The 83rd and 84th Report for the

Alameda County Mosquito Abatement District

2014-2015

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Dedicated to the Memories of:

Jim Golden
Board Member 1995-2013
Died April 2015

Jerry Brown
Employee 1950-1993
Died October 2015

Bob Knowles
Employee 1970-1999
Died December 2014

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DISTRICT UPDATES

Arrivals and Departures

The Alameda County Mosquito Abatement District (ACMAD) went through an uncharacteristic transition during 2014 to 2015 with changes in management, trustees, staff, regulations, procedures, technology, and the introduction of an invasive mosquito species.

Dr. Chindi Peavey

District Manager Dr. Chindi Peavey served ACMAD from mid-2012 until early 2015. Dr. Jan Washburn, who resigned his position as a Trustee representing the City of Berkeley for over 21 years, accepted the position of Interim Manager for six months until a permanent manager was chosen. He then returned to the Board representing the City of Oakland.

Dr. Jan O. Washburn

Ryan Clausnitzer, who also came from the Board of Trustees representing the City of Alameda, became the sixth District Manager in July of 2015. Besides his knowledge of the District as a Trustee and former Board President, he also has experience in mosquito and vector control in the Bay Area, most recently in Environmental Health with the San Francisco Department of Public Health.

There were also many changes among the Board of Trustees and District staff during the last few years. Fifty percent of the current fourteen members of the Board of Trustees were appointed in the past two years. Similarly, fifty percent of the sixteen permanent staff were replaced during this period; long-time employees Sharon Mead (30 years), Greg Wood (16 years), and Lyle Cain (12 years) retired from the District in 2014 and 2015.

Regulatory Updates

Before retiring in 2012, former District Manager John Rusmisel prepared the District for future challenges by initiating a Programmatic Environmental Impact Report (PEIR). Nearing the completion stage, this project has taken almost four years to finalize. The Notice of Availability of a Draft PEIR was issued on July 16, 2015, and the District held a public hearing for comments on the Draft PEIR on August 5, 2015. The PEIR (to identify the document is NOT the Notice of Availability) thoroughly analyzes the

District's integrated pest management program and will help protect the District's ability to control mosquitoes effectively and in an environmentally conscious manner.

New Challenges

Another major hurdle that faced the District, and the State, was the arrival of two invasive mosquito species, *Aedes albopictus* and *Aedes aegypti*. In May of 2015, District staff discovered two female *Ae. aegypti* mosquitoes at the District headquarters in Hayward. No further specimens were found after extensive surveillance and treatments. Besides the pestiferous and difficult to control nature of these species, they also vector serious diseases such as dengue fever, chikungunya, and Zika virus.

This discovery changed the way the District must survey and treat for mosquitoes. Detection of these invasive species relies both on the District's laboratory's surveillance strategy and on input for an informed public. The route by which mosquito abatement information is received and processed is transitioning from print media and brochures to social and digital media.

Looking Forward

In order to adjust to the future challenges of government regulations and invasive species, mosquito control technology must adapt by researching and evaluating newly-developed and novel options such as unmanned aircrafts (drones) and genetically modifying mosquitoes. Improvements in the District laboratory already allow a more rapid response to public health threats by the use of RT-QPCR testing for mosquito-borne diseases. The laboratory also utilizes a diverse array of adult mosquito surveillance traps, such as AGOs and BG Sentinels.

Besides technological advancements in the District laboratory, operational equipment upgrades include increased capacities in mosquitofish production, aerosolizing spray equipment for underground treatments, and GPS-coordinated adulticiding. Though the use of adult fogging is historically rare for ACMAD, it is an important tool for adult mosquito control and was utilized in the Tri-Valley area in 2014 and in San Lorenzo in 2015.

In order to adjust to the extensive changes in staff and leadership, increased government regulations, and equipment and laboratory upgrades, the District requires a strong financial backbone. The District provides additional (other) post-employment benefits (OPEB) for its retirees, and this fund is currently fully funded. The District also strives to control unfunded pension liabilities, produces a balanced budget, distributes funds into appropriate reserve categories, and does so while only requesting half of its potential benefit assessment revenue.

The District proudly approaches its 86th year of service to the people of Alameda County with a dedicated and professional staff who are supported to face the challenges of mosquito abatement by an engaged and thorough Board of Trustees.

GOVERNING BOARD

The Alameda County Board of Supervisors and each of the elected councils of the 13 cities within the District appoint one trustee to represent its constituency on the governing board of the Alameda County Mosquito Abatement District. The Board of Trustees consists of individuals dedicated to community service and willing to accrue the knowledge required to govern a mosquito abatement district effectively. The District board members possess a variety of skills and expertise in academia, agriculture, art, business, chemical engineering, education, electrical engineering, entomology, environmental health and safety, insurance, finance, government, general contracting, human resources, mechanical engineering, scientific research, and water quality.

The diversity of knowledge possessed by the trustees provides a broad, conceptual framework within which the Board decision-making occurs. In these ever-changing times, the knowledge base provided by the trustees is an invaluable resource to the effective and efficient operation of ACMAD.

The Trustees serve two-year terms without compensation; however, they do receive allowances for expenses incurred in attending business meetings of the Board. The regular Board meetings are held on the second Wednesday of each month at the District office, 23187 Connecticut Street, Hayward at 5:00 pm; all Board meetings are open to the public.

Trustees for the years 2014 & 2015

Trustee	**Representing**	**Years of Service**
Dennis Bray	County-at-large (2014)	11.0
Scott Paulsen	County-at-large (2014 & 2015)	1.5
Ryan Clausnitzer	Alameda (2014)	2.5
Wendi Poulson	Alameda (2015)	.5
Jan O. Washburn	Berkeley	21.5
Richard Guarienti	Dublin	2.5
Scott Donahue	Emeryville (2015)	1.0
George Young	Fremont	3.0
Barbara Halliday	Hayward (2014)	4.0
Elisa Marquez	Hayward (2015)	1.0
James N. Doggett	Livermore	38.0
Elizabeth Anders	Oakland (2014)	.5
Jan O. Washburn	Oakland (2015)	.5

Trustees for the years 2014 & 2015 (continued)

Trustee	Representing	Years of Service
William Spinola	Newark	33.0
Robert Dickinson	Piedmont	2.0
Kathy Narum	Pleasanton	2.5
James Prola	San Leandro (2014)	8.0
Ursula Reed	San Leandro (2015)	1.0
Ronald E. Quinn	Union City	14.0

Current Committee Assignments

Financial Committee

Purpose: A standing committee tasked with reviewing the annual budget, assessing the District's long term capital needs, making recommendations for designating reserves, and evaluating the allocation of the OPEB Trust.

Membership: Trustees Young, Quinn, Dickinson, and Narum

Status: Between April and June the committee will review the budget for the 2016-17 fiscal year, while reviewing the asset allocation of the OPEB Trust and selecting an auditing firm in the late summer.

Policy Committee

Purpose: A standing committee charged with evaluating the District's policies and updating and adding policies as needed. All District policies must be approved by a majority of the Board.

Membership: Trustees Doggett, Guarienti, and Marquez

Status: The Municipal Resource Group and staff are currently completing a review of District policies prior to proposed changes being presented to the committee. In order for policies to be enacted, they must have two readings and Board approval.

Manager Evaluation Committee

Purpose: A standing committee with the primary task of evaluating the District Manager. The evaluation takes place in June and contract adjustments are based on this evaluation.

Membership: Past, present, and future Board Presidents include Trustees George, Guarienti, and Narum

Status: This committee replaces the Ad Hoc Committee on Long Term Planning that was created to recruit and review the District Manager during the first year of employment. Further changes to the salary and contract can be recommended annually.

West Nile Virus (Public Health Emergency) Committee

Purpose: A standing committee that meets with the District Manager and/or staff in order to review District surveillance and treatment information pertaining to current or emerging public health threats. This committee makes recommendations to the Board if necessary.

Membership: Trustees Washburn, Doggett, and Poulson

Status: This committee only meets on an as needed basis.

Personnel Committee

Purpose: A standing committee that meets if personnel issues rise to the level of an appeal to the Board.

Membership: Board Officers – Guarienti, Narum, and Dickinson are members.

Status: This committee only meets on an as needed basis.

DISTRICT PERSONNEL
2014 - 2015

Name of Employee	Position	Years of Service
Dereje Alemayehu	Vector Biologist (Zone 3 & 4) Biological Specialist	14
Nick Appice	Mosquito Control Technician (Zones 2 & 3)	1.5
John Busam	Vector Biologist (Zone 9 & 10)	13.5
Ryan Clausnitzer	District Manager	.5
Lyle Cain	Vector Biologist (Zones 5 & 7)	15
Cornelius Campbell	Vector Biologist (Zone 8)	12
Miguel Cardenas	Mosquito Control Technician (Zone 2, Zone 6)	3
Erika Castillo	Environmental Specialist	13.5
Sarah Erspamer	Mosquito Control Technician (Zone 1)	.5
Robert Ferdan	Systems Specialist	.5
Eric Haas-Stapleton	Entomologist	.5
Joseph Huston	Field Operations Supervisor	24
Michelle Izumizaki	Mosquito Control Technician (Zone 1) Biological Specialist	6
Bruce Kirkpatrick	Entomologist	17
Clarence Lam	Administrative/Financial Manager	13
Gregory Leipzig	Vector Biologist (Zone 6)	9
Tom McMahon	Vector Biologist (Zone 10)	15
Sharon Mead	Systems Specialist	30
Chindi Peavey	District Manager	2.5
Ben Rusmisel	Vector Biologist (Zone 3 & 4)	.5
Jeremy Sette	Mosquito Control Technician (Zones 5 & 7)	.5
Jan Washburn	Interim District Manager	.5
Mark Wieland	Mechanical Specialist	1
Gregory Wood	Mechanical Specialist	16

