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The Impact of Temperatures from Los Angeles County on the Life History Traits of *Aedes albopictus*: Studies with High Temperatures

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BACKGROUND AND METHODS

Aedes albopictus, the Asian tiger mosquito, recently has become a global public health problem associated with the international trade of used tires and ornamental plants such as Lucky Bamboo. In addition to being a biting nuisance, *Ae. albopictus* is an excellent vector for several arboviruses, including dengue and chikungunya (Hawley 1988; Benedict et al. 2007). Since 2011 this species has been found in the Los Angeles basin in the towns of El Monte and Arcadia (S. Kluh personal communication). This latest finding is of considerable concern because it could mean that the population is now established permanently in Southern California, extending the risk for exotic arbovirus introduction and transmission.

The purpose of our studies is to understand the influence of the temperatures found in Los Angeles County on the life history traits of this species. This area of the state is characterized by daily high temperatures with a range of 20s°C to 30s°C, and lows between 9°C and 20s° C; rain occurs mostly during the winter months along with mild temperatures, and during the fall and summer the climate is dry (Harrison et al. 1971). This pattern seems to be appropriate for the establishment of *Ae. albopictus*, as it has been established in the southern areas of Spain and Italy, places with a comparable Mediterranean climate and similar temperatures (Aranda et al. 2006; Roiz et al. 2010)Aedes albopictus, has colonized nearly all the regions of Italy as well as other areas of Europe. During the summer of 2007 the tiger mosquito was responsible for an outbreak of Chikungunya in Italy, when this virus was brought in by a tourist of Indian origin returning from an endemic area. To increase the knowledge of tiger mosquito population dynamics, a survey was carried out from April to November 2008 in the municipalities of Arco and Riva del Garda (northern Italy.

Using an *Ae. albopictus* mosquito colony from the infested area, we tested the hypothesis that the heterogeneous climate and landscape of the Los Angeles basin impact the biology of the invading population and have prevented the extension of the infestation zone. Data on life history traits such as larval development rate and survival, adult longevity and effect of temperature on adult body were gathered for *Ae. albopictus* using constant temperatures spaced at 4ºC increments between 22 and 34ºC and a constant humidity of 70%. Temperatures and humidities were maintained using environmental chambers (Binder KBF 115, Tuttlingen, Germany) and confirmed using HOBO data loggers (Onset, Cape Cod, MA) placed inside the chambers. All of our experiments were conducted at the UC Davis Center for Vectorborne Diseases Biosafety Level 3 laboratory.

Each temperature treatment (22, 26, 30 and 34°C) had 15 replicate cups with 20 larvae per cup. Larvae were counted every day until development to pupal stage. Once larvae started pupating they were scored every 12 hours for 22 and 26°C, and at 6 hour intervals for 30 and 34°C due to faster development at these 2 temperatures. Once immature mosquitoes reached adulthood, we randomly selected 24 males and females from each temperature treatment and paired them in a pint carton to determine adult survival. Adults were offered 10% sucrose water daily, and the females were collected at death. Wings were removed and measured using Automontage Imaging software (Cambridge, UK).

RESULTS AND CONCLUSIONS

Our results show that *Ae. albopictus* larvae take 5-7 days to develop to pupal stage at 34°C, between 6 and 7 days for 30°C, 7 to 9 days for 26°C and 10 to 12 days for 22°C. Adult survival was inversely related to temperature with both males and females living longest at 22C and shortest at 34 °C. Mean larvae development time to pupa and adult (male and female) survival times are shown in Figures 1 and 2.

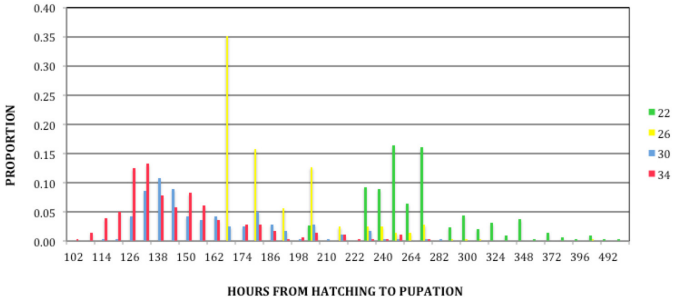

Figure 1. *Aedes albopictus* developmental time from larva to pupa at constant temperature treatments.

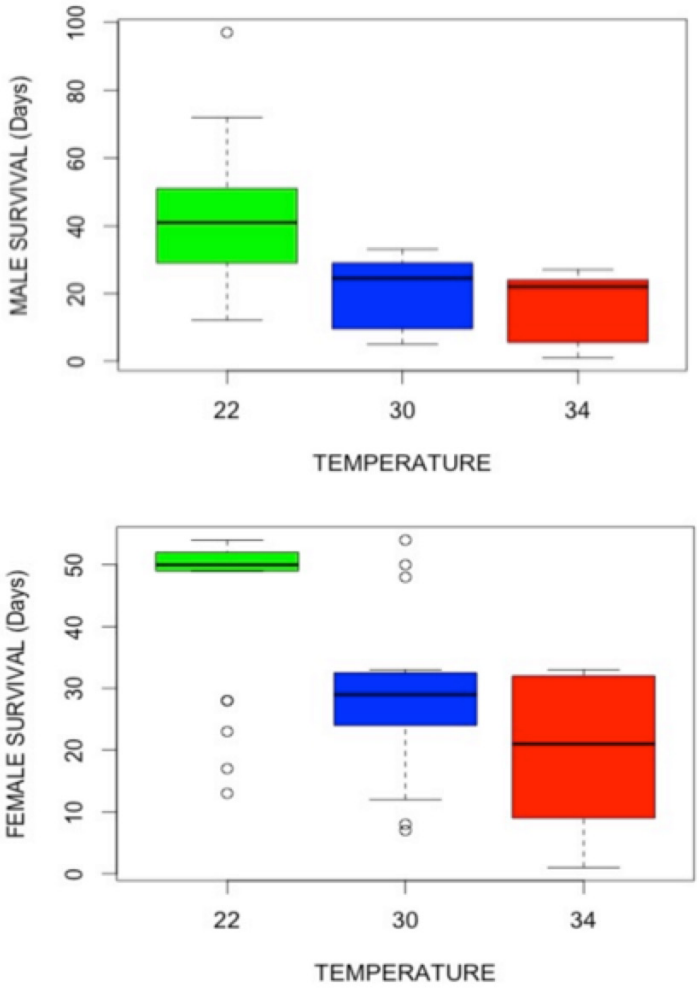

Figure 2. Mean adult survival for male and female *Ae. Albopictus* (error bars show + standard error of the mean).

We determined that temperature has an effect on the size of adult females. Mean values for each temperature are shown in Figure 3. At 22°C wings averaged 2.83 mm, compared to 2.73, 2.63 and 2.477mm for 26°C, 30°C and 34°C respectively.

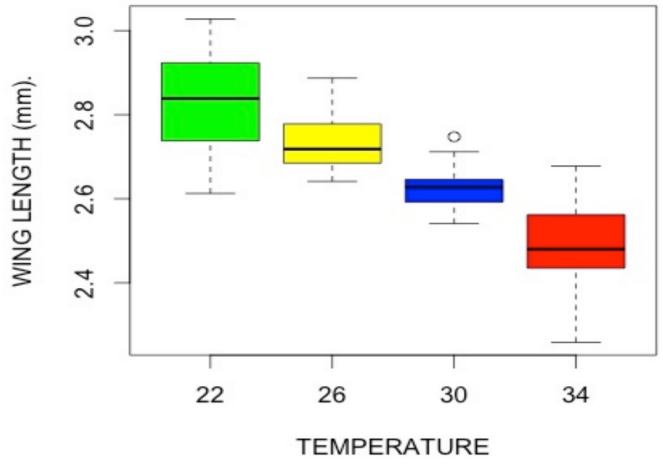

Figure 3. Influence of temperature on adult size of female *Ae. Albopictus* (error bars show + standard error of the mean).

In conclusion, our preliminary results showed that temperature was positively correlated with developmental rate. However, adult survival was inversely correlated with temperature; our data showed that both males and females lived longer at 22°C and the least number of days at 34°. Female longevity at all 3 temperatures was longer than male longevity. Similar patterns have been found in studies using *Ae. albopictus* populations from other regions (Delatte et al. 2009; Alto and Juliano 2001).

The results presented here are the first set of experiments designed to obtain data that will be used to construct degree day models to define minimal and maximal tolerance thresholds for the population of *Ae. albopictus* found in Los Angeles County. We will validate our degree day models derived from these constant temperatures by using cycling conditions of spring and summer temperatures and humidity measured from three areas in Los Angeles County.

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Using *Gambusia affinis* in Storm Water Structures

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ABSTRACT: The Coachella Valley Mosquito and Vector Control District examined the effectiveness of *Gambusia affinis* in storm water structures that are difficult to survey for mosquito breeding. Mosquitofish are visual predators and may not be effective at controlling mosquitoes in dark catch basins. We examined the impact of darkness on mosquitofish feeding on larval mosquitoes in laboratory and semi-field trials. Results showed mosquitofish were just as effective in consuming larvae in normal light, partial light, or darkness. Mosquitofish were introduced into a homeowner's association with 10 deep catch basins. The mosquitofish populations in each basin were confirmed by placing baited minnow traps in the basin for 24 hours. Adult mosquito populations were monitored by placing CO_2 traps inside the basin and larvae populations were examined using a 2-dip method with a standard dipper. Water parameters (temperature, pH, conductivity and dissolved oxygen) were measured every other visit. Results showed minimal to no breeding in all sites and confirmed that *Gambusia affinis* can provide adequate control for mosquito breeding in storm water structures.

INTRODUCTION

A number of storm-water structures within the District are deep and difficult to survey for mosquito breeding. Vector Control Technicians may be unable to inspect these deep basins with their standard dippers making it difficult to apply effective chemical treatments and evaluating their performance. Mosquitofish are effective control agents and good at preventing mosquitoes from laying eggs (Walton et al. 2009). They belong to a family of fishes that do not lay eggs; fertilization is internal and the female bears live young. Reproduction is rapid, and the young fish are ready to eat soon after birth. They prefer shallow, calm lakes, ponds and streams; their up-turned mouths make them ideal surface feeders. Mosquitofish are diurnal and visual predators, and so, they may not be good at controlling mosquitoes in catch basins that receive little or no light. This study examined the feeding behavior of mosquitofish during partial and complete darkness in a laboratory setting using 38 L (10 gal) aquariums, semi-field microcosm ponds, and storm water catch basins at a residential community.

MATERIALS AND METHODS

Laboratory. Three 38-L (10-gallon) aquariums were set up to simulate the light levels that could be encountered in the field. Each aquarium had a heater set at 27°C (80°F) and an air-stone immersed for aeration. The first aquarium was covered on all sides with cardboard for complete darkness. With the second aquarium, 3 sides were covered with cardboard and the front was partially covered (75%) to simulate a catch basin (partial darkness). The third aquarium was left uncovered (normal light).

Two trials were performed with the experimental aquaria. In the first trial, 10 males were stocked in each aquarium and then acclimated for a 24 hour period. 500 *Cx. quinquefasciatus* larvae were added, and the fish were allowed to feed for 7 hours. This was repeated with 10 females, and then 10 fish of mixed generations and sex.

In the second trial, 5 males were stocked in each aquarium and then acclimated for a 14 day period. 500 *Cx. quinquefasciatus* larvae were added, and the fish were allowed to feed for 7 hours. This was repeated with 5 females, and then 5 fish of mixed generations and sex.

Any remaining larvae after each trial were recorded.

Microcosm Ponds. Nine 852-L (225-gallon) ponds measuring 91cm W x 152cm L x 61cm D (3ft W x 5ft L x 2ft D) and a surface area of 1.4 m^2 (15 ft^2) were lined with window screen to make collection at the end of the study easier (Figure 1). The water level was maintained with automatic make-up valves.

Figure 1. Microcosm pond with window screen lining. The screen lining was used to concentrate larvae at the end of the trial.

In order to simulate partial and complete darkness, covers were created from 0.6 cm (¼ in) clear plexiglass and custom fitted to the dimensions of each pond.

Three of the covers were painted completely with dark green spray paint, three were partially painted with dark green spray paint leaving a clear center strip, and three were painted with clear satin spray paint for the control ponds (Figure 2). Twenty-five mosquitofish were stocked in each pond and allowed to acclimate for 2 weeks. The pond covers were secured and fish were fed 1.5 g (0.1 oz) fish meal at the beginning of each week. After the 2 weeks, 1,000 *Cx. quinquefasciatus* larvae were introduced into each pond, and the pond covers resecured. After 24 hours, the pond covers were removed. The corners of the net were slowly pulled up until roughly 15 cm (6 in) was still immersed the water. A fine-mesh bag net was then used to scoop out all mosquitofish and any remaining larvae. The contents of the bag net were placed into a shallow plastic tray, and the number of fish and larvae were recorded.

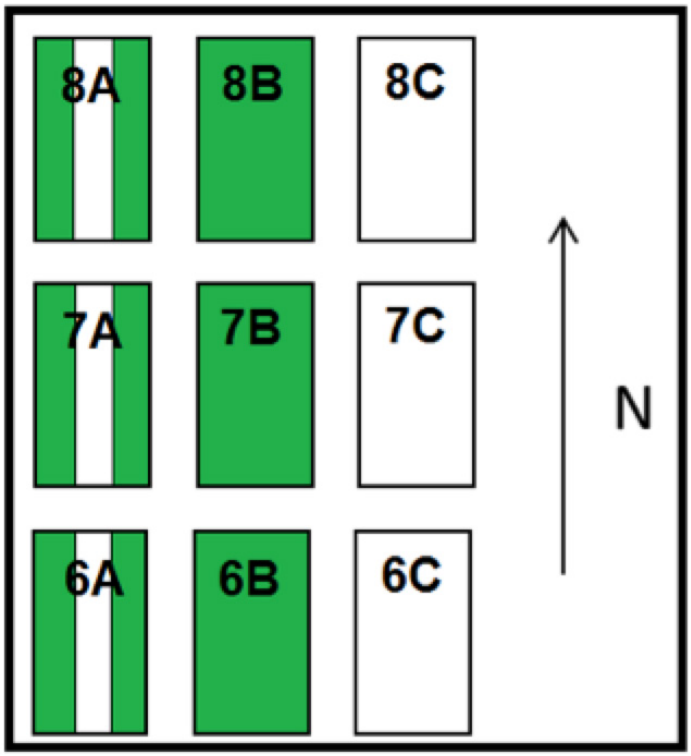

Figure 2. Layout of ponds. Green represents areas of covers that were painted green to provide partial (row A) or complete (row B) darkness.

Catch Basins. Ten deep catch basins in a gated community were selected for the study (Figure 3). The basin's length, width and depth were measured using an 8-m (25-ft) measuring tape. An LED headband flashlight was used to survey the inside of the basin for any visible adult mosquitoes resting on the inside walls. Adult mosquito traps (CO_2) were placed inside and hung on the basin's ladder. The traps were set as late in the afternoon as possible and picked up the next morning. The average afternoon air temperatures for the city were recorded at each visit (Wunderground, 2014). Traps were set every other week for 4 months (August through December 2013).

Figure 3. Location map of ten catch basins selected for fish stocking in a homeowners association in the Coachella Valley.

Larvae were surveyed using a standard dipper with multiple telescoping poles taped together. Two dips were taken per basin at each visit. Water parameters including temperature °C(°F), salinity (ppt), dissolved oxygen (ppm) and pH, were taken with a YSI 556 probe; water quality was measured for ammonia levels (ppm), nitrite levels (ppm) and nitrate levels (ppm) using multi-indicating test strips. The depth of standing water was measured with an 8-meter (25-ft) measuring tape and recorded.

The number of fish stocked at each basin varied on whether the site had mosquito larvae. Fish are known to discourage *Cx. quinquefasciatus* from laying eggs (Walton et al. 2009). If no mosquito breeding was occurring during the initial inspection, mosquitofish were stocked at a preventive rate of 60 fish per 9.3 m^2 (100 ft^2). If mosquito breeding was occurring, mosquitofish were stocked at the control rate of approximately 150 fish per 9.3 m^2 (100 ft^2). The fish were lowered in a bucket into the basin by rope and then poured into the water.

The fish were allowed to acclimate for 1 week before installing a minnow trap that was used to estimate the fish population in each basin. A 20 cm (8 in) piece of "swimming pool noodle" foam was placed inside the trap so it would float just beneath the water surface (Walton 2007). The traps were baited with six catfish chow pellets, lowered into the basin and then anchored with string tied on the basin's ladder. Fish were counted and returned to the basin. Mosquitofish movement and predation on immature mosquitoes within the basin were evaluated every other week for 4 months (September through December).

RESULTS

Laboratory. The mixed populations consumed nearly all available larvae in either acclimation period (Figures 4 and 5). Female mosquitofish performed as well as mixed populations after the longer acclimation period. Male mosquitofish did not do as well as the all-female group or the mixed group.

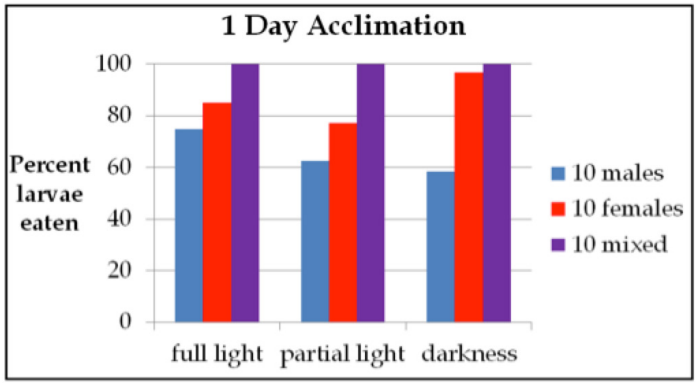

Figure 4. Percent of larvae eaten by fish in aquaria after being allowed to acclimate for one day.

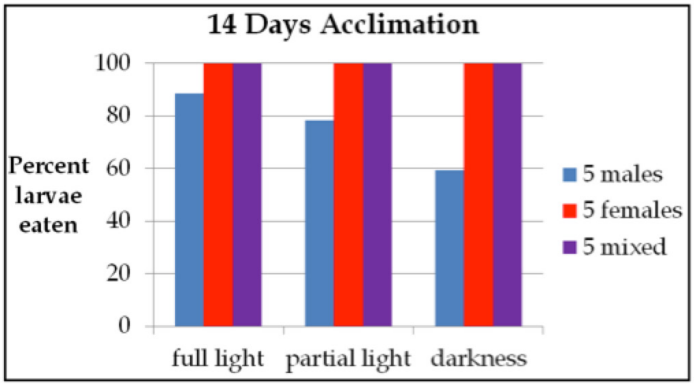

Figure 5. Percent of larvae eaten by fish after being allowed to acclimate to light conditions for 14 days.

Microcosm Ponds. Mosquitofish did not seem to be impacted by the lack of light as nearly all larvae were consumed in every treatment (Figure 6).

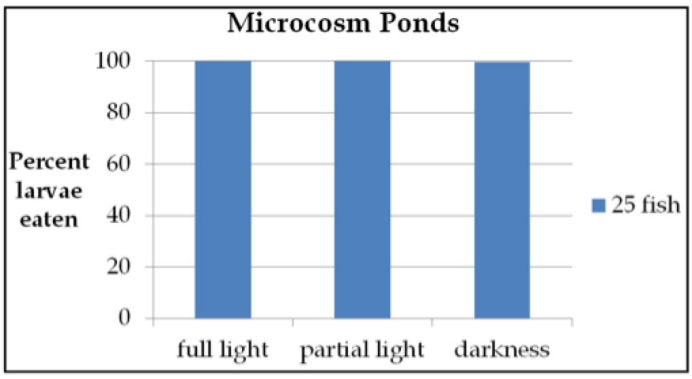

Figure 6. Percent of larvae consumed by 25 fish in different light conditions from each microcosm pond.

Catch Basins. Mosquitofish were effective at controlling immature mosquitoes within deep catch basins after a 7-day acclimation period (Figure 7), and the temperature in this range was not a factor in the fish's ability to control immature larvae. Mosquitoes caught with CO_2 traps before the fish were added were pale and appeared to be newly emerged from the catch basin. After the 7-day fish acclimation period, few adult mosquitoes were trapped for the 8 weeks (Figure 8), and air temperature was also not a factor.

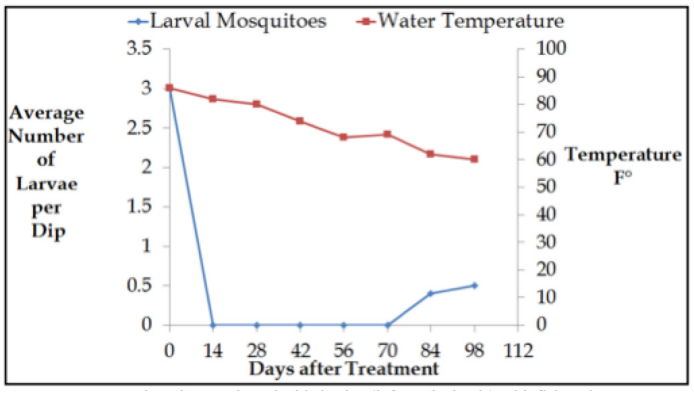

Figure 7. Average number of larval mosquitoes inside basins (left vertical axis) with fish and water temperature (right vertical axis). Larvae at day 0 are the pre-treatment larval densities.

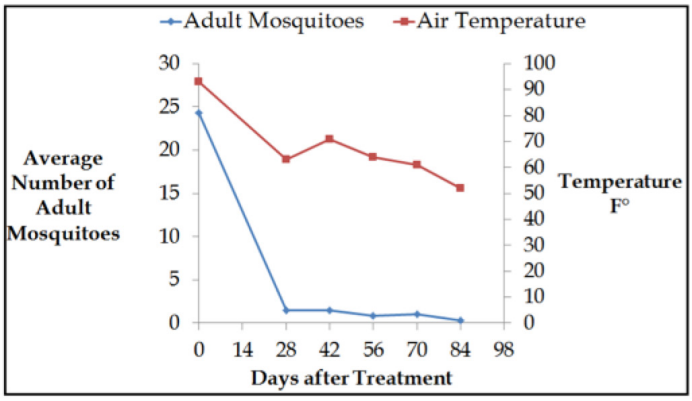

Figure 8. Average number of adult mosquitoes captured in CO_2 traps within the basins (left vertical axis) and air temperature (right vertical axis). Adults at day 0 are the pre-treatment adult densities. Traps were not set in September due to rain events that filled all basins to above the lids of the basins.

DISCUSSION

The feeding behavior of mosquitofish appears to be unaffected by low or no levels of light. In laboratory and semi-field conditions, mosquitofish consumed nearly all of the available mosquito larvae. The light levels measured in the catch basins were 0.012 lumen/m^2 (003 Fc) and in the microcosm ponds with dark covers 0.032 lumens/m^2 (001 Fc), indicating that the fish were able to function in low light.

The mosquitofish had a significant impact on controlling or deterring mosquitoes within the basins. During the trial (September – December), water temperature did not impact the fish's ability to control. Few mosquitoes were caught, and those caught appeared to have recently emerged. Thus, these basins did not appear to be used as refuge by adult mosquitoes during the time frameof this study.

Some variables may lead to the loss of mosquitofish such as heavy rain events that cause overflow of the basins, washing out fish. Basins that have three inches of water or less with a heavy organic load may stress and deter the fish from normal activity. Technicians should visually examine and confirm fish movement in basins that have low water levels and re-stock if necessary.

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Quality Control Procedures for Vector Borne Disease PCR Diagnostics, County of San Diego

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ABSTRACT: Quality control is an important component of laboratory testing in which procedures are established to ensure optimal and standardized testing conditions and to minimize test variability. In the County of San Diego, the Vector Disease & Diagnostic Laboratory (VDDL) routinely tests ticks, rodents, birds and mosquitoes for vector borne diseases including tularemia, Lyme disease, hantavirus, West Nile Virus, St. Louis encephalitis virus, and Western equine encephalitis virus. Because of sample diversity and the inherent differences that exist in testing procedures, the VDDL has implemented various methodologies to ensure quality control of the assays. Quality control procedures include: optimization and validation of polymerase chain reaction (PCR) assays using primer and probe matrices; the use of positive and negative controls during nucleic acid extraction (extraction controls); annual tests of reagents to validate performance; positive and negative PCR amplification controls; internal positive controls (IPC) to assess PCR inhibition; and calculating amplification efficiencies to monitor test performance. Additionally, a plasmid containing *Francisella tularensis* and *Borrelia burgdorferi* gene targets as well as non-tick DNA was constructed to use as a positive control for tularemia and Lyme disease PCR assays. This multi-target plasmid reduces the need for multiple positive controls and contains a tracer element to rule out contamination during amplification. These quality control procedures facilitate optimal diagnostic testing for vector borne diseases and help to maximize reliability of results.

Climatic Drivers of Seasonal Variation in *Culex tarsalis* Abundance

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INTRODUCTION

Culex tarsalis is a common vector of West Nile virus in California and typically found in rural areas (Reisen et al. 2004). Its abundance is in part dependent on climatic factors such as temperature and precipitation (Reeves 1990; Wegbreit and Reisen 2000). Increased abundance of *Culex tarsalis* has been associated with warmer temperatures and wetter conditions (Pecoraro et al. 2007; Reisen et al. 2008; Chuang et al. 2011). However, adult and larval survival may decline at climatic extremes, such as very high temperatures that can increase mortality (Hagstrum and Workman 1971; Walton et al. 1990; Reeves et al. 1994) or extreme amounts of water that can destroy larval habitat (Reisen et al. 1989). In this study, we tested the hypotheses that the responses of *Cx. tarsalis* to climatic factors would not be linear, and in particular, that the warmest temperatures and unusually wet years would be associated with reduced abundance

METHODS

This work was based on nearly 20,000 records of New Jersey light trap (NJLT; Mulhern 1942) collections from 26 Mosquito and Vector Control Association of California member districts (Table 1).

Climate Division	Agency (Abbreviation)	Spring Season Trapping Periods	Summer Season Trapping Periods
North/Central Coast	Alameda County MAD (ALCO)	227	229
North/Central Coast	Contra Costa MVCD (CNTR)	317	324
North/Central Coast	Marin-Sonoma MVCD (MARN)	462	469
North/Central Coast	Northern Salinas Valley MAD (NSAL)	439	441
North/Central Coast	San Mateo MAD (SANM)	283	285
North/Central Coast	Solano County MAD (SOLA)	299	295
Sacramento	Butte County MVCD (BUCO)	555	554
Sacramento	Burney Basin MAD (BURN)	89	90
Sacramento	Colusa MAD (CLSA)	57	57
Sacramento	Glenn County MVCD (GLEN)	184	181
Sacramento	Lake County VCD (LAKE)	40	41
Sacramento	Sacramento-Yolo MVCD (SAYO)	820	814
Sacramento	Shasta MVCD (SHAS)	288	292
Sacramento	Sutter-Yuba MVCD (SUYA)	823	840
Sacramento	Tehama County MVCD (TEHA)	276	284
San Joaquin	Consolidated MAD (CNSL)	501	507
San Joaquin	Delano MAD (DLNO)	236	240
San Joaquin	Delta VCD (DLTA)	335	336
San Joaquin	Fresno MVCD (FRNO)	166	165
San Joaquin	Fresno Westside MAD (FRWS)	415	414
San Joaquin	Kern MVCD (KERN)	754	756
San Joaquin	Merced County MAD (MERC)	428	427
San Joaquin	San Joaquin County MVCD (SJCM)	741	742
San Joaquin	Tulare MAD (TLRE)	201	201
San Joaquin	Turlock MAD (TRLK)	429	457
Southeast Desert	Coachella Valley MVCD (COAV)	516	517

Table 1. Mosquito and Vector Control Association of California agencies, by climate division, and the number of seasonal trap observations included in the study.

