared to 13.8 at the treatment sites ($p=0.0057$, $t(13)=3.306$). These results align with other studies on WALS efficacy (Harris et. al. 2021, Morgan et. al. 2021, Grippin et. al. 2021). DMVCD first used WALS to control *Ae. aegypti* at the Visalia Public Cemetery in 2020. In the previous cemetery study, *Ae. aegypti* abundance rapidly

Figure 1.—The BG-Sentinel 2 trap set with the 12v 22ah SLA battery, and the sugar yeast solution container.

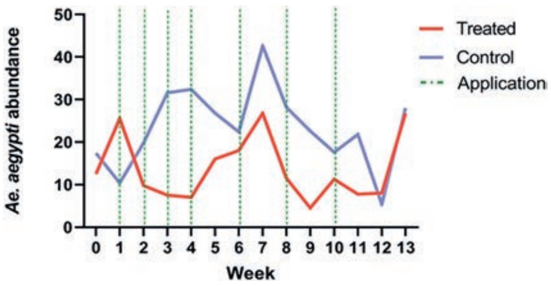

Figure 2.—The mean *Ae. aegypti* abundance per night for control and treatment sites after VectoBac® WDG applications with a truck-mounted Wide Area Larviciding System (WALS).

decreased during the application period but abundance rebounded to previous levels three weeks after the final treatment (Grippin et. al. 2021).

The 2022 WALS applications had a less pronounced effect than the 2020 cemetery applications (Fig. 2). Abundance was reduced for three weeks after the initial application when compared to the control sites. Abundance remained lower than control sites for two weeks following the final treatment (Figure 2). Common neighborhood obstacles such as fences, trees, and houses likely reduced the amount of product reaching sources in backyards (Morgan 2021). Abundance fluctuations could be attributed to residents in treatment areas creating or destroying mosquito breeding sources during the application period. Wind speeds of less than 2.2 m/s during applications may have prevented the product from traveling the desired 91.4 m (Valent BioSciences 2019). The inclusion of larvicide bioassay cups throughout the treatment area would have allowed for a better gauge of application efficacy (Harris 2021).

Conclusion

VectoBac WDG applications using truck-mounted WALS reduced *Ae. aegypti* abundance. By correctly timing WALS applications, Districts can effectively reduce *Ae. aegypti* in historic areas of high abundance.

Acknowledgements

We thank the District Manager, Mustapha Debboun, and the District Board of Trustees for their continued support.

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A valiant attempt at surveying mosquito district practices of California

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Introduction

Mosquito control programs throughout California use diverse methods for mosquito surveillance, control, and vector-borne disease agent testing. Variations in methods exist due to differing regional needs, climates, budgets, staffing, and the availability of resources. Mosquito surveillance data plays a critical role in operational planning to mitigate vector-borne disease risk to communities. However, this data is often not shareable between districts due to insufficient key information about protocol differences in surveillance and control practices. The CDC's National Public Health Framework discusses challenges facing financially stressed vector control organizations such as the need for improvement in core capacities, the lack of communication and coordination between vector control organizations and public health officials, and the lack of interconnected quality data (CDC 2020). There are many reasons that methods and techniques between vector control agencies vary widely, including but not limited to: differing regional needs, budget, staffing, trapping strategies, different mosquito species, how data is measured, data management, and interpretation (Brown et. al. 2021). District and agency data is often uploaded in a standard format to the VectorSurv database; however, trends across the data are not easily comparable because of these variations. Identifying gaps in knowledge can open discussions on practices to improve integrated vector management (IVM) control methods.

Methods

A 19-question survey was emailed on November 2022 to 73 California mosquito districts and environmental health agencies. The survey was re-sent two weeks later to acquire additional response. Districts that had not completed the survey by early-December were called to verify that they received the survey. The survey was emailed a third time if the agency indicated interest.

Results and Discussion

Of the 73 agencies to which the survey was sent, 71.2% (52/73) responded, nine were unresponsive, and 12 had no routine mosquito control activities. As expected, responses varied widely across the state because of variation in climate, staffing, methods, budget, resources, data collection, and community needs.

Across California, *Culex tarsalis* (23.7%) was reported as the most common species, followed by the *Cx. pipiens/ quinquefasciatus* complex (20.6%), with Cx. *erythrothorax* (10.3%) and *Culiseta incidens* (10.3%) tied for third.

Just under half of the districts, 48.0% (25/52), reported setting less than 50 mosquito traps a week, whereas 28.8% (15/52) set 50-100 traps, 15.3% (8/52) set 101-200, and only 7.6% (4/52) set over 201. This agreed well with the National Association of County and City Health Officials (NACCHO) survey that found about half of the 914 nationwide surveyed organizations were lacking in routine surveillance, species identification abilities, and treatments based on surveillance (NACCHO 2017).

A little over half of California's respondents, 53.8% (28/52), do not do any kind of insecticide resistance testing, whereas only 21.1% test both larvae and adults. These results are better than the 14% of districts and agencies nationwide that reported insecticide resistance testing in the 2017 NACCHO survey.

Programs like VectorSurv and ArboNET help standardize the reporting of mosquito arbovirus infections by location. However, there are limitations due to the variety of practices and collected data at the local level. Attempting to compile data across regions, without an understanding of individual variations, can lead to biases, misinterpretation, and errors (Brown et. al. 2021). For example, not having routine trap sets in the same location or not entering zero counts into the database can cause the data to be unknowingly skewed, especially when conducting computer modeling or drawing statewide comparisons (Brown et. al. 2021).

Conclusion

A deeper understanding of the strategies used will aid in district decision making processes and facilitate the development of comparable program data across the state. There are many areas that can be improved and discussing these issues at the association level can help lead towards standardization where possible. Results from this preliminary survey will help validate and improve questions for future surveys.

Acknowledgements

We thank the District Manager, Dr. Mustapha Debboun, and the District Board of Trustees for their continued support. We thank all of the mosquito districts and environmental health agencies of California for their help with the survey. Without them, this would not have been possible.

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Evaluation of adulticide application in vegetation dense areas using an unmanned aerial system

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Introduction

Unmanned aerial systems (UAS) are a new tool for mosquito surveillance and control in areas that were previously inaccessible using traditional methods or too expensive to treat using manned aircraft (Carrasco-Escobar et al. 2022, Faraji et al. 2021). High mosquito abundance and high West Nile virus (WNV) activity in several corn fields bordering urban areas has been a recurring problem for the Delta Mosquito and Vector Control District (DMVCD). The tall canopy and dense vegetation provide ideal harborage for mosquitoes and reduced water evaporation after the fields are flood irrigated prolongs mosquito production. Truck-mounted adulticide equipment cannot reach the center of the fields without crop damage, while proximity to urban areas made aerial applications unfeasible. To better control these and other hard to reach areas, DMVCD implemented a UAS Program for pesticide applications in 2022. The present study evaluated results from DMVCD's first UAS adulticide application.

Materials and Methods

Pyronyl^{TM} 525 Oil Concentrate (Central Life Sciences, 5% Pyrethrins, 25% Piperonyl Butoxide) was applied at a rate of 0.87 ounces (25.7 mL) per acre over 160 acres (64.7 ha) using a PrecisionVision 35X UAS from Leading Edge (Figures 1 & 2).

Adult mosquito abundance was monitored using three dry-ice baited Encephalitis Virus Surveillance (EVS) traps placed in the application area and four EVS traps placed in the control area the night before and the night after the adulticide application.

Application efficacy was estimated using Abbotts-corrected percent mortality in 10 sentinel field cages, each containing 25 to 35 wild-caught *Culex quinquefasciatus* Say and *Cx. tarsalis* Coquillett mosquitoes that were placed in the treatment area prior to adulticide application.

Percent control in the treatment area was estimated using Mulla's formula (Mulla et al. 1971, Reisen 2019). WNV activity was estimated using the minimum infection rate (MIR) (Biggerstaff 2009, CDC 2022).

Results and Discussion

Adulticide application began at approximately 0600 h and ended at 0615 h. Wind was from the east, northeast and at a speed of 0 to 3 mph (0-4.8 kph) at the time of application (Figure 2). Percent control, determined from pre- and post-application abundance per trap night, was estimated to be 13.4% for all female mosquito species in the treated area. This was in line with mortality in the sentinel field cages. The Abbott's-corrected mortality for the sentinel field cages was 16.7% at 8 hours post application, but only 12.2% after 24 hours. Sentinel field

Figure 1.—The PrecisionVision 35X UAS is capable of both adulticide and larvicide applications.

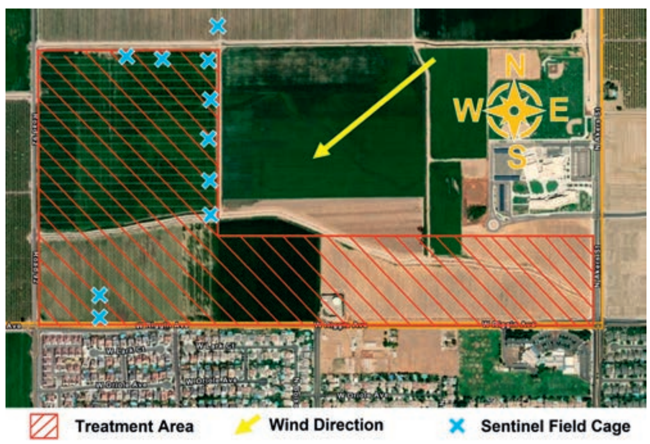

Figure 2.—The UAS adulticide treatment area showing the deployment of sentinel field cages and prevailing wind direction.

cage placement may have affected observed mortality, because the cumulative volume distribution of droplets was within product label requirements. Although wind speed was low during application, the direction was from the opposite direction expected. However, insecticide resistance also is likely to be an issue in the treatment area due to the low mortality of the wild-caught mosquitoes exposed in the field cages.

When analyzed by species, percent control was estimated to be 24.0% for *Cx. Quinquefasciatus*; no effect was seen on *Cx. tarsalis* abundance. Percent control could not be estimated for the other species because they were only collected in the control or treatment areas. Post-application, the WNV MIR was 25.9 per 1,000 female mosquitoes tested in the control area compared to 18.0 per 1,000 in the treatment area, for an insignificant infection rate difference of 7.9 (95% CI: −13.2, 29.1).

Conclusion

UAS can be used to quickly apply adulticides in vegetation dense areas that are difficult to access through traditional techniques. However, UAS applications in this first trial failed to markedly reduce mosquito abundance, perhaps due to resistance in the target populations. Additional adulticide products with crop labels are needed to combat existing insecticide resistance.

Acknowledgements

We thank the District Manager, Dr. Mustapha Debboun, and the District Board of Trustees for their continued support.