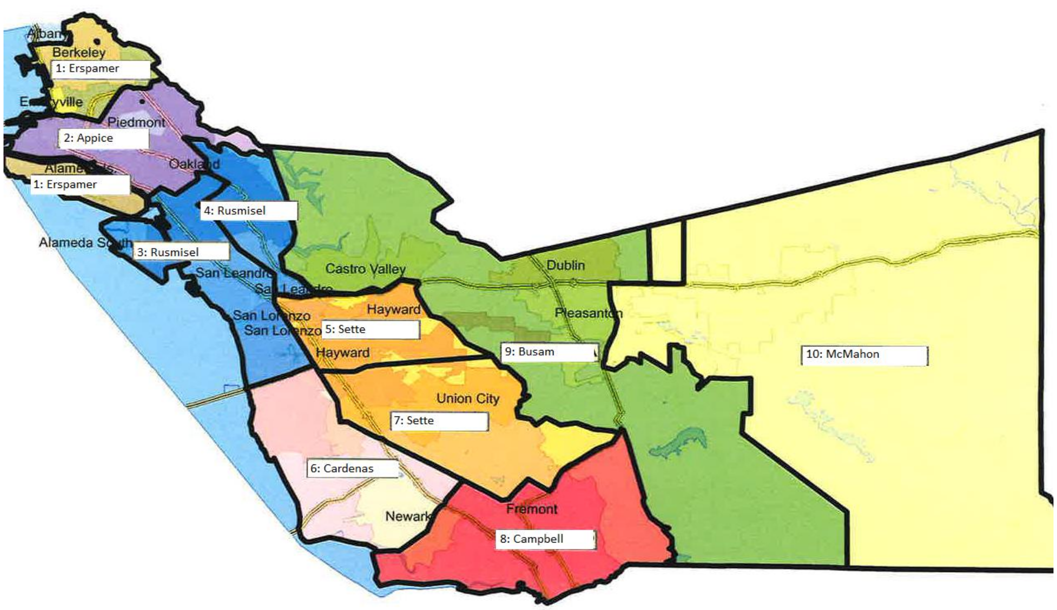
Seasonal Employees

2014	**2015**
Kevin Huffstutler	Jacob Ferdan
Gilberto Martinez	Kevin Huffstutler
Michelle Matthes	Michelle Matthes
Nobo Namata	Miguel Munoz
	Jason Young

2015 ACMAD Staff

<u>Standing:</u> Dereje Alemayehu, Jeremy Sette, Erika Castillo, Mark Wieland, John Busam, Eric Haas-Stapleton, Ben Rusmisel, Nick Appice, Sarah Erspamer, Neil Campbell, Robert Ferdan, Ryan Clausnitzer
<u>Kneeling:</u> Tom McMahon, Clarence Lam, Miguel Cardenas, Joseph Huston

2015 ACMAD Zone Boundaries



OPERATIONAL DATA 2011-2015

	2011	2012	2013	2014	2015
Physical control operations					
Maintenance of ditches (lineal feet)	8,515	15,440	0	0	0
Mosquitofish operations					
Total # of sites stocked with *Gambusia*	787	792	761	691	606
Total number of fish planted	17,118	15,663	15,986	13,445	10,664
Chemical control operations					
Pyrenone 25-5 adulticide (oz)	7	0	2	820	159
Skeeter Abate granules (lbs)	44	0	0	0	0
Surface Agents					
Golden Bear 1111 larvicidal oil (gal.)	111	3.4	0	0	0
BVA2 larvicidal oil (gal.)	1,255	876	1,937	1,540	2,170
Cocobear (gal.)	0	0	0	0.3	0.42
Agnique MMF monomolecular film (oz)	0.6	1.5	0	0	0
Biorational larvicides					
Bacteria based					
Bacillus thuringiensis israelensis					
Vectobac12AS liquid concentrate (gal.)	100	40	54	58	103
Vectobac GS (lbs)	0	0	0	0	481
Vectobac G granular (lbs)	4,496	2,874	2,741	2,464	3,923
Bacillus sphaericus					
Vectolex CG (lbs)	3,375	1,005	1094	659	1,460
Vectolex WSP (lbs)	57	23	16	6	34
Vectolex WDG (lbs)	194	41	54	108	140
FourStar 180 day Briquets (lbs)	188	29	93	54	5
Bacillus thuringiensis israelensis and *Bacillus sphaericus*					
Vectomax WSP (lbs)	0	0	0	0	2
Vectomax FG (lbs)	0	0	0	0	4,927
Vectomax CG (lbs)	181	31	0	0	0
Spinosad					
Natular XRT (lbs)	531	491	153	581	1,277
Natular G30 (lbs)	75	150	916	29	1
Insect growth regulator (methoprene)					
Altosid Liquid Larvicide 20% (oz)	683	222	311	275	626
Altosid Briquets (each)	1,684	1,478	1,903	1,686	3,072
Altosid XR Briquets (each)	611	1,042	247	3,911	2,510
Altosid Pellets (dry oz)	3,150	6,687	3,094	6,369	2,289
Altosid WSP (dry oz)	0	178	0	0	0

OPERATIONS REPORT

Material Usage

The Alameda County Mosquito Abatement District is dedicated to a control program that utilizes biorational materials aimed at eliminating larval populations of mosquitoes. Even though environmental conditions were well outside the norm during 2014 and 2015, the District was still able to maintain its focus on larval rather than adult control. Because of the ongoing drought and restrictions on water use, mosquito breeding patterns varied from the normal patterns observed in previous years. The relative scarcity of standing water forced mosquitoes to utilize smaller and more cryptic water sources, often making it difficult to locate larval populations and to treat them effectively. During the past two years, District operations have observed a change in the temporal pattern of mosquito emergence during the spring months as well as an increase in the rate of development of several species. These species include both nuisance biters as well as vectors of important human diseases such as West Nile virus (WNV).

Because ACMAD is responsible for mosquito control throughout the entire County of Alameda, it is necessary for District personnel to work cooperatively with other government agencies including the East Bay Municipal Water District, the Federal Fish and Wildlife Service, the East Bay Park District and other entities. When materials aimed at larval control are applied to environmentally sensitive landscapes, control efforts must be carefully orchestrated with various stakeholders. For example, in March of 2015, high populations of larval mosquitoes, (primarily *Culex tarsalis* and *Culiseta inornata*) were found breeding in marshes within Coyote Hills Regional Park. Park staff reported that mosquito biting was significantly higher than in previous years, and numerous visitors complained about the problem. Because mosquito breeding was occurring in a public park within sensitive marsh habitat, helicopter application of biorational larvicides was required. For safety considerations, the park was closed during application, and District staff worked closely with park personnel to monitor the application and make sure the park was cleared of visitors. Staff from the office of the Alameda County Department of Agriculture were on site during the application in order to ensure that all procedures followed State mandated regulations. To assess the efficacy of the treatment, District operation staff quantified the larvicide application rates by placing collection pans (N=10) throughout the marsh. Pans were placed in both open sites and in sites covered with vegetation. After aerial treatment, pans were returned to the ACMAD Lab, and application rates were quantified by counting the number of treatment pellets per pan. Pre- and post-application mosquito densities were determined from standard dip samples taken with the breeding areas. Results from the post-treatment analysis showed that the larvicide was applied at rates recommended by the manufacturer in both open areas of the marsh and within vegetation cover.

Moreover, dip samples taken one week after treatment indicated that larval mosquito densities were reduced between 95 and 100% within the marsh, and park personnel reported a significant (and welcome) reduction in the number of biting mosquitoes. Notably, with careful planning and execution of the abatement protocol, the Coyote Hills park was able to reopen at 11:00 am on the day of the treatment. This example highlights how the District focuses on biorational mosquito control while working cooperatively with the public and other government agencies.

The District closely monitors the threat of familiar mosquito borne pathogens such as West Nile virus as well as emerging diseases such as Zika. When viral activity is determined in either mosquitoes or reservoir hosts such as birds (for WNV), prompt mitigation procedures are enacted. A combination of WNV positive birds and WNV positive mosquitoes in several sections of the County in 2014 and 2015 posed a severe enough threat to public health and safety that adulticiding treatments by hand and via truck mounted fogger were utilized. This has been a rare occurrence with ACMAD in recent decades and despite the these few isolated treatments, adulticide chemical usage remains a small percentage of the overall treatments conducted by the District as depicted in Figure 1.

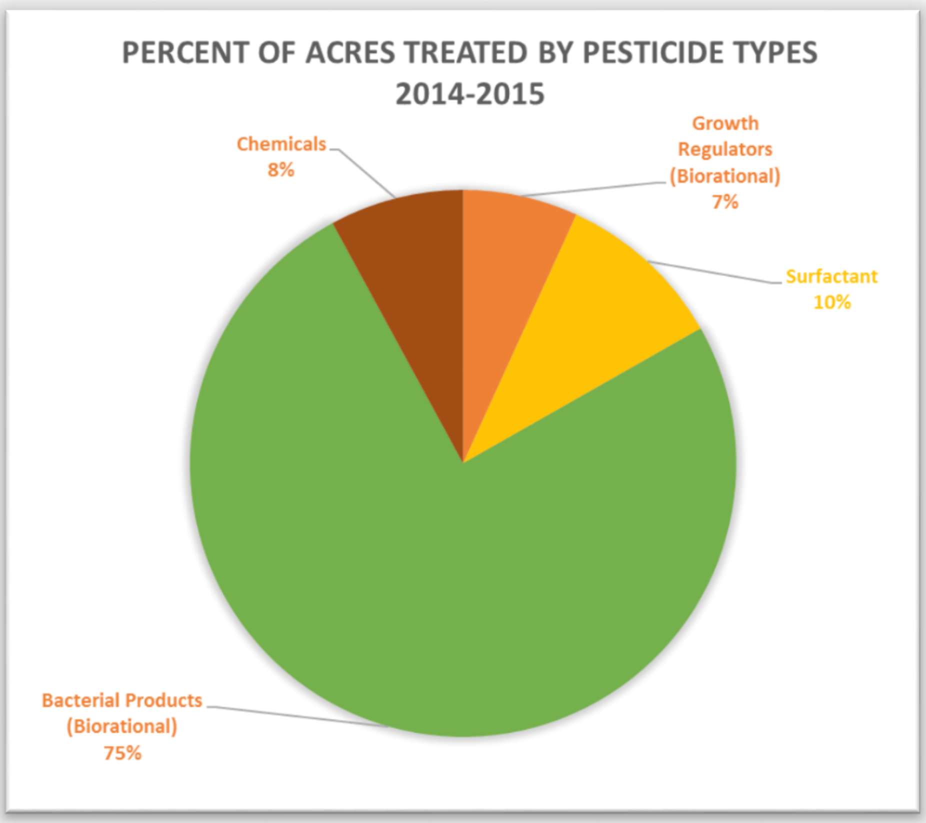


Figure 1. Acres treated by type of pesticide used.

Physical Control Operations

Ditching is the principal form of physical control utilized by the District to reduce and eliminate mosquito breeding sources. Having water flow into and out of our many marsh sources greatly reduces breeding of several of our most aggressive day biting mosquito species. It also saves a great deal of resources by eliminating and/or reducing the need to treat these sources with larvicides (e.g., biorationals and surfactants). The lack of external agency approval of the regional source reduction permits, however, brought ACMAD's physical control program to a standstill. The completion of the District's PEIR is a major step in securing this regional permit once again. The PEIR will help the permitting agencies to evaluate the impact, or lack thereof, of the District's physical control activities and will allow our critical ditching program to resume

Service Requests

Figure 2 depicts the five types of service requests taken by the District during 2014 and 2015. Mosquito fish requests comprise the highest number of calls (43%) received by the District; this proportion is consistent with previous years in which fish requests have been the most frequently requested District service.