Each agency was assigned spatially (ESRI 2011) to one of five National Oceanic Atmospheric Administration climate divisions (Guttman and Quayle 1996): Sacramento, San Joaquin, Southeast Desert, North Coast and Central Coast. Sites operated

by agencies in the North Coast and Central Coast regions were clustered near the shared border of the two regions; therefore, we assigned them to a single North/Central Coast climate division, leaving us with a total of four divisions. Counts of female *Cx. tarsalis* from traps operated for up to two weeks were included in the study, and counts were aggregated separately for the spring and summer seasons of each year.

Climate data were matched to trap sites spatially by location and temporally by season. Daily temperature data from NASA's Terrestrial Observation and Prediction System (TOPS; Nemani et al. 2007) were averaged within each season to get mean winter, spring and summer minimum (nighttime low) and maximum (daytime high) temperatures. Precipitation records (PRISM Climate Group 2013) were also geospatially matched, then summed across the winter and spring seasons to represent the seasons' water conditions. Summer precipitation in California is typically negligible and was not considered in our models.

Results were analyzed using JAGS software (Plummer 2003) in R (R Core Team 2013), integrated using the R2jags package (Su and Yajima 2012). We created a Bayesian hierarchical model to explain seasonal trap counts adjusted for total trap-nights. Each model included precipitation and one of the temperature variables. We created one model using concurrent seasonal climate variables and one model using lagged climate variables from the prior season. Because of the minimal summer precipitation in California, spring precipitation was included as the water variable for both summer models.

RESULTS

There was a large degree of variation in the responses of spring *Cx. tarsalis* to changes in climate variables (Figure 1).

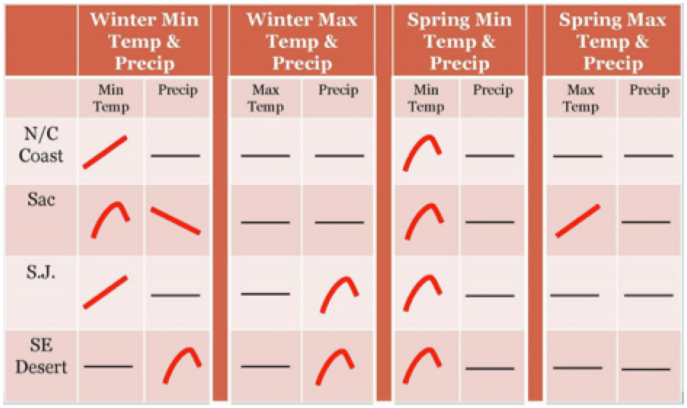

Figure 1. The shapes of the relationships of spring *Cx. tarsalis* with winter and spring climate variables. Relationships could be non-significant (flat line), linear (straight rising or falling line) or nonlinear (rising then falling over the range of the climate variable).

Most models showed only a credible relationship of abundance with temperature or water, but not both. Some nonlinear relationships implied that abundance increased along with the climate variable in question until a certain point, then began to decline in the presence of increasing temperature or precipitation. Temperature was a more consistent predictor of abundance (i.e., credible in more models) than precipitation. Spring abundance in the Sacramento climate division had credible relationships with both winter minimum temperature and precipitation (Figure 2).

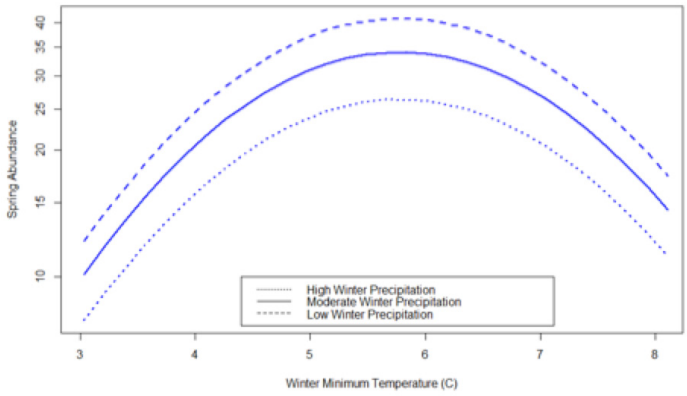

Figure 2. Model-based expectations for spring *Cx. tarsalis* abundance in the Sacramento climate division, as predicted by average daily winter minimum temperature, at the 25th (low), 50th (moderate) and 75th (high) precipitation percentiles.

Summer *Cx. tarsalis* abundance was less predictable from climate variables (i.e., fewer credible associations were identified) than in spring models with no credible associations found with summer temperatures or spring precipitation. However, our models did suggest that spring temperatures in the Central Valley (Sacramento and San Joaquin climate divisions) impact *Cx. tarsalis* abundance, with nonlinear relationships that were similar to those seen in spring models: abundance increased at warmer temperatures until a tipping point above which *Cx. tarsalis* counts were reduced.

The hierarchical nature of our model allowed us to look at variation in the associations between climate and abundance among agencies and sites. Sites within an agency were centered about that agency's coefficients, and agencies were then centered about their climate division coefficients (example shown in Figure 3).

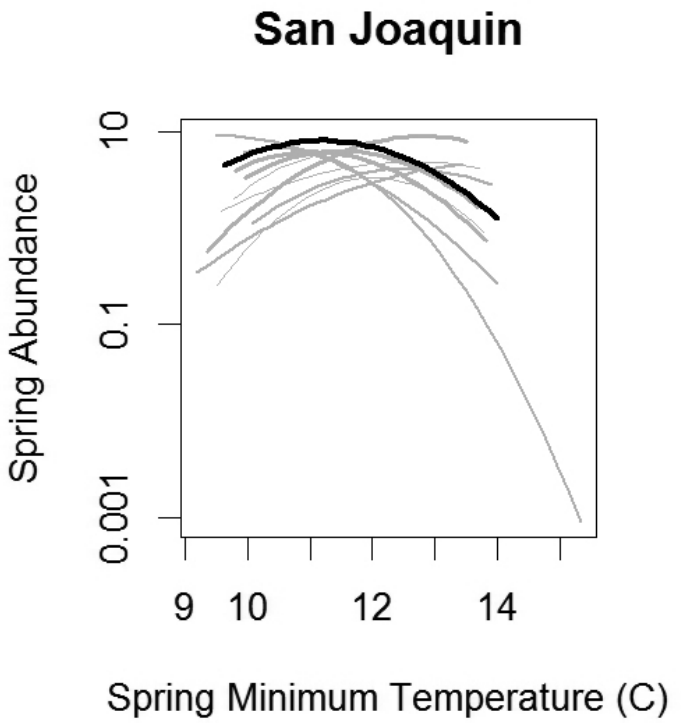

Figure 3. Example showing inter-agency variation in model-based expectations for summer *Cx. tarsalis* abundance within the San Joaquin climate division as predicted by spring minimum temperature. The divisional average is represented by the black line, and agencies within the climate division are represented by gray lines with line widths indicating relative sample sizes that dictate the contribution of each agency to estimates of the divisional average.

For each statistically significant climate division-level coefficient, we found that inter-agency variability was smaller than the inter-site variability, implying that there was a large amount of heterogeneity in climate responses at fine scales. However, the responses were more similar when compared across agencies within a climate division.

CONCLUSIONS

In summary, we found that spring *Cx. tarsalis* abundance was more predictable, based on climate, than summer abundance. In addition, for both spring and summer models, temperature was a better predictor of abundance than precipitation. This may be due to the fact that water availability in California is controlled largely by human management, such as agricultural irrigation and not by precipitation. In addition, spring temperatures were significant predictors of both spring and summer *Cx. tarsalis* population counts, particularly in the Central Valley, which could be useful when predicting future abundance a few months in advance. For most of the credible temperature models, relationships with mosquito counts suggested a point above which warmer temperatures correlated with declines in abundance that indicates that warmer weather is not always better for *Cx. tarsalis*. It is important to note that the "tipping point" temperatures identified here may also relate to broader climatic conditions associated with the high temperatures and do not necessarily imply a threshold for decreased survival based on temperature alone.

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Larvicidal Activity of Coffee and Caffeine Solutions against *Culex quinquefasciatus* L. (Diptera: Culicidae)

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ABSTRACT: Coffee is widely known for its stimulant properties due to the naturally-occurring caffeine found in coffee beans, and studies have shown that methylxanthines (such as caffeine) demonstrate pesticidal and insect anti-feeding activity. Coffee also contains many other chemical compounds found naturally in the raw coffee bean as well as compounds produced during the roasting process. It has been shown that coffee, decaffeinated coffee and pure caffeine solutions have all been effective as larvicidal agents in laboratory testing against *Aedes albopictus*. This study explores the larvicidal activity of caffeine, unroasted coffee, roasted coffee and decaffeinated roasted coffee against *Culex quinquefasciatus* mosquitoes.

INTRODUCTION

Caffeine is the most well known compound in coffee and it has demonstrated inherent pesticidal and anti-feeding qualities against many species of insects (Nathanson 2006). Specifically, caffeine and coffee solutions also have demonstrated activity against *Aedes aegypti* and *Ae. albopictus* in laboratory evaluations (Laranja 2003). However, coffee contains hundreds of compounds other than caffeine, some of which are found only in the raw unroasted coffee bean and some which are created solely during the roasting process. Some of the other chemicals in coffee include aromatic and nitrogenous compounds, alkaloids, melanoidins, and acids (Kreicbergs 2011). With the shift in public demand towards "green" pest control products, exploring the possibilities and uses of caffeine and coffee for its larvicidal potential may provide a solution that meets their demands for natural alternatives. This paper is an overview of preliminary exploratory trials in which caffeine, unroasted coffee, roasted coffee and decaffeinated roasted coffee with or without a piperonyl butoxide (PBO) synergist, were evaluated for larval toxicity.

MATERIALS AND METHODS

One pound bags of unroasted, French roast and decaffeinated French roast coffee beans were purchased from Jesus Mountain Coffee Company in Stockton, CA. The coffee beans were the Arabica variety, grown in Nicaragua. Caffeine of 99% purity was purchased from Alfa Aesar (Ward Hill, MA). Coffee beans were finely ground immediately before preparation of the stock solutions. Stock solutions were brewed with 10 g of ground coffee beans in 1000 mL of distilled water at a temperature of 90º C for 30 minutes on a stir plate. Solutions were allowed to cool overnight and then filtered through a stockinette sleeve to remove visible particulate matter. Glass beakers (250 mL) were filled with the appropriate amount of distilled water for their respective test groups and ten 2nd instar *Cx. quinquefasciatus* larvae from the San Joaquin susceptible laboratory colony were added to each beaker via a plastic disposable pipette. For the coffee groups, the stock coffee solutions were added to each beaker to obtain the desired concentrations and a total volume of 100 ml. If the treatment group was to contain PBO, 5 µl of PBO was added to the 100 ml solutions in each beaker to produce a 0.5 ppm concentration of PBO. If the treatment group was to contain caffeine, the appropriate amount of caffeine was measured by mass and added to 100 ml distilled water in each beaker, stirring gently until dissolved. A minimum of three replicates were run for each test group. Each beaker of larvae was fed daily with approximately 5 mg mosquito larvae food (mixture of ground Purina® Puppy Chow®, Purina® Rabbit Chow®, and Brewer's Yeast), and solutions were agitated daily to discourage film development on the surface. Larvae were observed daily for mortality, pupation and emergence; tests were carried out until the final living larvae died or pupated. Raw data was entered into POLO Plus for calculation of lethal dose.

RESULTS AND DISCUSSION

In these trials, caffeine showed larvicidal activity against *Cx. quinquefasciatus* larvae at all doses tested, and there was a positive correlation between caffeine and larval mortality, with increasing mortality as caffeine dose increased. Of the larvae reared in caffeine solutions, at 10 days there was an average of 54.8% mortality in the 6.25 mg group, 63.3% in the 12.5 mg group, 87.1% in the 25 mg group and 100% in the 50 mg group (Table 1).

	Larval mortality (%)			Pupation	Emergence
	3 Days	5 Days	10 Days	(%)	(%)
UTC	0.0	3.3	10.0	80.0	66.2
Caf. 6.25 mg	0.0	12.9	54.8	15.0	15.0
Caf. 12.5 mg	0.0	13.3	63.3	15.0	15.0
Caf. 25 mg	9.7	35.5	87.1	0.0	0.0
Caf. 50 mg	28.1	81.3	100.0	0.0	0.0
PBO	26.5	50.0	79.4	38.0	26.6
Caf. 6.25 mg+PBO	6.7	10.0	56.7	20.0	20.0
Caf. 12.5 mg+PBO	9.7	12.9	54.8	4.8	0.0
Caf. 25 mg+PBO	9.7	32.3	90.3	0.0	0.0
Caf. 50 mg+PBO	51.7	79.3	100.0	0.0	0.0

Table 1. Toxicity of caffeine and piperonyl butoxide (PBO) against *Culex quinquefasciatus* larvae.

The LD50 was calculated to be 5.41 mg and the LD90 was calculated to be 25.67 mg. With the addition of PBO, the average percent mortality of the larvae did not increase significantly. Pupation success and emergence in the caffeine solutions was just 15.0% in the 6.25 mg and 12.5 mg groups, and no pupation occurred in the 25 and 50 mg groups. There was 79.4% mortality observed in the PBO only group, indicating that PBO has some larvicidal properties on its own. The PBO, however, did not increase the effectiveness of the caffeine solutions; PBO addition to distilled water, however, eventually resulted in some larval death. Since the larvae were introduced to the test systems as 2nd instars, it is likely that the PBO is inhibiting the normal function of their cytochrome P450 system, affecting their metabolism and inhibiting their normal development into adult mosquitoes. Caffeine is a compound metabolized in mammals by enzymes in the cytochrome P450 system so it seemed likely that PBO would alter the activity of caffeine; however, this was not observed, and there was no significant difference between survival of larvae reared in pure caffeine solutions and caffeine solutions with PBO.

French roast coffee solutions also demonstrated larvicidal activity while additionally delaying the development of the larvae; trials had to be carried out 27 days, compared to 10 days for the caffeine trials, until all larvae had either died or pupated and emerged as adults. The two highest concentrations tested, 75% and 100%, resulted in 100% mortality, and the lower concentrations resulted in larval mortalities ranging from 34.6 to 76.5% (Table 2).

	Larval mortality (%)				Pupation	Emergence
	1 Day	7 Days	13 Days	27 Days	(%)	(%)
UTC	0.0	0.0	3.6	7.1	81.8	65.5
Roasted 6.25%	3.9	15.7	29.4	64.7	43.1	33.3
Roasted 12.5%	1.9	28.8	32.7	34.6	61.5	55.8
Roasted 25%	1.9	44.2	51.9	61.5	26.8	32.7
Roasted 50%	9.8	64.7	74.5	76.5	22.2	23.5
PBO	0.0	3.6	30.9	40.0	41.8	27.3
Roasted 6.25%+PBO	0.0	7.4	20.4	31.5	79.6	68.5
Roasted 12.5%+PBO	5.3	21.1	22.8	24.6	77.2	73.7
Roasted 25%+PBO	5.6	31.5	44.4	48.1	63.0	51.9
Roasted 50%+PBO	19.6	66.7	80.4	84.3	27.5	15.7

Table 2. Toxicity of French roast coffee solutions and piperonyl butoxide (PBO) against *Culex quinquefasciatus* larvae.

The LC_{50} for French roast coffee was calculated to be 8.09% and the LC_{90} was 71.76%. With the addition of PBO to the French roast coffee solutions, in comparison to the groups without PBO, larval mortality did not increase significantly and in some cases decreased. When added to coffee, it is possible that the PBO prevents the metabolism of the coffee particles, and perhaps some of the chemicals in roasted coffee are more toxic in a metabolized state (just as most organophosphates are pro-insecticides which become toxic once metabolized and activated by P450 enzymes of the insects). Although PBO did not increase the activity of the roasted coffee, there was again significant mortality in the PBO only group as was also observed during the caffeine trials.

Decaffeinated French roast coffee required a higher concentration than French roast coffee to observe 50% mortality (LC_{50} of 29.35%) (Table 3).

	Larval mortality (%)			Pupation	Emergence
	1 Day	3 Days	10 Days	(%)	(%)
UTC	0.0	0.0	6.7	81.0	71.4
Decaf 25%	0.0	33.3	43.3	50.0	45.0
Decaf 50%	0.0	46.7	76.7	14.6	14.6
Decaf 75%	3.3	70.0	93.3	6.7	6.7
Decaf 100%	16.7	86.7	100.0	13.3	0.0

Table 3. Toxicity of decaffeinated French roast coffee solutions against *Culex quinquefasciatus* larvae.

However, when targeting a higher overall kill, 59.74%, a concentration lower than that of the French roast coffee is sufficient to achieve an LC_{90}. Like the French roast, the decaffeinated French roast also reduced pupation and emergence and prevented emergence entirely at the 100% concentration. However, the decaffeinated French Roast did not appear to delay development with the final mortalities obtained by day ten.

The unroasted coffee solutions were most effective of the coffee preparations tested with an LC_{50} of only 5.18% and an LC90 of 47.82% (Table 4).

	Larval mortality (%)			Pupation	Emergence
	3 Day	7 Days	16 Days	(%)	(%)
UTC	3.3	6.7	10.0	80.0	77.5
Unroasted 25%	26.8	29.3	68.3	32.5	27.5
Unroasted 50%	40.0	60.0	93.3	10.0	6.7
Unroasted 75%	46.7	70.0	96.7	0.0	0.0
Unroasted 100%	60.0	66.7	100.0	0.0	0.0

Table 4. Toxicity of unroasted coffee solutions against *Culex quinquefasciatus* larvae.

Pupation success was reduced to <32.5% with no pupation occurring in the 75% and 100% concentrations. Adult emergence was also reduced to < 27.5%. The unroasted coffee solutions also delayed development, and these trials were carried out for 16 days. It was observed that the unroasted coffee solutions had a fresh, sharp, plant-like odor but developed a cloudy appearance and a pungent odor over time.

The caffeine and coffee solutions tested all showed some degree of larvicidal activity against *Cx. quinquefasciatus* larvae with the most effective being caffeine and unroasted coffee. This could be due to the different profiles of chemicals presented in different solutions, and the key chemicals responsible for the different toxicities could be identified by comparing their chemical profiles. Unroasted coffee and decaffeinated coffee were not tested with PBO due to time constraints and can be evaluated in the future. Other areas of interest to explore are the potential of DEF as a synergist for coffee and caffeine solutions as esterases have been observed as playing a role in the metabolism of caffeine in *Ae. aegypti* (Laranja 2003). Additional studies comparing the pH of the different solutions and testing other chemicals and carcinogenic compounds which are found in coffee (IARC 1991) could elucidate the role of specific chemicals in reducing mosquito populations.

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	Larval mortality (%)			Pupation	Emergence
	3 Day	7 Days	16 Days	(%)	(%)
UTC	3.3	6.7	10.0	80.0	77.5
Unroasted 25%	26.8	29.3	68.3	32.5	27.5
Unroasted 50%	40.0	60.0	93.3	10.0	6.7
Unroasted 75%	46.7	70.0	96.7	0.0	0.0
Unroasted 100%	60.0	66.7	100.0	0.0	0.0

Surveillance for Mosquito-borne Encephalitis Virus Activity in California, 2013

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ABSTRACT: In 2013 the California surveillance program for mosquito-borne encephalitis virus activity tested humans, horses, mosquitoes, sentinel chickens, dead birds and tree squirrels to detect arbovirus activity. West Nile virus was the only arbovirus detected by this program; 433 human infections were identified, and significant enzootic activity was detected among dead birds, mosquitoes and sentinel chickens.

INTRODUCTION

The California Arbovirus Surveillance program is a cooperative effort of the California Department of Public Health (CDPH), the University of California at Davis Center for Vectorborne Diseases (CVEC), 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 2013, the surveillance program components included the following:

(1) Diagnostic testing of specimens from human patients exhibiting symptoms of encephalitis, aseptic meningitis, acute flaccid paralysis, or with unexplained febrile illness of more than seven days.

(2) Diagnostic testing of specimens from horses exhibiting clinical signs of viral neurologic disease compatible with western equine encephalomyelitis virus (WEEV), WNV and other arboviruses as appropriate.

(3) Monitoring abundance and testing of mosquitoes for the presence of St. Louis encephalitis virus (SLEV), WEEV, WNV and other arboviruses as appropriate.

(4) Serological monitoring of sentinel chickens for SLEV, WEEV and WNV antibodies.

(5) Reporting and WNV diagnostic testing of dead birds and tree squirrels.

(6) Monthly reporting of arbovirus test results to ArboNET, the national arbovirus surveillance system.

(7) Weekly reporting of arbovirus activity in the CDPH Arbovirus Surveillance Bulletin and on the California WNV website: www.westnile.ca.gov.

(8) Data management and reporting through the web-based California Surveillance Gateway.

For the 6th consecutive year, West Nile virus was the only arbovirus detected by this system; a summary of WNV activity by county is in Table 1.

County	Humans[a]	Dead Birds[b]	Mosquito Pools	Sentinel Chickens	Dead Squirrels	Horses
Alameda	1	22	0	0	0	0
Alpine	0	0	0	0	0	0
Amador	0	0	0	0	0	0
Butte	24	42	33	57	0	0
Calaveras	0	0	0	0	0	0
Colusa	2	1	0	8	0	0
Contra Costa	5	68	13	7	1	1
Del Norte	0	0	0	0	0	0
El Dorado	1	0	0	0	0	0
Fresno	8	12	66	0	0	0
Glenn	9	8	16	9	0	0
Humboldt	1	1	0	0	0	0
Imperial	1	0	0	0	0	0
Inyo	0	0	0	0	0	0
Kern	30	2	181	12	0	2
Kings	1	12	78	0	0	0
Lake	0	10	62	6	0	0
Lassen	0	0	0	0	0	0
Los Angeles	172	313	417	151	3	0
Madera	4	7	16	2	0	0
Marin	4	7	0	1	0	0
Mariposa	0	0	0	0	0	0
Mendocino	0	2	0	0	0	0
Merced	0	39	8	21	0	0
Modoc	0	0	0	0	0	0
Mono	0	0	0	0	0	0
Monterey	0	0	0	0	0	0
Napa	1	2	0	0	0	0
Nevada	0	6	0	2	0	0
Orange	12	42	48	0	1	0
Placer	6	39	89	13	0	3
Plumas	0	0	0	0	0	0
Riverside	40	22	81	44	0	1
Sacramento	13	179	384	6	0	3
San Benito	0	0	0	1	0	1
San Bernardino	17	37	249	29	1	0
San Diego	0	9	0	0	0	0
San Francisco	1	0	0	0	0	0
San Joaquin	10	34	163	0	0	0
San Luis Obispo	0	1	1	0	0	0
San Mateo	0	0	0	0	0	0
Santa Barbara	1	0	0	0	0	0
Santa Clara	2	77	25	2	0	0
Santa Cruz	0	1	1	0	0	0
Shasta	1	38	15	21	1	0
Sierra	0	0	0	0	0	0
Siskiyou	0	0	0	0	0	0
Solano	1	15	1	12	0	0
Sonoma	0	37	5	2	0	0
Stanislaus	19	13	158	8	0	2
Sutter	10	16	61	35	0	0
Tehama	5	3	0	8	0	0
Trinity	0	1	0	0	0	0
Tulare	9	7	97	5	0	0
Tuolumne	0	1	0	0	0	0
Ventura	2	8	0	0	1	0
Yolo	6	106	246	12	0	0
Yuba	14	11	14	11	0	0
State Totals	**433**	**1,251**	**2,528**	**485**	**8**	**13**

[a]Includes asymptomatic infections detected through blood bank screening; no associated illness reported
[b]Does not include chronic infections

Table 1. Infections with West Nile virus in California, 2013

HUMAN DISEASE SURVEILLANCE

Serological diagnosis of human infection with WNV and other arboviruses was performed at the CDPH Viral and Rickettsial Disease Laboratory (VRDL) and 26 local public health laboratories. Local laboratories tested for WNV using an IgM or IgG immunofluorescent assay (IFA) and/or an IgM enzyme immunoassay (EIA). Specimens with inconclusive results were forwarded to the VRDL for further testing with a plaque reduction neutralization test (PRNT) or reverse transcriptase-polymerase chain reaction (RT-PCR). Additional WNV infections were identified through testing performed at blood donation centers.

A total of 379 symptomatic and 54 asymptomatic infections with WNV were identified in 2013, an 18% decrease in infections compared to 2012 (Table 2). Of the 379 clinical cases, 138 (36%) were classified as West Nile fever and 241 (64%) were classified as West Nile neuroinvasive disease (i.e. encephalitis, meningitis or acute flaccid paralysis). Case-patients were residents of 30 counties and 236 (62%) were male. Incidence was highest (31.7 cases per 100,000 persons) in Glenn County (Figure 1).

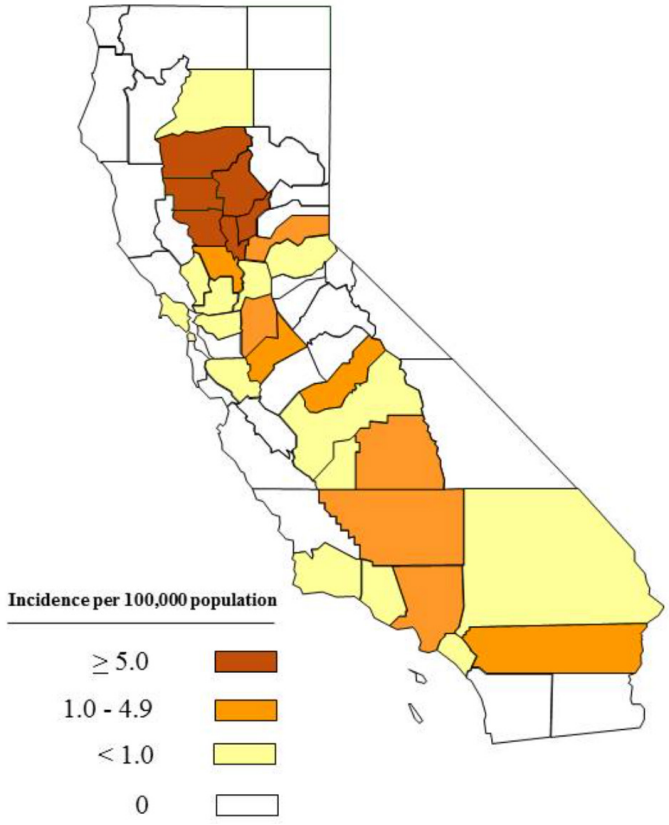

Figure 1. Incidence of human cases of West Nile virus in California, 2013.

The median ages for West Nile fever and neuroinvasive cases were 57 years (range, 5 to 92 years) and 56.5 years (range, 1 to 93 years), respectively. The median age of the 15 WNV-associated fatalities was 74 years (range, 55 to 92 years). Dates of symptom onset ranged from May 22 – November 7, with the peak occurring week 37 (September 8 – 14).

EQUINE SURVEILLANCE

Serum or brain tissue specimens from horses displaying neurological signs were tested for WNV at the California Animal Health & Food Safety Laboratory (CAHFS). West Nile virus infection was detected in 13 horses from 7 counties (Table 1). Five of the horses died or were euthanized as a result of their infection.