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Tailoring communication strategies to respective communities within the Delta Mosquito and Vector Control District

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Introduction

The Delta Mosquito and Vector Control District (DMVCD) serves 12 communities covering over 1,100 km^2 of northern Tulare County in Central California. It is home to a wide variety of mosquito sources, ranging from residential backyard sources such as bird baths, trash, plant saucers, and unmaintained swimming pools to large agricultural sources, such as dairy lagoons and flood irrigated pastures. Many outreach materials developed in California for *Aedes aegypti* contain a large list of mosquito sources, implying a one size fits all approach to source reduction (CDPH 2016; MVCAC n.d). However, many of the most productive mosquito-breeding sources listed in the available *Ae. aegypti* outreach material actually were not significant sources at DMVCD (Grippin et al. 2021). After the initial discovery of *Ae. aegypti*, outreach efforts should be changed to better fit the common sources found in yards (Hulcr et al. 2019; Christiano and Neimand. 2017). This poster displayed the common mosquito-breeding source types found during property inspections in 2020 and their contribution to overall *Ae. aegypti* production within the District. With proper planning and implementation, DMVCD personnel will tailor a strategy that will positively impact the reduction in mosquito populations, thus lowering the risk and transmission of mosquito-borne diseases.

Methods

Mosquito sources found during property inspections were categorized into 15 source types (Table 1). They were classified as positive or negative for *Ae. aegypti* larvae found at each inspection. The percent positive (percent of source type among inspections positive for immature *Ae. aegypti*) and percent contribution (percent of inspections of each positive source type that were positive for immature *Ae. aegypti*) were calculated for all of DMVCD (Figure 1A) and five major cities within the District (Figure 1B–F).

Results and Discussion

In 2020, DMVCD conducted 3,178 residential property inspections, categorizing mosquito-breeding source types found and collecting larvae samples for identification. Communities within the District varied widely in the types of sources that were most found positive (Figure 1A–F). Overall, the entire DMVCD showed plant trays (23.7%), other sources** (22.5%), yard drains (15.0%), and

Table 1.—Common source types found during 2020 property inspections and their contribution to *Ae. aegypti* breeding.

Source Type	Inspections (N)	Inspections with *Aedes*	% Breeding Source Type	Upper-Lower 95% CI	% Contribution to overall breeding	Upper-Lower 95% CI
Miscellaneous	2,094	122	5.8	4.9 - 6.9	14.0	11.8 - 16.5
Plant Trays	1,387	185	13.3	11.6 - 15.2	21.2	18.6 - 24.1
Decorative Items	1,094	16	1.5	0.9 - 2.4	1.8	1.1 - 3.0
Pet Dishes	1,034	60	5.8	4.5 - 7.4	6.9	5.4 - 8.8
Drains	885	116	13.1	11.0 - 15.5	13.3	11.2 -15.7
Fountains	720	84	11.7	9.5 - 14.2	9.6	7.8 - 11.8
Trash Cans	718	17	2.4	1.5 - 3.8	1.9	1.2 - 3.1
Toys	673	13	1.9	1.1 - 3.3	1.5	0.9 - 2.5
Birdbaths	582	46	7.9	6.0 - 10.4	5.3	4.0 - 7.0
Plants	464	47	10.1	7.7 - 13.2	5.4	4.1 - 7.1
Tarps	305	4	1.3	0.5 - 3.3	0.5	0.2 - 1.2
Tires	245	2	0.8	0.1 - 2.9	0.2	.04 - .08
Other**	668	150	22.5	19.5 - 25.8	17.2	14.8 - 19.8

****Other:** Sources include irrigation valves, water meter boxes, outdoor household appliances/items like bathtubs, toilets, and sinks, garden items like watering cans, wheelbarrows, hose holders, rooting plants, over watered plants, and spas.

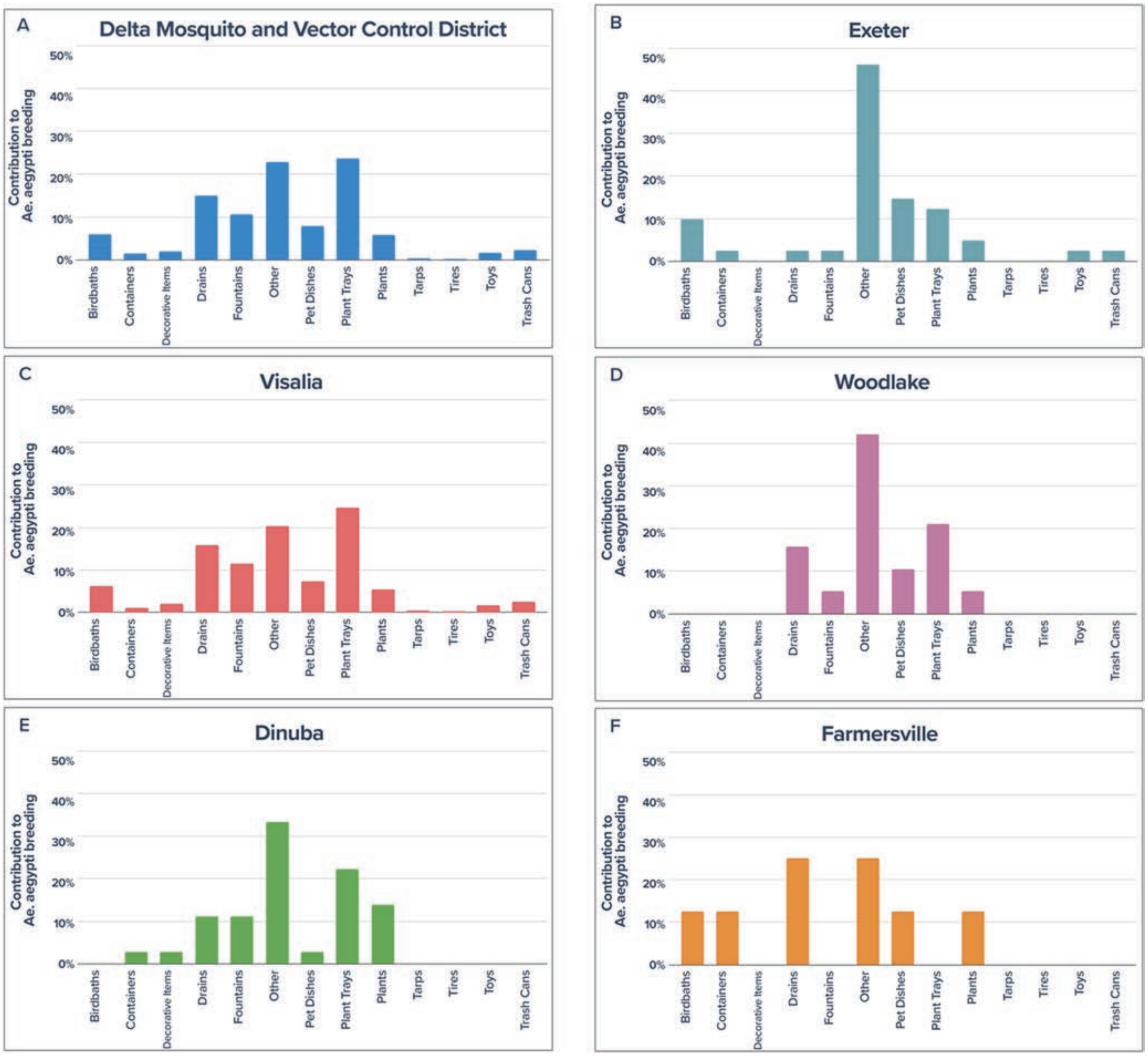

Figure 1A-F.—Percent contribution of common *Ae. aegypti* source types by city within the District.

fountains (10.6%) were most frequently positive for larvae (Figure 1A). These sources are listed in the outreach materials that are commonly used by vector control agencies when *Ae. aegypti* are first discovered in the area (CDPH 2016; MVCAC n.d). The sources with the least number of larvae present were tires (0.8%), tarps (1.3%), toys (1.9%), and trash cans (2.4%), which are also listed as top sources for *Ae. aegypti* (Figure 1A). The top three sources varied among communities. Birdbaths in Woodlake and Dinuba did not contribute to *Ae. aegypti* compared to 6.0% in Visalia (Figures 1C, D, and F). Promoting cleaning bird baths weekly in the communities where birdbaths are not contributing to the problem, would not reduce the *Ae. aegypti* population simply because the residents do not have them. Farmersville had no larvae in plant trays, a source that is very common everywhere else within the District (Figure 1F). In summary, the top four mosquito-breeding sources found within the District matched the sources listed in available *Ae. aegypti* outreach material. Yet, all other sources listed had little to no larvae present in the District.

Next Steps

This data will allow DMVCD to create a tailored strategy to effectively educate residents on which specific mosquito-breeding sources to address on their property. In addition, these specific education materials may help to recruit residents to eradicate mosquito- sources and thereby lower population levels, prevent mosquito bites and lower the risk of mosquito-borne illnesses spreading within the communities.

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Flea-borne typhus surveillance in unhoused resident camps in Santa Clara County

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Introduction

Unhoused individuals who reside in tents and RVs may live near other persons who are in similar situations, creating sizeable homeless encampments at numerous locations. These encampments unintentionally provide food, water, and harborage to commensal rodents, and may support large populations of these animals. Commensal rodents may carry fleas, which are implicated in many different pathogen transmission cycles, and this has the potential to cause illness in the humans that live alongside them. *Rickettsia typhi* is the causative agent for murine typhus, which is endemic in Los Angeles County and Orange County in California. *Rickettsia felis* is another potential agent that causes flea-borne spotted fever and has been detected in fleas from Sacramento County and Contra Costa County. The current study determined whether *R. typhi* and *R. felis* were present in fleas collected from homeless encampments within Santa Clara County.

Methods

Five homeless encampment sites in San Jose (Figure 1) were identified to have Norway rat populations due to the presence of food, water, harborage, and rodent burrows. Rats were trapped bi-weekly for two consecutive nights from these sites from February to October 2022, using Tomahawk Live Traps that were baited with peanut butter and canned cat food. Placement of the traps was informed by the presence of fresh rodent droppings, active burrows, rub marks, and suggestions from encampment residents. Traps were set in the early morning, left out overnight, and recovered the

Figure 1.—Locations of the five unhoused resident encampment sites in San Jose that were sampled in this study.

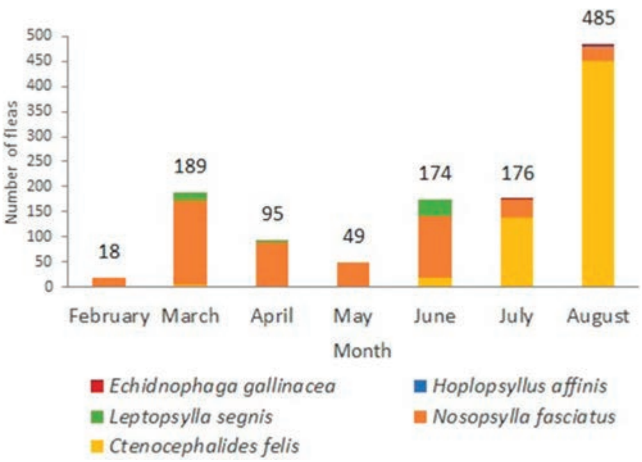

Figure 2.—Flea abundance by month and species. A total of 1,188 fleas were collected between the months of February and August.