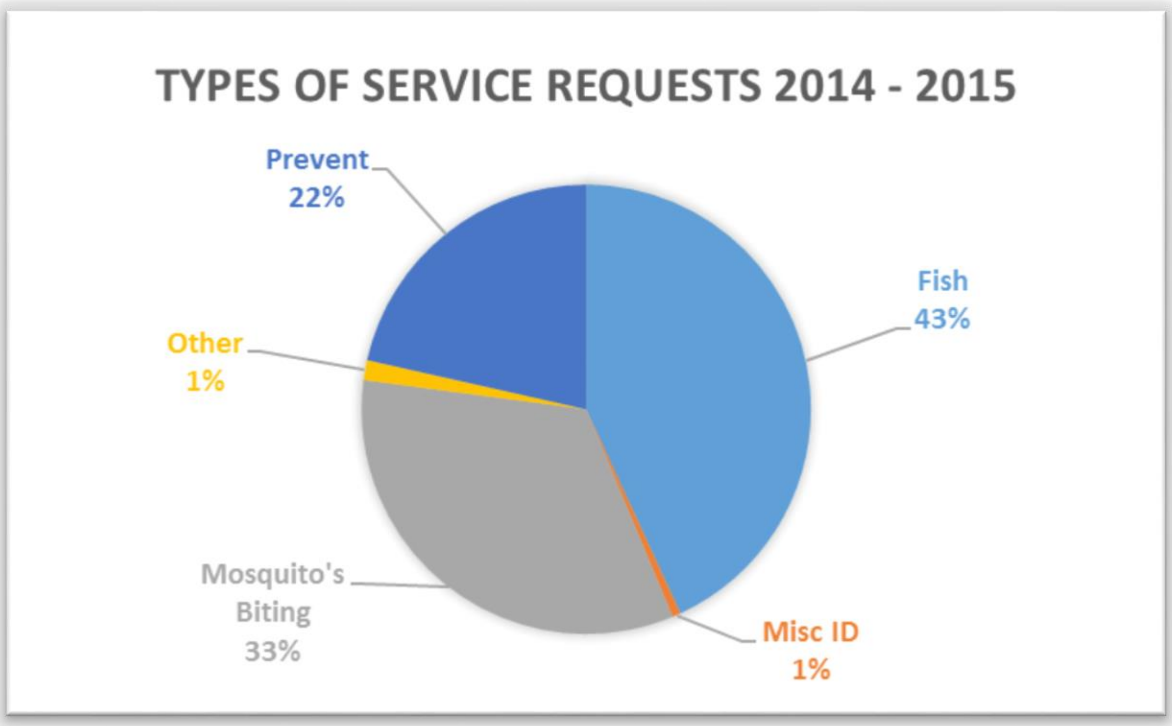


Figure 2. Types of service requests.

Planting mosquito fish has proven to be an important tool to prevent mosquito emergence from backyard ponds, swimming pools, horse troughs and other water filled containers. The second highest percentage of service requests (33%) was from callers indicating that they were either seeing or were being bitten by mosquitoes. For the two years combined, 22% of calls concerned sources of standing water that callers felt could be breeding mosquitoes. Notably, the two aforementioned types of service requests are often at least partially driven by the amount of coverage mosquitoes and mosquito related disease receive in local and national media. When mosquitoes are in the news, the District receives more service calls. Requests for various insect identification and “other” requests accounted for 1 % each of the service request calls or emails received by the district in 2014-2015.

The number and type of service request received from each city and area ACMAD serves are shown in Figure 4. These numbers are primarily driven by population of a given city, with cities having higher populations generating more service requests. Other factors that impact the type and relative number of service requests from a city include citizen awareness and/or concern with mosquito related issues and the amount and proximity of local sources in various regions of the County.

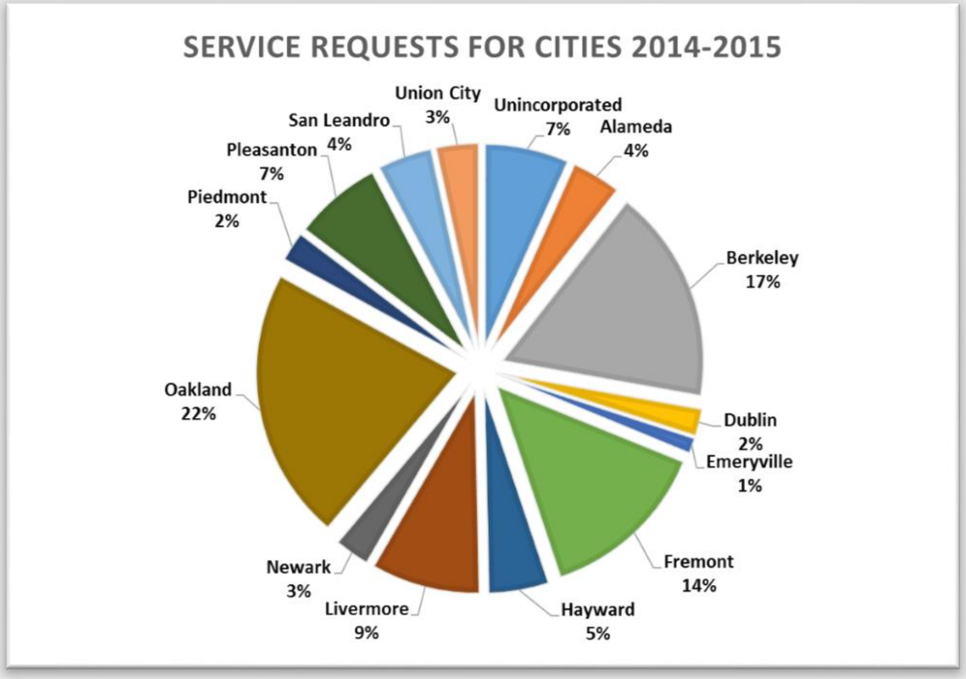


Figure 3. Proportion of service requests received per city within the District.

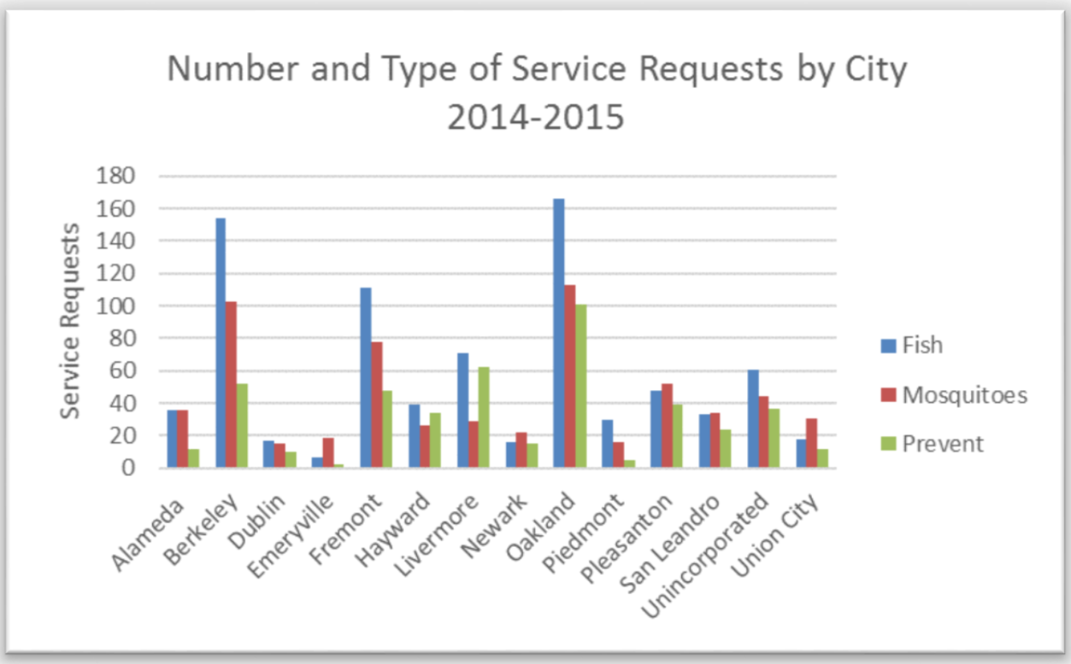


Figure 4. Number and type of service requests by city.

EQUIPMENT UPGRADES

Underground Larvicidal Aerosolizer (ULA)

The introduction of an underground larvicidal aerosolizer (ULA) proved to be a valuable asset to assist the District's rigorous storm drain and catch basin mosquito control program (Figure 5).

The ULA aerosolizes liquid mosquito larvicides and produces droplets larger than that of an ultra-low volume (ULV) fogger, accompanied by a more direct force, or push, resulting from the compressed air. This allows the larger droplets to fall quickly, treating the immediate area. The smaller, lighter droplets are carried by natural and induced air currents further down the storm drains, reaching areas that may have trapped water or areas where water travel is constricted.

Figure 5. ULA mounted in the back of a District truck (left). District staff using the ULA to treat a catch basin for breeding mosquitoes.

The ULA consist of a gasoline engine powered air compressor, trigger and wand assembly with venturi, and valve selected chemical tanks (Figure 5). There are two trigger and wand assemblies with adjoining tanks that accommodate water based and oil based solutions separately. Operators can choose between the two with the selector valve located behind the chemical tanks. The storage box contains the catch basin containment rubber mats and safety gear.

Fish Program

The District improved the Fish Program with the purchase of two 800 gallon rectangular tanks serviced by an integrated pumping system. The system was purchased by the District in 2015 and arrived outfitted with sectional dividers, a UV algal management system, water heater, and an isolation tray for young fry. All these features are essential for encouraging a self-sufficient aquatic environment suitable for maintaining a healthy and reproducing population of mosquito fish. Of course, with this improvement, the increased population of fish required additional protection from predators (e.g., birds); therefore, a new canopy covering was installed over the tanks. In addition to structural improvements, sensors and software contribute to the observation of trends and maintenance schedules. Several staff members contributed to the assembly and construction of the new fish production system.

Figure 6. Two new fish tanks with pump system (top left) and fish fry tray in the tank (top right). Protective shade cloth and netting added to the tank system (bottom left). Water quality monitoring software displays (bottom right).