County	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	Ten-year incidence per 100,000 person-years
Alameda	0	1	1	0	1	0	1	0	2	0	< 0.1
Alpine	0	0	0	0	0	0	0	0	0	0	0.0
Amador	0	3	0	0	0	0	0	1	0	0	1.1
Butte	7	24	31	16	6	2	1	3	10	24	5.6
Calaveras	0	2	0	0	1	0	0	0	0	0	0.7
Colusa	0	2	4	2	1	0	0	0	3	2	6.5
Contra Costa	0	11	8	3	4	5	4	3	4	5	0.4
Del Norte	0	0	0	0	0	0	0	0	0	0	0.0
El Dorado	0	1	2	0	1	1	0	1	0	1	0.4
Fresno	11	59	11	17	3	13	23	9	24	8	1.9
Glenn	3	13	12	7	1	0	2	1	7	9	19.4
Humboldt	0	1	0	0	0	0	0	0	0	0	0.1
Imperial	1	1	1	3	0	0	0	0	1	0	0.4
Inyo	0	0	0	0	0	0	0	0	0	0	0.0
Kern	59	67	49	140	2	18	15	18	25	25	4.9
Kings	0	32	1	7	2	3	1	1	3	1	3.4
Lake	1	0	2	0	0	0	0	0	1	0	0.6
Lassen	1	0	0	0	0	0	0	0	0	0	0.3
Los Angeles	306	40	13	36	156	20	4	58	163	151	1.0
Madera	0	18	0	2	0	1	7	2	3	3	2.4
Marin	0	0	1	0	0	0	0	0	0	2	0.12
Mariposa	0	0	0	0	0	0	0	0	0	0	0.0
Mendocino	0	0	0	2	0	0	0	0	0	0	0.2
Merced	1	25	4	4	1	4	1	1	13	0	2.1
Modoc	0	0	2	0	0	0	0	0	0	0	2.1
Mono	0	0	1	0	0	0	0	0	0	0	0.7
Monterey	0	0	0	0	0	1	0	0	1	0	< 0.1
Napa	0	0	1	1	0	0	0	0	0	1	0.2
Nevada	0	4	1	0	0	0	0	0	0	0	0.5
Orange	62	17	6	9	71	4	1	10	42	10	0.8
Placer	1	35	8	4	6	0	3	1	12	6	2.1
Plumas	0	1	0	0	0	0	0	0	0	0	0.5
Riverside	109	103	4	17	62	3	0	7	19	35	1.6
Sacramento	3	163	15	25	13	0	12	4	29	11	1.9
San Benito	0	0	0	0	0	0	0	0	0	0	0.0
San Bernardino	187	33	3	4	36	2	5	4	33	13	1.5
San Diego	2	1	1	15	35	4	0	0	1	0	0.2
San Francisco	0	2	0	0	0	0	1	0	1	1	< 0.1
San Joaquin	2	34	8	10	12	10	6	5	13	8	1.6
San Luis Obispo	1	0	1	0	0	0	0	0	0	0	0.1
San Mateo	0	1	0	0	0	0	0	0	0	0	< 0.1
Santa Barbara	0	2	0	0	1	0	0	1	0	1	0.1
Santa Clara	1	5	5	4	1	0	0	1	0	2	0.1
Santa Cruz	0	0	0	0	0	0	0	1	0	0	< 0.1
Shasta	5	1	4	9	1	0	0	0	1	1	1.2
Sierra	0	0	0	0	0	0	0	0	0	0	0.0
Siskiyou	0	0	0	0	0	0	0	0	0	0	0.2
Solano	0	5	8	1	1	0	0	0	2	1	0.4
Sonoma	0	1	0	1	0	0	0	0	0	0	< 0.1
Stanislaus	0	84	11	21	17	14	12	11	26	17	4.1
Sutter	0	9	12	3	0	0	0	0	8	10	4.4
Tehama	10	4	6	4	4	0	0	1	4	5	6.0
Trinity	0	0	0	0	0	0	0	0	0	0	0.0
Tulare	3	56	6	10	5	4	12	11	7	5	2.6
Tuolumne	0	1	0	0	0	0	0	0	0	0	0.2
Ventura	2	1	3	1	0	0	0	0	7	2	0.2
Yolo	1	11	27	2	1	2	0	0	10	6	2.9
Yuba	0	6	5	0	0	1	0	3	4	13	4.4
Total WNV Cases	**779**	**880**	**278**	**380**	**445**	**112**	**111**	**158**	**479**	**379**	**1.1**
Asymptomatic Infections	51	55	14	29	53	17	20	18	48	54	
Total WNV infections	**830**	**935**	**292**	**409**	**498**	**129**	**131**	**176**	**527**	**433**	**1.1**

Table 2. Reported West Nile virus human cases by county of residence and year in California, 2004 – 2013.

MOSQUITO SURVEILLANCE

A total of 777,619 mosquitoes (29,363 pools) collected in 37 counties were tested at the University of California, Center for Vectorborne Diseases (CVEC) or at one of seven local agencies by a real-time (TaqMan) reverse transcriptase-polymerase chain reaction (qRT-PCR) for SLEV, WEEV and/or WNV viral RNA. Four local agencies also tested an additional 13,425 mosquitoes (615 pools) for WNV using a commercial rapid assay-RAMP® (Rapid Analyte Measurement Platform, Response Biomedical Corp) (Table 3).

County	Agency	No. mosquitoes tested[b]	No. mosquito pools tested	WNV + pools	No. flocks	No. chickens[c]	No. WNV positive flocks	WNV + sera
Alameda	Alameda Co. MAD	1,835	60	0	3	21	0	0
Alpine		0			0			
Amador		0			0			
Butte	Butte Co. MVCD	7,069	147	33	7	77	7	57
Calaveras	Saddle Creek CSD	0			1	10	0	0
Colusa	Colusa MAD	0			1	10	1	8
Contra Costa	Contra Costa MVCD	12,729	453	13	5	54	3	7
Del Norte		0			0			
El Dorado		0			0			
Fresno	Consolidated MAD	17,773	520	42	0			
Fresno	Fresno MVCD	2,210	50	5	0			
Fresno	Fresno Westside MAD	10,156	266	19	0			
Glenn	Glenn Co. MVCD	2,100	42	16	1	11	1	9
Humboldt		0			0			
Imperial		0			0			
Inyo	Owens Valley MAP	88	2	0	0			
Kern	Delano MAD	1,694	56	17	2	20	2	12
Kern	Kern MVCD	22,838	543	97	0			
Kern	Westside MVCD	8,767	205	67	0			
Kings	Consolidated MAD	22	2	1	0			
Kings	Kings MAD	12,571	424	77	0			
Lake	Lake Co. VCD	15,223	427	62	2	14	1	6
Lassen		0			0			
Los Angeles	Antelope Valley MVCD	1,000	35	5	10	60	7	25
Los Angeles	Greater LA Co. VCD	95,655	2,396	391	7	70	7	50
Los Angeles	Long Beach VCP	9,217	207	5	3	30	3	10
Los Angeles	Los Angeles Co. West VCD	13,524	320	15	18	108	10	51
Los Angeles	San Gabriel Valley MVCD	3,345	197	1	10	40	4	15
Madera	Madera Co. MVCD	1,849	88	16	2	12	2	2
Marin	Marin-Sonoma MVCD	2,764	534	0	1	6	1	1
Mariposa		0			0			
Mendocino		0			0			
Merced	Merced Co. MAD	1,605	136	8	7	42	6	21
Merced	Turlock MAD	1,081	34	0	0			
Modoc		0			0			
Mono		0			0			
Monterey	North Salinas Valley MAD	720	16	0	2	21	0	0
Napa	Napa Co. MAD	2,401	141	0	0			
Nevada	Nevada Co. Agric. Dept.	0			2	20	1	2
Orange	Orange Co. VCD	40,892	1,803	48	0			
Placer	Placer Co. MVCD	31,705	1,967	89	4	24	4	13
Plumas		0			0			
Riverside	Coachella Valley MVCD	69,763	2,033	43	10	100	4	25
Riverside	Northwest MVCD	8,436	300	10	6	18	3	6
Riverside	Riverside Co. EH	27,940	671	28	5	60	3	13
Sacramento	Sacramento-Yolo MVCD	75,458	4,558	384	3	25	3	6
San Benito	San Benito Co. Agric Dept.	0			1	10	1	1
San Bernardino	San Bernardino Co. VCP	21,863	680	10	8	88	5	20
San Bernardino	West Valley MVCD	33,605	1,582	239	8	18	5	9
San Diego	San Diego Co. EH	7,808	202	0	2	20	0	0
San Francisco	Presidio Trust	183	7	0	0			
San Joaquin	San Joaquin Co. MVCD	35,643	1,676	163	0			
San Luis Obispo	Santa Barbara Co. MVMD	1,454	38	1	0			
San Mateo	San Mateo Co. MVCD	3,101	174	0	2	20	0	0
Santa Barbara	Santa Barbara Co. MVMD	17,474	401	0	5	50	0	0
Santa Clara	Santa Clara Co. VCD	9,719	631	25	7	49	2	2
Santa Cruz	Santa Cruz Co. MVCD	1,228	54	1	2	20	0	0
Shasta	Burney Basin MAD	0			2	12	0	0
Shasta	Shasta MVCD	7,153	262	15	5	40	5	21
Sierra		0			0			
Siskiyou		0			0			
Solano	Solano Co. MAD	2,879	75	1	3	38	2	12
Sonoma	Marin-Sonoma MVCD	9,836	908	5	3	18	1	2
Stanislaus	East Side MAD	9,948	357	16	2	16	2	8
Stanislaus	Turlock MAD	39,490	1,166	142	0			
Sutter	Sutter-Yuba MVCD	10,722	336	61	6	42	6	35
Tehama	Tehama Co. MVCD	0			3	30	3	8
Trinity		0			0			
Tulare	Delano MAD	954	24	5	1	10	1	3
Tulare	Delta VCD	16,198	538	78	1	10	1	2
Tulare	Kings MAD	273	11	2	0			
Tulare	Tulare MAD	2,611	80	12	0			
Tuolumne		0			0			
Ventura	City of Moorpark VC	0			1	8	0	0
Ventura	Ventura Co. EH	1,534	34	0	4	40	0	0
Yolo	Sacramento-Yolo MVCD	51,921	1,997	246	3	25	3	12
Yuba	Sutter-Yuba MVCD	3,017	112	14	2	14	2	11
Total		**791,044**	**29,978**	**2,528**	**183**	**1,431**	**112**	**485**

[a]No mosquito pools or sentinel chickens were positive for SLEV or WEEV in 2013.
[b]Tested by University of California at Davis Center for Vectorborne Diseases or local mosquito/vector control agency.
[c]Reflects planned standard number of chickens per flock. Actual number may vary due to mortality or replacement of seroconverted chickens.

Table 3. Mosquitoes and sentinel chickens tested for St. Louis encephalitis[a], western equine encephalomyelitis[a], and/or West Nile viruses in California, 2013.

West Nile virus was detected in 2,528 mosquito pools from 27 counties; 2,509 were positive by RT-PCR and 19 were positive by RAMP only (Tables 1 and 3). Statewide, the minimum infection rate (MIR), calculated as 1,000 times the number of infected mosquito pools divided by the number of mosquitoes tested, of WNV in all mosquitoes tested was 3.2; the MIR was highest (8.7) in Madera County (Figure 2, Table 3). Since 2004, the MIR of WNV in California has ranged from a low of 1.1 to a high of 3.2 (Figure 3).

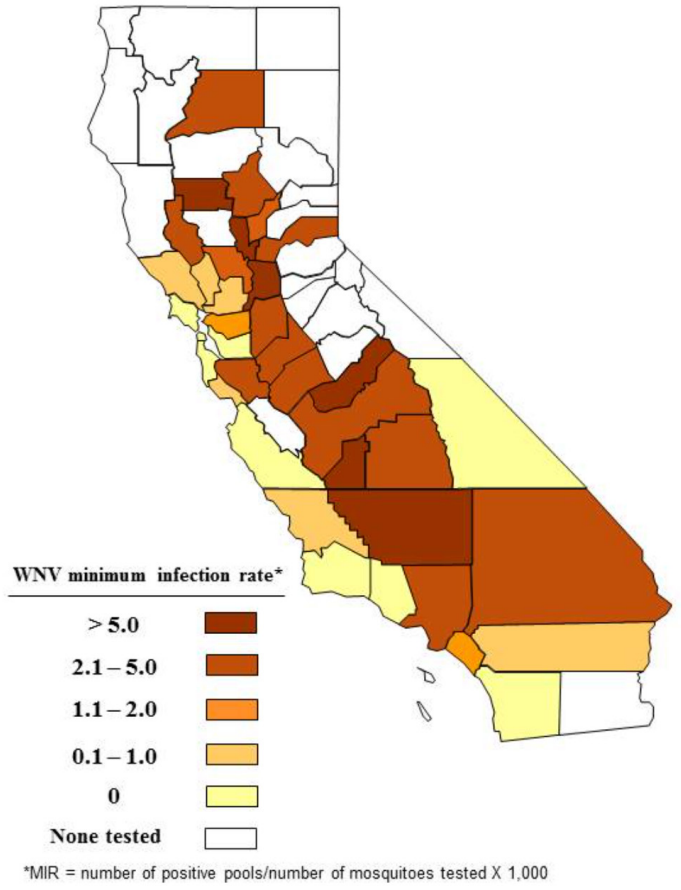

Figure 2. West Nile virus minimum infection rate of mosquitoes in California, 2013.

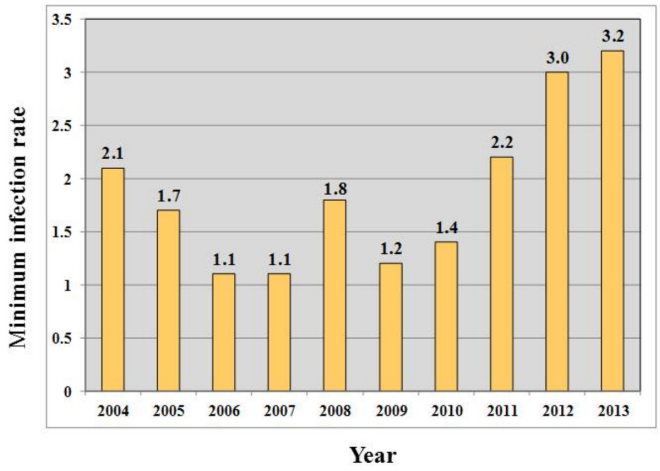

Figure 3. Annual minimum infection rate of West Nile virus in mosquito pools in California, 2004 – 2013.

West Nile virus was identified from six *Culex* species (*Cx. erythrothorax*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. stigmatosoma*, *Cx. tarsalis* and *Cx. thriambus)* and one other species (*Anopheles freeborni*) (Table 4). Positive mosquito pools were collected from May 8 – November 27, with the peak occurring in August. The first and last detections of WNV in mosquitoes were from *Cx. quinquefasciatus* pools collected in Tulare and San Bernardino counties, respectively.

***Culex* species**	**Pools**	**No. mosquitoes**	**WNV +**	**MIR[a]**
Cx. boharti	3	3	0	0.0
Cx. erythrothorax	1,634	54,921	20	0.4
Cx. pipiens	7,349	125,339	523	4.2
Cx. quinquefasciatus	8,098	247,833	935	3.8
Cx. restuans	2	50	0	0.0
Cx. stigmatosoma	622	5,345	23	4.3
Cx. tarsalis	11,610	341,303	1,021	3.0
Cx. territans	1	7	0	0.0
Cx. thriambus	101	184	1	5.4
unknown	19	72	1	13.9
All *Culex*	**29,439**	**775,057**	**2,524**	**3.3**

***Anopheles* species**	**Pools**	**No. mosquitoes**	**WNV +**	**MIR[a]**
An. franciscanus	7	114	0	0.0
An. freeborni	57	2,673	1	0.4
An. hermsi	50	1,435	0	0.0
All *Anopheles*	**114**	**4,222**	**1**	**0.2**

***Aedes* species**	**Pools**	**No. mosquitoes**	**WNV +**	**MIR[a]**
Ae. aegypti	13	214	0	0.0
Ae. albopictus	1	12	0	0.0
Ae. dorsalis	30	568	0	0.0
Ae. melanimom	18	654	0	0.0
Ae. squamiger	6	137	0	0.0
Ae. taeniorhynchus	2	61	0	0.0
Ae. vexans	16	700	0	0.0
Ae. washinoi	21	922	0	0.0
All *Aedes*	**107**	**3,268**	**0**	**0.0**

Other species	**Pools**	**No. mosquitoes**	**WNV +**	**MIR[a]**
Coquillettidia perturbans	1	50	0	0.0
Culiseta incidens	205	4,479	0	0.0
Culiseta inornata	25	229	0	0.0
Culiseta particeps	34	1,089	0	0.0
Unknown	53	2,650	3	1.1
All other	**318**	**8,497**	**3**	**0.4**

[a] Minimum Infection Rate (MIR) = (No. pools positive/No. mosquitoes tested) X 1000

Table 4. Mosquitoes tested for West Nile virus in California, 2013.

Aedes aegypti, a primary vector of dengue and chikungunya viruses, was detected for the first time this year in Fresno, Madera and San Mateo counties; *Ae. albopictus* has been detected in Los Angeles County since 2011. Accordingly, CVEC began testing both *Ae. aegypti* and *Ae. albopictus* for dengue and chikungunya viruses, in addition to WNV. A total of 214 *Ae. aegypti* and 12 *Ae. albopictus* tested negative for all three viruses.

CHICKEN SEROSURVEILLANCE

Forty local mosquito and vector control agencies in 34 counties maintained 183 sentinel chicken flocks (Table 3). Blood samples were collected from chickens every other week and tested for antibodies to SLEV, WNV and WEEV by an EIA at the CDPH Vector-Borne Disease Section Laboratory (VBDS) or at one of two local agencies. Positive samples were confirmed at the VBDS laboratory by IFA or western blot. Samples with inconclusive results were tested by PRNT at the VRDL.

Out of 16,849 chicken blood samples that were tested, 485 seroconversions to WNV were detected among 112 flocks in 26 counties (Tables 1 and 3, Figure 4). Statewide, 34% of sentinel chickens seroconverted to WNV. Since 2004, the percentage of WNV seroconversions in chickens has ranged from a low of 14%

to a high of 34% (Figure 5). Seroconversions occurred from May 29 – November 14, with the peak occurring in August. The first and last WNV seroconversions were detected in Los Angeles and Riverside counties, respectively.

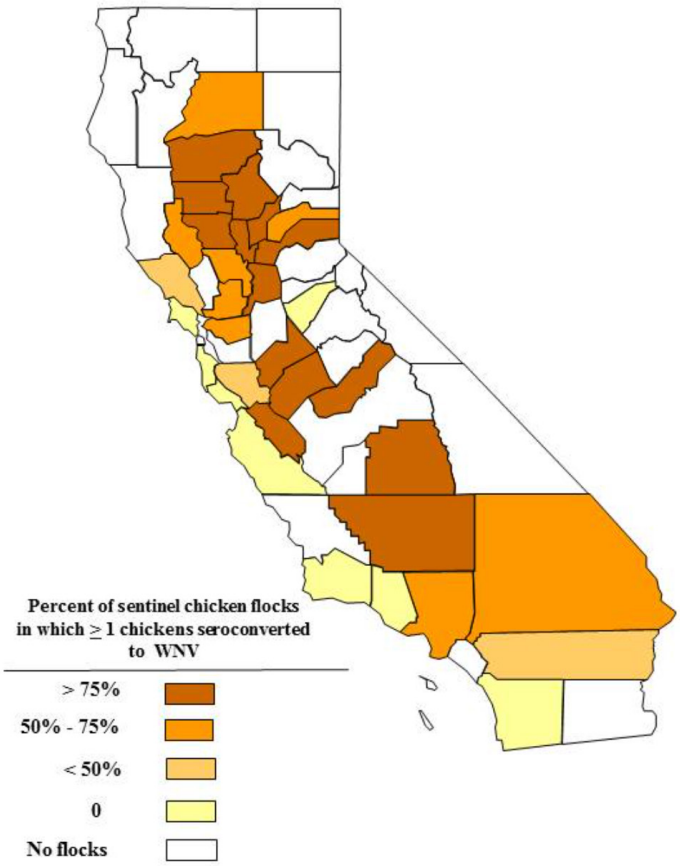

Figure 4. West Nile virus seroconversions in sentinel chicken flocks in California, 2013.

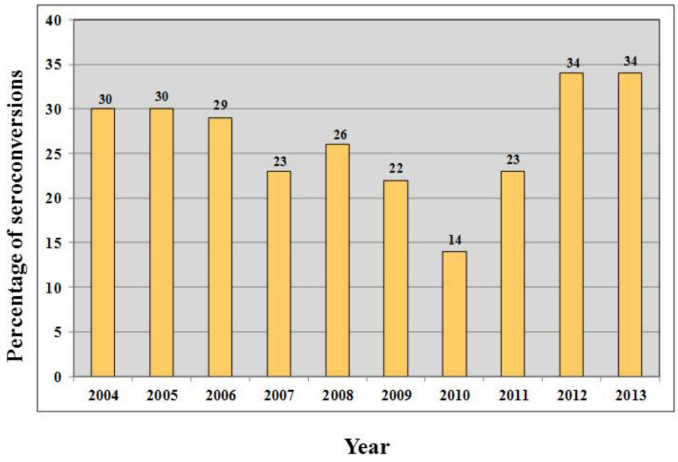

Figure 5. Annual percentage of sentinel chicken seroconversions to West Nile virus in California, 2004 – 2013.

DEAD BIRD AND DEAD SQUIRREL SURVEILLANCE

The WNV hotline and website received 14,727 dead bird reports from the public in 57 counties (Table 5). Oral swab or tissue samples taken from dead bird carcasses were tested either at CVEC by RT-PCR, at CAHFS by RT-PCR or IHC, or at one of 13 local agencies by RT-PCR or RAMP. Of the 3,306 carcasses deemed suitable for testing, WNV was detected in 1,416 (43%) carcasses from 40 counties; 1,251 tested as acute infections (i.e., recent within current surveillance season) from 39 counties, and 156 tested as chronic infections (i.e., exposed at an undeterminable time in the past, Anderson et al. 2012) from 32 counties (Tables 1 and 5, Figure 6). Since 2004, the prevalence of WNV positive dead birds has ranged from a low of 19% to a high of 56% (Figure 7). Of the acute infections, 1,145 were confirmed positive by RT-PCR, 105 by RAMP and 1 by IHC. Positive birds were collected from February 4 – November 26, with the peak occurring in July. The first and last positive dead birds were American Crows from Los Angeles and Santa Clara counties, respectively.

County	Reported	Tested	Positive-acute	Positive-chronic
Alameda	516	77	22	6
Alpine	3	0		
Amador	23	1	0	0
Butte	390	82	42	5
Calaveras	23	1	0	0
Colusa	24	2	1	0
Contra Costa	1,396	123	68	6
Del Norte	0			
El Dorado	145	25	0	1
Fresno	412	67	12	7
Glenn	23	10	8	0
Humboldt	25	3	1	0
Imperial	2	0		
Inyo	8	1	0	0
Kern	148	21	2	2
Kings	93	22	12	2
Lake	117	24	10	0
Lassen	2	0		
Los Angeles	2,640	522	313	34
Madera	65	13	7	3
Marin	266	33	7	1
Mariposa	7	2	0	0
Mendocino	34	4	2	0
Merced	302	60	39	3
Modoc	4	0		
Mono	6	1	0	0
Monterey	67	14	0	1
Napa	93	4	2	0
Nevada	86	17	6	1
Orange	215	318	42	0
Placer	358	186	39	9
Plumas	7	0		
Riverside	467	42	22	2
Sacramento	1,495	459	179	19
San Benito	27	3	0	0
San Bernardino	452	100	37	4
San Diego	137	107	9	0
San Francisco	72	7	0	0
San Joaquin	417	91	34	4
San Luis Obispo	79	17	1	2
San Mateo	317	62	0	4
Santa Barbara	47	9	0	1
Santa Clara	1,046	234	77	14
Santa Cruz	169	19	1	2
Shasta	118	73	38	4
Sierra	1	0		
Siskiyou	1	0		
Solano	336	26	15	0
Sonoma	486	74	37	3
Stanislaus	379	38	13	4
Sutter	175	33	16	1
Tehama	37	7	3	1
Trinity	6	1	1	0
Tulare	161	35	7	2
Tuolumne	14	3	1	0
Ventura	234	38	8	2
Yolo	463	175	106	5
Yuba	91	20	11	1
Totals	**14,727**	**3,306**	**1,251**	**156**

[a]Tested by University of California at Davis Center for Vectorborne Diseases or local mosquito/vector control agency

Table 5. Dead birds reported, tested[a] and positive for West Nile virus in California, 2013.

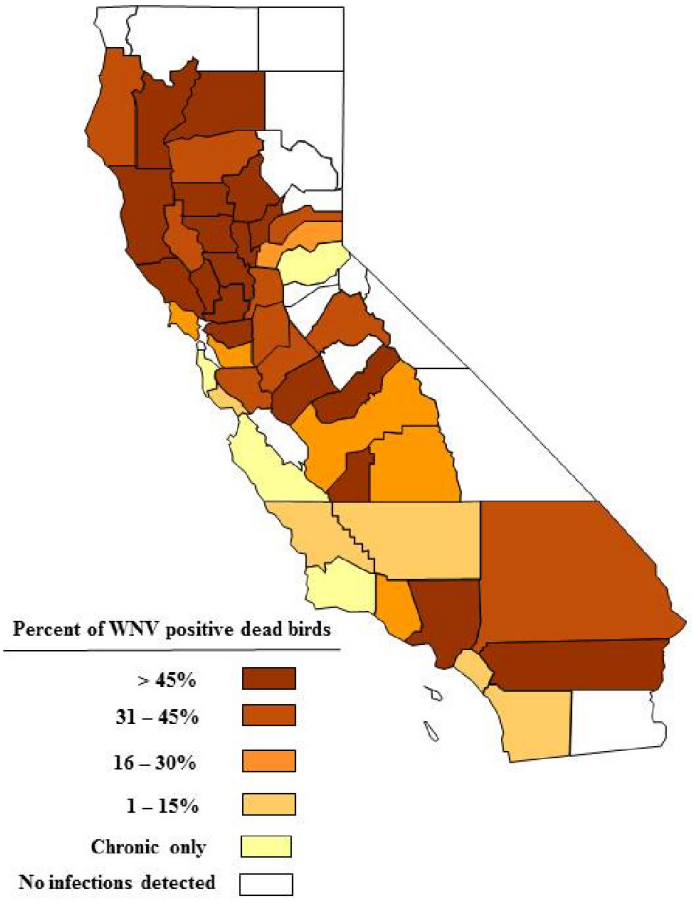

Figure 6. West Nile virus infection prevalence in dead birds in California, 2013.

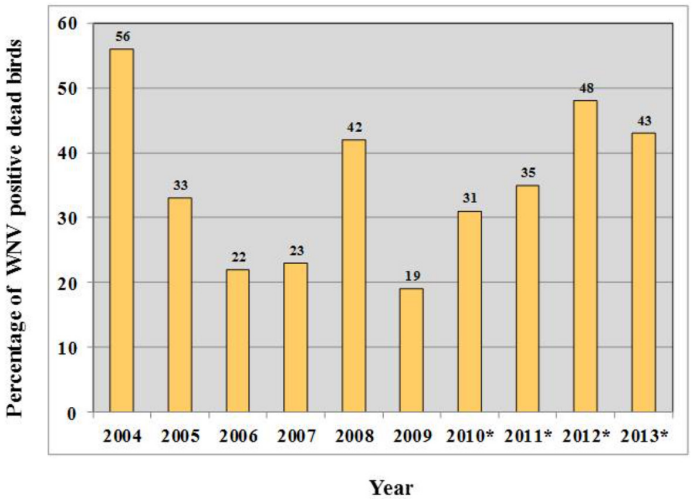

Figure 7. Annual percentage of WNV infection in dead birds in California, 2004 – 2013. Data include chronic and recent infections.

A total of 461 dead squirrels were also reported through the WNV Hotline; 97 squirrel carcasses were tested and WNV RNA was detected by RT-PCR in 8 (8.2%) carcasses from seven counties (Table 1). These included seven fox squirrels (*Sciurus niger*) and one western gray squirrel (*S. griseus*).