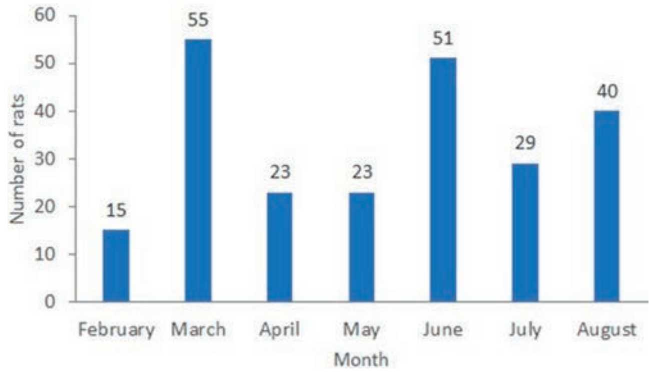

Figure 3.—Norway rat (*Rattus norvegicus*) abundance. A total of 236 rats were collected between the months of February and August.

following morning. Rats were transported back to the Santa Clara County Vector Control District (SCCVCD) laboratory and euthanized using CO_2, according to American Veterinary Medical Association protocol (Underwood and Raymond, 2020). To collect their ectoparasites, the rats were individually bagged, sprayed with PT 565 Plus XLO, a pyrethrin insecticide, and left for at least 30 minutes for the pesticide to take effect. After, rats were combed with a fine bristle brush and the collected ectoparasites were stored in vials of 70% ethanol. Ectoparasites were identified using a taxonomic key (CDC, 2003), and fleas were washed with molecular grade water and sorted by species, sex, rat identification number and placed in pools of up to five fleas per vial. DNA was extracted with the MagMax DNA Ultra 2.0 Kit following manufacturer's recommendations and tested for the presence of *Rickettsia typhi* and *Rickettsia felis* via duplex PCR following the protocol in Rangel et al. (2019).

Results

A total of 1,188 fleas (Figure 2) were collected from 236 rats (Figure 3) during the course of the study. Flea species collected were *Ctenocephalides felis*, *Nosopsyllus fasciatus*, *Leptopsylla segnis*, *Echidnophaga gallinacea*, and *Hoplopsyllus affinis*. *Rickettsia typhi* was not detected in any of the flea pools. *Rickettsia felis* was found in 91 of 175 *Ctenocephalides felis* pools, 1 of 4 *Echidnophaga gallinacea* pools, and 2 of 236 *Nosopsyllus fasciatus* pools.

Conclusions

Rickettsia typhi was not detected in the fleas sampled in this study. *Rickettsia felis* was found in cat fleas, sticktight fleas, and northern rat fleas from rats living in encampments in Santa Clara County (Figure 4). Our results set the stage for ongoing flea-borne disease surveillance in our District and though monitoring the ectoparasite abundance and diversity, we hope to be able to prevent any disease outbreaks within the vulnerable population residing in homeless encampment sites in the county.

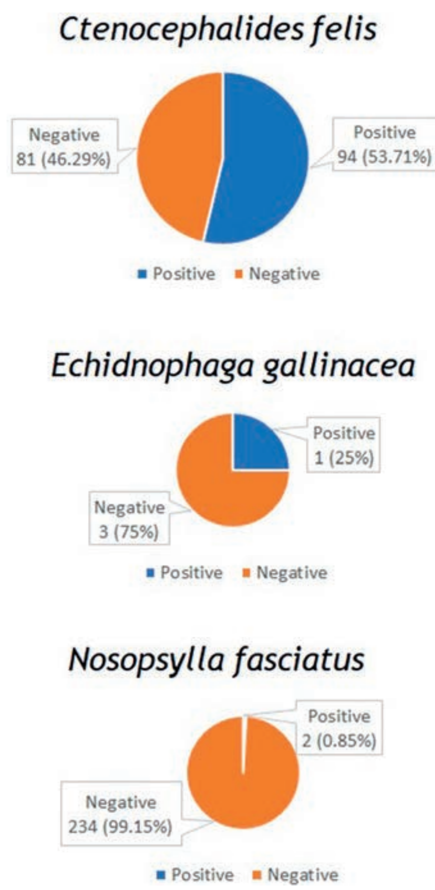

Figure 4.—Prevalence of *R. felis* in different flea species. *R. typhi* was not detected in any of the flea pools.

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Does the diversity of West Nile virus positive dead bird species detected during outbreak and non-outbreak years impact the transmission to humans?

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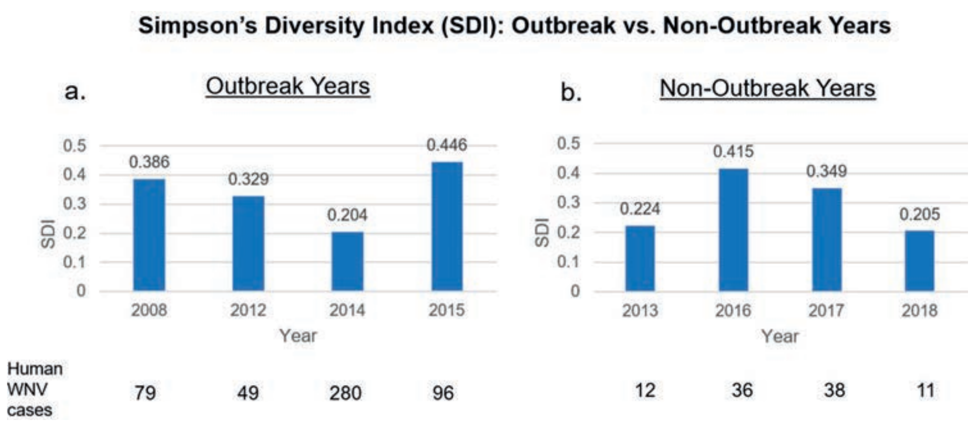
Introduction

West Nile virus (WNV) is a member of the family *Flaviviridae* (Colpitts et al. 2012). Infection with WNV can cause flu-like symptoms in humans and in 20% of cases, could result in more serious symptoms, such as paralysis, brain and spinal cord inflammation, and even death. WNV in nature is maintained in a cycle involving birds and mosquitoes. Humans, horses, and other animals are "dead-end" hosts for the virus. Humans acquire WNV by a bite from a mosquito harboring the virus. WNV cases in humans have been tracked in Orange County since 2004 by the Orange County Health Care Agency (OCHCA; OC Health Care Agency. 2019, 2021). Monitoring the transmission of WNV to the human population is important in directing mosquito control to keep infection rates low to non-existent. One method of monitoring the risk of WNV transmission to humans is to evaluate dead birds for infection with WNV. The Orange County Mosquito and Vector Control District (OCMVCD) has been performing dead bird monitoring within Orange County every year since 2003. In 2008, OCMVCD reported that 37 species of dead birds were positive for WNV (Krueger et al. 2009). This raised the possibility of bird species diversity influencing transmission of WNV to the human population. We posited that higher bird species diversity would correlate with increased WNV cases in humans. Our hypothesis was that dead bird species diversity will be higher during WNV outbreak years in humans than non-outbreak years.

Methods

OCHCA's West Nile virus human infection database (OC Health Care Agency 2019, 2021) was analyzed and four WNV outbreak and four non-outbreak years were identified. An outbreak year was defined in this study as having ≥ 40 WNV human cases. Briefly, the database from OCMVCD's Dead Bird Surveillance Program for Orange County was analyzed comparing the outbreak and non-outbreak years. The database contained all the birds that were collected or submitted to the program each year from 2003-2021. For each year, the number of different bird species tested for WNV was tabulated and bird species diversity was calculated using the Simpson's Diversity Index (SDI) (Simpson 1949). SDI is a measure of the diversity within a population, and the values range from 0



Figures 1.—a) Bird species diversity in the OCMVCD Dead Bird database between WNV Outbreak Years (2008, 2012, 2014, 2015) and b) Non-Outbreak Years (2013, 2016, 2017, 2018), as determined by the SDI. The number of reported human WNV cases are displayed for each year.

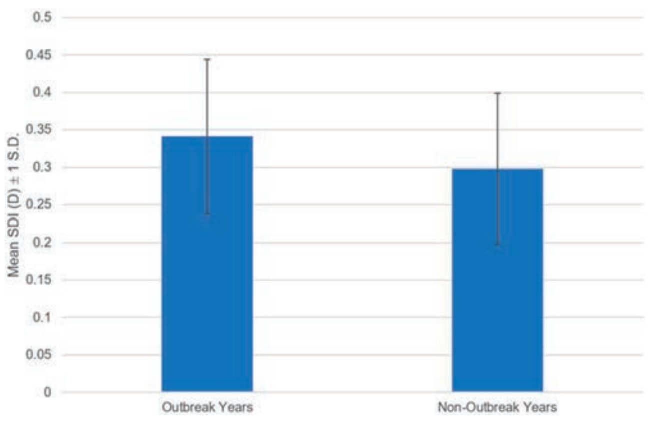


Figure 2.—The mean Simpson's Diversity Index (SDI) and ± standard deviation (S.D.) for Outbreak (0.341, s.d. ± 0.103) and Non-Outbreak (0.298, s.d. ± 0.101) years.

to 1, with a larger value indicating greater diversity in the population.

SDI is calculated as follows:

$$\mathrm{SDI} = 1 - \frac{\sum n(n-1)}{N(N-1)}$$

where n is the number of individual birds of one species, Σ is the sum of, and N is the total number of all bird species for that particular year. The data was tabulated and the resulting SDIs, mean SDIs, and ±1 standard deviation were calculated in Microsoft® Excel. The bird species diversities were compared between outbreak years and non-outbreak years using the SDI values to determine whether the data supported the hypothesis.

Results and Discussion

Bird species diversity as measured by SDI during outbreak and non-outbreak years in the human population in Orange County was not significantly different (Figures 1 and 2).

If several species tested positive for WNV, it did not correspond to higher bird species diversity. Although there were several bird species that tested WNV-positive, only 3-4 species had a sufficient number of positives (Figure 3). Most species were represented by only 1-2 birds, < 1% of all bird species tested. SDI accounts for both "species richness" (total # of different species in a population) and "species evenness" (the relative abundance of each species). We observed high species richness but low species evenness, which resulted in low SDI values or low bird species diversity.

American crows (*Corvus brachyrhynchos*) were the dominant bird species submitted to the OCMVCD Dead Bird Surveillance Program each year, regardless of whether it was an outbreak or non-outbreak year. American crows represented at least 73% of all WNV-positive dead birds each year.