LAB REPORT

Overview of ACMAD Lab Activities

The ACMAD Lab is focused on supporting the activities of Operations by assessing mosquito abundance, determining the prevalence of arboviruses (arthropod-borne viruses) in birds and mosquitoes, and conducting research that supports District activities. Mosquito abundance is assessed by collecting mosquitoes in a variety of trap types that are placed throughout the County and subsequently identifying the mosquitoes in each collection to species. Arbovirus prevalence is assessed by testing dead birds that are reported to ACMAD by the public and the California Department of Public Health (CDPH). Mosquito species that are known disease vectors are collected in traps and tested for the presence of West Nile virus (WNV), Saint Louis encephalitis virus (SLEV), and Western equine encephalitis virus (WEEV). Research priorities are determined by the needs of Operations (e.g. assessing the efficacy of new treatment technologies) and to gain knowledge of local mosquito populations that will improve mosquito control practices (e.g. quantifying pesticide resistance in mosquitoes collected throughout the District).

Mosquito Abundance Monitoring

Overview of Abundance Monitoring. The ACMAD Lab monitors mosquito abundance within the county by analyzing the contents of traps that are placed in the field and designed to capture adult mosquitoes or the eggs they lay (i.e. oviposit) onto surfaces. Four types of traps which employ different mosquito attractants are used by the District: New Jersey Light Traps (NJLT; light attractant), dry ice-baited CDC EVS traps (EVS uses a CO_2 attractant), CDC autocidal gravid ovitrap (AGO; oviposition site attractant), and BG-Sentinel trap (BG uses human scent attractant). Mosquito egg abundance is monitored using ovi-cup traps that attract gravid female mosquitoes; this allows us to determine whether invasive species of mosquito are present in the County (e.g. the yellow fever mosquito (*Aedes aegypti*)).

Monitoring with NJLT. Fourteen NJLT were deployed at sites that were identified by Operations to be of high importance for regular monitoring of mosquito abundance. The locations of these traps are indicated on the trap site map by lightning bolt icons (Figure 7). Using the NJLT, mosquito abundance was monitored weekly during each month of 2014 and 2015 (Figure 8). For 2014, a total of 12,626 mosquitoes were collected from the NJLT and identified to species. For 2015, 1.95-fold more mosquitoes were collected in NJLT and identified to species (24,719 mosquitoes). The data from NJLT suggest there was increased mosquito abundance in the County for 2015 relative to 2014. For 2014, data from individual NJLT sites showed highest mosquito abundance in North Berkeley, Fremont (adjacent to Coyote Hills Regional Park), and Livermore (indicated by red and dark orange circles in Figure 9A). Very low mosquito abundance was observed during 2014 at Mountain House, southeast Fremont, and south Oakland (indicated by green circles in Figure 9A). In contrast, highest mosquito abundance for 2015, as measured using NJLT, occurred in the southwest region of Fremont, near Coyote Hills Regional Park (Fremont), and Union City (Figure 9B). Low mosquito abundance for 2015 was observed using NJLT in Mountain House, Pleasanton, east Fremont, and throughout Oakland (Figure 9B).

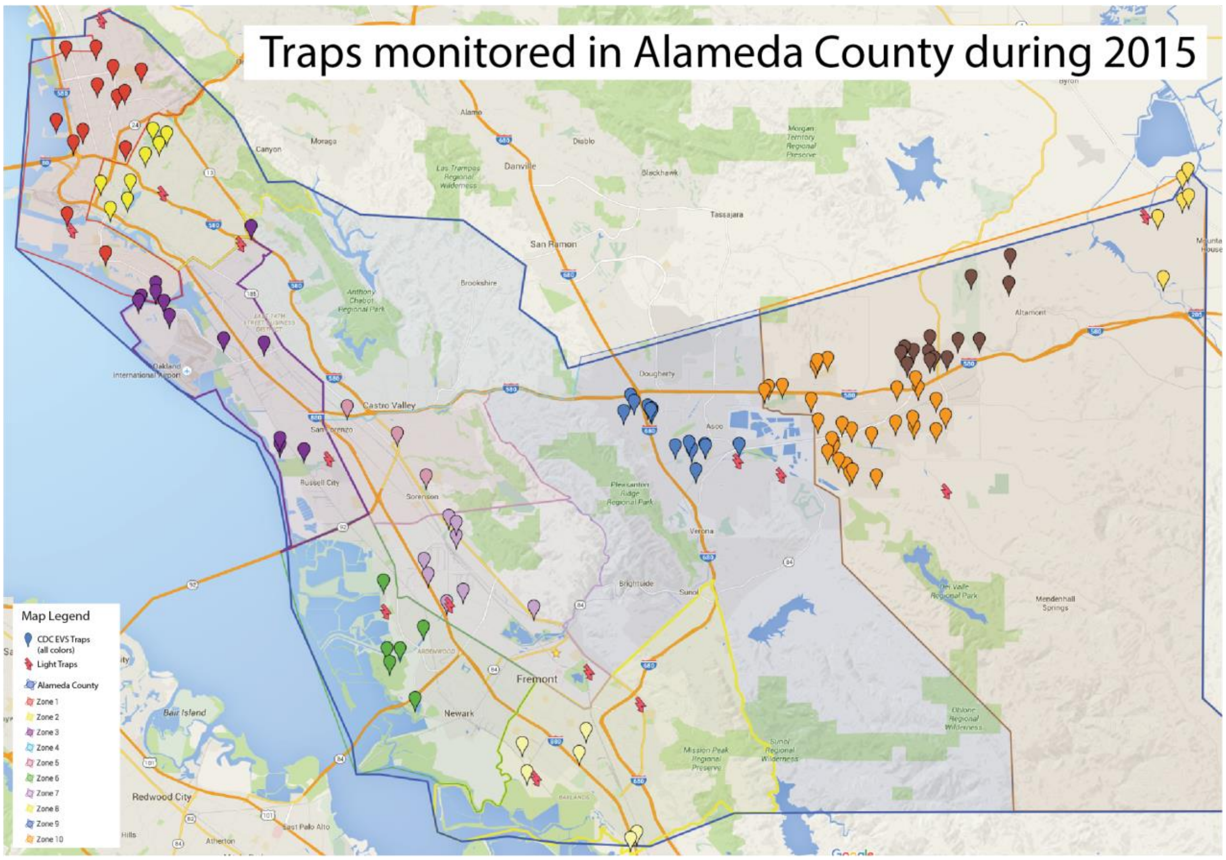


Figure 7. Location of CDC EVS Traps (*i.e.* CO_2 traps). CDC EVS trap locations are indicated by inverted tear-drop icons (all colors) while NJLT locations are indicated by red lightning bolts. The Alameda County boundary is within the large blue polygon, while zones serviced by Operations Staff are indicated by smaller colored polygons.

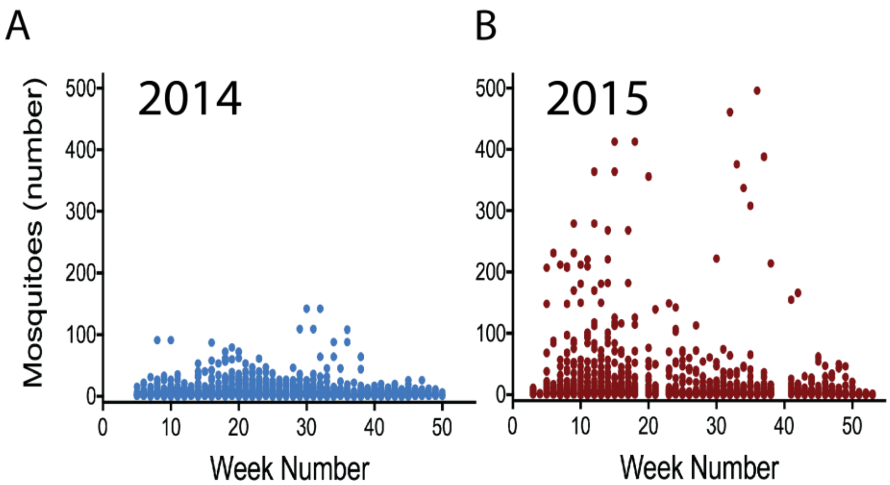


Figure 8. Number of Mosquitoes Collected in NJLT. Aggregate number of mosquitoes collected in all NJLT in the County for each week during 2014 (A) and 2015 (B). More mosquitoes were collected in NJLT during 2015 relative to 2014.

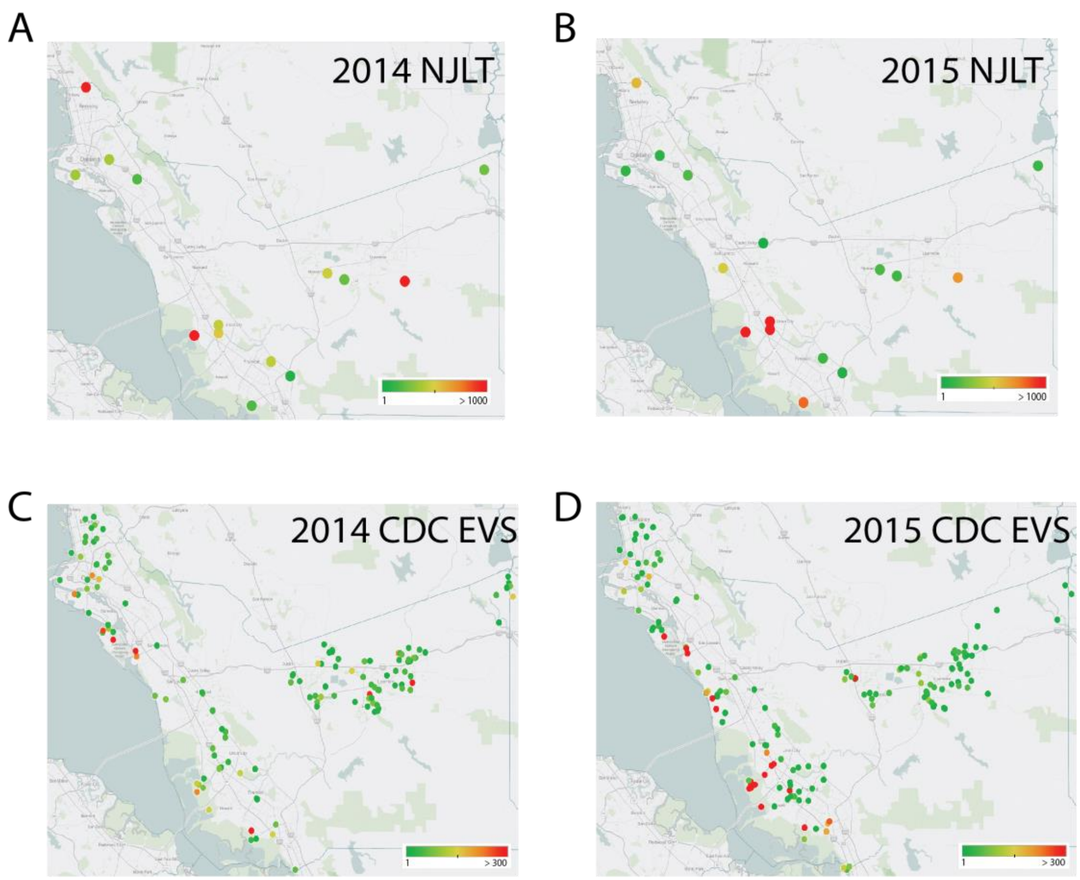


Figure 9. Mosquito abundance in NJLT (top) and CDC EVS traps (bottom) for 2014 (left) and 2015 (right). Highest mosquito abundance is indicated with red and dark orange circles, with colors progressing from yellow to green indicating lower abundance. Scale bars for the NJLT range from 1 to > 1000 mosquitoes per trap site, while scale bars for CDC EVS traps range from 1 to > 300 mosquitoes per trap site.