SUMMARY

In 2013, 433 human WNV infections, including 15 fatalities, were reported from 33 counties, a decrease of 18% compared to 2012 (Tables 1 and 2). The statewide case incidence per 100,000 persons was 1.0. The highest number of cases was reported from Los Angeles County with 172 cases, although case rates were greatest within the northern Sacramento Valley region (Figure 1, Table 2). The proportion of WNND cases among all reported cases in California was 64%; in comparison, 51% of nationally reported WNV cases were classified as neuroinvasive disease (U.S. Centers for Disease Control and Prevention).

Ecological surveillance continued to detect high levels of enzootic WNV activity throughout the state. Although the number of reported human cases was only the 5th highest in the last 10 years (Table 2), the statewide minimum infection rate of WNV in mosquitoes was higher in 2013 than in any other surveillance year (Figures 3 and 5). Additionally, the percentage of sentinel chicken seroconversions in 2013 was equivalent to 2012, both years being all-time highs (Figure 5). There was a slight decrease in the prevalence of WNV in dead birds, although this may have been due to mid-season funding reductions in the bird surveillance program, decreasing the number and species of birds that were tested. Regardless, the prevalence of WNV in dead birds was still the third highest detected between 2004 and 2013 (Figure 7). These data, along with the high proportion of neuroinvasive disease cases, suggest that there is significant underreporting of WNV illness in California.

Although the ecological surveillance data documented WNV activity throughout the year, most WNV detections occurred from June through October, with peak activity in August. Human cases followed the ecological indicators, occurring primarily during the months of July through October, and highlight the importance of environmental surveillance to determine the level of risk for WNV transmission and to direct mosquito control efforts. West Nile virus is now endemic in California, and for the 6th consecutive year, the only arbovirus detected by this system.

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Environmental Determinants of *Anopheles gambiae* Aquatic Habitat Productivity

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ABSTRACT: Recent studies have suggested that land use changes such as deforestation strongly enhance the productivity of malaria vectors, and thus malaria transmission. However, the mechanism for habitat productivity enhancement by deforestation is not clear. The present study examines a metagenomics analysis of *Anopheles gambiae* mosquitoes to determine the impacts of deforestation on bacterial and algal diversity of the larval habitats and subsequent habitat productivity. The metagenomic analysis was based on high-throughput next-generation pyrosequencing of microbial 16S and 23S DNA directly extracted from field-collected water and mosquito specimens and provided a comprehensive view of microbial community diversity. Results of habitat productivity in different land use scenarios demonstrated a significant effect of land cover on larval development and adult mosquito productivity. Metagenomic analysis of algal communities by pyrosequencing of 23S DNA revealed a selective feeding on green algae species (Chlorophyta), which were far more abundant in the larval gut when compared to the available potential resources. This study provided strong evidence that algal community shifts resulting from deforestation enhanced habitat productivity for *An. gambiae* mosquitoes. The implications of microbial community dynamics in the scope of malaria vector control are discussed.

INTRODUCTION

Over the past three decades, as a result of increasing population and agricultural development, the western Kenya highlands have undergone considerable environmental changes (Himeidan and Kweka 2012). Environmental changes such as temperature, humidity and habitat availability can significantly affect mosquito abundance, which in turn affects malaria transmission intensity (Minakawa et al. 2005). Past studies have suggested that land use changes, such as deforestation, strongly enhance the productivity of malaria vectors and thus malaria transmission (Walsh et al. 1993). This is because deforestation exposes aquatic habitats to sunlight, resulting in increased water temperatures. Further, exposure to sunlight may induce changes in the microbial communities that mosquito larvae use for nutrition. However, there is little knowledge of how microbial communities in aquatic habitats are regulated by exposure to sunlight and organic nutrients, and how mosquito larvae respond to microbial community changes in larval habitats (Merritt et al. 1992, Merritt et al. 1992).

In the current study, we examined how chemical and physical characteristics of larval habitats under variable canopy coverage affect larval habitat productivity and assessed the importance of canopy coverage independent of temperature. The mechanisms of larval population regulation have recently received renewed interest (Himeidan and Kweka 2012). We hypothesize that *Anopheles gambiae* are largely regulated by physical habitat characteristics and the resultant availability of nutritional resources. We utilized two additional experiments to test the hypothesis that reduced canopy coverage, with or without variation in temperature, would lead to increased larval habitat productivity due to the resulting increase of photosynthetic microbes that *An. gambiae* use for food. A third experiment was conducted to test the effects of food addition in all experimental treatments. One important technique we used was the metagenomic analysis of the larval habitats and *An. gambiae* mosquito larvae, which offers a comprehensive view of microbial communities (Wooley et al. 2010, Wang et al. 2011). This approach was based on the high-throughput next-generation pyrosequencing of microbial 16S and 23S DNA extracted directly from field-collected water and mosquito specimens, thus avoiding the bias associated with inability to culture some bacteria or algae required by the traditional methods for detecting and identifying microbes (Wooley et al. 2010). The metagenomics approach provides a more powerful tool to test the hypothesis that larval gut contents would demonstrate preferential feeding by larvae upon the photosynthetic microbes in the surface microlayer of their aquatic habitats.

METHODS

We established an array of artificial habitats, or microcosms, in Iguhu, Kakamega District, western Kenya (1,500 m). Three sub-sites were chosen for deforested, semi-forested and forested habitats (un-shaded, partially shaded and heavily shaded, respectively). Eight microcosms were distributed at each sub-site, and one hundred first instar larvae were added into each. Habitats were monitored daily for survivorship and life table data (instar proportions); adult wing length was also measured in newly emerged mosquitoes. Temperature was logged hourly throughout the duration of the experiment. Pooled 50 ml surface water samples (< 2 mm in depth) were taken weekly from each sub-site in triplicate using a needle and syringe, centrifuged at 10,000 *g* for 20 minutes, and the resultant pellet was used for DNA extraction. For each canopy coverage level, triplicate samples of eight larval guts were removed under a dissecting microscope using sterilized dissection devices.

Temperature-controlled microcosms were constructed using perforated baskets lined with thin (1 mil) polyethylene

plastic sheeting to allow for maximum heat exchange between microcosms and pools. Replicates were assigned shade or sunlit treatment to mimic forested and deforested habitats. Shades were constructed with wooden frames and 1.5 mil opaque black plastic. All microcosms were placed into approximately 4 x 2 x 0.5 meter pools that were filled with stream water to match the water level in the microcosms.

DNA was extracted from larval guts and water sample pellets using the UltraClean® Soil DNA Extraction Kit (Mo Bio Laboratories, Carlsbad, CA, USA). To characterize the bacterial communities, the variable 16S rDNA region V1-3 was amplified. Algal communities were characterized using the Domain V of the 23S plastid rRNA gene (Sherwood et al. 2008). 16S PCR, 23S PCR and pyrosequencing were conducted at Research and Testing Laboratory (Lubbock, TX) using the Roche Titanium 454 FLX pyrosequencing platform as described previously (Callaway et al. 2010).

Food supplementation with approximately 100 mg of TetraMin Fish Food (Spectrum Brands, Inc., Madison, WI, USA) was added daily to eight additional replicates of all treatments in experiment one and two.

For all experiments, differences in *An. gambiae* larval survivorship, larval development time and adult wing length among habitats were compared using analysis of variance (ANOVA) (JMP Statistical Discovery Software, version 5.1; SAS Institute, Cary, NC). Stepwise regression was used to analyze the effects of environmental variables on larval survivorship and development time, and indirect effects were analyzed using path analysis and performed by using SPSS Amos (Amos Development Corporation, Meadville, PA 16335, USA). For pyro-sequencing data, bacterial species richness and diversity analyses were conducted using the program MOTHUR (Schloss et al. 2009). We used MOTHUR to generate matrices of genetic distance and group sequences into operational taxonomic units (OTUs); for each treatment, we used MOTHUR to calculate Chao1 estimates of richness and ACE estimates of diversity (Wooley et al. 2010).

RESULTS AND DISCUSSION

The current study examined the relationship between habitat vector productivity, microbial food resources and deforestation. Bacterial and algal communities are dependent on temperature in water and soil, but drastic changes in microbial communities, particularly photosynthetic microbes, also result from sunlight proliferation to the habitat surface as a consequence of disforestation. The semi-natural, temperature and light variable treatments confirm previous findings that larval success is limited by canopy coverage (Afrane et al. 2007). Temperature-controlled experiments were designed to control water temperatures while subjecting habitats to variable sunlight levels. Despite temperatures conducive to high larval survivorship in the shaded, temperature-controlled habitats, pupation rates were low, presumably due to the lack of available food resources in the sunlight poor environment (Fig. 1). These conclusions are further supported by the results of food supplementation. When shaded habitats were supplemented with food, larval performance was nearly identical when compared to food supplemented sunlit habitats (Figure 1).

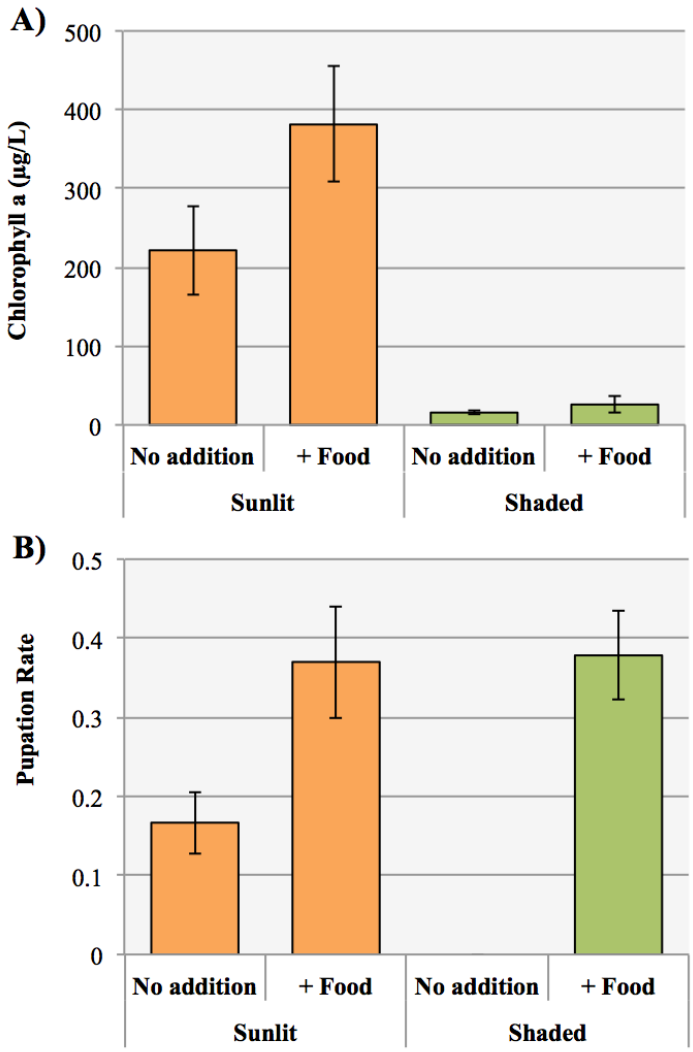

Figure 1. Chlorophyll a concentration (A) and pupation rates (B) of *Anopheles gambiae* in temperature controlled microcosms, with and without food supplementation. Food supplementation in shaded microcosms resulted in nearly identical pupation rates to sunlit microcosms ($P = 0.93$). Food supplementation also resulted in increased chlorophyll a and larval pupation rates in all semi-natural treatments.

Bacterial and algal communities are thought to be of most importance to *An. gambiae* larvae (Merritt et al. 1992, Kaufman et al. 2006). While foraging on biofilms has been shown to be of particular importance to *Aedes albopictus* and *Ae. aegypti*, algal abundance has been suggested as an important factor for anophelines (Kaufman et al. 2006, Garros et al. 2008).

The Proteobacteria were detected in highest abundance in all samples from both the water SuM and LGC; however, a notable shift in Proteobacteria class representation was observed.

The Enterobacteriaceae (Gammaproteobacteria) were shown to be effective colonizers of the larval gut, and previous work has demonstrated that the adult gut selectively favors the colonization of Enterobacteriaceae (Wang et al. 2011) (Figure 2).

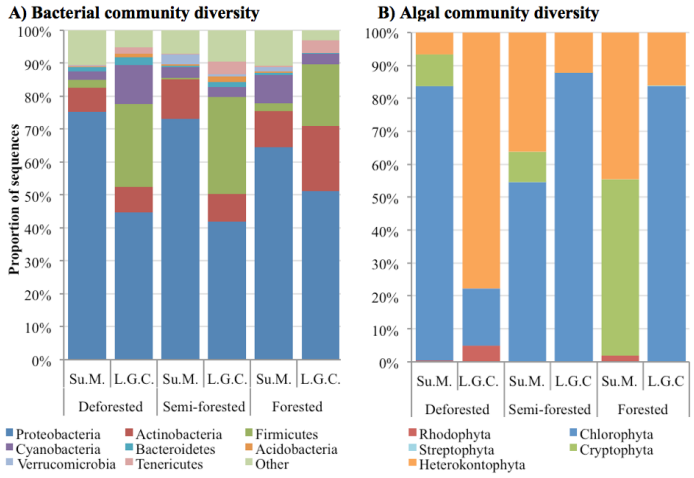

Figure 2. Bacterial (A) and algal (B) diversity at the phylum level revealed by pyrosequencing. Major bacterial phyla are presented and remaining taxa and unclassified bacteria are pooled and referred to as Other. SuM: water surface microlayer; LGC: third-instar larval gut contents.

The mechanisms for differential ingestion of certain microbial groups by *An. gambiae* larvae are not clear. One potential mechanism is larval behavioral modification in response to resource availability. For example, it has been shown that *An. quadrimaculatus* larvae employ a suite of behaviors to discriminate between available food resources on or near the surface and in the water column (Merritt et al. 1992). Feeding intensity can also be up-regulated by the presence of phagostimulants produced by microbial sources (Dadd 1970).

Productive anopheline habitats have long been strongly correlated with sunlight and the presence of algae (Coggeshall 1926, Howland 1930, Gimnig et al. 2002, Tuno et al. 2006). Further, Tuno et al. (2006) found an association between the green alga, *Rhopalosolen* species (Chlorophyta), and *An. gambiae* abundance and body size. Although no Chlorophyta sequences were obtained from the forest water samples, the gut contents of larvae reared in the forest were dominated by chlorophytes (≈ 84%) (Figure 2). This distinction is likely due to the very low abundance of chlorophytes in the habitats and further reduction by larvae. Alternatively, larvae may be employing atypical foraging techniques (i.e., scraping, shredding) in the forested habitats with low algal growth.

These results demonstrate that sunlight proliferation to habitats and the resultant growth of photosynthetic microbes are critical to the success of *An. gambiae* larvae. Further, this work suggests that larval habitat productivity is up-regulated by the eutrophication of aquatic habitats associated with agriculture. The combined effect of sunlight proliferation due to canopy removal and the use of fertilizers contribute to habitat eutrophication and algal blooms that will be beneficial to larval populations. This study enhances our ability to identify or predict aquatic habitats suitable for malaria vector production, and thus facilitates mosquito larval control targeted at the most productive habitats.

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Evaluation of 4 Genes for Detecting and Identifying Spotted Fever group rickettsia in the County of San Diego

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ABSTRACT: Taxonomic classification of members of the order *Rickettsiales* was originally based on relatively few phenotypic criteria and was divided into three groups: spotted fever, typhus and scrub typhus groups based on their clinical disease. Over the past thirty years, however, the taxonomic classification of the Order *Rickettsiales* has undergone many changes based on their genetic sequences. To clarify rickettsial phylogeny, a multigenic approach based on simultaneous analyses of several gene loci is used, including 16S rRNA, 17kDa protein gene, the citrate synthase encoding gene (*gltA*), and the *Rickettsia*-specific *ompA*, *ompB*, *sca1*, *sca2*, and *sca4* genes, encoding autotransporter proteins. Molecular typing of the variable intergenic regions (IGR) has also proved to be a high resolution typing method. At the San Diego Vector Disease Diagnostic Laboratory (VDDL) multi locus sequence typing (MLST) was carried out for the species identification of Spotted Fever group rickettsia (SFGR) using *ompA* (semi nested), *gltA*, IGR RR0155-*rpmB* (nested) and IGR RR1240-*tlc5* (nested)(Figure 1). In our laboratory, *ompA* and IGR RR0155-*rpmB* proved to be the most reliable targets to be able to detect and differentiate between *Rickettsia rickettsii*, *R. philipii*, Hlp and *R. rhipicephali* all of which are found in the County of San Diego. Samples with lower Ct values were likely to be identified as *R. philipii* while the samples with higher Ct values were likely to be identified as *R. rhipicephali.* Samples with a Ct value higher than 37 were likely to fail conventional PCR and sequencing (Figure 2).

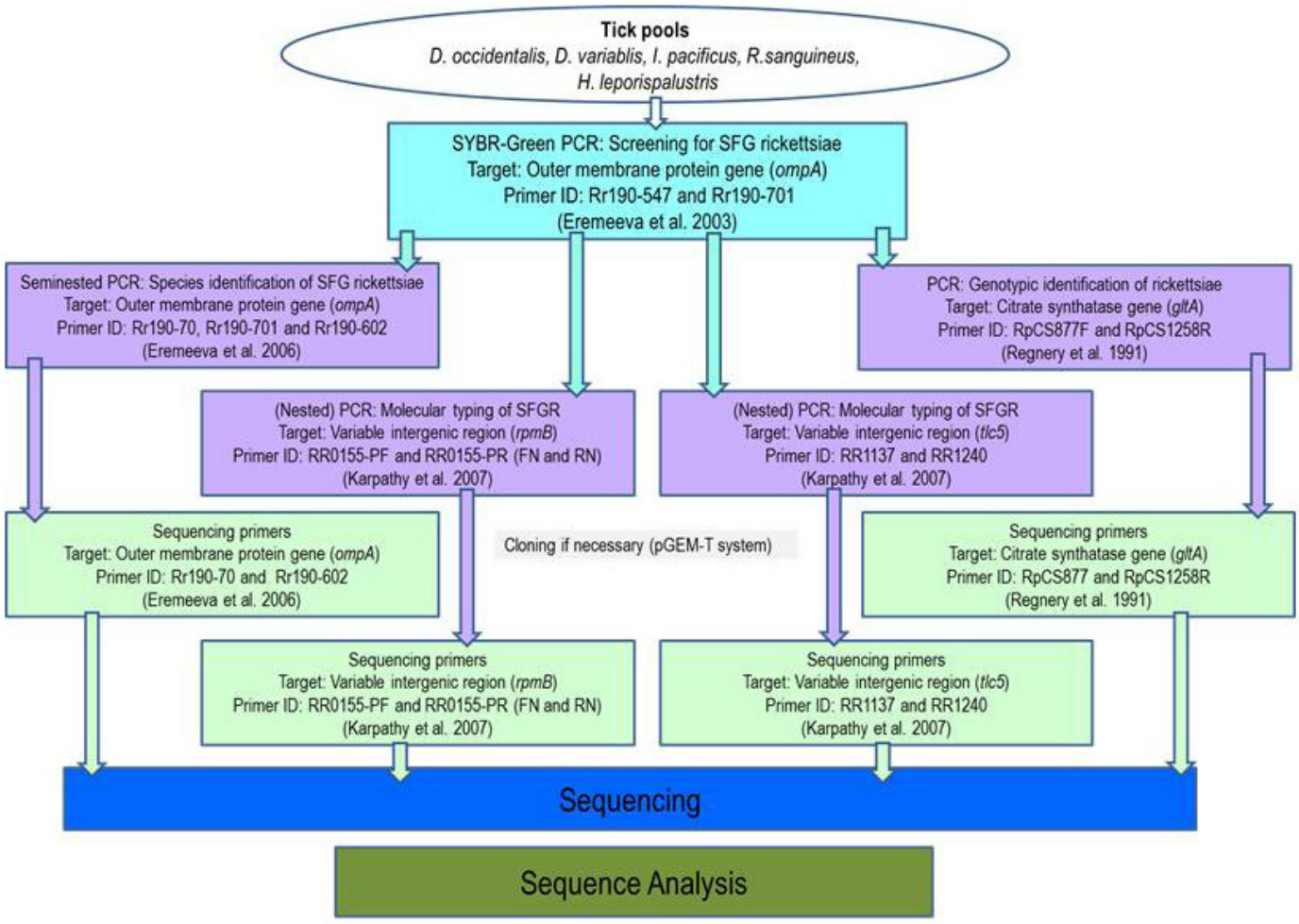

Figure 1. Polymerase Chain Reaction and Sequencing workflow.

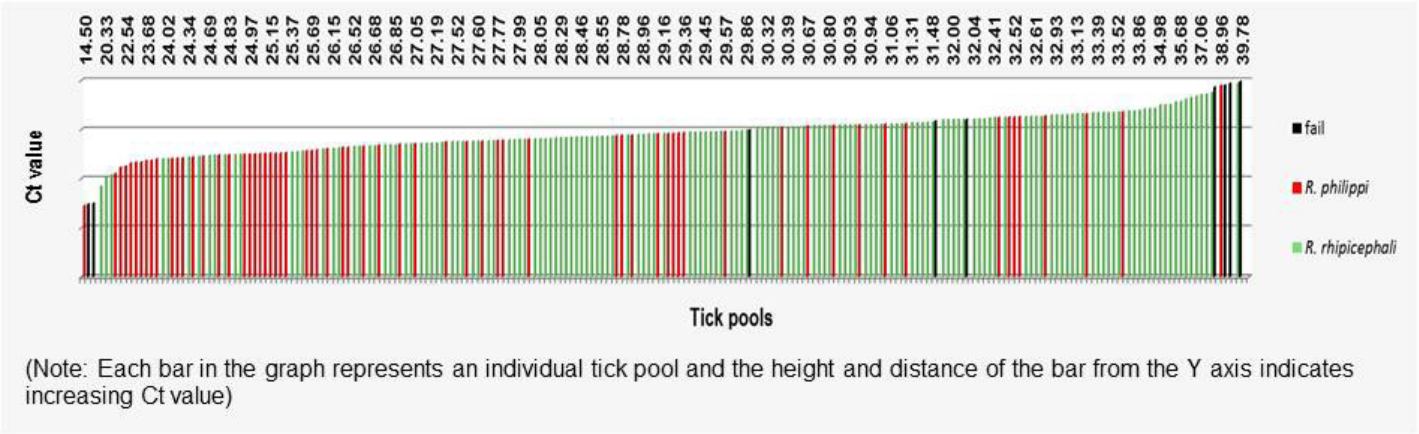

Figure 2. Analysis of sequencing based on PCR Ct values.

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Rickettsia 364D (proposed *Rickettsia philipii*) in San Diego

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A new rickettsial disease was described in California in 2010 which caused swelling, erythema and ulceration at the site of a presumed tick bite (Shapiro et al. 2010). Biopsy of the affected skin revealed the genetic material of a rickettsia closely related to *Rickettsia rickettsii*, an organism called 364D, that was first detected by Bell in Ventura County, CA, in 1966 (Philip et al. 1978). Recently, four additional pediatric cases of 364D-induced disease have been described and 13 cases in California at the time of this writing have been reported (Johnson et al. 2013, personal communication, respectively). Its name has been proposed to be changed to *Rickettsia philipii*. In order to assess the potential risk of 364D to public health, a study was conducted in 2011 to determine if 364D was present in San Diego County.

County Vector Control Program staff used traditional flagging methods to collect *Dermacentor occidentalis* ticks from 33 tick-infested sites throughout the County. Multiple tick collections were performed at each location during winter and early spring in 2011. One thousand six hundred fifty four male (49%) and one thousand seven hundred seventy seven female (51%) *D. occidentalis* ticks were collected and pooled into 187 male and 197 female pools, respectively, containing 1-11 ticks per pool (mean 9.2, mode 10, stdev 2.2). The pools were tested for Spotted Fever Group Rickettsia (SFGR) using a group-specific SYBR green real-time PCR test for the rickettsial *ompA* gene (Eremeeva et al. 2003). One hundred seven male (57%) and one hundred seven female (54%) tick pools were positive for SFGR. *Rickettsia* species identification was obtained by sequencing positive pools for the *ompA* gene and intergenic region locus (IGR) identified by primer pair RR0155-*rpm*B (Karpathy et al. 2007). Of the male tick pools, 32 (29.9%) contained 364D, 74 (69.2%) contained *R. rhipicephali*, and one pool contained *R. rickettsii*, the agent of Rocky Mountain Spotted Fever. This is only the fourth report of *R. rickettsii* found in California; it was detected near the Green Valley Falls fire road (32.90151, -116.58315). The prevalence of rickettsia-positive female tick pools was similar with 35 (32.7%) pools positive for 364D, 72 (67.3%) positive for *R. rhipicephali* and none positive for *R. rickettsii*. The overall prevalence of 364D and *R. rhipicephali* in individual ticks was calculated to be 2.1% (95% CI: 1.6-2.6%) and 5.2% (95% CI: 4.4-6.1%), respectively. No mixed infections were found. The infection rates for both 364D and *R. rhipicephali* were lower than found in a study of other Southern California counties performed in 2006-2007 (Wikswo et al. 2008). In that study, 24.7% of *D. occidentalis* ticks were infected with *R. rhipicephali* and 7.7% were infected with 364D.

Interestingly, 54% of SFGR-positive tick pools collected from Lopez Canyon (Sorrento Valley) were infected with 364D (14/26) whereas in the adjacent Los Peñasquitos and McGonigle canyons, 22% (6/27) were infected with 364D (p=0.024). This is in contrast to the Escondido Creek tick collection area where only 3% (1/32) of positive tick pools were infected with 364D ($p<0.05$); the remainder of positive tick pools from all locations were infected with *R. rhipicephali*. In three other County tick collection locations with at least 17 or more tick pools, the percentage of 364D-positive pools were 35% (Escondido Calle Messina), 31% (Mission Trails) and 47% (Tierrasanta) (figure 1).

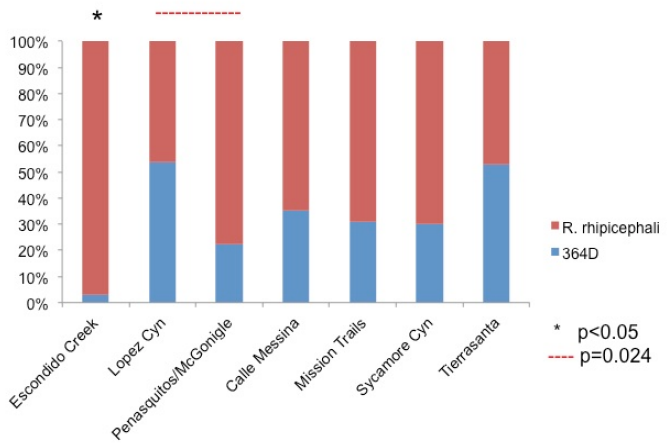

Figure 1. Distribution of 364D and *R. rhipicephali* in San Diego County. Percentages of positive SFGR pools of *D. occidentalis* ticks from different locations where at least 11 or more pools were collected.

Tick collections from 2012-2014 are being analyzed to determine if the distributions and prevalence found in 2011 are stable and what factors might be associated with the presence of 364D.