Conclusions

After analyzing OCMVCD's Dead Bird Database and OCHCA's database for human WNV cases in Orange County, we rejected our hypothesis that the diversity of dead birds submitted for testing that tested positive for WNV was an indicator of increased WNV transmission to humans. The preponderance of dead American crows in the sample population each year likely influenced these results. It would be interesting to compare Orange County dead bird data with data collected from other counties and states to determine whether the latter would support our conclusions with regards to bird diversity and WNV transmission to humans. The dead bird monitoring program should also be reviewed for effectiveness (e.g., possible flaws of citizen research: Ward et al. 2006) and the influences of host dynamics (e.g., birds that get infected with WNV but do not succumb to infection; birds developing resistance to WNV: Hopf et al. 2022) should be evaluated. Dead bird data should also be compared to other data sets (e.g., mosquito data; mosquito blood meal data:

Bird species ranking: % of Total WNV+ Dead Birds

Outbreak Years

2008 (Total species = 37)	
American Crow	77.90
House Finch	8.10
House Sparrow	2.46
Mourning Dove	1.59
Common Pigeon	1.45
Western Bluebird	1.18
Several (n=31)	≤0.58

2012 (Total species = 11)	
American Crow	81.65
House Finch	6.42
House Sparrow	4.59
Several (n=8)	≤0.92

2014 (Total species = 16)	
American Crow	89.11
House Finch	4.22
Northern Mockingbird	1.11
Several (n=13)	≤0.88

2015 (Total species = 12)	
American Crow	73.81
House Finch	10.71
Cooper's Hawk	2.38
Western Bluebird	2.38
Western Scrub Jay	2.38
Several (n=7)	≤1.19

Non-Outbreak Years

2013 (Total species = 3)	
American Crow	87.80
House Finch	9.76
Cooper's Hawk	2.44

2016 (Total species = 13)	
American Crow	76.29
House Finch	5.15
Cooper's Hawk	4.12
Red-Shouldered Hawl	3.09
Common Raven	2.06
Mourning Dove	2.06
Several (n=7)	≤1.03

2017 (Total species = 9)	
American Crow	80.70
House Finch	5.26
Western Bluebird	3.51
Several (n=6)	≤1.75

2018 (Total species = 3)	
American Crow	89.47
Cooper's Hawk	5.26
House Sparrow	5.26

Figure 3.—Bird species ranking (% of total WNV-positive dead birds) for each year comparing Outbreak Years and Non-Outbreak Years. Calculated SDIs and the number of reported human WNV cases are displayed for each year.

Molaei et al. 2010, Thiemann et al. 2012, Wheeler et al. 2021) that may shed light on WNV transmission in nature. Finally, another line of research worth pursuing is "What makes a bird an ideal host reservoir for WNV that supports virus transmission to humans?"

Acknowledgements

The authors would like to thank Robert Cummings and colleagues at OCMVCD for their helpful scientific discussions and collaboration.

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Topographic-based assessment for density of adult *Ixodes pacificus* and *Dermacentor occidentalis* and prevalence of *Borrelia burgdorferi* and *Borrelia miyamotoi* on hiking trails in Santa Clara County

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Abstract

A hiking smart phone application, GAIA GPS Inc. (Berkeley, CA), was implemented in tick surveillance to capture topographic data regarding elevation, slope and distance traveled during transect sampling. During two successive winter seasons, 70 trails within 16 parks in Santa Clara County were sampled by flagging between November 2017 and March 2019, during which 2,670 female and 2,315 male *Ixodes pacificus* and 1,104 *Dermacentor occidentalis* were collected. *Ixodes pacificus* were individually tested for *Borrelia burgdorferi* sensu strictu and *Borrelia miyamotoi* using duplex RT-PCR. Overall, 45 adult female *I. pacificus* were found to be infected, 29 with *B. miyamotoi* and 16 with *B. burgdorferi,* whereas 27 males were infected, 18 with *miyamotoi* and nine with *burdorferi;* three males tested positive for both borrelia species. General linear model (GLM) tests showed tick density varied significantly among parks and trails for each tick species. Slope or delta elevation was found to have a significant ($P<0.05$) association with density of male and female *I. pacificus*, but not with *D. occidentalis* based on GLM and Pearson Correlation Tests. Based on Fisher's LSD Multiple-Comparison Test on natural log-transformed data, the density of female *I. pacificus* was significantly ($P<0.05$) greater on Southwest facing aspects compared to that of Northeast facing aspects.

Introduction

In California, *Ixodes pacificus* and *Dermacentor occidentalis* are two predominant tick species of concern for disease transmission and biting-nuisance to the public (Kramer and Beesley 1993, Padgett et al. 2014). These two species differ in pathogens transmitted and preferred habitats; *Ixodes pacificus* is the primary vector of Lyme disease and is found in oak woodland habitat, whereas *Dermacentor occidentalis* transmits rickettsias and other pathogens (Furman and Loomis 1984) and prefers open grassland and chaparral (McDonald 2018). Despite these habitat differences, the two species are often found sympatric in distribution and often sampled along the same transects.

Tick species distribution and density have been modeled based on abiotic topological features such as elevation, slope and aspect (Diuk-Wasser et al. 2010, Lane et al. 1985, Cadenas et al. 2007, Medlock et al 2008, Gilbert 2010, Van Horn et al. 2018). In Ozark forests, the Lone Star tick (*Amblyomna americanun*) was found to have greater abundance in valleys and north-facing aspects (Van Horn et al. 2018). In the Eastern United States, altitude was found to be a strong predictor for nymphal density of *Ixodes scapularis* (Diuk-Wasser et al. 2010). In California, *D. occidentalis* was more abundant on south than on north facing slopes (Lane et al. 1985). In Switzerland, Cardenas et al. found greater densities of questing *Ixodes ricinus* on the south facing slope compared to north facing slopes between 620 and 1,070 meters elevation (Cadenas et al. 2007). In Gower, south Wales, *I. ricinus* was found to be associated with topographic features that reduce exposure to cold northerly winds and hot midday sun (Medlock et al. 2014).

One challenge to the conduct of adult tick sampling has been the accurate measurement of transect length for determination of tick abundance or density estimates. The Santa Clara County Vector Control District (District) experimented with pedometers as a measuring device, but with little success (unpublished data). A smart phone application called GAIA GPS (Trailbehind Inc.) was tested for accuracy in distance measurement by comparing results with a measuring wheel along a one-mile trail and found to be 99% accurate (unpublished data). That application did not require a constant telephone network connection, but used satellites to record a "bread crumb trail" of elevation points and produced audible alerts when the set distance had been achieved.

To assess abiotic topological effects on tick density and tick infection rate, park hiking trails in Santa Clara County were sampled by transect measured using the GAIA GPS application, followed by geographic information system

(GIS) analysis for hillside aspect and calculation of slope. Sampled ixodid ticks were individually tested for *B. burdorferi* and *B. miyamotoi*. Topological trends were assessed using general linear modelling with density of male and female *I. pacificus* and *D. occidentalis* as well as for ixodid infection rates with *B. burgdorferi* and *B. miyamotoi* as dependent variables.

Materials and Methods

Field Sampling

Five Vector Control Technician staff were assigned 14 parks to sample adult ticks starting the Fall of 2017 with the goal of obtaining a sample size of about 150 ticks per park. Sampling continued until April of 2019. Standard 1x1 m tick flags were made of flannel material and attached to either wooden rods or hiking poles using metal binder clips. Wet flags were replaced with dry ones as needed. Tick drags were conducted along the trail edges.

To provide increased accuracy in sampling distance measurements, a smart phone application, GAIA (gaiagps.com) was compared to that of a measuring wheel and found to be within 99% accuracy. The app was set to produce audible alerts for 250 m sampling increments that was chosen as a standard sampling transect distance for this study. Other features of the app were automated recording of track and elevation and an option to save markers at each 250 m increment that were utilized to record tick sample vial numbers. After each collection, the track was saved and shared via email for later GIS investigation.

Laboratory Testing

Ticks were identified to species, gender and immediately placed inside grinding tubes that were filled with 400 μL of lysis/binding solution and 2 ceramic beads (Omni Inc.). The lysis/binding solution was prepared according to MagMax Core protocol by adding 1:1 lysis to 98% isopropanol. The samples were stored at 4°C until they were homogenized using Omni Bead Ruptor 24 (Omni International Inc.) by vibrating the samples at speed 5 for 3 minutes. The process was repeated three times with 7 sec intervals in between vibrations. Immediately after homogenization, samples were centrifuged at 5,500 rpm for 3 min at room temperature.

Extraction was conducted using MagMax 24 machine. 70 μL supernatant of sample was mixed with 20 μL Magnetic Beads, 10 μL Proteinase K, and 100 μL of Isopropanol. Then, samples were washed twice, each with 150 μL of Wash 1 and Wash 2, before the clean materials were eluted in 50 μL of elution buffer. The final products were kept at −80°C before being screened by PCR.

DNA extracts were tested by qPCR, as described in Tsao et al. (2004), Barbour et al. (2009), and Jobe et al. (2016), with probes for the 16S rDNA that differed between *B. burgdorferi* and *B. miyamotoi*. Forward and reverse primers were, respectively, 5′GCTGTAAACGATGCACACTTGGT and 5′GGCGGCACACTTAACACGTTAG. The corresponding dye-labeled probes were 6FAM-TTCGGTACTAACTTTTAGTTAA and VIC-CGGTACTAACCTTTCGATTA with 3′ ends modified with a minor groove binding (MGB) protein (Applied Biosystems) for *B. burgdorferi* and *B. miyamotoi*, respectively. Positive samples for tamplification were obtained from previous years.

The PCR conditions were 50°C for 2 min and 95°C for 10 minutes, followed by 45 cycles of 95°C for 15 seconds and 63°C for 1 minute, using TaqMan Universal.

GIS Harvesting of Topographic Data

Tracks (for example: see Figure 1) generated by the GAIA app were converted from GPX and KMZ format to ArcGIS features using ArcTools. In ArcGIS, elevation data was retrieved for each track segment or vial collected by selecting track points corresponding to each 250 m sampling interval and recording statistics for average, minimum and maximum. The slope of each interval was calculated based on change in elevation (delta) divided by 250 m. Aspect was obtained by using ArcGIS Spatial Analyst feature called Aspect and a slope shapefile provided by the Santa Clara County Planning Office. That module compared the downslope direction of the maximum rate of change in value from each cell to its neighbors and reported a value ranging from zero the 360 (due north). North was defined as 0-22.5; Northeast as 22.5-67.5; East as 67.5-112.5; Southeast as 112.5-247.5; South as 157.5-202.5; Southwest as 202.5-247.5; West as 247.5-292.5; Northwest as 292.5-337.5 and North as 337.5-360.

Statistical Analysis

Data were analyzed using SYSTAT 13©. Parks and trail topography was averaged using descriptive statistics. General linear models were used to determine significant associations for dependent variables, tick abundance and infection rate and independent variables, elevation, slope, aspect, park, tick species and tick gender. Dependent variables were checked for normality, skewness and kurtosis and transformations were not needed.

Results

Sixteen parks sampled for ticks within Santa Clara County had average elevations ranging from 145 m to 485 m above sea level. Trail slope or change in elevation and aspect were averaged for each park trail (i.e. transect) sampled (Table 1). Within the 16 parks, 70 trails were sampled for ticks yielding a total of 2,315 male *I. pacificus*, 2,670 female *I. pacificus* and 1,104 *D. occidentalis*.

Borrelia burgdorferi sensu stricto was found in 16 adult female and 9 adult male *I. pacificus* and *B. miyamotoi* was found in 29 adult female and 18 adult male *I. pacificus*. Dual infections for both *B. burgdorferi* and *B. miyamotoi* were found in three male *I. pacificus*. Overall infection rates of female *I. pacificus* were 0.599 and 1.09 for *B. burgdorferi* and *B. miyamotoi*, respectively. In males, infection rates were found to be 0.390 and 0.777 for *B. burgdorferi* and *B. miyamotoi*, respectively.