Monitoring with CDC EVS traps. In 2015, 119 CDC EVS traps were deployed throughout the county to assess mosquito abundance and the prevalence of mosquito-vectored disease in mosquitoes (e.g. WNV). During 2014, more than 95 sites were regularly monitored for mosquito abundance by District personnel (not shown). The locations of the CDC EVS traps deployed during 2015 and regularly monitored for mosquito abundance are indicated on the trap site map by the inverted-drop-shaped icons (Figure 7). Additional CDC EVS traps were placed to monitor mosquito abundance in areas where birds or mosquitoes were found to contain WNV (trap locations not shown). Highest mosquito abundances for 2014, as measured using CDC EVS traps (Figure 9C), were in southwest Oakland, southwest Fremont (near Baylands), and Livermore. For 2015, highest mosquito abundance was observed on and Oakland (near Bay Farm Island), west Hayward, Fremont (in Coyote Hills Regional Park), Union City, south Dublin, and Fremont (central and southwest regions; Figure 9D). Of note, relatively low mosquito abundance was observed in 2014 for Fremont with relatively high abundance in Livermore. In contrast, high abundance was observed in 2015 in some areas of Fremont, with low abundance in Livermore. This shifting pattern of mosquito abundance is notable because it correlated with presence of WNV-positive birds in these regions: 2014 had higher numbers of WNV-positive birds in Livermore (eastern Alameda County) with substantially fewer observed in Fremont, while in 2015 WNV was detected only in the western regions of the County (i.e. no WNV detected in Livermore; Figure 10)

Monitoring with AGO, ovi-cup and BG-Sentinel Traps. Two species of non-native invasive mosquito have been found recently in California: *Ae. aegypti* (yellow fever mosquito) and *Ae. albopictus* (Asian tiger mosquito). These species originated in Africa and Southeast Asia, respectively, but have been inadvertently transported and established in tropical and sub-tropical regions throughout the world. AGO, ovi-cup, and BG-Sentinel traps are used to monitor for these invasive mosquito species in Alameda County, and none of these traps collected *Ae. albopictus* or *Ae. aegypti* during 2014 or 2015.

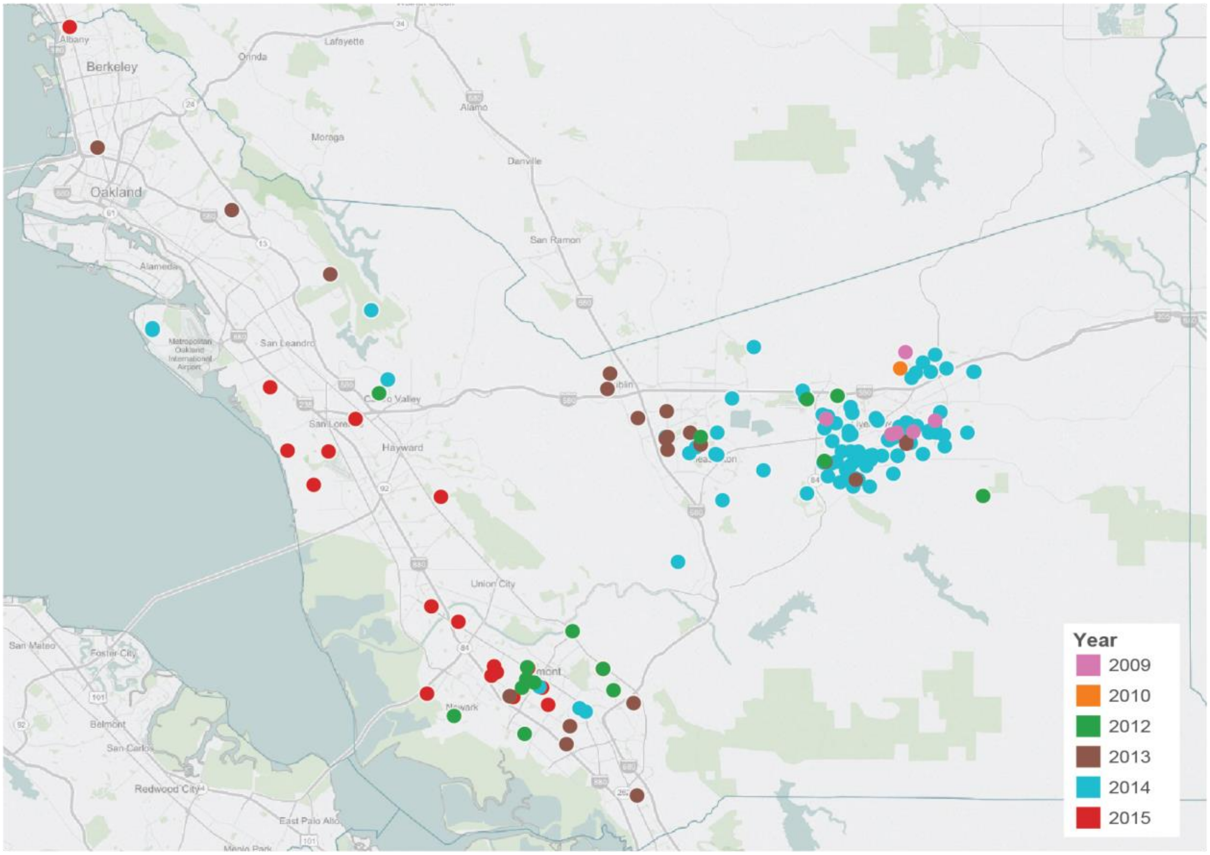


Figure 10. Location of birds that were collected in the County from 2009 – 2015, and found to contain WNV. The location of birds containing WNV are indicated with colored circles that correspond to the year shown in the figure legend. WNV was not detected in any bird during 2011. To date, no bird in the County has been found to contain SLE or WEE.

Arbovirus Surveillance in Birds and Mosquitoes

Improvements in Arbovirus Surveillance. During 2014 and until July of 2015, corvid birds (e.g. crows) were tested in the ACMAD Lab for WNV infection using a rapid analyte measurement platform test (*i.e.* the RAMP test, an immunoassay). Non-corvid birds and mosquitoes were sent to the Center for Vector-borne Diseases at UC Davis Center (CVEC) and tested for WNV, SLEV, and WEEV using reverse transcription - quantitative polymerase chain reaction (RT-QPCR). Since August of 2015, birds and mosquitoes have been tested for the presence of WNV, SLEV and WEEV in the ACMAD Lab using the RT-QPCR method employed by CVEC. We now directly report the results of our testing to the California Vector-borne Disease Surveillance Gateway (http://gateway.calsurv.org/). Testing birds and mosquitoes for these viruses in the ACMAD Lab has reduced the testing cost and the timespan between mosquito collection and providing test results to Operations and CDPH (e.g., previously, from 2- 5 days to now, as little as 3 hours). The reduced testing time substantially improves the response of Operations to WNV detected in birds or mosquitoes. For example, when a WNV-positive bird is identified, we set approximately 30 CDC EVS traps within one mile of where the bird was found, and the mosquitoes collected in the traps are tested in the ACMAD Lab for WNV, SLEV and WEEV. This has allowed us to identify with greater precision the areas where Operations should focus mosquito control efforts. Additionally, species of mosquitoes that are known to transmit WNV are routinely tested for the virus when they are collected in the CDC EVS traps. Overall, we test more than 90 % of known vector-competent species that are collected. Finally, we are providing arbovirus testing services to Alameda County Vector Control Services District for the mosquitoes they collect in Albany, CA.

Arbovirus surveillance in birds. Because WNV can amplify and cause severe disease in some species of bird (e.g. corvids), and some species of mosquito acquire blood meals from both birds and humans (e.g. *Culex pipiens*), birds can serve as a reservoir for arboviruses that may then be transmitted to humans by mosquitoes. Consequently, the presence of unusually high numbers of dead birds may indicate sustained transmission of arboviruses between birds and mosquitoes in a particular locale, thereby increasing the risk of transmission to humans. Regular testing of dead birds for the presence of arboviruses can provide early warning of increased risk for arbovirus transmission to humans and allows Operations to focus mosquito control efforts on specific high-risk areas. Dead birds in the District are reported by the public to the CDPH. Those that can be tested for WNV, SLEV or WEEV are retrieved by ACMAD Operations Staff and brought to the ACMAD Lab for testing. In 2015, 494 dead birds were reported, and of the 82 birds that could be tested, 3.8 % contained WNV (Figure 11). Birds that were not tested have typically been dead for too long making them unsuitable for virus detection. All of the WNV-positive birds in 2015 were collected in the western regions of the county, with most found in or near Fremont and Hayward (Figure 10). Higher numbers of dead birds were reported during 2014 (n = 856), the proportion of tested birds found to contain WNV was substantially higher (11.3 %; Figure 11), and all were collected in Livermore (Figure 10). A comparison of the number of birds tested by the ACMAD Lab and the number of birds that were reported to CDPH indicates there was no significant difference in the efforts made by the ACMAD Lab in testing dead birds during 2014 or 2015 relative to the prior 5 years (Fisher's exact test; $P = 0.5140$ and 0.5992, respectively).