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Developing a Predictive Risk Model for West Nile Virus Activity based on Mosquito Breeding Sources, Environmental, and Socioeconomic Factors for Orange County, California

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ABSTRACT: Since the introduction of West Nile virus (WNV) in southern California in 2003, Orange County has been recognized as a hotspot of WNV activity. Most of the county's WNV activity (WNV-positive dead birds and mosquitoes, along with notable clustering of human cases) has been found in north Orange County, which suggests the existence of specific factors that increase the risk for WNV infection in this area. We built a comprehensive geo-database from different data resources and relied on advanced data mining and GIS technology to determine the spatial correlations among reported human WNV infections, known mosquito breeding sources, WNV-positive dead birds and mosquitoes, elevation and socioeconomic variables within Orange County from 2004 - 2013. We applied a multivariate logistic regression model in R to determine the significant factors for accurate prediction of WNV risk areas in the county. We corroborated the model with a 10-fold cross-validation method based on known locations of locally-acquired, human WNV infections. Our results showed that the model's accuracy was approximately 84.4% in R. Of 14 variables, the risk factors that were found to be important in characterizing a WNV risk area were: an elevated vector index, high population density, a relatively large proportion of the population > 60 years old, an abundance of sites with stagnant water in street gutters, and low elevation. Of these, the vector index was particularly significant, while low elevation was weakly associated with human cases. To visualize the WNV risk areas, a new method was developed to identify the areas based upon interpolation of the predicted WNV risk values; from this method, a high resolution map was made that captured 82.2% of the WNV prediction rate within risk areas. Retrospective follow up on human WNV clusters has provided insight into finding previously unknown mosquito breeding sources, such as stagnant water in street gutters and problematic underground stormwater drainage systems.

INTRODUCTION

Since the introduction of West Nile virus (WNV; family Flaviviridae, genus *Flavivirus*) to southern California in 2003, California has become one of the most affected states for WNV epidemics in the USA (Lindsey et al. 2010, Liu and Weng 2012). A great number of WNV infections have been detected in birds, mosquitoes and some mammals in the state since its arrival (*http://www.westnile.ca.gov/*). Southern California, with its warm Mediterranean climate, provides a favorable habitat for mosquito vectors and avian hosts, and was recognized early by the California Department of Public Health (CDPH) as a high risk area for WNV (Figure 1) (Reisen et al. 2004).

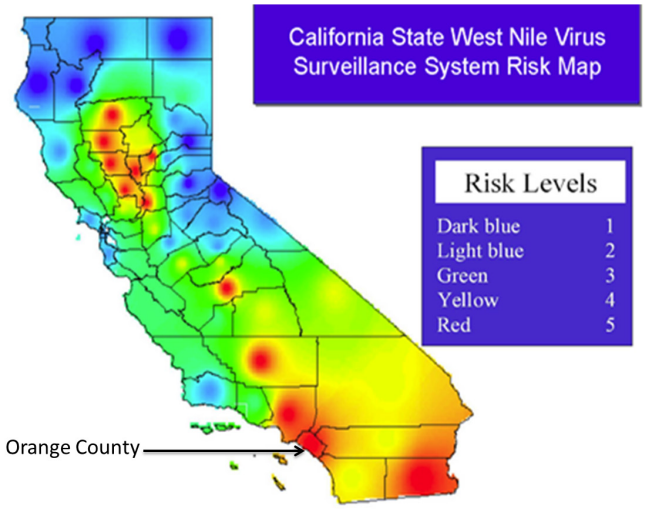

Figure 1. California State West Nile Virus Surveillance System Risk Map, 2005.

WNV is transmitted and maintained in southern California primarily in urban landscapes through a bird-mosquito cycle, with the peridomestic-breeding southern house mosquito, *Culex quinquefasciatus* Say, and several species of passerine birds, such as the house finch (*Haemorhous mexicanus* Muller), house sparrow (*Passer domesticus* L.) and American crow (*Corvus brachyrhynchos* Brehm), as the primary enzootic and epizootic drivers of WNV activity in Orange and the surrounding counties of southern California (Kwan et al. 2010, Molaei et al. 2010, Kwan et al. 2012, Reisen 2013).

Orange County has recorded 251 human WNV infections with 9 deaths during 2004 - 2013 (OC Health Care Agency, *http://ochealthinfo.com*) and has been recognized as a hotspot of WNV activity, with positive mosquito pools, dead birds, seropositive wild birds and human infections (Figure 2) occurring every year since its introduction. Temporally, most of the county's human WNV infections have occurred during August – September and have varied annually through apparent four-year cycles (Figure 2). In its most active year, Orange County had the third highest number of reported human infections (79) per county in the United States during 2008 (USGS, *http://diseasemaps.usgs.gov*). WNV is endemic in the county and is expected to be a public health concern indefinitely (OC Health Care Agency, *http://ochealthinfo.com*). The Orange County Vector Control District (OCVCD) routinely recommends all residents and visitors to take preventative measures to avoid being bitten by mosquitoes, especially during warm months of the year (April – November) when arboviral disease transmission is most likely to occur.

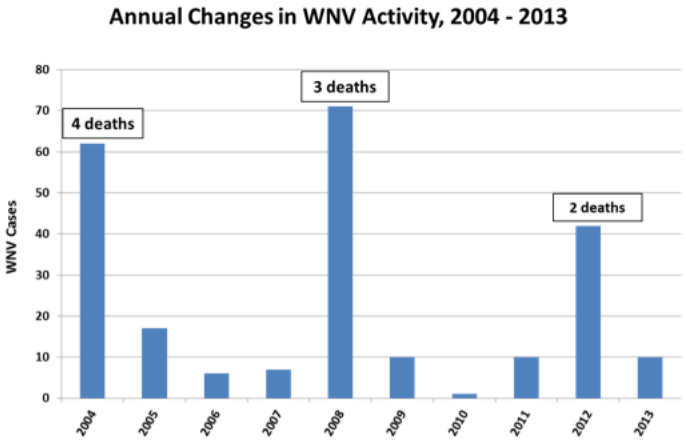

Figure 2. Number of WNV cases per year, Orange County, CA, 2004 – 2013.

Within Orange County, the areas with the highest cumulative human WNV cases and most pronounced incidence rates are clustered primarily in the north, especially in the cities of Fullerton and Anaheim (Figure 3). Although separated by years, many human WNV infections have occurred in clusters (two or more infections) of within-cluster distances of less than a quarter mile apart (some cases have appeared on the same streets). This discovery created an interest to develop an empirically based model to account for human WNV case-related factors and to assess their importance in explaining the possible processes that may have led to these patterns.

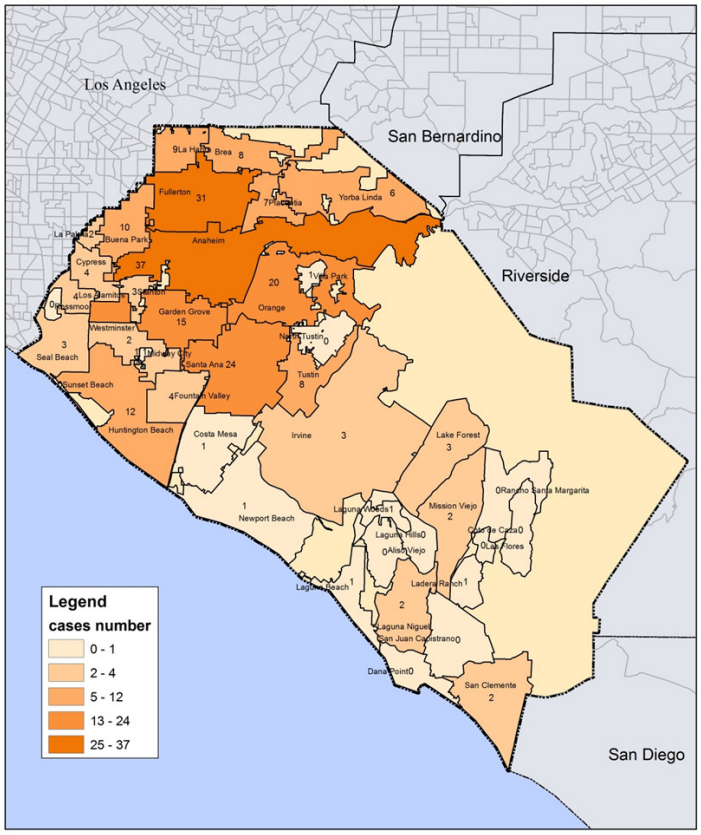

Figure 3. Locations (by city) of WNV cases in Orange County, CA 2004-2013.

Previous Studies Assessing WNV Risk Areas. Many researchers have demonstrated the effectiveness of using environmental factors to investigate WNV disease patterns. With the advent of powerful geographic information systems (GIS) technologies, landscape epidemiology has proven very effective at predicting both spatial and temporal distributions of certain diseases, especially for vector-borne diseases transmitted by mosquitoes, ticks or other animals (Reisen 2010). Researchers have used such variables as vegetation indices, land surface temperature, land use and land cover within a GIS framework to correlate with disease occurrence in the upper Midwest of the U.S. (Ruiz et al. 2010, Liu and Weng 2009). Winters et al. (2008) used GIS-derived environmental data and regression analysis to develop spatial models for predicting high-risk areas of exposure to WNV in western and eastern Colorado based on human cases from 2002 to 2006. In highly urbanized areas, socioeconomic conditions (population age structure, housing age, per capita income) were shown to be the determinant risk factors for human WNV infections in the greater Chicago area (Ruiz et al. 2004); in Orange County, Harrigan et al. (2010) demonstrated with random forest modeling that income was the most important socioeconomic factor for human WNV case prediction.

During the first years following the WNV invasion in California, the DYCAST (Dynamic Continuous Space-Time) system was used for predicting human cases based on public reporting of dead birds (Carney et al. 2011). DYCAST initially had a WNV case sensitivity rate of 80.8%, but prediction rates decreased as public calls for dead birds declined in later years. Pan et al. (2008) developed another data mining method using a neural network model for analysis of WNV risk factors to find the significant variables that contributed to WNV infection rates in humans. On a nationwide scale, Young et al. (2013) developed a WNV prediction model using environmental variables by data mining with Cubist (RuleQuest Research) to compute disease incidences for many U. S. counties; recently, Harrigan et al. (2014) used climate change models to predict future WNV hotspots across the U.S.

Most studies, however, have focused primarily on environmental variables and/or socioeconomic factors in developing WNV disease models (Ezenwa et al. 2006, Rios et al. 2006, Ruiz et al. 2007, Harrigan et al. 2010, Rochlin et al. 2011), but relatively few investigations (Reisen et al. 2008, Harrigan et al. 2010) have incorporated mosquito breeding source information as a contributor to WNV outbreaks, and none has included comprehensive mosquito data in spatial analyses of WNV transmission across landscapes. Since the Orange County Vector Control District (OCVCD) is in the process of digitizing many of its known mosquito breeding sources, the question arose as to the insights our agency would gain by developing a WNV risk map based not only on a variety of socioeconomic, environmental and human WNV infections, but also on mosquito data (particularly the vector index) and locations of mosquito breeding sources.

As part of a preliminary study, we built a comprehensive geo-database from different data resources and relied on advanced data mining and GIS technology to determine the spatial relationships among reported WNV cases, known mosquito breeding sources, WNV prevalence levels in dead birds and mosquitoes, environmental, and socioeconomic variables to accurately identify WNV hotspots within Orange County, California. Using a set of selected variables, we developed a new prediction method and mapping technique based on an individual case-level study.

METHODS

Study Area. Orange County occupies a large portion of the Greater Los Angeles Basin and has an estimated population of 3,010,237 people living in a mostly metropolitan area of 790.6 square miles; the population density is approximately 3,808 persons per square mile and is the third most populous county in the state (U.S. Census Bureau, 2010. *http://quickfacts.census.gov/qfd/states/06/06059.html*). Most of Orange County's population lives within two shallow, urbanized coastal plains in the Santa Ana and Saddleback valleys (Figure 4). The region's climate is Mediterranean, moderated by easterly winds from the Pacific Ocean, with mean annual rainfall of 13.59 inches and mean annual temperature of 64.6° F. (Orange County Weather, 2014, *http://www.usa.com/orange-county-ca-weather.htm*). The weather is typically warm and dry from May – October, with precipitation occurring mainly during November – April.

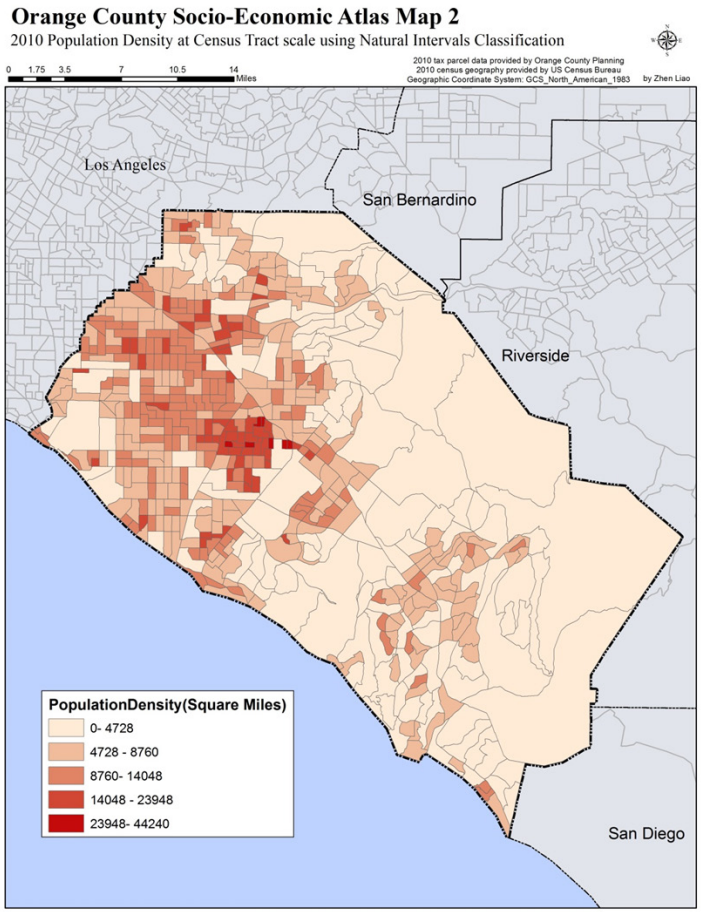

Figure 4. Orange County Population Density Map.

Mosquito breeding sites in the county consist largely of sources associated with human development, such as water-filled residential containers, improperly maintained swimming pools and ponds, and slow-flowing water from residential irrigation runoff in street gutters, catch basins, and drainage channels (Su et al. 2003).

Software Programs and Tools. ESRI's (Environmental Systems Research Institute) ArcGIS™ 10.1 was used for data collection, processing, analysis and for final visualization of a WNV risk map. The statistical computing software R2.15.3, with data exported from ArcGIS™ 10.1 databases, was used for data exploration, predictive model formation, logistic model calculation and model verification. Microsoft Office Excel® was used to calculate simple ratios such as the vector (i.e., mosquito) index (VI) (Biggerstaff 2009, Nasci et al. 2005).

Data. A variety of factors considered potentially important in understanding WNV risk to humans were included in the analyses. These consisted of environmental, socioeconomic, WNV infection data for dead birds and mosquitoes, and locations of mosquito breeding sources. Socioeconomic data were summarized and analyzed at the census tract level (582 census tracts were used). Economic and housing parcel data were obtained from the United States Census Bureau (2010) (*http://quickfacts.census.gov/qfd/states/06/06059.html*) and the County of Orange (*http://www.ocgov.com*), respectively.

WNV surveillance data for 2004 – 2013 were used in this study. Mosquitoes were collected in modified CO_2 – baited, encephalitis virus surveillance (EVS) traps (Rohe and Fall 1979) and gravid traps (Cummings 1992), identified to species (Meyer 2003), enumerated and pooled. Dead birds were collected in response to public calls, identified to species, necropsied by OCVCD staff, and kidney specimens were harvested (Velten et al. 2010). Mosquito and tissue samples were stored at -80° C and tested by real time RT-PCR (Lanciotti et al. 2000).

The vector index (VI) was calculated using the method outlined by Nasci et al. (2005). Infection rates were calculated for *Cx. quinquefasciatus* females only, since this species is widely abundant and is considered the most important WNV vector in Orange County (Molaei et al. 2010). Other mosquitoes (*Cx. tarsalis* Coquillett and *Cx. stigmatosoma* Dyar) were not included in the VI because of their low counts, infrequency of collection, and relatively low WNV infection rates. In total, data from 36 consistently-trapped sites for 2004 – 2013 were used in the analyses. Interpolation techniques in ArcGIS™ 10.1 were required to generate comprehensive VI values for the areas between mosquito trap locations in the county. Global positioning system (GPS) locations of 1,513 WNV-positive dead birds collected in the county during 2004 – 2013 were used in this study. American crows constituted 82.7% (1,252) of the WNV-positive dead birds.

The geographic center points of large mosquito breeding sites were used to assign their GPS locations. Large breeding sources, such as flood control channels and underground drains, were given the same relative value as smaller point sources, such as neglected swimming pools that were producing mosquitoes.

The cumulative numbers of points per square mile for each mosquito breeding source type were used in the calculations.

For 2004 – 2013, the Orange County Health Care Agency reported a total of 230 confirmed human cases of WNV to the OCVCD. The residential address of each WNV case was geocoded separately to the street level and was used as the exposure site in the analysis. An equal number of control points (230) were generated in ArcGIS™. Of the 251 reported human WNV infections in Orange County, a total of 21 occurred in asymptomatic blood donors or in patients with out-of-county travel history and were not used in the study. Datasets (Table 1) were aggregated to individual case level and compiled into data tables for R software.

Name	Unit	Min	Mean	Max	Standard deviation
Socioeconomic Variables					
Mean per Capita Income	census tract	14	37631	145045	18055.62
Age rate > 60 years	census tract	0	11.65	82.80	9.155158
Population Density	persons/sq. mi.	13	6171	44240	5304.861
Housing Density	units/sq. mi.	0	2071	10640	1608.557
Housing Age	census tract	13	20	21	1.7
Density of Mosquito Breeding Sources					
Channels (342)	points/sq. mi.	0	1.761	17.468	3.43606
Ditches (460)	points/sq. mi.	0	0.9934	13.5815	2.193283
Street Gutters (8,886)	points/sq. mi.	0	21.856	220.819	29.79977
Underground Drain Hotspots (586)	points/sq. mi.	0	1.1668	24.3713	2.85368
Neglected Swimming Pools (760)	points/sq. mi.	0	1.821	33.453	3.779544
Environmental Variable					
Elevation	feet	0	134.6	1534	212.8812
WNV Infection Variables					
Dead Birds (1,513)	points/sq. mi.	0	3.630	28.784	5.038971
Vector (Mosquito) Index (36 sites)		0	0.15914	0.33894	0.1088647

Table 1. List of variables.

Modeling Approach. Figure 5 shows the modelling approach. Our model was constructed using multivariate logistic regression. Such machine learning methods can be used in place of simple statistical techniques like linear regression to model complex relationships with multiple interacting variables. Logistic regression allows for setting up a multivariate regression relation between a dependent variable and several independent variables when the variables are either continuous or categorical, or any combination of both types, or when the dependent variable is binary where the number of available categories is two (Hair et al. 1998). In this study, the dependent variable was a binary variable representing the presence or absence of WNV cases. Through the use of a logistic regression model, risk factors could be collected and assessed for significance of association with the presence of WNV while simultaneously controlling for the effect of other variables (Ruiz et al. 2004, DeCarlo et al. 2011, Rochlin et al. 2011). The relationship between the case occurrence and its dependency on several variables was expressed as:

$p = e^z / (1+e^z)$, where $z = b_0 + b_1x_1+b_2x_2+b_3x_3+\ldots..+b_nx_n$ when p =1, case occurring; when p=0, no case occurring

Once a spatial relationship between the location of human WNV case occurrence and WNV-related factors was calculated, logistic regression equations were developed and used to calculate the probability that each control point would be in a cluster.

Model processing. The above-mentioned datasets were aggregated to the individual case level and compiled into data tables for R software. WNV human case data served as the dependent variable in the equation. The environmental, socioeconomic, WNV infection data for dead birds and mosquitoes, and mosquito breeding sources served as independent, or explanatory, variables in a manner conceptually similar to multiple regression. Model selection (stepwise method) was performed to select the most relevant variables.

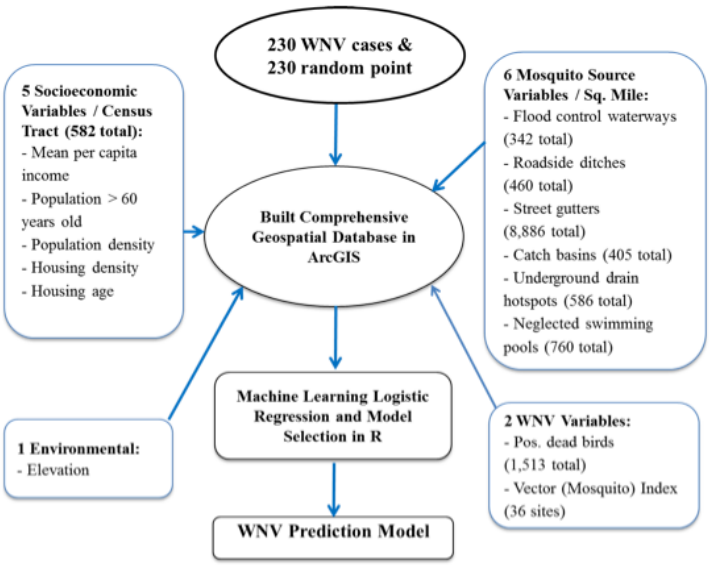

Figure 5. Components of the multivariate logistic regression model.

Table 2 shows the model selection results. The risk of WNV infection in humans was associated with the VI (Vector Index), population density, proportion of population > 60 years of age, proximity to living by poorly-draining street gutters and low elevation. We found no significant association among human WNV cases and certain socioeconomic variables (mean per capita income, housing density, housing age) and living near a variety of mosquito breeding sources (flood control channels, catch basins, underground drains, neglected swimming pools), or proximity to WNV-infected dead birds. According to the Akaike Information Criterion (AIC) score, the rank of significant variable importance was: VI > population density > proportion of the population over 60 years of age > street gutter mosquito breeding sites > elevation.

Step: AIC=442.25			
N ~ Gutter + Population Density + Age Rate + Elevation + Vector Index			
	Degrees of Freedom	**Deviance**	**AIC**
		430.25	442.25
Elevation	1	434.88	444.88
Street Gutter	1	441.05	451.05
Age Rate > 60	1	442.82	452.82
Population Density	1	445.49	455.49
VI (Vector Index)	1	459.33	469.33

Table 2. Final model selection result.

We further examined the exact association between the WNV case probabilities and the selected variables. Table 3 shows the coefficients and significance level of the variables. The final logistic prediction model was based on the maximum-likelihood method and was found to be:

z = -2.5312327 + 0.0161854*street gutter + 0.0001278 *pop density + 0.0490451*age rate > 60 + 6.7459826*VI -0.0029495 *elevation

Of the significant variables, four (VI, population density, proportion of population > 60, proximity to street gutter breeding sources) had a positive effect with human WNV cases, while elevation had a slightly negative influence. This analysis revealed that an area is more likely to include one human WNV case when it has a higher population density, comprises a higher percentage of people in a census tract over 60 years' old, has an abundance of locations with poorly-draining street gutters, and has a high WNV infection rate in mosquitoes.

	Coefficient	**Estimate Std. Error**	z value	**Pr(>|z|)**	
(Intercept)	-2.531e+00	4.678e-01	-5.411	6.27e-08	***
Gutter	1.619e-02	5.406e-03	2.994	0.002754	**
Population Density	1.278e-04	3.591e-05	3.560	0.000371	***
Age Rate	4.905e-02	1.556e-02	3.152	0.001624	**
ELE	-2.949e-03	1.595e-03	-1.849	**0.064415**	.
VI	6.746e+00	1.280e+00	5.272	1.35e-07	***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					
Null deviance: 637.70 on 459 degrees of freedom					
Residual deviance: 430.25 on 454 degrees of freedom					
AIC: 442.25					

Table 3. Coefficients and levels of significance for the variables.

Model Evaluation. Cross-validation is a model validation technique for estimating how accurately a predictive model will perform in practice; ten-fold cross-validation was used in this study. In the original sample, 460 points (230 WNV human cases and 230 random points) were randomly partitioned into ten equally-sized subsamples. A single subsample was retained as validation data for model testing. The remaining nine subsamples were used to form a new logistic regression model with the five selected variables. This was repeated for each subsample. For this logistic binary classification model, each case in the validation set was either predicted correctly or incorrectly. In this situation, the misclassification error rate was used to summarize the fit. Here our average prediction error rate was 0.1561486 in R, and the model accuracy was found to be 84.4%. This value indicates that the model "fits" the real world data very well and is in effect, a good predictive model.

Visualization. For data visualization, the interpolation function of ArcGIS 10.1™ was used to generate a WNV risk map for Orange County based on the analyses. A regular grid map for the entire county was generated. R software was used to compute the predicted human WNV disease infection rate for each control point in the dataset. These predicted incidence values were re-imported into ArcGIS 10.1™ and joined back to the Orange County grid data via the Federal Information Processing Standards (FIPS) code for mapping (Figures 6 and 7).

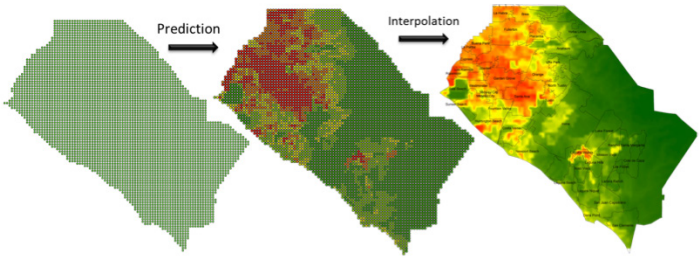

Figure 6. Steps in visualizing the WNV risk map.

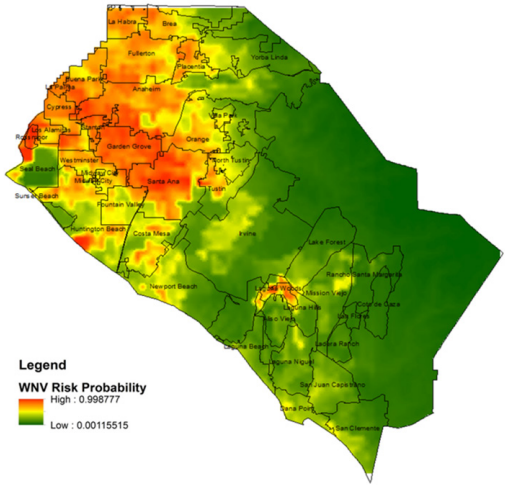

Figure 7. WNV risk map for Orange County, CA.

When the locations of the 230 human WNV cases were over-laid on the model, the model successfully captured 82.2% of the cases (n =189) in the high risk "red" areas depicting WNV infection rates > 0.5 (Figure 7). The areas of Orange County with the highest WNV risk were found to be in the northwestern part of the county encompassing the cities of Fullerton, west Anaheim, and Garden Grove.

DISCUSSION AND CONCLUSION

This study uniquely combined machine learning with GIS technology to achieve an improved local-scale model and used a new visualization method to identify variables and conditions that could be applied specifically for Orange County (or to other highly urbanization areas). As part of a preliminary study, we built a comprehensive geo-database from different data resources and relied on advanced data mining and GIS technology to determine the spatial correlations among reported WNV cases, known mosquito breeding sources, WNV prevalence levels in dead birds and mosquitoes, and socioeconomic variables to accurately identify WNV hotspots within the county. The significance of this study is that it produced an accurate and reliable disease surveillance visualization method by incorporating mosquito data, socioeconomic and landscape metrics as potential factors of WNV intensity. The main difference between this study and previous WNV risk assessment efforts is that a large number and variety of mosquito breeding sources were applied in the prediction model. Also, the use of individual WNV case locations helped to capture differences in spatial characteristics and avoided restrictions dependent on administrative boundaries (census tracts, county boundaries) found in other studies.

Our multivariate logistic regression model was substantiated and cross-validated for the prediction of WNV risk. As an individual case-level study, our model's prediction error in R was 0.1561486, indicating that the model "fits" the real world data very well and is in effect, a good spatial model with a prediction rate of 84.4% for WNV cases. Of the 4 major factors contributing to human WNV cases (VI, street gutter sources, population density, age rate > 60 per area), the Vector Index was the strongest predictor of WNV infection, as also found by Jones et al. (2011). In contrast, many socioeconomic factors were not significant when mosquito breeding locations were taken into consideration in the risk model.