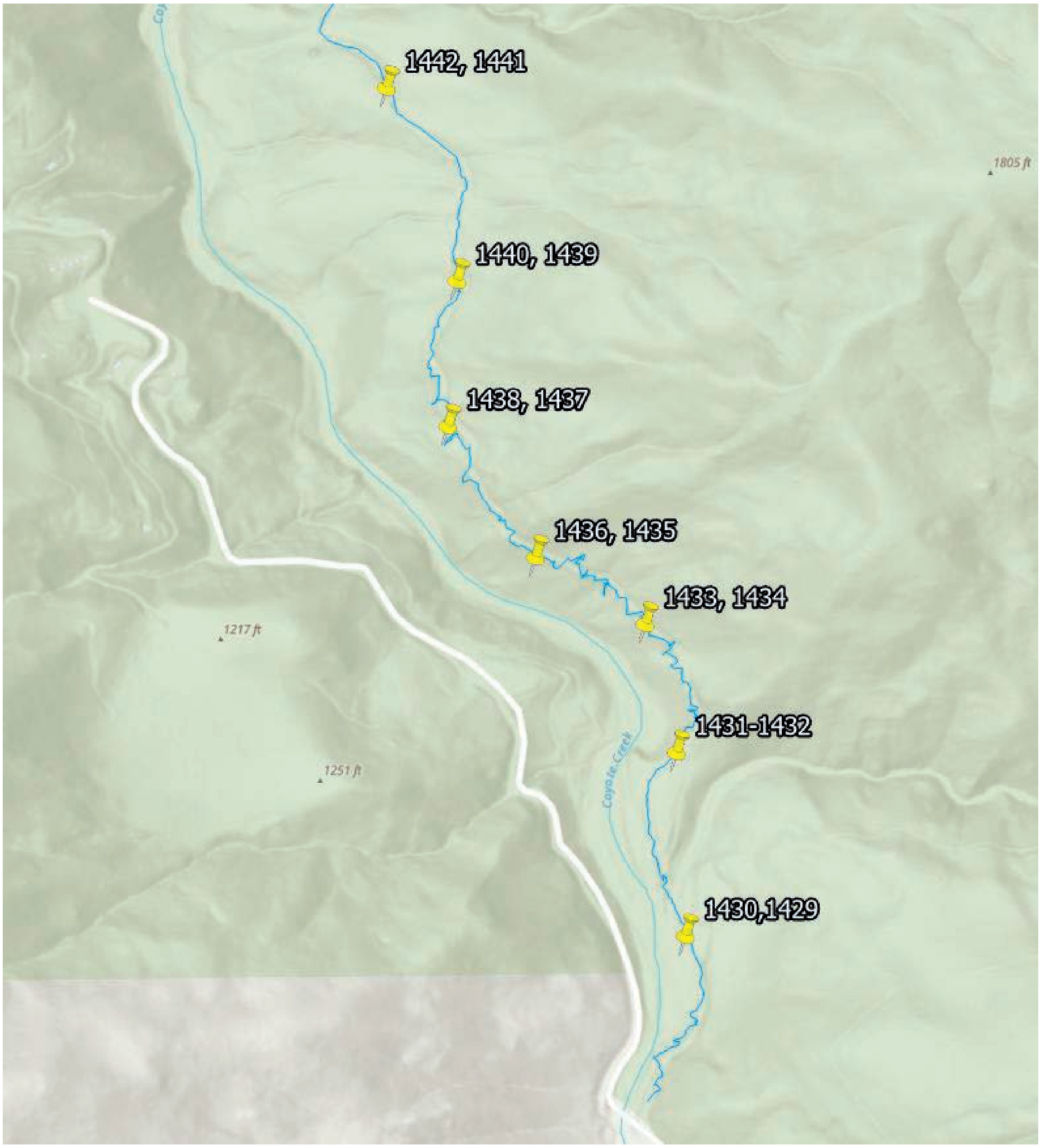

Figure 1.—Example of tick transect collection points (yellow push pins) along trail in Henry Coe State Park at Gilroy Hot Springs showing blue "breadcrumb" trail of elevation points and tick vial numbers for male and female *Ixodes pacificus* created using GAIA GPS.

Analysis of topographic and other features using General Linear Modelling indicated significant ($P<0.05$) association of male and female *I. pacificus* densities with the independent variables of Park, Trail, Trail Slope, Change in Trail Elevation and Month sampled (Table 2). In adult *D*. occidentalis, significant ($P<0.05$) associations were found for Park, Trail and Month (Table 2). Infection rates were significantly ($P<0.05$) associated with Park, Trail and Aspect (cardinal direction) for *Borrelia* (both species combined) (Table 3). *Borrelia burgdorferi* was associated with Trail; *B. miyamotoi* infections were associated with Trail, Aspect (degrees) and Aspect (cardinal direction) (Table 3).

Table 1.—Park topological features by park sampled for ticks. Values were derived from GAIA GPS captured data.

Park Name	Average Elevation (m)	Average stdelev	Average Min-elev (m)	Average Max-elev (m)	Average Aspect	Avg delta	Average of slope
Almaden Quicksilver	241.72	7.17	221.76	252.90	168.05	31.14	0.12
Alum Rock	178.00	8.50	156.22	191.86	169.33	35.63	0.14
Calero	176.00	5.55	158.73	185.73	135.30	27.00	0.11
Coyote	265.00	5.11	248.92	273.00	193.75	24.08	0.10
Foothills Park Palo Alto	251.49	7.78	231.80	265.00	194.46	33.20	0.13
Fremont Older	223.29	7.24	200.86	235.83	158.79	34.98	0.14
Grant Ranch	485.90	5.28	471.59	496.17	137.00	24.59	0.10
Heintz OSP	157.38	8.63	136.23	172.67	204.47	36.44	0.15
Henry Coe Park	480.78	6.88	460.86	491.82	174.69	30.96	0.12
Lexington	253.19	7.95	228.41	266.23	182.88	37.82	0.15
Mount Madonna	395.09	6.74	376.30	411.34	143.89	35.04	0.14
Rancho Canada del Oro	252.50	7.35	237.25	265.50	204.50	28.25	0.11
Rancho San Antonio	145.31	7.60	120.04	162.65	154.73	42.62	0.17
Santa Teresa	147.79	7.84	130.50	160.70	170.00	30.20	0.12
Sierra Azul	514.71	5.71	495.47	523.68	160.69	28.21	0.11
Stevens Creek	146.49	7.38	121.39	157.21	183.97	35.82	0.14

General Linear Modeling indicated that Parks and Trails vary significantly in terms of male and female *I. pacificus* and *D. occidentalis* density (Table 2) and overall *Borrelia* infection rate (Table 3). *Borrelia burgdorferi* and *B. miyamotoi* each varied significantly by Trail, but not significantly (P>0.05) by Park. Transect slope or change in elevation was significantly (P<0.05) associated with density of male and female *I. pacificus*, but not significant (P>0.05) for D. occidentals (Table 2). Transect elevation (minimum, maximum and average) was not significantly (P>0.05) associated with tick density or tick infection rate (Tables 2 and 3). Sampling Month was found to be significantly associated with tick density, but Year was not (Table 2); tick infection rates were not found to be associated with Month or Year (Table 3).

Transect Aspect was not found to be significantly associated with tick density (Table 2), but tick infection rate for *Borrelia* species was significantly associated with Aspect reported as cardinal direction (Table 3, Figure 2). Due to lower count of transects in the cardinal direction North and a pool of one positive tick, a 100% infection rate was calculated and caused an average infection rate of 8%. Removing that outlier indicated slightly higher infection rates in the South, Southeast and East and lower rates in Northeast, Southwest and West.

Table 2.—General Linear Model P-Values for *Ixodes* and *Dermacentor* transect counts based on independent variable.

	Ixodes pacificus		
Independent Variable	Female	Male	*Dermacentor*
Park Name	0.000	0.000	0.008
Trail Name	0.000	0.000	0.002
Slope	0.035	0.016	0.405
Avg Elevation	0.528	0.827	0.611
Min Elevation	0.439	0.685	0.635
Max Elevation	0.571	0.888	0.564
Aspect (degrees)	0.557	0.328	0.870
Aspect (cardinal)	0.904	0.804	0.904
Delta Elevation	0.035	0.016	0.405
Month	0.005	0.002	0.001
Year	0.184	0.711	0.470

Discussion

Utilization of the GAIA GPS application greatly improved adult tick surveillance by providing accurate transect distance measurement and capture of precise location and elevation data for GIS analysis. Audible alerts aided the staff in sample collection and recording of collection vial numbers to associate GIS data with tick collection and test result data.

Compared to other studies modeling tick distribution using topological features, our study found South, Southeast and Southwest facing slopes to have highest abundance of *Ixodes* ticks, in accordance with Cardenas et al. (2007) where *I. ricinis* was found more often on south-facing mountains. Our data did not support observations by Lane et al. (1985) where *D. occidentalis* was found predominantly on south-facing slopes.

Table 3.—General Linear Model P-Values for *Ixodes* male and female total infection rate and for *Borrelia burdorferi* and *B. miyamotoi* based on independent variable.

	Infection rate		
Independent Variable	Borrelia spp	B. burg.	B miya.
Park Name	0.038	0.087	0.773
Trail Name	0.004	0.000	0.000
Slope	0.916	0.189	0.489
Avg Elevation	0.699	0.424	0.259
Min Elevation	0.698	0.410	0.268
Max Elevation	0.702	0.457	0.243
Aspect (degrees)	0.131	0.269	0.016
Aspect (cardinal)	0.027	0.960	0.002
Delta Elevation	0.916	0.189	0.489
Month	0.173	0.206	0.170
Year	0.835	0.636	0.480

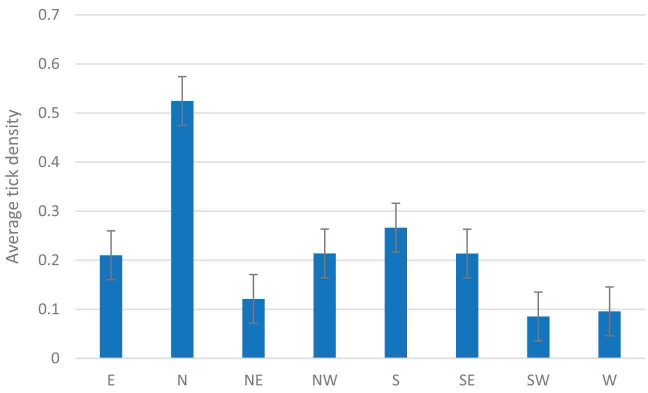

Figure 2.—Average density of adult ticks (± standard error) per 250 m transect by average hillside aspect using cardinal direction.

Statistical challenges to analyzing any effects of topological features during tick sampling include highly variable numbers of ticks collected along transect length, low infection rates, and estimation of hillside aspect along winding trails may lack accuracy depending on where along transects ticks were collected.