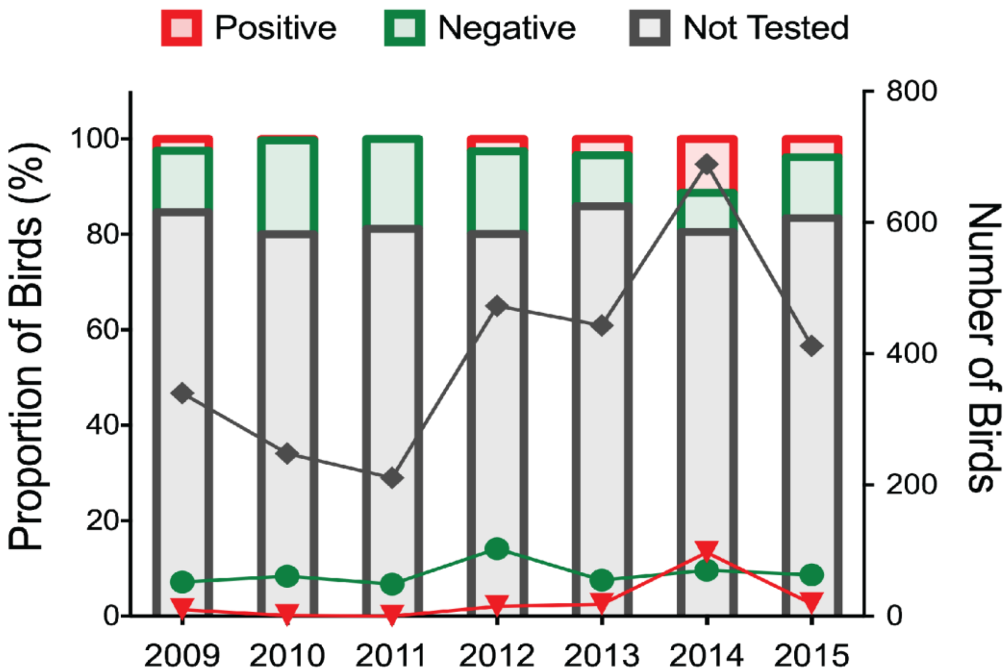


Figure 11. Proportion and number of dead birds reported by CDPH to the District that were positive or negative for WNV, or could not be tested from 2009 - 2015. The proportion of birds that did or did not contain WNV, or were not tested is shown on the left y-axis, and the number of birds for each year is shown on the right y-axis.

Arbovirus surveillance in mosquitoes. Since August of 2015, the ACMAD Lab has conducted routine arbovirus surveillance of all mosquitoes captured in the CDC EVS traps placed throughout the County. More than 90 % of arbovirus-competent species of mosquitoes were tested in the ACMAD Lab for the presence of WNV, SLEV, and WEEV using RT-QPCR. When dead birds were found to be infected with WNV, the ACMAD Lab placed 25 – 30 CDC EVS traps in an area no more than one mile from where the dead bird was found, and we tested all vector-competent species of mosquitoes that were collected in the traps for the presence of WNV, WEEV, and SLEV.

When testing mosquitoes for arbovirus infection, it is not economical to test each mosquito individually. Instead, the mosquitoes collected in a single trap or group of nearby traps are pooled together into groups of up to 50 mosquitoes and tested for arboviruses. During 2014, 213 mosquito pools were tested, and 16 (7.5%) of the pools were found to contain WNV (Figure 12). Similar to what was observed for WNV-positive birds during 2014, all of the WNV-positive mosquito pools detected during 2014 were collected in Livermore. Increased numbers of mosquito pools were tested in 2015 (n = 398), yet a similar number of WNV-positive mosquitoes were detected (n = 17; 4.4 % positive mosquito pools; Figure 12). As was observed for WNV-positive dead birds in 2015, the mosquitoes from all of the WNV-positive pools were collected in the western region of the county. Of note, 12 % of the WNV-positive mosquito pools were detected because of the routine arbovirus surveillance of mosquitoes that were collected in CDC EVS traps and not because of a mosquito trapping response to WNV-positive birds. To date, neither WEEV nor SLEV has been detected in any mosquito collected in Alameda County.

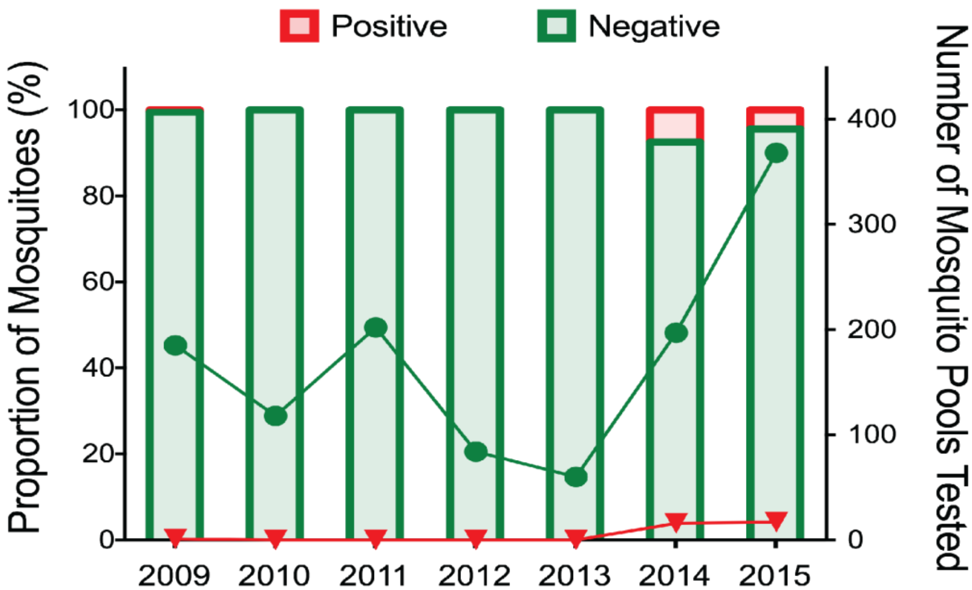


Figure 12. Proportion and number of mosquito pools collected from CDC EVS traps that were pooled and tested for WNV. The proportion of mosquito pools that did or did not contain WNV for each year is shown on the left y-axis, and the number of mosquito pools tested during each year is shown on the right y-axis.

Mosquito Research

Research Overview. During 2015, research in the ACMAD Lab focused on three goals: (1) Compare the efficacy of broken dry ice blocks with pellets in CDC EVS traps with the goal of improving trap performance and increasing employee safety, (2) Assess the impact of aerosolized BVA 2 (a mineral oil-based larvicide) on adult mosquito abundance in storm drains, and (3) Compare approaches for isolating RNA from mosquitoes with the goal determining which platform the ACMAD Lab should adopt when transitioning to an automated RNA isolation system. Finally, the ACMAD Lab began to lay the foundation for District staff to enter into collaborative research agreements with local academic institutions with the intent of recruiting undergraduate research interns and graduate students for mosquito-related research that supports the mission of the District. To this end, a research plan is described herein that is aimed at assessing pesticide resistance in *Culex pipiens* collected throughout the District.

Comparison of dry ice blocks or pellets for CDC EVS traps. The rationale for this study was to improve safety as technicians were hammering dry ice blocks into pieces that could fit into the CDC EVS traps (a distinct hazard to the eyes, and a time-consuming endeavor). District staff believed that broken dry ice blocks allowed the CO_2 traps to function for longer periods of time and thus improved trap performance. The results of the study showed that there was no significant difference in how much dry ice could be placed in the traps or the quantity of dry ice that remained in the traps after being in the field for 18 hours (Figure 13). CDC EVS traps collect most mosquitoes during the early evening. Because CDC EVS traps are typically placed in the field between late morning and early afternoon, the quantity of dry ice pellets remaining in the CDC EVS traps should be sufficient for collecting mosquitoes throughout the entire trap day. Moreover, use of dry ice pellets over broken blocks improves employee safety while reducing time needed to prepare the CDC EVS traps for placement and helps standardize the attractant among traps. Consequently, the District staff now use dry ice pellets for the CO_2 traps whenever possible.

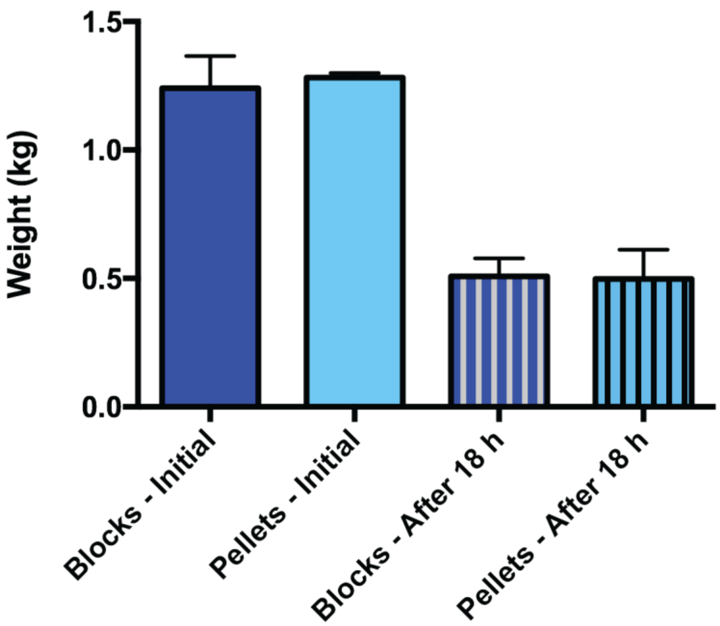


Figure 13. Comparison of dry ice blocks and pellets in CDC EVS traps. The dry ice receptacle of CDC EVS traps were filled completely with pellets of dry ice or blocks of dry ice that were crushed with a hammer (n = 3 per treatment). The mass of dry ice in each was measured before and after being placed outside for 18 hour (h).

Assessment of aerosolized BVA 2. We conducted a preliminary 128-day study in Pleasanton to assess the efficacy of aerosolized BVA 2 oil to reduce local abundance of adult mosquitoes in catch basins and storm drains. The Underground Larvacidal Aerosolizer (ULA) is a truck-mounted instrument that generates BVA 2 droplets which are deposited near the site that they are released, or are distributed moderate distances within storm drains to extend the range of treatment for mosquito control (please see Equipment Upgrades for more information regarding the ULA). Smaller aerosolized BVA 2 droplets remain suspended for a short duration in the storm drain and may contact adult mosquitoes in residence. If adult mosquitoes are coated with sufficient quantities of BVA 2, they may be unable to fly, thus providing immediate control of adult mosquitoes that are residing in storm drains treated with aerosolized BVA 2. Briefly, CDC EVS trapping data showed increasing adult mosquito abundance in the area around Val Vista Park in Pleasanton (day 8, Figure 14), and inspections of nearby properties indicated that mosquito breeding was likely occurring in the storm drains. Mosquito abundance at Val Vista Park was compared to abundance at the nearby Pleasanton Waste Water Treatment Plant (less than 100 meters from aerosolizer treatment sites) where the aerosolized BVA 2 was not applied. On day 32, aerosolized BVA 2 was applied to storm drains in the area (15-30 seconds of treatment per drain, 8.3 ml / second) and mosquito abundance assessed on day 35 (Figure 14). The results show a 92 % reduction in mosquito abundance two days after the treatment. Continued monitoring of the area showed an increase in mosquito abundance on day 57, with substantially higher mosquito abundance on day 77. Consequently, a second aerosolized BVA 2 treatment was applied to the storm drains in the area. Mosquito abundance was reduced by 68 % within a week of treatment, and within two weeks, abundance was reduced further to 94 % of the pre-treatment levels (Figure 14). Notably, mosquito abundance remained low in the area for the remainder of the study (51 days after the second treatment; Figure 14) and for the remainder of 2015 (not shown). The results of a more limited study of aerosolized BVA 2 in Fremont also showed reduced mosquito abundance after the treatment (not shown). Our findings suggest that BVA 2 aerosolized into storm trains may be highly effective for localized control of adult mosquitoes that reside in these environments. Expanded studies to assess the efficacy of aerosolized BVA 2 in storm drains are intended for 2016 and 2017.