With the high resolution risk map, WNV hotspots were delineated for OCVCD for the purpose of spatially-focused mosquito control (Figure 6). (Because of Health Insurance Portability and Accountability Act [HIPAA] regulations, the Orange County Health Care Agency prohibits OCVCD from displaying the locations of the 230 human WNV cases in the map). Retrospective follow up on human WNV clusters has provided insight into disease risk and helped OCVCD staff find previously unknown mosquito breeding sources, such as poorly-draining street gutters and underground storm drains, in WNV-impacted neighborhoods.

Elevation had a slightly negative association with human WNV cases ($p = 0.064425$), but was kept in the final logistic prediction model. With 10 fold cross-validation, we generated a slightly lower prediction error in R using the elevation variable with the 4 significant variables than without it in the final model (0.1561486 vs. 0.1567439). In many low-lying urban areas of Orange County, small differences in elevation between the inlet and discharge points for runoff water in many water conveyance structures contribute to mosquito breeding in these systems, potentially enhancing WNV activity for the area.

Limitations and Areas for Future Study. Unfortunately, mosquito breeding sources were not "weighted" as to their importance, nor were all known breeding sources plotted in our study. More GPS data points should have been included for large mosquito breeding sources based on their multiple treatment locations and factored in the analyses. For example, if location data for all larvicidal treatment sites used in treating underground stormwater drainage systems, such as the drop inlets (i.e., manholes), had been incorporated in the model, then this type of urban mosquito breeding source might have been identified as a significant contributor to human WNV infections in the model.

OCVCD plans to continue with geocoding mosquito breeding sites and incorporating more data in future geospatial WNV risk models. It should also be noted that other machine learning programs and techniques, including neural networks, are available and might model complex relationships between the environment and WNV risk better than multivariate logistic regression, as described in this study.

ACKNOWLEDGEMENTS

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Cost and Efficiency Analysis of West Nile Virus Surveillance Methods in California

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INTRODUCTION

The surveillance methods used to detect West Nile virus (WNV) determine the location and timing of viral amplification in order to inform control efforts. To ensure an optimal surveillance approach, one must consider the balance between a method's ability to detect virus activity and the cost of its use. We have examined this balance for the commonly used methods for WNV surveillance in mosquitoes, chickens and dead birds by comparing their effectiveness after equalizing the number of sampling occasions in time and space, and equalizing total cost.

MATERIALS AND METHODS

Surveillance data collected in Sacramento-Yolo, Kern and Coachella Valley Mosquito and Vector Control Districts (MVCDs) during the period from 2004-2012 were used in the comparisons. The surveillance methods included testing of the primary mosquito vectors, *Culex tarsalis* and *Culex pipiens* complex, for presence of viral RNA by RT-PCR; testing for antibodies against WNV in sentinel chicken flocks; and the testing of public-reported dead birds for viral RNA. To equalize the methods in time and space, each sampled flock was paired with the nearest CO_2-baited or gravid trap sampled in the same week (Healy et al. 2013). The average distance between the paired flock and trap site (1.5 km) was used as the area of inclusion for reported dead birds because dead bird sampling effort depends on passive detection via public reporting and could not be strictly controlled in the study. From these data, the week of first detection and the week of highest viral activity in a season detected by each method was compared using the Wilcoxon Signed-Rank test to compare the timeliness of virus detection, and the frequency of virus detection by each method was used to compare relative efficiency.

To equalize and compare the methods in terms of cost, we asked each of the three participating agencies to estimate their costs associated with collections, maintenance, testing and personnel time for each method. Costs for collections and maintenance included personnel time, typical costs of supplies for mosquito traps and sentinel flocks, the average cost per tested dead bird for maintaining a state-wide reporting hotline, and shipping and necropsy of birds prior to testing. Testing costs included those incurred for either in-house testing or centralized testing at the UC Davis Center for Vectorborne Diseases, depending on the approach of the particular agency. These costs were averaged across the agencies, resulting in an average cost per unit of surveillance (i.e., a mosquito trap, sentinel flock or dead bird), which was multiplied by the average number of surveillance units tested per week, yielding a total cost for each week and surveillance type. To compare the methods after equalization, the number of positive surveillance units per week was divided by the total costs per week to yield the number of positive units per $1,000 spent on each surveillance method, and the paired student's *t*-test was used to evaluate significance.

RESULTS AND DISCUSSION

At an equal density in space and time, testing of dead birds and mosquitoes tended to detect WNV earlier than sentinel chickens, although the results for dead birds were strongly influenced by Sacramento-Yolo MVCD's data due to the larger number of birds sampled compared to the other agencies. These results agree with earlier findings, even though previous studies did not necessarily account for the variability in sampling effort in both time and space (Cherry et al. 2001, Kwan et al. 2010, Patnaik et al. 2007, Unlu et al. 2009). Dead bird and mosquito testing typically detected the onset of virus activity 2 - 5 weeks earlier than sentinel flocks and detected activity in mosquitoes and dead birds peaked 3 weeks before sentinel flocks (Table 1). During the surveillance weeks in which at least one surveillance method detected activity (524 total positive surveillance-weeks), mosquito traps most frequently detected early season activity (April-June), detecting 71% of the positive weeks. During the typical peak period of activity (July-August) and late season period of waning activity (September-October), sentinel flocks detected WNV most frequently, with 62% and 72% of positive weeks detected in each time period, respectively. The lag in detection by sentinel chickens and predominance of chicken positives in the latter part of the season may be due to the delay between the infectious mosquito bite and the time when antibodies become detectable, which can be 7 to 10 days post-infectious bite (Patiris et al. 2008, Senne et al. 2000), as well as the bleeding schedule for sentinel chickens which is typically performed every other week. The combination

of antibody delays and bleeding schedule could delay detection by >3 weeks, depending on the exact timing of the transmission event in relation to the sampling schedule.

	Median difference in week of first detection	P-value	Median difference in week of peak detection	P-value
Mosquitoes v Chickens	2	<0.001	3	<0.001
Dead Birds v Chickens	5	0.005	3	<0.001
Mosquitoes v Dead Birds	0	0.73	0	0.98

Table 1. Difference, in weeks, between detection of onset and peak activity by each surveillance method.

Among the surveillance methods, testing of dead birds was most cost-effective in detection of virus activity over a season. The average cost per unit was $65 for dead birds, $72 for mosquito traps and $111 for sentinel flocks. Comparison of the number of positive surveillance units per $1,000 spent on surveillance showed that dead birds were the most cost-effective indicator of WNV occurrence over the entire season, yielding approximately 3 more positives per $1,000 than both sentinel flocks and mosquito traps (Table 2). Between mosquitoes and sentinel chickens, the number of positives per $1,000 varied over the season, with mosquito traps yielding slightly more positives during late spring and sentinel flocks yielding more positives per $1,000 during the latter part of the season. When results were stratified by vector control agency, we found a strong influence of Sacramento-Yolo and Kern MVCDs, which have higher numbers of reported dead birds, while Coachella Valley had very few dead birds detected during the study period due to the relatively low abundance of bird species susceptible to mortality by WNV infection.

Part of Season	Chicken flocks		Mosquito traps		Dead birds	
	No.	(95% CI)	No.	(95% CI)	No.	(95% CI)
April-June	0.2	(0.2, 0.3)	0.6	(0.5, 0.7)	1.6	(1.4, 1.8)
July-August	2.7	(2.5, 2.9)	2.6	(2.5, 2.7)	7.7	(7.5, 8.0)
September-October	2.4	(2.2, 2.5)	1.0	(0.9, 1.0)	5.6	(5.4, 5.8)
Entire season	1.6	(1.4, 1.8)	1.4	(1.2, 1.5)	4.4	(4.0, 5.0)

Table 2. The average number of positive surveillance units per $1,000 by surveillance method and district, 2004-2012.

In conclusion, our analysis shows that a surveillance system that utilizes testing of dead birds reported by the public will detect early viral activity efficiently in terms of both effort and costs, so long as susceptible bird species that experience a high mortality rate from infection with WNV, such as corvids, are present in urban areas. Testing of the mosquito vectors is both efficient and cost-effective for detection of virus activity in the early season, and sentinel chicken testing detects the most viral activity in the peak and late season and is also slightly more cost-effective than testing of mosquitoes during these late-season periods, with the caveat that some of the detections in chickens may represent transmission events that occurred 1 - 3 weeks prior.

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*The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Biological Control Organism Program at Coachella Valley Mosquito and Vector Control District

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Adapting to a changing environment has been a function of the Coachella Valley Mosquito and Vector Control District since its inception. The District was established March 12, 1928 by the Riverside County Board of Supervisors to control a growing eye gnat problem. When the Coachella Valley Channel brought water to the valley, bringing with it mosquito breeding sites, the District was expanded to conduct mosquito control in 1951. In 1995 the District expanded to a full vector control agency with its current name, and in 2005 the Red Imported Fire Ant program was added. The District opened its BioControl Facility in 2006. Personnel at the BioControl Facility have examined organisms that can reduce the populations of larval mosquitoes in the Coachella Valley. District staff have examined mermethid nematodes, tadpole shrimp and mosquitofish as viable mosquito control agents.

Nematodes (Romanomermis iyengari). Nematodes parasitize mosquito larvae, killing the mosquitoes as they exit. A single inoculation of two outdoor ponds in July 2009 produced nematodes for more than a year. Staff examined whether the substrate in local detention basins was appropriate for the adult nematodes to reproduce. Only when the substrate was gravel were mosquito larvae parasitized by nematodes. Most of the Coachella Valley consists of soil or sand substrate, so the District did not continue to pursue nematodes as a control agent.

***Tadpole Shrimp* (*Triops newberryi*)**. Tadpole shrimp are crustaceans found naturally in the Coachella Valley. These organisms live in temporary pools of water and have diapausing eggs which makes the agricultural habitats ideal places to use these organisms. While the tadpole shrimp can be a pest in rice, they have not been harmful in citrus and date groves. The District produces and stocks tadpole shrimp in organic date gardens, distributing tadpole shrimp egg laden soil in rows as a bio-control agent of *Psorophora* and *Culex* spp. mosquitoes. When the Bio-Control Facility was constructed in 2006, the District attempted rearing tadpole shrimp outdoors; since then, rearing has moved indoors. In 2013 approximately 100,000 tadpole shrimp eggs were produced in the District's indoor rearing facility. Approximately 30,000 eggs were stocked in a local organic date garden during the spring of 2013.

***Mosquitofish* (*Gambusia affinis*)**. Mosquitofish are a highly effective biological control agent for mosquito control. District staff regularly stock mosquitofish in man-made water retention structures such as neglected pools, duck clubs, drainage ditches and private ponds. District biologists are evaluating mosquitofish effectiveness in street side catch basins that are continually filled with water from "urban drool" or runoff from poor irrigation designs in suburban landscapes of the Coachella Valley. In 2013 more than 180,000 fish were produced at the District's indoor and outdoor rearing facility and nearly 140,000 fish were stocked in duck club ponds, man-made water retention structures, and non-functioning swimming pools and ornamental ponds (Figure 1).

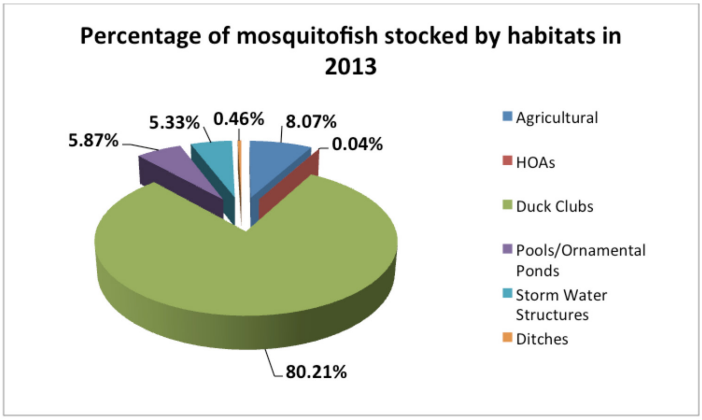

Figure 1. Placement of mosquitofish by habitat stocked in 2013

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Fighting the Bite One Scout Patch at a Time

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ABSTRACT: Since the arrival of West Nile virus (WNV) in San Diego County in 2004, the San Diego County Vector Control Program (VCP) has developed a wide spectrum of outreach tools to teach residents how to protect themselves from WNV. In order to reach high risk outdoor enthusiast youth groups and their families, the VCP developed a participatory scout patch program in partnership with the Boy and Girl Scouts of San Diego-Imperial Councils. The scout patch program uses interactive online games and quizzes to impart basic knowledge of WNV and disease prevention strategies, allowing Boy and Girl Scouts to earn a "Fight the Bite" WNV participatory patch to be worn on the back of their sash or vest. Details of the program are discussed in this paper.

INTRODUCTION

The County of San Diego has an estimated 1,355,896 residents (US Department of Commerce, 2014). The Boy and Girl Scouts of America, San Diego-Imperial Councils have approximately 46,547 scouts. Of these, 8,700 Girl Scouts are between ages 6 - 12 years and 8,598 Cub Scouts (a junior branch of the Boy Scouts) are between the ages of 7 - 10 years (BSA Annual Report, Imperial County Council, 2013; About Girl Scouts, San Diego-Imperial, 2014). The VCP targeted scouts in this age bracket due to the size of this group as well as their high risk of WNV exposure during outdoor activities. Additionally, because scouts have strong ties to their communities and must perform community service, they have the potential to use their knowledge to educate neighbors about WNV, to change behaviors to reduce the risk of WNV infection and to protect public health.

METHODS

The VCP developed outreach materials that appeal to youth 6 -12 years of age. The materials include a "SD Swat Team" website designed for children at the 2nd - 8th grade reading levels (www.sdswatteam.com). On the website, kids can play an educational online game called "Hazard Hunt" where players learn about the mosquito habitats and lifecycle. Players then identify hazards around the home in the form of mosquito breeding sites. Upon completion of the game, a participatory certificate can be printed out and presented to their scout leader to receive the "Fight the Bite" patch (Figure1).

Figure 1. "Fight the Bite" Participatory Scout Patch.

A professional marketing company was hired to develop and create the scout patches. The VCP created flyers to distribute to Boy and Girl Scout troops to teach them about the game and how to earn the "Fight the Bite" patch. The VCP introduced the Scout Patch Program to the Boy and Girl Scouts of San Diego Imperial Councils in 2012, and the program was officially launched in March 2013.

RESULTS

Two hundred and fifty patches were given to the Boy and Girl Scouts. The majority of the Girl Scouts that received the Scout Patch were Brownies (grades 2nd – 3rd) and Daisies (kindergarten – 1st grade). The Boy Scouts that participated most were Cub Scouts (grades 1st-5th). Since the program was launched, the SD Swat Team website has had 1,018 views with a total of 335 sessions. The number of views peaked on November 27, 2013 with 59 visits. This coincided with the release of the Girl Scouts bi-weekly newsletter that promoted the Scout patch program.

DISCUSSION

The Boy and Girl Scouts had a lot of positive comments regarding the "Fight the Bite" scout patch program. Parents and scouts showed great interest in the program when they attended outreach events provided by organizations throughout the community. Since the program had positive preliminary results, the VCP will continue to promote the "Fight the Bite" Scout patch program. Each troop leader will record the age and gender of the scout receiving the patches as well as their scout level. To improve the success of the program, pre and post patch questionnaires will be submitted to the troop leaders. Scouts will be encouraged to fill out the questionnaires in order to measure and improve the effectiveness of the Scout Patch Program in heightening their knowledge of WNV prevention.

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Developing a Predictive Risk Map for West Nile Virus Activity based on Mosquito Breeding Sources and Socioeconomic Factors in Orange County, California

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ABSTRACT: In this preliminary study, we built a comprehensive geo-database from different data resources and relied on advanced data mining and GIS technology to determine the spatial correlations among reported human West Nile virus (WNV) infections, known mosquito breeding sources, WNV-positive dead birds and mosquitoes and socioeconomic factors within Orange County, California from 2004 - 2012. We applied a multivariate logistic regression model in R to determine the significant factors for accurate prediction of WNV risk areas in the county. We corroborated the model with a cross-validation method based on known locations of locally acquired, human WNV infections during 2004 – 2012. Our results showed that the model's accuracy was approximately 84% in R. Based on the cross-validation method, a group of socioeconomic factors were shown to be more important determinants for human WNV infection than living in close proximity to mosquito breeding sources, WNV-positive dead birds or infected mosquitoes. However, of any single factor, standing water in street gutters was found to be frequently associated with infection. Finally, we developed a new technique for the location selection method, or suitability analysis, to highlight areas for high disease risk, and then developed a new method called "Information Ball" to solve data compatibility and software problems to visualize areas in Orange County with the highest risk for WNV transmission to humans.

INTRODUCTION

Since the introduction of West Nile virus (WNV; family: *Flaviviridae*, genus: *Flavivirus*) to southern California in 2003, Orange County has recorded 240 human WNV infections with 9 deaths during 2004 - 2012 (OC Health Care Agency, http://ochealthinfo.com) and has been recognized as a "hotspot" of WNV activity with positive mosquito pools, dead birds, seropositive wild birds and human infections occurring every year since its introduction (Figure 1). Most of the county's human WNV infections have occurred during August – September (Figure 2) and have varied annually through distinct four-year cycles (Figure 1). In the peak year of 2008, Orange County had the third highest number of reported human infections (79) per county in the United States (USGS, http://diseasemaps.usgs.gov).

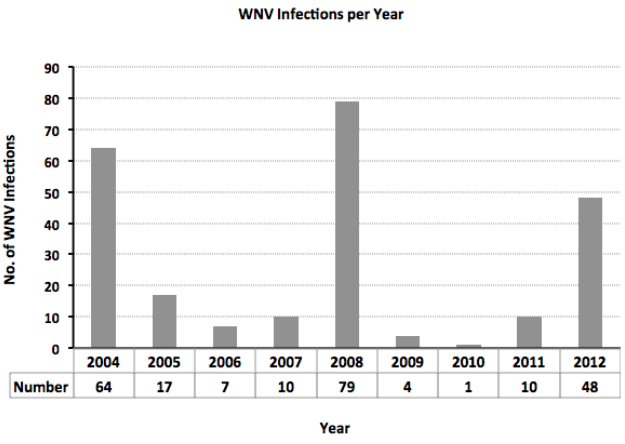


Figure 1. Number of WNV infections per year, 2004 – 2012.

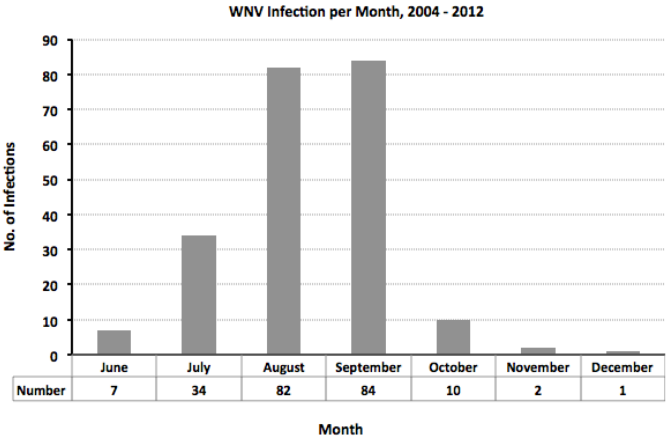


Figure 2. Number of WNV infections (n = 200) by month of onset, 2004 – 2012. Note: 40 of the 240 known infections do not have verifiable onset dates.

WNV is endemic in the county and is expected to be a public health concern indefinitely (OC Health Care Agency, http://ochealthinfo.com). The Orange County Vector Control District (OCVCD) routinely recommends all residents and visitors to take preventative measures to avoid being bitten by mosquitoes, especially during warm months of the year (April – November) when arboviral disease transmission is most likely to occur (http://ocvcd.org).

WNV is transmitted and maintained in southern California primarily in urban landscapes through a bird-mosquito cycle, with the peridomestic-breeding southern house mosquito, *Culex quinquefasciatus* Say. Several species of passerine birds such as the house finch (*Haemorhous mexicanus* Muller), house sparrow (*Passer domesticus* L.) and American crow (*Corvus brachyrhynchos* Brehm) serve as the primary enzootic and epizootic

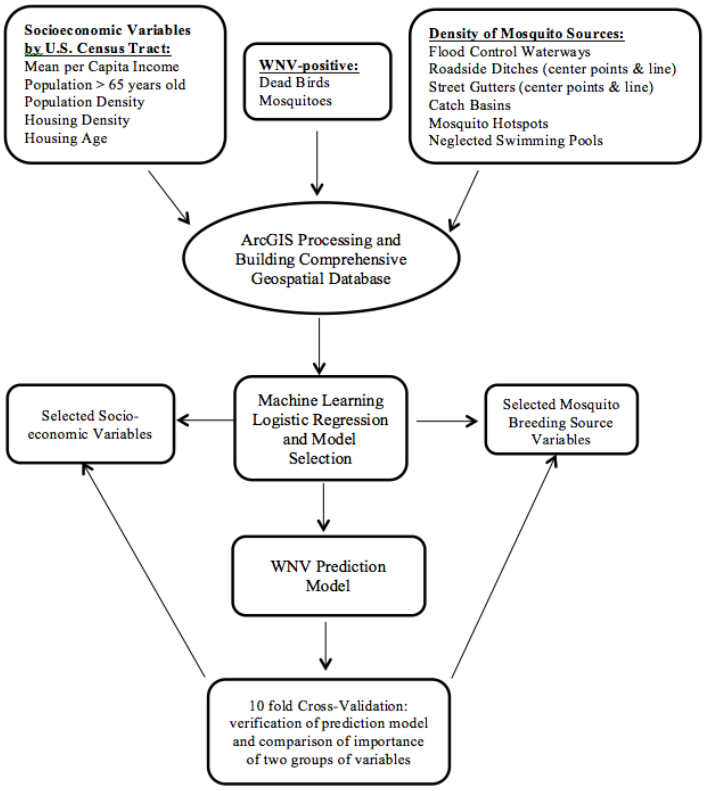
drivers of WNV activity in Orange and the surrounding counties of southern California (Kwan et al. 2010, Molaei et al. 2010, Kwan et al. 2012, Reisen 2013). With the recent advent of powerful geographic information systems (GIS) technologies, landscape epidemiology has proven very effective at predicting both spatial and temporal distributions of certain diseases, especially for vector-borne diseases- transmitted by mosquitoes, ticks or other animals (Reisen 2010). While a number of these studies have focused on environmental variables and/or socioeconomic factors (Ezenwa et al. 2006, Rios et al. 2006, Ruiz et al. 2007, Harrigan et al. 2010 ,Rochlin et al. 2011) in developing WNV disease models, only a few investigations (Reisen et al. 2008, Harrigan et al. 2010) have incorporated any mosquito breeding source information as a contributor to WNV outbreaks. No reports, however, have included detailed data of known mosquito breeding sources into spatial analyses of WNV transmission across landscapes. Since the OCVCD is in the process of digitizing many of its known mosquito breeding sources, the question arose as to the insights our agency would gain by developing a WNV risk map based on a variety of mosquito breeding sources and WNV human infection data. As part of a preliminary study, we built a comprehensive geo-database from different data resources and relied on advanced data mining and GIS technology to determine the spatial correlations among reported WNV cases, known mosquito breeding sources, WNV prevalence levels in dead birds and mosquitoes, and socioeconomic variables to accurately identify WNV hotspots within Orange County, California.

METHODS

Study Area. Orange County occupies a large portion of the Greater Los Angeles Basin, and has an estimated population of 3,010,237 people living in a mostly metropolitan area of 790.6 square miles; the population density is approximately 3,808 persons per square mile and is the third most populous county in the state (US Census Bureau, http://quickfacts.census.gov/qfd/states/06/06059.html). Most of Orange County's population lives in two low-lying urbanized areas in the coastal Santa Ana and Saddleback valleys. The region's climate is Mediterranean, moderated by easterly winds from the Pacific Ocean, with mean annual rainfall of 13.59 inches and mean annual temperature of 64.6° F. (Orange County Weather, http://www.usa.com/orange-county-ca-weather.htm). The weather is typically warm and dry from May – October, with precipitation occurring mainly during November – April.

Mosquito breeding sites in the county consist largely of sources associated with human development, such as water-filled residential containers, improperly maintained swimming pools and ponds and slow-flowing water from residential irrigation runoff in street gutters, catch basins and drainage channels (Su et al. 2003).

Prediction Variables. Simplified flowchart of the materials and methods employed.



Data Analysis. Multivariate logistic regression was used to predict the relative ranking and significance of the variables (Pradhan 2010). The model was then subjected to a 10-fold cross-validation to estimate how accurately it would perform in practice. ArcGIS ver10.1® was used to process the data separately by Kernel density and line density calculation. Of the listed 240 human WNV infections received from the Orange County Health Care Agency, 238 were used (2 cases contracted the virus from unknown locations). Each infection location became a "positive point" while another 238 random point locations were generated from ArcGIS and used as negative points.

We calculated the middle point for the length of the various street gutters, roadside ditches and flood control channels and used these locations to calculate the number of points per square mile for each of these linear mosquito breeding sources. The numbers of neglected swimming pools and catch basins per square mile were also calculated for each source type based on their locations in the county. These data were then correlated to the 238 WNV-positive points and 238 negative points to extract information separately from the 14 variable layers (socioeconomic = 5, WNV positive dead birds& mosquitoes = 2 and breeding sources = 7) in ArcGIS. Finally, by collecting data from AcrGIS® and other resources, the geodatabase was built. From this, the Akaike Information Criterion (AIC) was used for final model selection.

RESULTS AND CONCLUSIONS

From these analyses, the variables of importance ($P < 0.05$) were selected and ranked from highest to lowest: street gutter > housing unit density > neglected swimming pools > mean per capita income > mosquito hot spot > ditches > housing average age. None of the other variables such as the census tract-based data for the proportion of the population over 65, population density, and locations of WNV-positive dead birds/mosquitoes, catch basins and flood control waterways were significant. Next, using this new group of significant variables, we used R software for a new logistic regression model to establish a new level of combined significance. From this, a final WNV risk model with a prediction error rate of 0.170968 was made based upon proximity to the following variables: street gutter + housing density + neglected swimming pools + mean per capita income + mosquito hot spot + roadside ditches + average housing age. For any single variable, proximity to known street gutter mosquito breeding sites was found to be the most significant in determining WNV risk. Collectively, three socioeconomic factors (housing density, housing age, and mean per capita income) had a lower prediction error rate (0.1876541) than the combination error rate (0.2058115) of the four mosquito breeding sources (street gutters, roadside ditches, neglected swimming pools and mosquito hot spots).

Visualization of Thematic WNV Risk Map. For data visualization, the interpolation function of ArcGIS 2010 was used to generate a WNV risk map for Orange County based on the analyses (Figure 3). Because of Health Insurance Portability and Accountability Act (HIPAA) regulations, the Orange County Health Care Agency prohibits the OCVCD from displaying the locations of the 238 human WNV infections in this paper. However, approximately 84% (n = 200) of the human WNV infections were captured within the red shaded areas, indicating high risk, as depicted in Figure 3. Of the remainder, 31 infections fell within moderate-risk areas (yellow shading), while only 7 infections were found to lie within low-risk, green-shaded areas.