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Resistance in the marsh: methoprene and *Aedes dorsalis*

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Introduction

Aedes dorsalis is a mosquito of great concern for Alameda County Mosquito Abatement District (ACMAD) due to its aggressive biting behavior and potential for very high abundance near marsh habitats. Service requests for this species can be high in the absence of control efforts, and substantial resources may be used to limit their reproduction. *Aedes dorsalis* is a warm-weather mosquito that is capable of maturing from egg to adult in a short amount of time within habitats of the eastern part of the San Francisco Bay. ACMAD often relies upon products that contain insect growth regulators such as methoprene (e.g., Altosid) to control early instar *Ae. dorsalis*. Resistance to methoprene in several species of mosquito have been reported throughout the state of California (Cornel et al. 2002, Su et al. 2021), and a neighboring District has found methoprene resistance in *Ae. dorsalis* using a bench-top bioassay (personal communication, Eric Haas-Stapleton).

Methods

Between the months of July and September of 2022, *Ae. dorsalis* and the water in which they were found were collected from multiple field locations in Alameda County. A minimum of two-hundred larvae were collected for each test. Altosid Liquid Larvicide (ALL) contains -(S)-Methoprene (CAS #65733-16-6) as the active ingredient with a concentration of 20% (v/v) according to the product label. ALL was first diluted 1:4 to in tap water to produce a 5% solution (5ALL). Following the protocol provided by Sacramento-Yolo Mosquito and Vector Control District. The 5-ALL was further diluted using the marsh water for final concentrations of 0, 0.05, 0.025, 1, or 5 parts per billion (ppb). The diluted 5-ALL was placed into 6 oz styrofoam cups (100 ml per cup) that were supplemented with four rabbit pellets before eight *Ae. dorsalis* larvae were added to each cup. The bioassay cups were placed in a humidified (75% relative humidity) chamber set at 28°C and monitored daily for adult mosquito emergence for ten days.

Results and Discussion

Preliminary trials using ALL concentrations at 5 ppb and higher resulted in no adult emergence. Methoprene concentrations for the remaining trials were reduced. The average adult emergence for each methoprene concentration of two replicated trials was: 87% at 0 ppb, 60% at 0.05 ppb, 23% at 0.25 ppb, 19% at 1 ppb, 0% at 5 ppb. A third trial had low emergence in all concentrations including the control group (not shown). Fewer *Ae. dorsalis* emerged as the methoprene concentration increased. The results of this study demonstrated that the *Ae. dorsalis* larvae that were tested in Alameda County were susceptible to methoprene concentrations that are commonly used in field applications.

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Outbreak of caterpillar-associated rash due to the Western tussock moth (Lymantriidae: *Orgyia vetusta*) at an Orange County elementary school, May 2022

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Investigation Summary

In April 2022, the Orange County Mosquito and Vector Control District (OCMVCD) received a service request for biting insects from a private elementary school in Laguna Beach, California. The Principal noted that during the previous two weeks students and staff reported insect bite-like rashes on their bodies. Some of the rashes were found on parts of the body normally covered with clothing, such as the abdomen and upper leg area, and some of the rashes were on exposed skin. The Principal stated that the frequency of students and staff reporting rashes was increasing and that bites were reported to occur indoors and outdoors. The elementary school was located within the Laguna Park Wilderness Preserve and a creek with year-round water traverses the school property (Figure 1). Within 24 h of the initial service request, OCMVCD Inspectors surveyed the creek for mosquitoes, observing *Culiseta incidens* larvae in rock pools, and applied a Bti larvicide to areas containing larvae. No adult mosquitoes were collected during the initial inspection and treatment of the creek. Two EVS CO_2-baited mosquito traps were set north and south of the school along vegetation overhanging the creek and two BG-Sentinel mosquito traps were set adjacent to school buildings.

A few hours later the Inspector who set the CO_2 mosquito traps in vegetation along the creek felt intense itching along their forearms and discovered raised red

Figure 1.—Aerial image of the elementary charter school and flood control channel traversing the property in Laguna Beach, CA.

Figure 2.—OCMVCD Inspector's forearm with a caterpillar associated-rash caused by true setae from the western tussock moth, *Orgyia vetusta*.

Figure 3.—A western tussock moth caterpillar, *Orgyia vetusta*, on an aggregation of cocoons and egg masses. Photo by Laura Krueger.

welts appearing like insect bites (Figure 2). Upon returning to the office, they changed out of their uniforms into street clothes. At home that evening, the red raised welts continued to appear and became larger with increasingly irritation. The largest bite-like rashes were found in the crook of their elbows which were covered by the work uniform during inspection to prevent potential skin exposure. The Inspector became increasingly worried that very small insects or mites were causing the reaction on their skin. The other Inspector present during the inspection and treatment, and did not set mosquito traps, was fine. The next day the traps were collected and contained a low abundance of mosquitoes. The Inspector scheduled an inspection of the school with the Vector Ecologist and Supervisor (herein the Inspection Team). During the inspection, students alerted the Inspection Team to the presence of caterpillars and masses of cocoons and adults underneath shade structures and awnings of school buildings along the creek (Figure 3). The aggregations of caterpillars, cocoons, and adult moths were found throughout the school campus. The students stated that those who picked up the caterpillars had the most severe rashes and that there was a large population of caterpillars this year as compared to other years. The focus of the investigation then shifted to the caterpillars and cocoons as the cause of the rash. Caterpillar associated rash, also called lepodopterism, are terms used for the variety of cutaneous and systemic reactions that result from contact with caterpillars, cocoons, and adult life stages of butterflies and moths that contain urticating setae (Mullen and Durden, 2009).

Cocoons were also located in the tree where the Inspector hung mosquito traps the previous week (Figure 4). The urticating hairs likely fell from the tree during the setting of the mosquito trap and embedded in the Inspector’s forearm through the opening of the uniform sleeve. The exposure demonstrates that setae can dislodge from the cocoons and travel through the air, causing symptoms without direct contact with the larvae or cocoons. Reports in scientific literature show that inhalation of setae results in coughing, shortness of breath, and asthma like symptoms, and that setae can embed in human eyes causing severe conjunctivitis (Battisti et. al. 2011).

The caterpillars were identified as the western tussock moth (Lymantriidae: *Orgyia vetusta*). All life stages of this species possess two types of setae that cause irritation in

Figure 4.—Location of CO2 trap in flood control channel on overhanging vegetation. Photo by Laura Krueger.

Figure 5.—Adult male western tussock moth, *Orgyia vestusta*. Photo by Laura Krueger.

humans; true setae that can imbed in the skin causing a topical dermatitis (or if breathed into the lungs, can irritate the respiratory system), and modified setae that contain a toxin that can cause reactions in sensitized hosts. These two types of setae can be seen on the caterpillar of *O. vetusta* (Figure 3). When full grown the caterpillars are 1.5 to 2.0 inches in length, gray in color, with numerous colored spots and four prominent tufts of white hair on the body. The caterpillars detach the setae from their body at pupation and incorporate them into their cocoon for protection. When observed under a microscope, the cocoons are comprised of the white tufts of urticating hairs and also the large black modified setae. The caterpillars aggregate cocoons in protected areas. Interestingly, there is incredible sexual dimorphism between male and female adult moths (Kratnzer 2015). The female moths are silver-gray and flightless, having short vestigial wings, whereas the male moth has grey to black wings with a white horizontal patch of scales (Fig. 5). Upon emergence from the cocoon, females mate and lay egg masses comprised of 125-300 eggs directly on the cocoon. The young larvae feed on the remaining egg mass and then produce a silk thread used as a 'balloon' for dispersal. It is reported that there are two generations of tussock moth in southern California, with the first generation emerging in early spring. The species is known to feed on most species of evergreen or deciduous trees and can cause economic damage to fruit trees in commercial agriculture (UC IPM 2017).

Recommendations to School

The Principal implemented the following recommendations immediately: 1) Prevent contact between students/staff and all life stages of the moth, 2) Notify parents/staff of the risk associated with exposure to all life stages of the moth, 3) Establish a log to record student and staff reports of caterpillar aggregations or stings, 4) Inspect all areas for aggregations of caterpillars, cocoons and adults 5) Provide staff the appropriate personal protective equipment (N-95 mask, safety glasses, wear long sleeve shirts and pants and wide brim hat) and training to remove moth life stages by power washing the shade structures and other areas with aggregations of the moth life stages, 6) Trim trees overhanging the shade structures to limit caterpillar movement, 7) Assess the program and if caterpillar rash continues, consider pesticide application to overhanging trees surrounding the school site. Implementation of these measures limited student and staff exposure to caterpillars and reduced, but not eliminated, the instances of rash within the school children.

Lessons Learned

Responding to an infestation of stinging caterpillars affecting students and staff at a school presents unique challenges. Lessons learned by the Inspection Team include:

- Infestations of *Orgyia vestuta* persist for many years so assistance to the site may be on-going.
- Host an educational meeting at the school site for students, parents, and staff to answer questions or concerns.
- Schedule follow-up inspections at the school to ensure recommendations are completed in a timely manner (removal of caterpillars and cocoons by power washing, trimming overhanging vegetation).

Conclusion

This is the first report of caterpillar associated rash, or lepidopterism, due to *Orgyia vetusta*, affecting residents of Orange County, CA. The first symptoms and signs of urticaria included local swelling, red discoloration, and possible wheal formation. These symptoms mimic reactions from biting arthropods and determination of the cause of the symptoms must be based on a careful investigation that rules out other potential causes.

Acknowledgements

Thank you to Kiet Nguyen and Kassie Reyes for creating the map in Figure 1.

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Newly emerging rickettsial disease 364D, *Rickettsia philipii*, in Santa Clara County

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Introduction

Until 2008, the primary Spotted Fever Group Rickettsia (SFGR) of concern to public health in California was *Rickettsia rickettsii,* the causative agent of Rocky Mountain Spotted Fever. In 2008, Pacific Coast Tick Fever (PCTF), a newly emerging tick-borne disease in Northern California caused by the bacterium *Rickettsia philipii*, was detected for the first time in a human in Lake County. This rickettsia is transmitted by the Pacific Coast Tick, *Dermacentor occidentalis* (Fig. 1). PCTF is characterized by an eschar (Fig. 2), fever, and headache (Padgett et al. 2016). Since 2008, there have been 21 cases of PCTF reported within California (Fig. 3). In addition to Santa Clara County, cases have been reported in nearby Alameda, Contra Costa, and Monterey counties. It is an emerging disease of concern and most cases likely are underdiagnosed and underreported.

The first case of PCTF within Santa Clara County was reported in 2011. In August 2021, the second human case of PCTF was reported in the same geographic area (Fig. 4). To monitor for public health risks, the Santa Clara County Vector Control District (SCCVCD) developed a plan to monitor Pacific Coast tick fever risk wherever cases were reported. Over a six-month period, from January to June 2022, SCCVCD personnel conducted monthly sampling at the resident's property and surrounding parks within a one-mile radius and tested collected ticks for the pathogen.

Figure 1.—Male and female adult Pacific Coast Ticks (*Dermacentor occidentalis*).

Methods

Three sites were selected for surveillance: the resident's home and two public parks with known tick presence within a one-mile radius of the property. Each site was visited monthly over a 6-month period, starting in January of 2022 (Fig. 4). Ticks were collected at each site by dragging, flagging, and dry ice baited traps. Tick flagging and dragging was conducted using a 1-m^2 cloth. Dragging was done in areas with deep leaf litter and flagging was conducted along trail edges and shrubbery. Dry ice baited traps consisted of 3.15 lbs of dry ice in the center of a 1 m^2 cloth surrounded by double sided carpet tape. Dry ice traps were left for 3-4 hours near dense shrubbery at the resident's property.