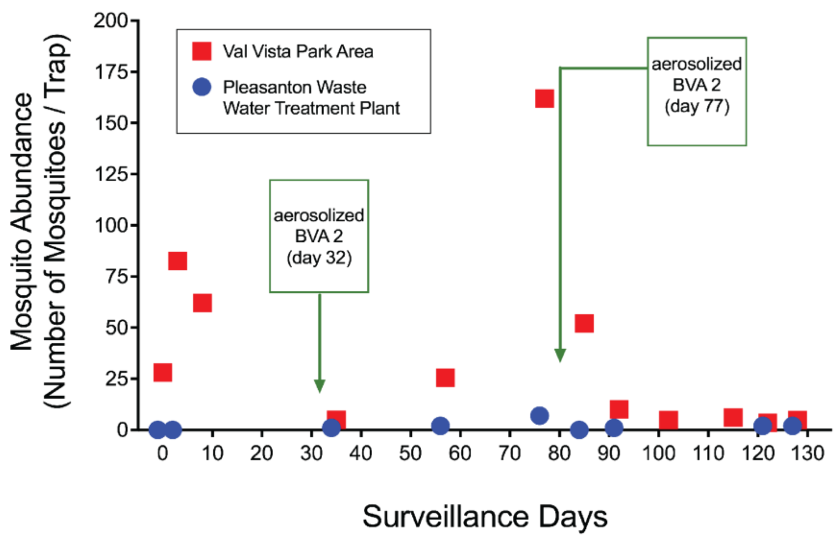


Figure 14. Proportion and number of mosquito pools collected from CDC EVS traps that were pooled and tested for WNV. The proportion of mosquito pools that did or did not contain WNV for each year is shown on the left y-axis, and the number of mosquito pools tested for each year is shown on the right y-axis.

Comparison of methods for isolating RNA from mosquitoes. Analyzing low quantities viral RNA isolated from mosquitoes is essential to assess the prevalence of arboviruses and the intensity of infection in mosquitoes. Two technologies predominate for isolating RNA from cells: silica membranes (RNeasy spin column, Qiagen) or silica conjugated to magnetic particles (MagMAX, ThermoFisher). The ACMAD Lab evaluated the relative quantity of viral RNA that was isolated from adult mosquitoes using RNeasy spin columns or an automated MagMAX system. Because the ACMAD Lab does not currently possess an automated MagMAX system for RNA isolation, we collaborated with Laboratory Staff at the San Mateo Mosquito and Vector Control District, who provided the necessary instrumentation. The optical density and quantity of the purified RNA were assessed as an indirect measure of specificity for each RNA isolation method. Briefly, pools of adult *Culex erythrothorax* mosquitoes (n = 0, 1, 5, 10, 25, or 50 mosquitoes per pool) and inactivated virus (WNV, SLEV, and WEEV) were added to lysis buffer, and the samples homogenized using a bead beater (n = 3 per treatment). Samples were subsequently centrifuged, and the RNA was isolated from the supernatant using RNeasy spin columns with a vacuum manifold or a MagMAX Express instrument, as described by the manufacturers. Identical sample and RNA elution volumes were used for each sample. The optical density (525 nm) and RNA concentration of the elutions were measured using a NanoDrop 2000 Spectrophotometer (ThermoFisher). Half of each eluted RNA sample was subsequently clarified with centrifugation to remove residual precipitates from the isolated RNA. Triplex TaqMan RT-QPCR was used to assess the relative quantity of WNV, SLEV, and WEEV in each RNA sample, and the quantity of virus that detected was reported as the cycle threshold value (Ct value). Clarification of the eluted RNA using centrifugation did not affect RNA concentration (Figure 15) but did reduce the quantity of brown precipitate in the eluted RNA samples (not shown) and improved virus detection in the RT-QPCR assay (Figure 16). Increasing the quantity of eluted RNA in the RT-QPCR assay from 2 µl to 10 µl improved the sensitivity for detecting WNV, SLEV, and WEEV (Figure 16). Increasing the number of mosquitoes in a sample tube (*i.e.* mosquito pool) from 1 to 50 did not significantly affect amplification of WNV, SLEV or WEEV in the RT-QPCR assay (Figure 16). In summary, because the Ct values from samples isolated using MagMAX were always significantly lower than those from RNA isolated using RNeasy columns (Figure 16; Two-way ANOVA, $P < 0.0001$), the MagMAX platform will be adopted by the ACMAD Lab for automated RNA isolation.

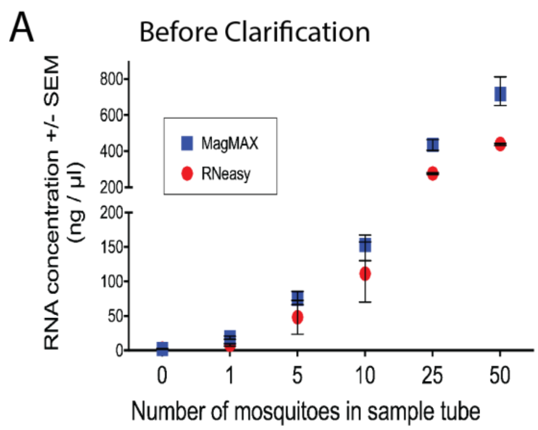


Figure 15. RNA concentration before (A) and after (B) clarification. There was no significant difference in RNA concentration of samples before and after clarification (Two-way ANOVA, RNeasy P = 0.8257, MagMAX P = 0.8790). Significant differences in RNA concentration were observed for samples containing 25 or 50 mosquitoes when extracts from RNeasy columns and MagMAX were compared (Unpaired t test, P < 0.01). The greatest reduction in RNA concentration (45 %) was observed for the 50 mosquito sample. RNA quality as measured by the ratio of absorbance at 260 and 280 nm was high for all samples (260/280 = 2.15 ± 0.147).

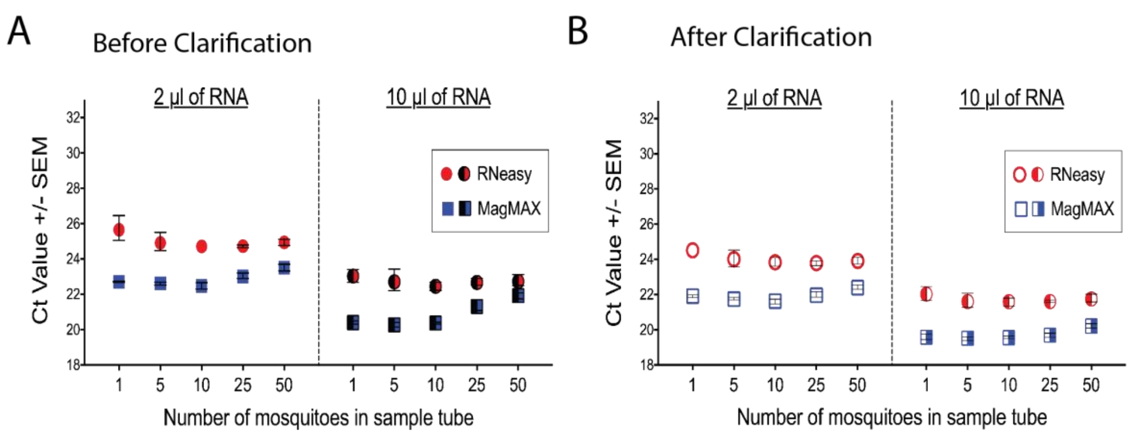


Figure 16. Triplex RT-QPCR amplification of WNV using 2 µl or 10 µl of eluted RNA that was isolated using MagMAX or RNeasy columns, before (A) and after clarification (B) using centrifugation. A similar distribution of Ct values was observed for amplification of WEE and SLE (not shown). When the RNA samples were clarified, there was a significant reduction in Ct values (Two-way ANOVA, P<0.001), suggesting that clarifying the samples may increase the sensitivity of the assay for detecting arboviruses in mosquitoes. Assays with 10 µl of RNA had higher Ct values relative to those with 2 µl of RNA (Two-way ANOVA, P<0.001), indicting that a higher volume of eluted RNA may enhance detection of arboviruses. When the number of mosquitoes in a sample was increased from 1 to 50, there was no significant difference in Ct values within each isolation method (Multiple t tests, P>0.1), suggesting that isolating arbovirus RNA from up to 50 mosquitoes in a single tube does not negatively impact the TaqMan assay. RNA isolated using MagMAX produced significantly lower Ct values in the arbovirus TaqMan assays relative to RNA isolated using RNeasy columns (Two-way ANOVA, P<0.001), suggesting that use of MagMAX may be more sensitive in screening mosquitoes for arbovirus infection.