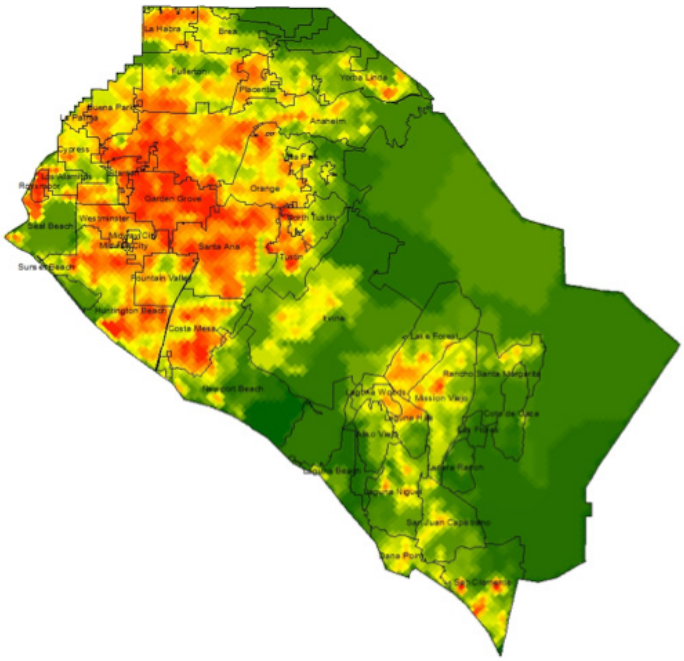


Figure 3. WNV risk map, Orange County, California, based on 238 WNV infections with onset dates from 2004 – 2012. Red shading indicates highest risk areas, yellow moderateß and green the lowest.

The OCVCD has used the retrospective compilation of WNV human infection data to re-examine many of its mosquito control practices. Although separated by years, many WNV infections have occurred in clusters (2 or more infections) of within-cluster distances of less than a quarter mile apart (some cases have appeared on the same streets). Detailed inspections of these areas have revealed several unrecognized mosquito sources, especially poorly-draining underground drains, in these impacted neighborhoods. The OCVCD plans on continuing with its geocoding of vector sites and incorporating more data in future geospatial WNV risk models.

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Use of modeling to predict the spread of *Aedes albopictus*

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INTRODUCTION

Aedes albopictus is a competent vector of numerous arboviruses, including West Nile and dengue viruses, as well as chikungunya virus, which have emerged rapidly in the Western Hemisphere during the past year. The relevance of *Ae. albopictus* for public health is heightened by its ability to invade and establish in new areas through movement of its desiccation-resistant eggs in containers. During the past 30 years, this species has expanded its range to include much of southern Europe (Mitchell 1995, Aranda et al. 2006, Carrieri et al. 2011, Munnoz et al. 2011, Roiz et al. 2011) and the eastern U.S., and it had invaded California repeatedly in the past without successful establishment (Kluh et al. 2002, Linthicum et al. 2002, Linthicum et al. 2003, Tietze 2003). Since 2011, however, it has been established in a small area of southern California focused in two cities within the Los Angeles Basin. In this study, we used a detailed spatial simulation model to consider two questions: (1) What are the spatial scales at which surveillance and control should be focused following invasion? and (2) What is the most likely mode of introduction into new areas?

MATERIALS AND METHODS

The study area covered the incorporated cities of El Monte (San Gabriel Valley MVCD) and South El Monte (Greater L.A. County VCD), two contiguous cities that have been the foci of surveillance and detection of *Ae. albopictus* since its discovery in 2011. Real estate parcels (n = 24,549 – typically consisting of individual households and associated land) were the spatial units chosen for modeling because they resolved habitats at a fine enough scale to represent mosquito flight patterns realistically.

Two models were constructed. The first was a receptivity model to capture heterogeneity among parcels in the probabilities that *Ae. albopictus* was present. This model was fitted to *Ae. albopictus* collection data, including trapping and inspections, from the respective vector control agencies between 2011 and 2013. The model was hierarchically structured, capturing variation in the probabilities of *Ae. albopictus* presence among real estate parcels within census blocks due to land use, greenness and the intensity of urban development. The resulting modeled receptivity surface was used to weight the probabilities of *Ae. albopictus* movement among parcels, with greater probability of moving to parcels with higher probabilities of presence.

The second model was the movement simulation model in which *Ae. albopictus* adult females remained within a parcel or moved to neighboring parcels within 200 m each day. The 200-m radius for each parcel's neighborhood was chosen as the range that most closely reproduced mark-recapture results (Marini et al. 2010) from movement simulations using our final model. For each model simulation, movement occurred randomly according to probabilities of staying or moving that were informed by the model-based receptivity model described above and the distances between parcels, with inverse-distance weighting so that movement to nearby parcels was more likely than movement to more distant ones. The model also simulated reproduction of *Ae. albopictus,* and females alternately fed and laid eggs at rates defined from literature (Hawley 1988 and references therein). Variation in air temperatures obtained from a local weather station was used to drive rates for gonotrophic and aquatic immature development (Delatte et al. 2009) through fitted rate equations (Sharpe and Demichele 1977).

RESULTS AND DISCUSSION

For the receptivity model, the best fit to *Ae. albopictus* occurrence data was achieved using the intensity of urban development within census blocks and the greenness of each parcel estimated by the maximum normalized difference vegetation index (NDVI) at 1-meter resolution from the National Agricultural Imagery Program. Using the receptivity model to define movement probabilities for all parcels (e.g., Figure 1) and literature-based biological parameters (e.g., Figure 2), we then simulated movement of *Ae. albopictus* following introductions in randomly-chosen parcels near the center of the El Monte-South El Monte area. The model was run for 100 stochastic simulations initiated at the mid-point of each season (winter = Feb 5, spring = May 5, summer = Aug 5 and fall = Nov 5), with random simulation of introduction site location and daily mosquito dispersal to estimate seasonal variation in the typical (median) and maximal ranges of *Ae. albopictus* movement as a function of days since the introduction.

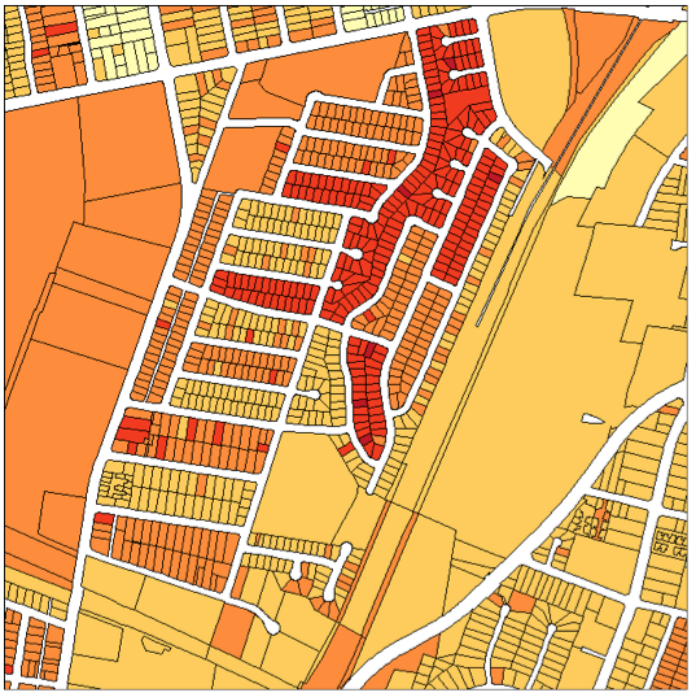

Figure 1. Example showing heterogeneity in receptivity (i.e., probability of *Ae. albopictus* presence) among real estate parcels in the study area.

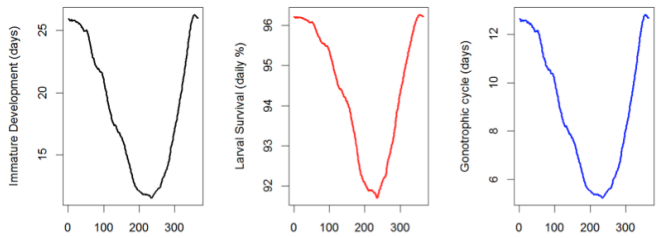

Figure 2. Model estimates for immature development times (egg hatch to adult emergence), daily larval survival, and gonotrophic cycle length based on fitted rate equations and detailed biological data (Delatte et al. 2009) for average temperatures in Los Angeles.

One week after introduction, some females had reached a distance of up to 300 m from the introduction point in some of the model simulations, but the typical female (represented by the median of all dispersal distances for each model simulation) had traveled shorter distances of 60-70 m. By two months after introduction, typical dispersal distances were centered around 100 m with a much wider range of 0 - 400 m among model simulations. Dispersal ranges varied seasonally, particularly for the maximum distance traveled, which was greater in summer than winter and presumably due to accelerated development rates in warmer temperatures. The maximum distance traveled over the simulated two-month period for any mosquito in any simulation was just less than 800 m, providing a conservative estimate of a potential radius for active surveillance and control following an *Ae. albopictus* introduction.

As reported previously (Barker et al. 2013), our model suggests that introductions of *Ae. albopictus* as a batch of unhatched eggs in a container would result in a greater probability of establishment than a single adult female transported in an automobile. This depends on average egg survival per day from literature, but we expect that natural egg survival would be lower due to the possibility of total egg loss following extended periods of desiccation or reduction in the potential for future egg flooding due to changes in container orientation during transport (e.g., rotation of tires). This is being considered in our ongoing model development.

It is important to note that detection of *Ae. albopictus* does not immediately follow its arrival in a new area, and increases in the amount of time that passes before detection by vector control personnel could substantially increase the area that would need to be targeted for surveillance and control. We are using our model to evaluate the impact of these detection delays as part of our ongoing research.

Our modeled dispersal estimates focus on mosquito movement and do not consider mechanisms for longer "jumps" in *Ae. albopictus'* range after the initial introduction. Such jumps could occur through human-aided dispersal of transported egg-laden containers or adult females in automobiles. Following a detection of *Ae. albopictus*, vector control personnel should additionally consider remote locations that are most likely to receive human-transported mosquitoes from the detection area (e.g., garden centers or used tire facilities) and consider these for further surveillance. Our estimates then provide a basis for the range of surveillance and control efforts that might be considered following each new detection.

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West Nile virus in Santa Clara County: 2008-2013

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ABSTRACT: In 2013 the Santa Clara County Vector Control District detected the highest level of West Nile virus activity in the county since 2007. This report outlines the district's WNV surveillance process and summarizes findings from 2008 - 2013 with an emphasis on the resurgence of virus detected in Santa Clara County in 2013.

INTRODUCTION

Santa Clara County is located at the southern end of the San Francisco Bay, covering 1,304 square miles and including a variety of ecological community types such as grasslands, oak woodlands, redwood forests and saltwater and freshwater marshlands. Approximately 1.68 million people inhabit its fourteen cities in predominately suburban neighborhoods (Wikimedia 2014). Since 1988 the Santa Clara County Vector Control District (SCCVCD) has served the residents of this county through the abatement of mosquitoes and mitigation of other vector-borne disease threats.

In the years immediately following the first arrival of West Nile virus (WNV) in California in 2003, the SCCVCD implemented a comprehensive WNV surveillance and response program (Tietze et al. 2008). Surveillance procedures included monitoring for WNV via dead bird reporting and testing, mosquito surveillance, sentinel chicken flocks and data and GIS analysis. Since 2003 there have been 19 human cases of WNV in Santa Clara County. The majority of these cases occurred in the first few years of virus detection in the county from 2004 to 2007. Subsequently, from 2008 through 2012, the District detected relatively low levels of WNV in the county, with the exception of elevated detection levels in 2013. This report summarizes changes in methodology and results of SCCVCD surveillance activities during the years 2008-2013.

MATERIALS AND METHODS

The SCCVCD has a multi-tiered surveillance and response program for the detection of WNV. The first level of surveillance consists of testing sentinel chickens and dead birds and squirrel carcasses reported to the District, either directly from the public or through the California Department of Public Health Dead Bird Hotline. A positive virus detection from any of these methods is followed by collecting adult mosquitoes using EVS traps from a one-mile radius around the initial animal detection. If any positive mosquitoes are detected in these collections, residential areas in an approximately one-mile radius of the trap location of the WNV-positive mosquito are subjected to enhanced mosquito control measures, including targeted delivery of educational materials and ground-based mosquito adulticiding.

Changes in Surveillance Methods. In recent years, modifications have been made to the surveillance protocols as described in Tietze et al. 2008. In 2006 and 2007, the SCCVCD began to limit WNV testing of reported carcass species to only corvids, raptors and tree squirrels. As a result of the changes in testing protocols due to budget restraints in the CDPH statewide WNV monitoring program during 2013, the District now only accepts corvids (i.e., crows and jays) for testing.

Over the past three years, the District has incorporated the use of real-time (TaqMan) reverse transcriptase-polymerase chain reaction (RT-PCR) for WNV testing. All mosquito samples and dead birds are now tested using in-house RT-PCR. The District uses the VectorTest™ as a rapid screening test for oral swabs taken from dead corvids, but only virus detections verified by RT-PCR testing are now considered "confirmed" positives by the CDPH. During the 2013 season, in keeping with state guidelines, the District also discontinued the use of the "chronic" positive category previously used for birds and squirrels in which relatively low levels of virus had been detected. These animals are now considered WNV-positive.

As this change in categorization potentially triggers additional mosquito trapping events, the SCCVCD began instituting a secondary RT-PCR test in 2013 to confirm whether these low-level virus indications are legitimate virus detections. All bird swabs are now initially tested via RT-PCR using the very sensitive Envelope (ENV) set of primers and detection probes (Lanciotti et al. 2000). If virus is detected at a level previously considered to be "chronic" (CT $\geq$ 30, per Reisen et al. 2013), the swab samples are re-tested through RT-PCR using a more specific, but less sensitive, set of Non-Structural Protein 1 (NS1) primers and probes (Shi et al. 2001). A positive result using NS1 indicates that the low-level detection is indeed evidence of WNV presence and not a result of contamination or other procedural error in the ENV test. All mosquito pools tested by the SCCVCD are also initially screened using ENV primers and probes, and low-level detections (CT $\geq$ 35) are confirmed by NS1.

Although this report is focused primarily on surveillance activities, it should be noted that after the publication of Tietze et al. 2008, the SCCVCD discontinued the community meetings that previously preceded WNV-triggered fogging events. The District now notifies residents of impending adulticiding operations via direct delivery of informational packets to affected households 24 - 48 hours in advance of the planned fogging event. During this same time period, the District also maintains a special phone hotline with extended hours to answer questions or address concerns from residents.

RESULTS

From 2008 through 2012, the District continued to detect WNV in dead birds, mosquitoes and other organisms surveyed (Table 1). Dead bird reports remained the most important early indicator for potential areas of high virus transmission risk. The numbers of birds testing positive varied from year-to-year, but were at relatively lower levels than in previous years. Only two human cases of WNV occurred during this five-year period.

Year	Human cases	Horses	Dead birds	Mosquito samples	Chickens	Squirrels
2008	1	0	13	1	0	0
2009	0	0	14	14	0	2
2010	0	0	32	10	0	6
2011	1	0	35	16	0	1
2012	0	0	20	3	0	2
2013	2	0	88*	25	2	0

Table 1. West Nile virus detections in Santa Clara County, 2008 - 2013. *Total from 2013 includes 11 birds that tested WNV-positive by VectorTest™ but were not tested by RT-PCR.

In 2013, the District saw a dramatic spike in virus activity, including detections of WNV in 88 dead birds, 25 mosquito samples and Santa Clara County's first two sentinel chicken seroconversions. Two human cases of WNV occurred in 2013, both in areas from which positive dead birds had been reported.

WNV in Birds. Although the numbers of WNV detections in birds was dramatically higher in 2013 than in the five preceding years, almost all detections were similarly during the warm summer months in Santa Clara County (Figure 1). The peak month of detection in 2013 was somewhat later than in other years, but in all cases virus activity fell off rapidly by the end of the year.

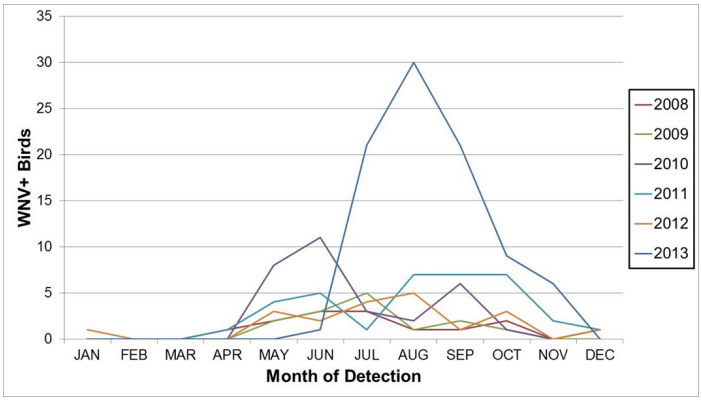

Figure 1. Seasonal Distribution of WNV-positive birds, 2008-2013.

The geographical distribution of the WNV bird detections had two definitive foci in 2013. Whereas in the preceding seasons positive bird findings were distributed in small clusters in San Jose and in the western suburban areas of the county, in 2013 San Jose became the major focus of West Nile virus activity, with an additional area of multiple WNV-positive bird detections in Milpitas on the east side of Santa Clara County (Figure 2 and Table 2).

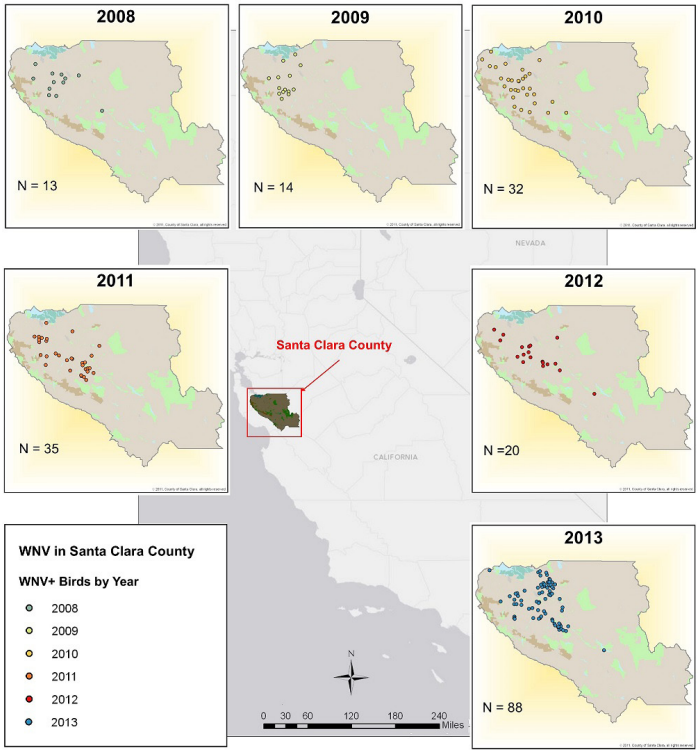

Figure 2. Multi-year Distribution of WNV-positive birds in Santa Clara County.

City	2008	2009	2010	2011	2012	2013	Total
Campbell			2	1			**3**
Cupertino			2	1		1	**4**
Los Altos	1	1	2		2		**6**
Los Gatos			1				**1**
Milpitas			1			6	**7**
Monte Sereno			1				**1**
Morgan Hill					1	1	**2**
Mountain View	2		1	1	2		**6**
Palo Alto/Stanford			4			1	**5**
San Jose	4	8	8	24	12	59	**115**
Santa Clara	4	1	6		3	1	**15**
Saratoga	1	2	2	1		4	**10**
Sunnyvale	1	2	2	7		4	**16**
Total	**13**	**14**	**32**	**35**	**20**	**77**	**191**

Table 2. WNV-Positive Birds by City, 2008 - 2013. Data retrieved from "westnile.ca.gov," and does not include VectorTest™-only detections from 2013.

WNV in Mosquitoes. A total of 69 WNV-positive mosquito collections were made in seven cities over 2008 - 2013 as shown in Table 3. The only city with positive mosquito detections every year was San Jose. San Jose was also the city with the highest number of WNV+ mosquito detections in any given year. Until 2013, WNV was found in mosquitoes outside San Jose in a scattering of cities on the south west side of San Francisco Bay, south of San Mateo County. Similar to the pattern of WNV detections in birds, the first detection of WNV in mosquito pools from the eastern suburban communities of the county occurred in 2013, in the city of Milpitas, just south of Alameda County.

City	2008	2009	2010	2011	2012	2013	Total
Campbell			1				1
Los Altos					1		1
Milpitas						2	2
Mountain View					1		1
San Jose	1	13	6	15	1	23	59
Santa Clara			3				3
Sunnyvale		1		1			2
Total	**1**	**14**	**10**	**16**	**3**	**25**	**69**

Table 3. WNV-Positive Mosquitoes by City, 2008 - 2013.

DISCUSSION

Improvements in in-house testing methods and public notification processes has led to greater confidence in test results and faster operational response times. Limiting carcass testing to only corvids allows for detection of areas of elevated virus levels with a minimum of excess collection and testing effort. As shown in Table 4, in-house RT-PCR testing in 2013 allowed the district to complete testing of mosquito pools within two days, in most cases. Between 2003 and 2007, the average time lag between WNV detection in mosquitoes and operational response was 12 days (Tietze et al. 2008). In 2013, the average delay between mosquito trap date and adulticiding action was down to seven days (Table 4). The average time from test detection to fogging was only 5 days.

Mosquito Traps Set	Mosquitoes Tested	Days to test	Fog Date	Days from Trapping to Fogging	Days from Testing to Fogging
7/3/2013	7/5/2013	2	7/11/2013	8	6
7/17/2013	7/22/2013	5	7/26/2013	9	4
7/25/2013	7/26/2013	1	8/1/2013	7	6
8/7/2013	8/9/2013	2	8/15/2013	8	6
8/14/2013	8/16/2013	2	8/21/2013	7	5
8/17/2013	8/19/2013	2	8/23/2013	6	4
8/20/2013	8/22/2013	2	8/26/2013	6	4
8/22/2013	8/23/2013	1	8/28/2013	6	5
8/28/1930	8/30/2013	2	9/4/2013	6	4
8/29/2013	8/30/2013	1	9/4/2013	5	4
9/6/2013	9/6/2013	0	9/11/2013	5	5
	Averages:	**2**		**7**	**5**

Table 4. Operational Response Times for WNV-Related Adulticiding Events in Santa Clara County, 2013.

The recurrent annual detections of WNV in Santa Clara County support the idea that the virus is well established in this area. However, the high variance in detection levels, timing and geographical distribution in certain years implies that the occurrence of the virus may be significantly affected by changes in weather or other environmental and ecological conditions. While the rest of California observed peak virus activity in 2012, Santa Clara County experienced much higher levels of WNV in 2013. Events such as the WNV outbreak in Texas in 2012 have demonstrated the potential for the epidemic spread of human disease in the absence of adequate vector monitoring and mosquito control (Garcia 2013). Although the mechanisms causing the variability in this endemic virus may be not well understood, the need for ongoing surveillance and rapid response by the SCCVCD clearly persists.

ACKNOWLEGEMENTS

Many thanks to Menou Thaopraseuth, Bill Shipway, Mike Stephenson, Caroline Dunkelberger, Mark Marden, Jeff Young, Noor Tietze and the entire staff of the SCCVCD for their hard work and assistance.

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Pyrethroid Pesticides in San Diego County Freshwater Streams

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Urban streams of San Diego have been impacted by the modernization of pesticide practices from agricultural, commercial and domestic use. Pyrethroids, commonly used for mosquito adulticiding, in recent studies have been shown to accumulate in freshwater sediments through urban runoff. Even though pyrethroids are not used by the San Diego County Vector Control Program, significant levels of pyrethroids associated with acute toxicity have been found in three urban streams of San Diego: Los Peñasquitos Creek, San Diego River and Forrester Creek. Sediment samples were collected between 2 - 10 days after rain events and were analyzed using the EPA's 10-day Whole Freshwater Sediment Acute Toxicity Invertebrate Test. Sediments from each site were tested *in vitro*, demonstrating survival rates of the indicator organism *Hyalella azteca*. Sampling captured from two rain events during the 2011 - 2012 rain season showed all three sites had survival rates ranging from 44 - 58% and 22 - 62%, respectively. A Toxicity Identification Evaluation (TIE) utilizing piperonyl butoxide (PBO), carboxylesterase and C18 columns were designed to identify pyrethroids as the main cause of mortality. These three different applications were applied to sediment samples that showed the lowest survival rate (22%) collected from Forrester Creek. PBO, a synergist of pyrethroids, was added to the sediment, increasing toxicity twofold over 48 hours. Carboxylesterase, an antagonist of pyrethroids, was also added to the sediment and improved survival rates to 98%. C18 columns were used to extract pyrethroids bound to sediment particles, which improved the survival rate of *H. azteca* to 98%. Lastly, chemical analysis conducted via Liquid Chromatography-triple Quadrupole Mass Spectrometry on all sediment samples also confirmed the presence of permethrin and bifenthrin pyrethroids. Permethrin and bifenthrin were found in dry weight concentrations ranging from as low as 1.9 – 26.0 µg/kg and non-detect to 53.0 µg/kg, respectively. This study demonstrates the importance of non-Vector Control Program pesticide use as a contributing source of excessive ecological toxicity. The ecological impact and any resistance that may develop within our native mosquito population should be considered before any countywide vector control efforts using pyrethroids are employed.

Getting in the Door: Alerting Residents to Increased WNV Activity in their Neighborhoods Using the USPS Every Door Direct Mail program

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ABSTRACT: A key component to Integrated Vector Management is Public Outreach with many programs using a mix of paid advertising, news media, events and community presentations to raise awareness about mosquito control and public health protection. One challenge with these channels is getting into every household in a targeted community to alert individuals quickly if there is increased West Nile virus (WNV) activity discovered in their neighborhood. This is a particularly difficult task in the diverse communities present in the Coachella Valley. This paper explores "The Yellow Postcard Pilot" carried out by the Coachella Valley Mosquito and Vector Control District September 11 - 14, 2013. We used the United States Postal Service's Every Door Direct Mail (E.D.D.M.) online tool to identify homes within a half-mile radius of traps where WNV-positive mosquito samples had been collected. We designed bright yellow postcards alerting residents to the increased disease transmission using the E.D.D.M.'s specifications. We then printed and dropped off the cards at the local Post Office in pre-bundled stacks of 100. The postcards were then distributed to 1,880 homes located on the streets we specified using the E.D.D.M. online tool. The total cost for postcard design, printing, and delivery was $0.32 per household (less than the cost of a postage stamp), and the total time passed between decision to send the postcard and delivery was three days. We also carried out a door-to-door survey in the delivery area to evaluate the impact of the postcard pilot.

INTRODUCTION

Every effective Integrated Vector Management program includes public outreach as one of the key components (WHO 2012). The challenge is identifying the most effective public outreach channels for a particular community. The Coachella Valley have a diverse population consisting of 439,000 people: 30% are >55 years old, and 28% are < 20; 37% are white and 55% Hispanic; median incomes ranges from $111,078 in the city of Indian Wells to $40,299 in the city of Coachella; and education levels among adults range from 24% with a Bachelor's or higher degree to 47% with at most a high school diploma (Coachella Valley Economic Partnership 2013). Raising awareness among the general population about the dangers of mosquito-borne illnesses and protection and prevention measures to reduce the risk of contracting these diseases can be carried out via the traditional communication methods in both English and Spanish. These methods include television, radio, newspaper, cinema and online advertising, as well as direct outreach at schools, city halls, community organizations, fairs and festivals.

The greatest challenge is reaching all residents in a specific neighborhood where increased mosquito populations or mosquito-borne disease transmission has been discovered. Traditional methods to reach small communities include door-to-door campaigns, leaving information on "door hangers" outside homes, compiling mailing lists for direct mailing and holding community meetings. A number of obstacles exist to these traditional methods, and given the Coachella Valley's diverse demographics, each neighborhood's obstacle may be different. Some neighborhoods are made up of gated communities or gated homes with no direct access to the residence. With a large community of part-time residents, door hangars left outside for days are a sign to robbers that no one is home, making this an unpopular method of communication. Some neighborhoods and communities have a network to organize meetings quickly, while others have no such built-in system. Identifying and buying lists of area addresses for a direct mail campaign can be costly and time intensive.