Ticks were collected with forceps, stored in 70% ETOH and returned to the SCCVCD laboratory. Vials with collected ticks were stored in the laboratory refrigerator set to 4°C until processed. Ticks were identified to species, sex, and life stage

Figure 2.—Eschar on human.

Figure 3.—Map of California. Green = counties where *D. occidentalis* ticks have tested positive for *R. philippi*; Blue = counties where *D. occidentalis* ticks have tested negative for *R. philipii*; White = counties where no ticks have been tested; Red dots = counties with confirmed cases, number is for number of cases.

using published keys (Keirans and Litwak 1989). Each tick was washed with distilled water to remove ethanol and placed on a Kimtech tissue paper to dry. Each tick was placed in a 2.0 ml grinding vial and incubated overnight for 16 to 18 h at 65°C with proteinase K to soften the exoskeleton. Ticks were then homogenized with 5 ceramic beads (2.8mm) using the Omni Bead Ruptor 24. DNA was extracted using the MagMAX DNA Multi-Sample Ultra 2.0 Kit following the manufacturers protocol. Rickettsia 364D was amplified using RT-PCR. A 7-bp deletion in the intergenic region between nusG and rplK was targeted as described in Karpathy et al. (2019).

Results

In total, we collected 134 *D. occidentalis*; 70 males and 64 females from 3 different sites using 3 collection

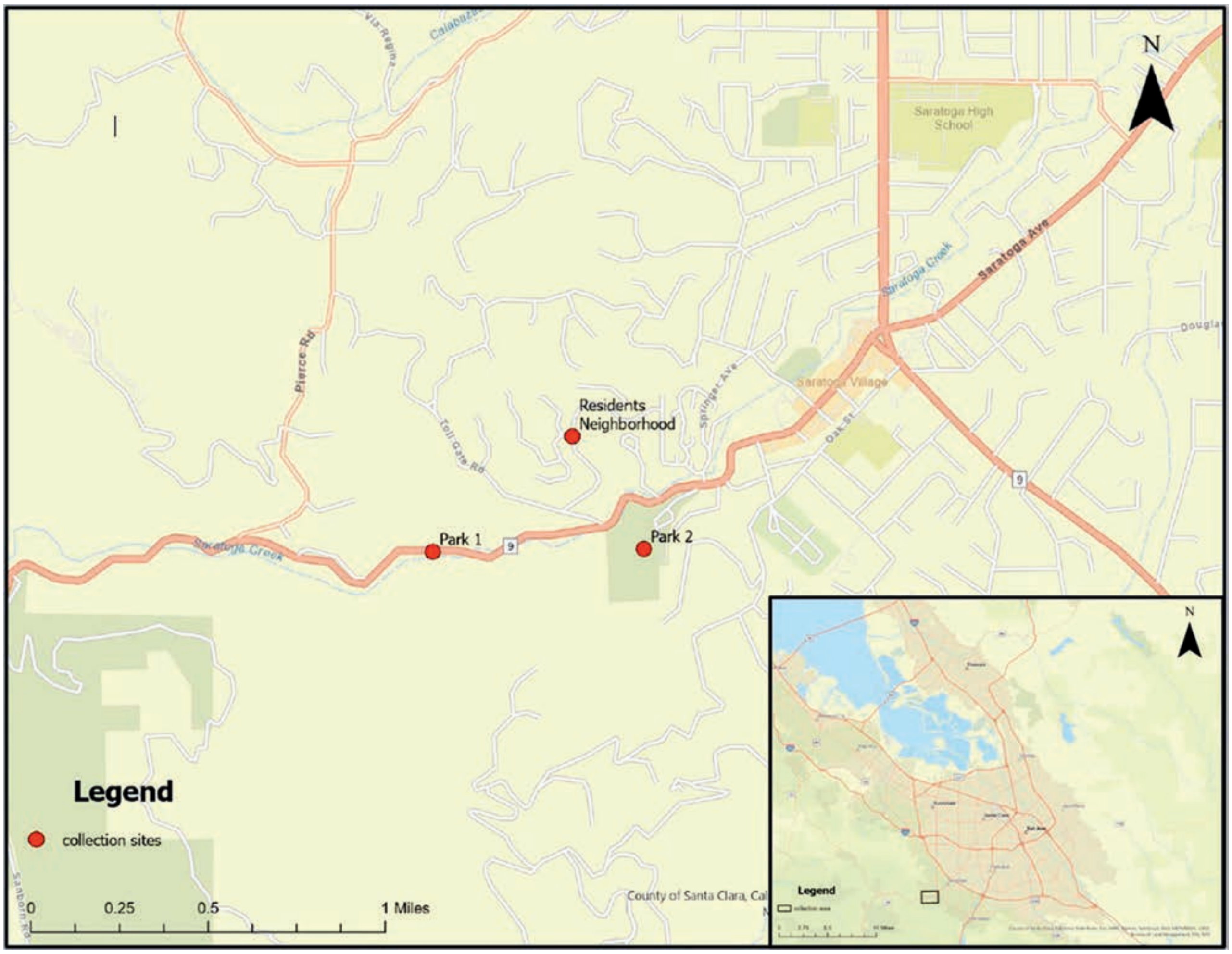

Figure 4.—Tick collection sites

methods and tested these for the presence of *R. philipii*, using Real-Time PCR. The pathogen was detected in 5.2% (7 of 134) of *D. occidentalis* tested: 1 from the resident's property and 6 from Park 1 (Fig. 4). These results need to be confirmed by sequencing. Assessing the prevalence and distribution of PCTF in ticks in Santa Clara County will require a larger study to sample and test ticks from throughout the county. Understanding the prevalence and distribution of the disease agent in the ticks in our county will be useful for determining measures that should be taken to reduce human exposure and infection.

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Effects of Spinosad and *Bacillus thuringeiensis var. israelensis* on mosquito larval density at a Santa Clara County water treatment plant

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Introduction

Sewer lagoons located at the San Jose-Santa Clara Regional Wastewater Facility are major sources of *Culex pipiens* and *Cx. tarsalis* populations during most of the year. In the past, the lagoons have been typically treated with *Bacillus thuringiensis var. israelensis* (*BTI*) products. We are currently implementing *Saccharopolyspora spinosa* A&D (*Spinosad*) as alternative product for wastewater lagoon treatment.

Methods

Eight sewer lagoons were selected for treatment using *BTI* or *Spinosad* (Fig. 1). A general dipping protocol was created for the eight Sewer lagoons sites (Fig. 2). These sites were checked biweekly or weekly depending on accessibility and weather conditions. Each dip was recorded via dipping sheets held by the Technicians involved with the project or recorded via data collection software.

Figure 1.—Lagoons treated with *BTI* in orange and *Spinosad* in yellow. Red Dots plot emphasize sample dip series (also see Figure. 2).

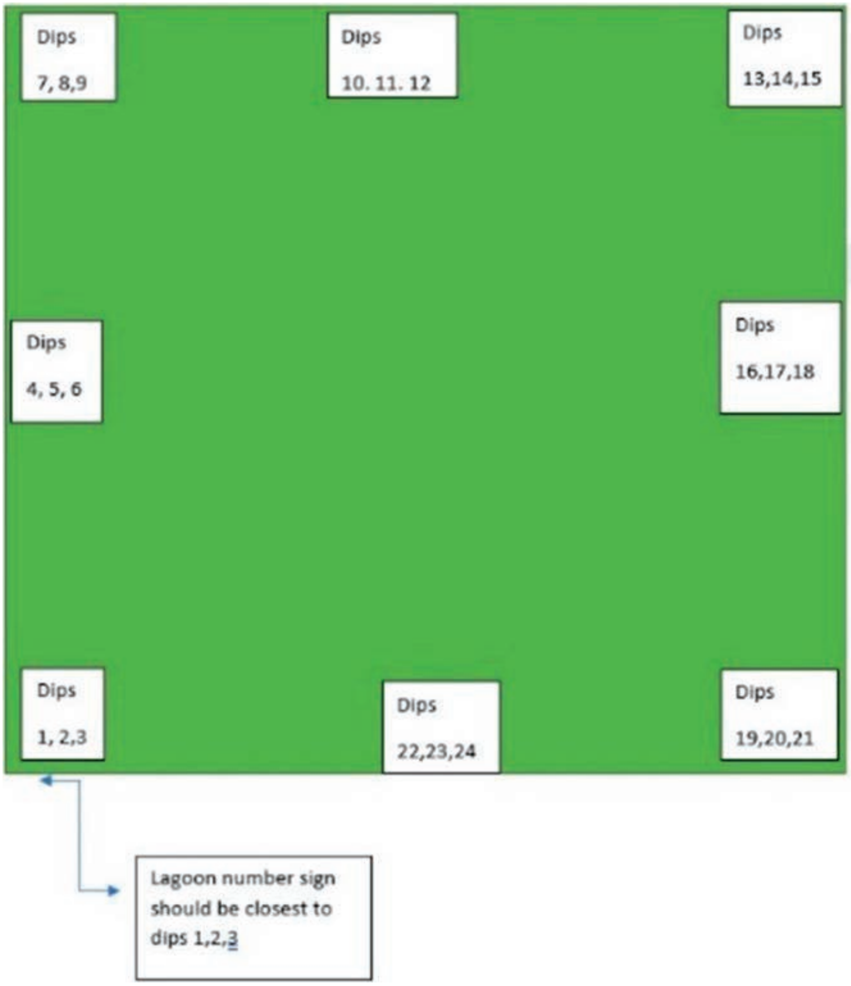

Figure 2.—Diagram showing protocol for dips taken per lagoon.

Results and Discussion

A main constraining variable was the morning to afternoon wind that ranged from two to twelve mph. This possibility affected mosquito flight range, area of treatment/swath width (how), and product placement. The constant of an evolving environment at RSM (Rural Sewage Management) due to maintenance work around the levee roads of the ALs warranted a switch of *BTI* product from liquid to solid in the *BTI* lagoons.

At the end of the study after dips were counted and processed, it seemed that the lagoons that were treated with *BTI* showed a higher number of larvae prior than Lagoons treated with *Spinosad* (Fig. 3). Larval showed a significant decline in abundance over using both treatments (Fig. 4). Unfortunately, due to time constraints and the multiple sources of variation stated above, we felt the the results were inconclusive.

Future evaluations will use only *Spinosad* and compare the efficacy of different treatment frequencies during 2023 with 2022, including data on time of treatment, cost, and labor. Overall abundance at trap locations in 2023 will be compared to 2022. These data will evaluate the pros and cons of switching to Spinosad.

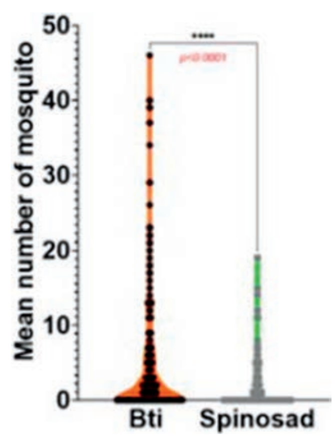

Figure 3.—Violin Plot showing difference in larval counts within *Bti* and. *Spinosad* treated lagoons.