Research plan to assess pesticide resistance in Cx. pipiens collected throughout the County. The geospatial allelic variation in genes known to mediate resistance to pyrethroid insecticides (e.g. knockdown resistance (*kdr*), acetylcholine esterase -1 (*ace-1*) and members of the cytochrome P450 (*CYP*) gene superfamily) will be assessed for *Cx. pipiens* collected in the County. Knockdown resistance (*kdr*) is a well-studied mechanism of resistance to pyrethroid insecticides and is known to occur in many insect species, including mosquitos that transmit pathogens such as WNV. Pyrethroids act on the insect nervous system by prolonging voltage-gated sodium ion channel opening to cause increased neuron activity and eventually death [1]. Resistance to pyrethroids is conferred by point mutations in the sodium channel that reduce their sensitivity to pyrethroids (*i.e.* the *kdr* gene). Pyrethroid resistance in mosquitoes is also mediated by mutations in the enzymes involved with neurotransmitter activity [e.g. *acetylcholine esterase – 1* (*ace-1*)][2] or oxidase enzymes that metabolize pyrethroids (cytochrome P450 (*CYP*) gene superfamily) [3,4]. The allelic variation in *kdr*-mediated pyrethroid resistance has been previously documented for mosquito species other than *Cx. pipiens* [5-8]. Additionally, a complex network of mechanisms that include point mutations in resistance genes (*e.g*. *ace-1* or *CYP*), changes in gene expression, RNA-editing, and other metabolic pathways contribute to resistance [9-11]. Consequently, only assessing the distribution of *kdr* alleles in mosquitoes that are native or invasive to Alameda County may not provide sufficient information on resistance to pyrethroids. Less well studied is the geospatial allelic variation in mutations of *ace-1* or *CYP* in mosquitoes. Thus, it is of value to analyze the allelic variation of *kdr*, *ace-1* and *CYP* in mosquitoes collected in Alameda County. Moreover, assessing the prevalence of resistance alleles for these genes may be of high operational value. For example, were an outbreak of a mosquito-vectored disease to occur (e.g. WNV), assessing the pyrethroid resistance of adult mosquitoes within the planned treatment area may help Operations determine whether the treatment has the potential to reduce adult mosquito populations substantially and limit disease transmission. For this study, *Cx. pipiens*, a vector of WNV, will be collected throughout Alameda County and tested for resistance to pyrethroid insecticides using functional, biochemical, and genetic assays. The functional test will be the bottle assay that is traditionally used to assess mosquito resistance to insecticides [12]. The biochemical tests will be performed on cellular lysates isolated from the collected mosquitoes to assess the activity of enzymes known to metabolize pyrethroids (e.g. α-esterase, β-esterase, oxidase, acetylcholine esterase, and glutathione-s-transferase). DNA isolated from the mosquitoes tested with the bottle assay will be analyzed using RT-QPCR to assess the geospatial distribution of resistance alleles for *kdr*, *ace-1* and certain *CYP* genes. This research will be conducted with students from California State University, East Bay.

PUBLIC OUTREACH

In 2014 the District overhauled its long standing website at [www.mosquitoes.org (Figure 17)](http://www.mosquitoes.org). While much of the content of the site stayed the same, a more streamlined look and navigational menu were incorporated into the new design. In addition, the public can now signup for quarterly newsletters, press releases, fogging notifications, and connect directly with the District's Facebook and Twitter accounts all via the website. Each page of the website also allows for the public to share, bookmark, or translate the content through a variety of modes such as social media (Facebook, Twitter, Tumblr, Pinterest, Digg, etc.), email, and google translate.

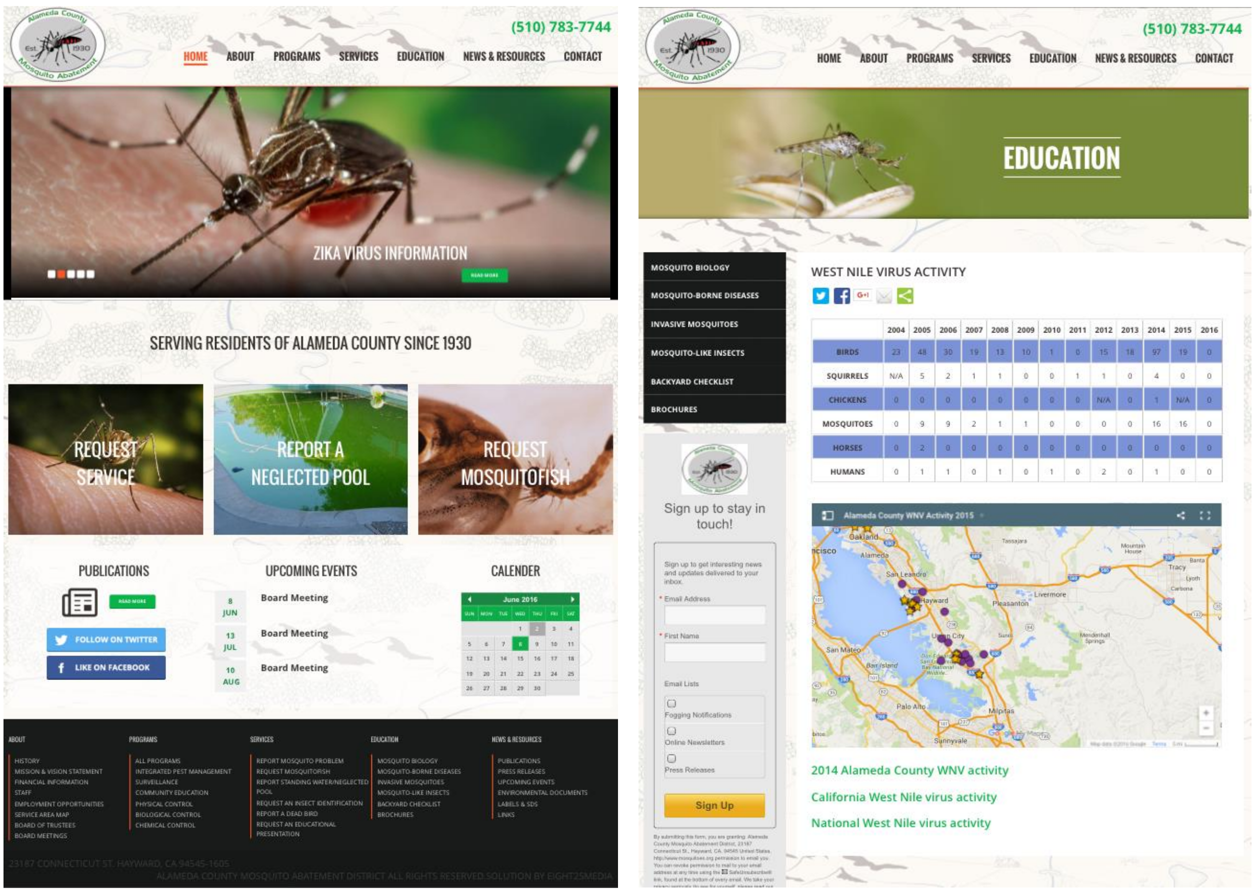


Figure 17. New website homepage design (left). The new website page layout features a signup tool on the left hand side and share buttons underneath the page headings (right).

As a part of the Districts attempt to reach out to the public on mosquito related issues through digital platforms, internet advertisements were incorporated into the District's overall media campaigns. These ads supplemented the existing advertisements in the PennySaver publication and posters in Bay Area Rapid Transit (BART) stations. All of the ads focused on draining standing water, reporting neglected swimming pools, and adopting personal preventive measures to avoid mosquito bites.

Figure 18. Alameda County Fair Display for 2015

Public outreach through participation in local fairs and community events continued to be a staple of the District's public education program. In 2014 and 2015, the District's display at the annual Alameda County Fair won numerous awards (including the exhibitor's choice award for two straight years; Figure 18). In addition, it attracted fairgoers to the mosquito prevention messaging that is a vital part of the District's overall program.

Shows and fairs the District participated in:

<u>2014</u>

- Alameda County Spring Home & Garden Show
- Dublin St. Patrick's Day Festival
- Oakland Earth Expo
- CSUEB World Health Day Info Fair
- Berkeley Bay Festival
- San Leandro Earth Day & Watershed Festival
- Alisal Elementary Science Fair
- Port of Oakland Earth Day Festival
- Chabot College Return of the Swallows Festival
- Peralta Colleges Sustainability Eco Festival
- Alameda Earth Day Festival
- Dublin Water Wise Workshop
- Alden Lane Nursery Fish Giveaway
- Hayward Cinco de Mayo Festival
- Palomares Elementary School Science Expo & Watershed Festival
- UCB Botanical Garden "Bug Days"
- Alameda County Fair
- Hayward Zucchini Festival
- Newark Days Festival
- Alameda County Fall Home & Garden Show

<u>2015</u>

- Alameda County Spring Home & Garden Show
- Dublin St. Patrick's Day Festival
- San Leandro Earth Day & Watershed Festival
- Oakland Earth Expo
- Berkeley Bay Festival
- Peralta Colleges Sustainability Eco Festival
- Alameda Earth Day Festival
- Hayward Cinco de Mayo Festival
- Alden Lane Nursery Fish Giveaway
- Palomares Elementary School Science Expo & Watershed Festival
- Niles Wildflower, Art, Garden, & Quilt Show
- Alameda County Fair
- Hayward Zucchini Festival
- Newark Days Festival
- Alameda County Fall Home & Garden Show
- CSUEB Discovery Day Science Fair

FINANCIAL REPORT

FOR FISCAL YEARS ENDING JUNE 30, 2014 AND JUNE 30, 2015

	2014	2015
Revenues :		
Property taxes	$1,597,083	$1,732,006
Redevelopment distribution	$165,563	$172,346
Special Assessments	$1,886,169	$1,899,118
Homeowners Property Tax Relief, State Subvention	$15,924	$15,714
Transfer from OPEB Trust	$0	$133,188
Interest	$9,958	$13,942
Miscellaneous	$24,619	$38,724
Total Revenues	$3,699,316	$4,005,038
Expenditures :		
Salaries and fringe benefits	$1,950,547	$2,086,888
Materials, supplies and services	$556,992	$807,706
Payment of CalPERS "side fund" & reduction of unfunded liability	$0	$825,406
Transfer to OPEB trust	$800,000	$500,000
Capital outlay	$135,589	$252,341
Total Expenditures	$3,443,128	$3,146,935
Net change in fund balances	$256,188	$858,103
Fund balances, beginning of period	$3,263,459	$3,519,647
Fund balances, end of period	$3,519,647	$4,377,750

Alameda County Mosquito Abatement District
Combined Balance Sheet For The Years
Ending June 30, 2014 and June 30, 2015

Assets	**June 30, 2014**	**June 30, 2015**
Current and Investments	$ 3,713,484.00	$ 4,592,660.00
Accounts receivable	$ -	$ -
Capital Assets (Net)		
Non-depreciable assets	$ 61,406.00	$ 61,406.00
Depreciable assets, net	$ 2,606,574.00	$ 2,627,985.00
Total Assets	$ 6,381,464.00	$ 7,282,051.00
	============	===========
Deferred Outflow	$ -	$ 163,799.00
Liabilities		
Account Payable	$ 54,908.00	$ 98,462.00
Compensated Absences	$ 138,929.00	$ 116,448.00
Net Pension Liability	$ -	$ 1,923,046.00
Total Liabilities	$ 193,837.00	$ 2,137,956.00
	===========	===========
Net Assets		
Invested in Capital Assets	$ 2,667,980.00	$ 2,689,391.00
Unrestricted	$ 3,519,647.00	$ 2,769,101.00
Total Net Assets	$ 6,187,627.00	$ 5,458,492.00
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