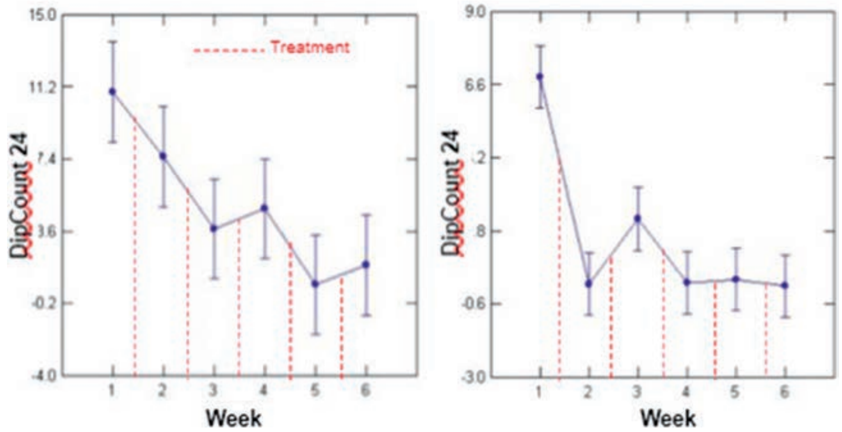

Figure 4.—Mean (+/−SE) larval counts per week in *Bti* (left) and *Spinosad* (right) treated ponds. Red dotted lines show treatments.

Outreach improvements during the high West Nile virus season in Santa Clara County

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Abstract

The County of Santa Clara Vector Control District (District) experienced high West Nile virus (WNV) activity during 2022, requiring increased outreach efforts to notify and educate the public regarding WNV treatments, the importance of disease prevention, and personal protection. Previously, the District heavily relied on door-to-door notifications through the use of doorhangers, but this ongoing outreach method was no longer performing adequately during a fast-paced WNV season. The combination of back-to-back WNV treatments and the time-consuming door hanger process, led to the reevaluation and planning of new outreach and education methods that allowed increased transparency and quick distribution to the public. Working together with the County's Office of Public Affairs team, the District modified its WNV communication methods and improved outreach materials. New methods included developing a more detailed WNV FAQ sheet, educational videos, increased social media activity, paid campaigns, and developing "back-pocket" responses to the community's concerns. Future improvements were discussed and included a dedicated WNV page easily accessible to the public and pro-active notifications regarding WNV activity and mosquito treatments before the start of the season.

Implementing a grid-based adult mosquito trapping scheme for Santa Clara County Vector Control District

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Abstract

In 2020, the Santa Clara County Vector Control District (District) created a grid-based sampling scheme for arbovirus surveillance largely following that of Orange County Mosquito and Vector Control District. Grid sites were created every 0.5 mile across the populated areas of the county (n=2,334 sites). Adult mosquito surveillance was conducted throughout the county by placing one EVS and one gravid trap at each of six grid sites along each trap route. The grid system facilitated District staff adult mosquito trapping efforts by "pre-registering" those sites in Calsurv, the state data repository for mosquito trap data. In 2022, grid-based adult mosquito trapping was conducted using 37 routes in an attempt to sample all the routes twice a month. In many instances, grid-based trapping collections detected WNV positive mosquito pools prior to that of positive bird detections that also triggered mosquito sampling. EVS and gravid traps both yielded positive WNV detections. During 2022 a supply chain shortage of dry ice precluded some EVS trapping, leaving gravid traps as the primary means of surveillance trapping.

Modification of the Gravid Trap for improved survival, collection, and quality of mosquitoes in support of mosquito surveillance program in Santa Clara County

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Abstract

It is essential to be able to trap and test mosquitoes to determine what steps should be taken to prevent the transmission of the pathogens they carry. The recent closing of BioQuip, a major supplier of mosquito traps, has negatively impacted many mosquito surveillance programs around the US. To circumvent this set back, the Santa Clara Mosquito Control District has implemented its own in-house trap construction and modification program. This program successfully addressed the trap shortage, repaired broken traps, and improved overall trap performance.

History, biology, and control of *Mus Musculus* in Alameda County

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Introduction

The house mouse (*Mus musculus* Linnaeus) originates from central Asia and was brought to the Americas during the sixteenth century (UC IPM, 2011). Since then, they have become one of the most damaging species of rodents in the US. They are very adaptable and can live in a large range of conditions. They prefer to live around humans and are often found around homes, fields, and other structures. House mice are small, generally 4-7 inches long as adults and weigh approximately half an ounce (Fig. 1). This allows them to squeeze into buildings through holes only slightly larger than ¼ inch (UC IPM, 2011). They generally prefer to eat cereal grains, but are not picky and will consume different types of foods. Once inside, they seek an area near food and water in which to nest. House mice do not need to leave the home for food and water and can live within walls or behind appliances such as ovens or refrigerators, only moving about 10-25 feet from their nests (University of Kentucky, 2022). Mice can reproduce very quickly, having a 20-day gestation period, 4-8 young per litter, and roughly 8 litters per year (UC IPM, 2011) or ca. 60 young produced per female mouse per year. Young mice are capable of reproduction at only 6 weeks of age, and adult females can mate immediately after giving birth, so a new generation of mice can be produced every 20-30 days (UC IPM, 2011). Mice infestations can cause a number of problems in structures, specifically droppings and urine left around the home leads to unsanitary conditions and can create a risk of contracting salmonella from contaminated foods (Fig. 2). They can be a host to mites (*Liponyssoides sanguineus* Hirst*)* that can bite and be a nuisance to humans, as well as potentially carrying Rickettsialpox (*Rickettsia akari*) (CDC 2023). Mice can gnaw on wires within walls possibly causing fires, and electrical problems, as well as plumbing and wood damage. Proper control of house mice requires exclusion, population reduction, and sanitation.

Methods

Trapping, as a method of population reduction, is very important indoors as it brings relief to the complainant and ensures control of the population. Traps should be placed every 2-3′ apart, and in pairs about an inch apart from one another. They should be placed perpendicular to the wall, baseboard or foundation, and baited to attract the mice. Using familiar food sources such as nuts, nut butters, seeds, and dried fruit such as raisins or dried apricots as bait can be useful. Mice gravitate to confined spaces, so areas such as cabinetry, closets, water heater closets, and crawl spaces are frequently found to have frequent mice activity.

Exclusion, which is the most important aspect for the overall control of mice infestations, is often overlooked as an abatement strategy. The first and most necessary aspect of mouse exclusion, is a very thorough inspection for entry points. The bodies of house mice are very pliable, and they are able to squeeze their bodies into very small gaps and spaces (UC IPM, 2011). Typically any holes larger than ¼″ inch must be closed to ensure the exclusion of all possible mouse-sized entry points (Fig. 3). A typical occurrence in infestations of house mice, is that the activity is seen

Figure 1.—House mouse (*Mus musculus*).

Figure 2.—Mouse droppings in an attic.

Figure 4.—An opening around a pipe leading into a crawl space vent screen.

roughly 15-25 ft from their nesting areas (Univ. of Kentucky, 2022). Therefore, when performing inspections and exclusion efforts, it is necessary to look in close proximity of activity for entry points to seal off. The proper sealing of entry points is extremely important to make sure that possible entry points cannot be re-opened and access re-gained (Fig. 4). The best materials for exclusion are wood, concrete, rodent control-grade steel wool fabric (e.g., Xcluder® fabric), aluminum flashing, and ¼″ inch galvanized steel mesh screen, which would be nailed, stapled or caulked into place to cover the entry point (Fig. 5). Many homeowners and property managers use expanding foam to cover entry points, which could be errant, as the mice can easily scratch and chew their way through foam leading to reinfestation (Fig. 6). Expanded foam should only be used as a place holder for proper materials such as Xcluder® fabric. Xcluder® though easy to use, should primarily be used to close gaps where attachment of mesh screen, flashing or wood would not be feasible. In situations where repairs are necessary, but there are conduit pipes or wires entering foundation vents or holes in walls, it is important to use the right materials to cover entry points, as improper materials could lead to potential fire hazards (Fig. 4 and 5).

The use of rodenticide baits outdoors is a good method of continual control. Baits such as Selontra® Cholecalciferol contain stop feed chemicals, which can allow for multiple mice to feed on one block of bait and can lead to the control of multiple mice around a structure. This can not only control of mice in the infested structure, but potentially have a positive impact on adjacent structures. A potential downside to baiting, may be the secondary poisoning of non-target animals. Therefore it is very important when baiting to be aware of the surroundings of the area being bated, as to not unintentionally expose people or animals to pesticides. Baits should never be used as the only means to control a mouse infestation. Baiting should be performed in conjunction with trapping and exclusion. Sanitation of infested spaces is important for the health and safety of people and animals that dwell in homes or buildings infested with mice.

Figure 3.—Rodent entry point leading into a garage.

Figure 5.—Example of repair made on Fig. 5.

Figure 6.—Expanding foam application that was unsuccessful, and entry was regained.

Conclusions

Generally, Alameda County Vector Control Services District recommends snap traps, baited with peanut butter or nuts, as they are the most humane way to dispose of mice. Glue traps can take several hours or even days for the mouse to expire when caught or you may even have to dispatch the captured mice. Poison baits are effective but should only be applied once the exterior openings into the building are secured. If mice are entering the walls or unreachable cramped spaces, poisoning may cause them to die out of sight and produce a strong odor and flies. Throughout the control effort, sanitation is helpful in getting rid of mice. Good sanitation reduces the probability of salmonella infection and mite bites. It can also aid in more effective trapping, as they are less likely to be attracted to an area with less competing food sources and nesting materials.

Control of *M. musculus* has the best results when the following steps are implemented: inspecting, trapping, exclusion, sanitation, and exterior trapping/chemical control. Following these steps help save time and money, lessen the risk of property damage, and lessen the likelihood of contact with harmful bacteria and pathogens that can be spread by the presence of house mice.

Acknowledgements

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Spatial and temporal trends in *Aedes aegypti* populations in the West Valley region of San Bernadino County

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Abstract

Invasive *Aedes* mosquitoes are a threat to public health because of their capacity to transmit arboviruses such as Zika, dengue and chikungunya. In California, these mosquitoes have spread rapidly in recent years. Here, we present the spatial and temporal trends of *Aedes aegypti* dispersal in the West Valley region of San Bernadino County. Between 2017 and 2022, mosquitoes were collected weekly using BG Sentinel traps. Weather data was obtained for the same period from local weather stations. Multivariate regression was done to determine the relationship between weather factors and *Aedes* mosquito counts. The results indicated that minimum daily temperature was the most significant factor that explained 46% of the variability in *Aedes* mosquito counts. The mean number of *Ae. aegypti* per trap-night increased nearly five-fold from 3.1 in 2017 to 15.3 in 2022. BG traps set due to resident service requests increased by six-fold from 103 in 2017 to 687 in 2022. Overall, nearly two-thirds of the annual invasive *Aedes* mosquito collections were captured between August and September. Incorporating In2Care® Traps into routine IPM strategies has shown promising results in controlling the population of invasive *Aedes* in the region.