OBJECTIVE

Our objective was to identify a method to inform all residents of a particular neighborhood or group of neighborhoods when: 1) There are increased mosquito populations where we are not able to find the breeding source, and we need help locating potential backyard sources, or 2) There is an increase of West Nile virus-positive mosquitoes in a certain area, and we want to encourage heightened protection practices. The approach should not require District staff to go door-to-door, especially in gated communities; should avoid leaving materials exposed outside of homes; should not involve developing or buying address lists and mailing at standard postage rates; and should be able to reach the community directly within five days of discovering increased mosquito populations or disease transmission.

METHODS

The United States Postal Service Every Door Direct Mail (E.D.D.M.) program is an online tool that permits users to mail exclusively to small areas, based on mail carrier routes at only $0.16 per mail piece. The user can map out a target area, use demographic data (e.g., age, household income and size) to select a delivery route, choose a mailing drop off date and pay online. The tool does not require identifying names or addresses, only choosing streets or blocks within pre-identified mail carrier routes (Figure 1).

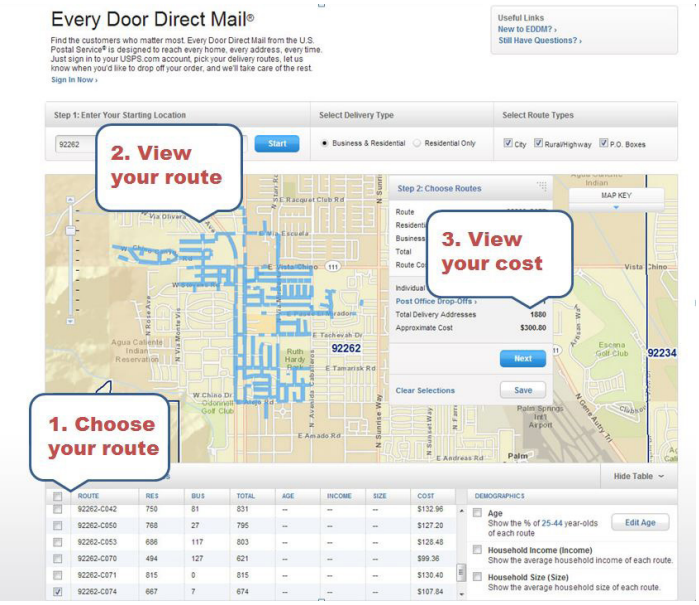

Figure 1. The United States Postal Service Every Door Direct program online tool makes it possible to target a population by neighborhoods.

We decided on September 11, 2013 to pilot the program when four mosquito samples in the same neighborhood of Palm Springs tested positive for West Nile virus over a five-week period, with the last positive discovered on September 9. We could not locate a breeding water source in the area and transmission in this part of the Valley was rare. Using the E.D.D.M. mapping tool, we identified 1,880 homes within a half-mile radius of the mosquito traps where WNV-positive mosquito samples had been collected. We then used the E.D.D.M. mail specification guidelines to design a bright yellow postcard alerting residents to the increased disease transmission in their area with information on breeding prevention and personal protection (Figure 2).

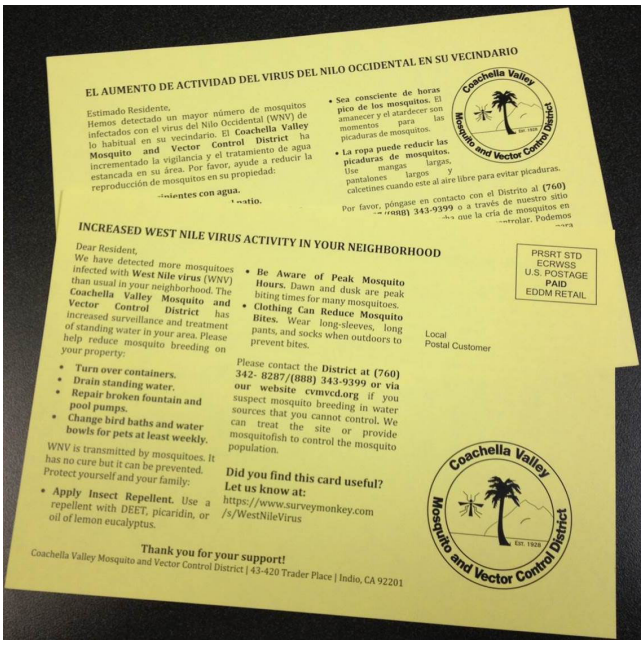

Figure 2. Postcards were created in English and Spanish to alert residents about disease transmission in their neighborhoods and then distributed through the Every Door Direct Program.

We contracted a printer to print, bundle in stacks of 100 (also per the specifications), and deliver the cards to the local Palm Springs Post Office. The postcards were then distributed to 1,880 homes located on the streets specified using the E.D.D.M. online tool. The postcards were designed and translated using District resources on September 12. The postcards were printed and delivered to the Post Office September 13 at a cost of $297.54. The mailing charge for the 1,880 homes was $301.60 and the postcards were delivered on September 14. The combined cost was $599.14, making the production and delivery of the public health notification to every home in our target area $0.32 per residence, or $0.14 less than the cost of a U.S. postage stampat that time.

RESULTS

Two weeks after the postcards were delivered, two District staff members visited the streets along the delivery route with a survey about the postcards. Out of 75 homes approached, most were not accessible without a security code, and of the remaining homes 17 people answered the door and agreed to do the survey. An additional person called the District and did the survey by phone. The survey asked the following questions: (1) How helpful did you find this postcard? (2) Would you like to receive this kind of public health notice again? (3) Did you share this information with your neighbors? (4) A comment box for anything else the resident wanted to share about the postcard (Figure 3).

Figure 3. District staff canvased two streets along the specified delivery route with a survey to gather feedback on the impact of the postcards.

Of the 18 people who participated in the survey, 72% remembered receiving the postcard, and 92% of those found the postcard to be either "Extremely helpful," "Helpful" or "Somewhat helpful" and responded they would like to receive this kind of public health notice in the future (Figures 4 and 5).

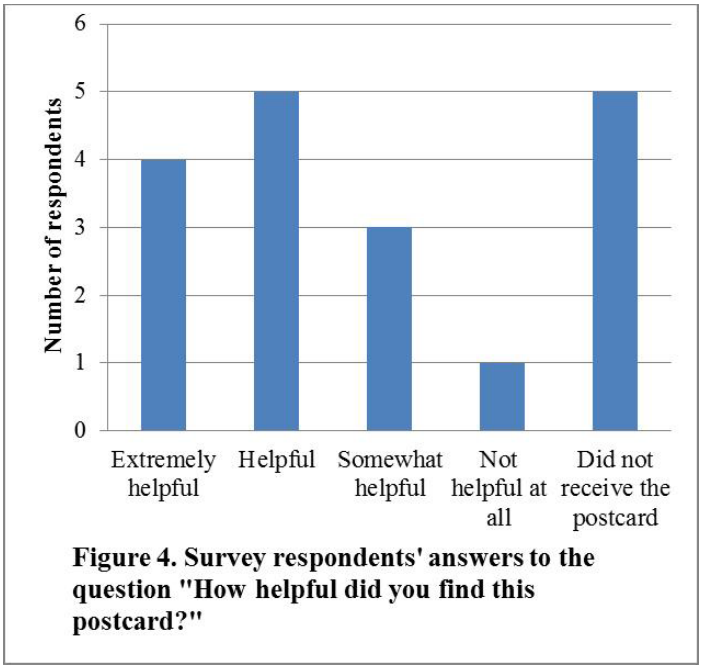

Figure 4. Survey respondents' answers to the question "How helpful did you find this postcard?"

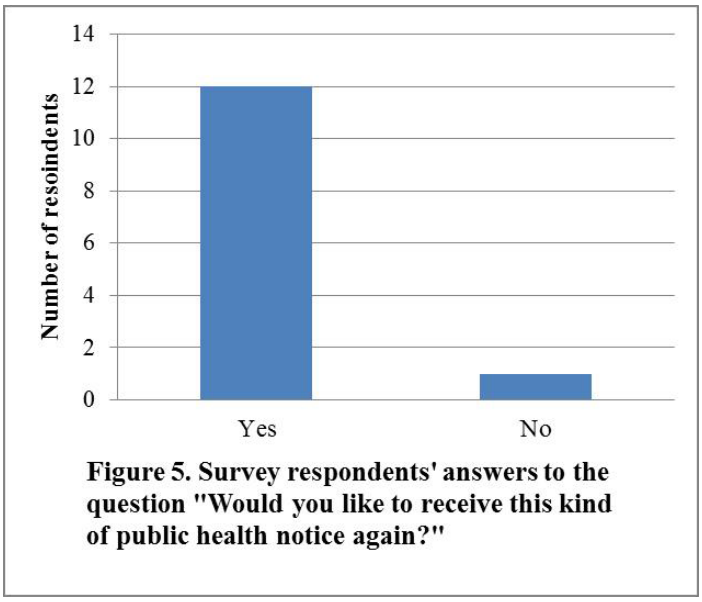

Figure 5. Survey respondents' answers to the question "Would you like to receive this kind of public health notice again?"

CONCLUSION

The E.D.D.M. program was fast, efficient and cost effective. The door-to-door survey we conducted to gather feedback about the postcard's impact underscored the difficulty vector control agencies face accessing some communities. While the sample size of survey participants was small to garner meaningful results, we were encouraged that most people received the postcards and remembered receiving them. We were also encouraged that 9 out of 10 people found the information on the postcards to be at least somewhat helpful. The E.D.D.M. program helped us achieve our intended objectives of reaching residents in a small defined area, without going door-to-door and without leaving materials on door handles outside of homes. We also did not require human or financial resources to develop address lists or to pay standard postage rates. We reached the community directly within three days of the decision to pilot the program which we found to be timely and faster than most communication channels.

To develop, translate, print and deliver materials as quickly as we did during this pilot, a few components need to be carried out prior to deciding on an E.D.D.M. mailing. (1) Prepare templates prior to an event so they just need to be tailored to the specific mailing; (2) Templates should adhere to the Post Office specification guidelines (dimensions, address and placement); (3) Identify a printer that can carry out the project within 24 hours; and (4) Work with local Post Offices to find out when retail flyers are delivered, as the Post Office will not deliver your mailer on the same day.

Survey participant comments gave us ideas for improved impact in the future, including adding a picture of a mosquito to the postcard, reducing the amount of text on the postcard, increasing the text size and including a map with the street names that border the area of concern.

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Evaluation of Biorational Larvicides in Storm Water Structures

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ABSTRACT: The Coachella Valley Mosquito and Vector Control District uses several biorational larvicides to treat breeding mosquitoes found in urban drool in storm water structures. Because these structures hold water continuously, briquettes and tablets with long residuals can potentially reduce the number of visits needed by technicians. We examined the efficacy of Fourstar® Sustained Release Microbial Briquets (180-day residual), Altosid® XR Extended Residual Briquets (150-day residual), and Natular™ XRT tablets (180-day residual) in catch basins and drywells. Initially, one briquette or tablet was suspended in each site. Sites were sampled for larval populations using a 2-dip method with a standard dipper. Lab-reared larvae were introduced into the site using modified BioQuip breeders, and breeders were replaced every two weeks. Water parameters (i.e., temperature, pH, conductivity and dissolved oxygen) were measured. Some sites required additional briquettes to provide adequate control. We found that Natular XRT tablets provided the most consistent control, whereas both the FourStar and Altosid XR briquettes were more variable in controlling larvae. Previous work showed that Altosid XR briquettes persisted for approximately 30 days when suspended instead of the 150 days on the label.

INTRODUCTION

The Integrated Vector Management (IVM) program controls the presence of mosquitoes in the Coachella Valley using physical, biological and chemical control methods. The use of long-term control products does increase the efficacy of the urban technician work flow, allowing for more sites to be covered by a single person. In urban areas, underground storm water structures provide habitats suitable for immature *Cx. quinquefasciatus* mosquitoes. The stagnant basins vary in the amount of water that they hold and the amount of trash that accumulates; both of these variables can impact product efficacy.

In this study, we evaluated the efficacy and the residual activity of three products labeled for long-term mosquito control: Fourstar® Sustained Release Microbial Briquets, Natular™ XRT tablets and Altosid® XR Extended Residual Briquets. FourStar briquettes use *Bacillus sphaericus* and *Bacillus thuringiensis israelensis* and are labeled for up to 180 days of control. Natular XRT tablets have spinosad as the active ingredient and may provide up to 180 days of control. Altosid XR briquettes are S-methoprene and are labeled for 150 days of potential control.

MATERIALS AND METHODS

Stagnant catch basins and drywells in urban areas were selected based on a history of mosquito breeding. Thirty-two sites were selected, sixteen in Palm Springs and sixteen in La Quinta; sites were similar in size and had less than 9.29 m² (100 ft²) surface area. Prior to treatment, sites were assessed for initial larval density. From August through November 2013, four control products were tested. Eight sites each received one of the following: FourStar® 180-day Briquet (old formulation), FourStar® 180-day Briquet (new formulation), Natular™ XRT 180-day tablet or Altosid® XR 150-day Briquet (Figure 1). The products were placed in a poly-mesh net bag with two recycled wine corks and suspended from the manhole cover, so that the product floated (Figure 2). The District typically floats briquette and tablet products to keep the active ingredient close to where *Cx. quinquefasciatus* are likely to encounter it. Sites were treated with one briquette or tablet per label directions (Central Life Sciences 2014, Central Life Sciences 2014, Clarke 2014).

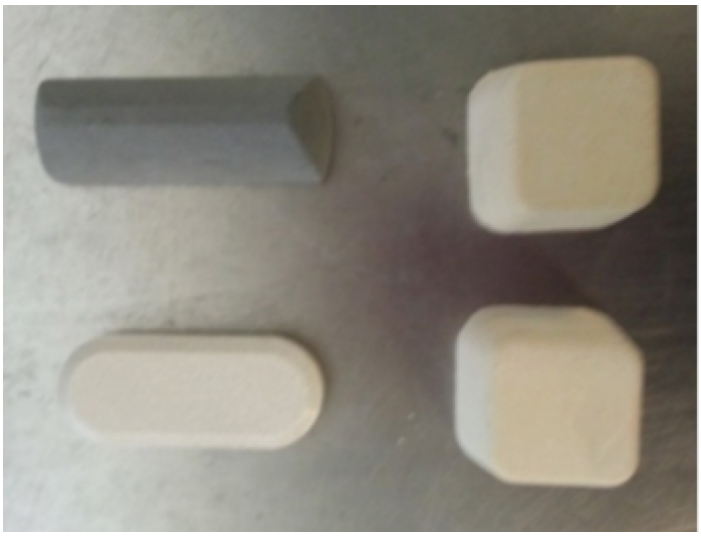

Figure 1. The four products used: top left – Altosid® XR 150-day Briquet; bottom left – Natular™ XRT 180-day tablet; top right – FourStar® 180-day Briquet (old formulation); and bottom right – FourStar® 180-day Briquet (new formulation).

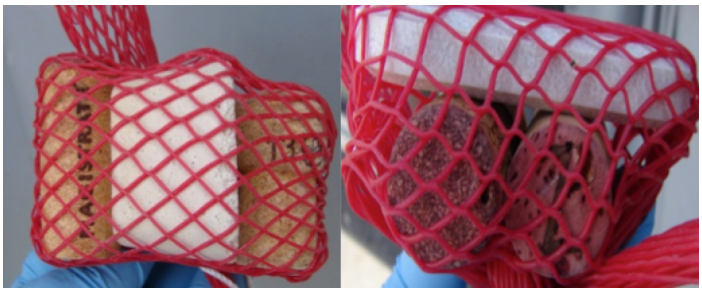

Figure 2. The District's standard operating procedure is to apply briquettes and tablets in poly-net mesh bags with cork so that the product floats near the surface where Cx. quinquefasciatus larvae live. Left: FourStar® 180-day Briquet. Right: Natular™ XRT 180- day tablet.

Floating larval cages (modified BioQuip breeders) were placed in each site. The bottom chambers were modified with screen to allow water to flow in and out, and the cages were placed in Styrofoam which served as floats (Figure 3). After treatment and at inspections, 25 laboratory reared, third instar, *Culex quinquefasciatus* larvae were added to each floating cage. Larvae in the cage were examined for survival to adulthood, and larval density outside of the floating cage (i.e., field population) was assessed. Two dips using a standard 350 ml dipper were taken at each site, and larval counts were averaged. When present in Altosid sites, late instar stages and pupae were collected along with a water sample and maintained for adult emergence in the laboratory. Water parameters (i.e., temperature, pH, dissolved oxygen, and conductivity) were measured using the YSI Multi-probe, and the data were recorded. If a tablet or briquette was completely depleted prior to the expected residual, it was replaced. If product failure occurred in a site with one briquette, an additional briquette was added, and if product failure persisted, then pupacide (Agnique MMF) was applied to knock down the population.

Figure 3. Floating larval cages were placed in each site, and the product was suspended from the manhole cover. Larvae were added to the cages and examined for survival.

RESULTS

The new formulation of FourStar briquettes required frequent retreatment in the eight sites (Figure 4). In La Quinta, two briquettes were applied per site because breeding was not controlled with a single briquette. In November several sites needed pupacide treatments to reduce the mosquito population.

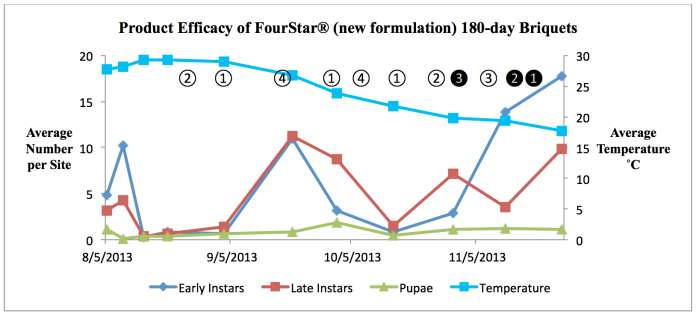

Figure 4. Efficacy of FourStar (new formulation) briquettes averaged for the eight sites. Sites required additional briquettes because the product depleted prior to the expected residual or breeding was occuring. Graph shows the number of additonal briquettes (clear circles) and the number of pupacide applications (black circles) made after the initial treatment.

The old formulation of FourStar performed similarly to the new formulation. Better control was seen early in the study, particularly at the Palm Springs sites. Two briquettes were placed in each catch basin in La Quinta in September (Figure 5) to reduce the breeding. Fewer pupacide treatments were needed.

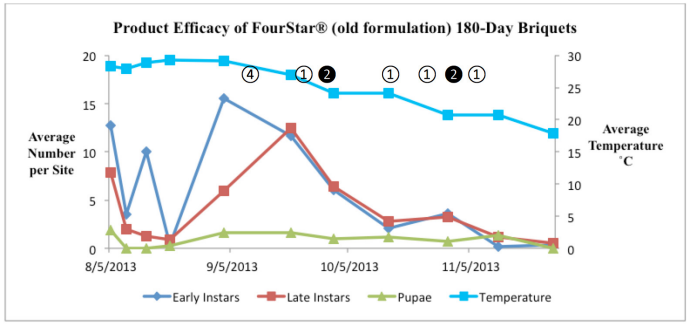

Figure 5. Efficacy of FourStar (old formuation) briquettes averaged for the eight sites. Sites required additional briquettes because the product depleted prior to the expected residual or breeding was occuring. Graph shows the number of additonal briquettes (clear circles) and the number of pupacide applications (black circles) made after the initial treatment.

Natular XRT tablets reduced the mosquito breeding throughout the study period. Fewer tablets were completely dissolved during the course of the study (Figure 6). No pupacide treatments were made to the Natular XRT sites. The Palm Springs sites were part of a West Nile virus focus area in September and October; one site received applications of Vectobac 12AS during enhanced larval surveillance.

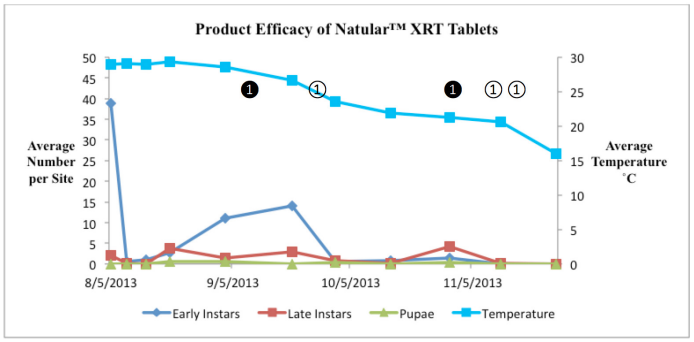

Figure 6. Efficacy of Natular XRT tablets averaged for the eight sites. Sites required additional briquettes because the product depleted prior to the expected residual or breeding was occuring. Graph shows the number of additonal briquettes (clear circles) after the initial treatment. One site in Palm Springs required two additional larvicide treatments (Vectobac 12AS) because it was located in the area of a West Nile Virus positive mosquito pool (black circles).

Altosid XR Briquets provided control throughout the study. Although they dissolved faster than the Natular XRT tablets, fewer reapplications were needed when compared to the FourStar briquettes (Figure 7). Two sites received three pupacide applications: one had excessive trash and mud which limited product efficacy while the other was breeding heavily at one inspection.

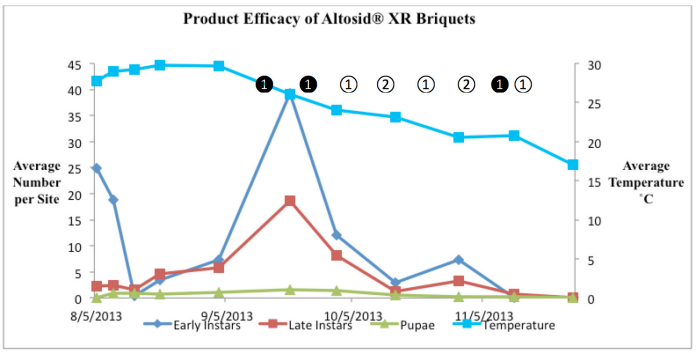

Figure 7. Efficacy of FourStar (old formuation) briquettes averaged for the eight sites. Late instars and pupae were collected and examined for adult emergence. Sites required additional briquettes because the product depleted prior to the expected residual or breeding was occuring. Graph shows the number of additonal briquettes (clear circles) and the number of pupacide applications (black circles) made after the initial treatment.

DISCUSSION

In this study, all of the products did not last as long as expected according to the label (Table 1). Although the products were labeled for use for up to 150 and 180 days, we observed that the residual activities were effective for only 90 days at best. Part of this is due to the fact that the product was suspended in the water column instead of sitting on the bottom of the catch basin as designed. By suspending the briquette or tablet, water was in contact with all sides instead of only a portion of the product. The reduced residual activity is also due in part to the nature of the sites; these catch basins received irrigation runoff daily, sometimes multiple times per day. Such a reduction in the effectiveness needs to be understood by technicians using the product as well as by those expecting the product to protect residents.

Product	La Quinta		Palm Springs	
	Same product	Pupacide	Same product	Pupacide
FourStar (new)	14	6	4	0
FourStar (old)	6	4	2	0
Altosid XR	4	1	3	2
Natular XRT	2	0	1	0

Table 1. Number of times briquettes/tablets were supplemented in a site or treated with pupacide (Agnique MMF).

In general, Natular XRT tablets lasted longer than Altosid XR or FourStar briquettes. On average, Natular XRT tablets were replaced every 90 days, Altosid XR Briquets every 30 days and FourStar every 60 days. Yet, the control provided by Fourstar was highly variable. Trash, debris and water inputs varied greatly in these sites, and it is possible that the FourStar briquettes would work better in sites not receiving regular inputs of water.

Location mattered greatly in this study (Table 1). Sites in La Quinta required more replacement of the product and more treatments with pupacide to control breeding than the Palm Springs sites. The sites in La Quinta were part of a homeowners association where some of the sites connect to a lake. The expanded amount of water present, even though the lake does not breed mosquitoes, could have caused the product to dissolve faster than expected and decreased the efficacy.

Finally, the Altosid XR Briquets used in this study were more than a year old. Central Life Sciences has since reformulated the briquettes (John Holick, personal communication), and staff will reevaluate this study with the new product.

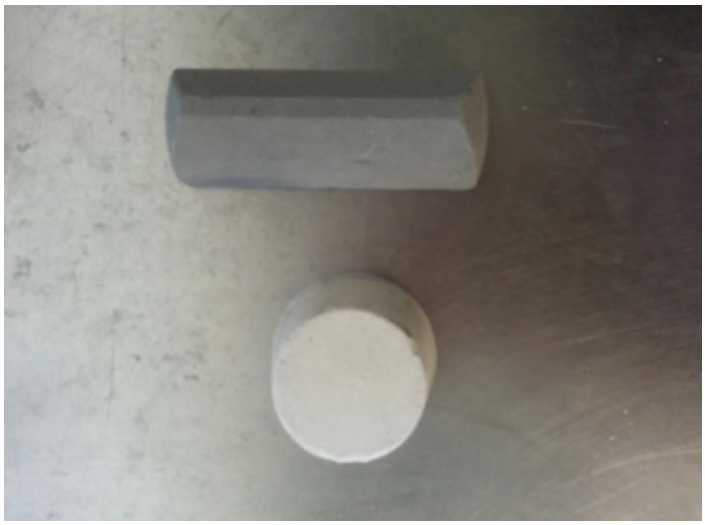

Figure 8. Top: Altosid® XR 150-day Briquet (old formulation). Bottom: Altosid® XR 150-day Briquet (new formulation).

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Natular™ XRT 180-day tablets chemical label. www.clarke.com/Clarke/May 22, 2014, http://www.clarke.com/images/pdf/Labels/2012Labels/natularxrt.pdf.

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Adapting Vector Control Educational Materials to the California Common Core and Next Generation Science Standards

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INTRODUCTION

The California State Board of Education approved the California Common Core Standards for Mathematics and English Language Arts and Literacy in History/Social Studies, Science and Technical Subjects in August of 2010 and the California Next Generation Science Standards in September of 2013. These standards were developed as part of a voluntary state-led effort to improve students' ability to succeed in college and the real world. With the adoption of the common core and next generation standards, California shifted the academic emphasis for K-12 students from simply learning about content to applying content and skills to real-life situations. This shift creates an excellent opportunity for vector control districts and agencies to provide curriculum that is aligned with the new standards, while educating school aged children about mosquitoes and raising West Nile virus awareness.

NEW STANDARDS

The California Common Core Math (CCSSM) and English Language Arts and Literacy in History/Social Studies, Science and Technical Subjects (CCSS ELA/Literacy) Standards and the California Next Generation Science Standards (NGSS) are designed to prepare students for college and careers in the twenty-first century. They emphasize critical thinking and problem solving, communication and the development of rational practices or "habits of mind" in the context of the real world (CDE 2014). Students are exposed to informational texts more often and at an earlier age than under the previous standards. A greater emphasis is placed on word problems and demonstrating conceptual understanding. Practical applications requiring analysis, evaluation and justification are introduced and designed to internalize rational thought processes to prepare students for adulthood.

An example of the shift in the emphasis of the standards can be illustrated by examining the elementary school level 1998 and 2013 standards for life cycles:

- **1998 Standard**
  "Plants and animals have predictable life cycles. Students know the sequential stages of life cycles are different for different animals, such as butterflies, frogs and mice" (CDE 2003).

- **2013 NGSS Standard**
  "Develop models to describe that organisms have unique and diverse life cycles but all have in common birth, growth, reproduction, and death" (CDE NGSS 2013).

The earlier standard focused on students knowing the life cycle stages of specific animals (core content idea) and recognizing that differences exist among the life cycles of different animals. The new standard expands the competency expectation to include a scientific practice (modeling) and the awareness of a universal concept (patterns). To successfully master this standard, the student must utilize higher level thinking skills beyond memorizing life stages.

The inclusion of these Scientific and Engineering Practices and universal or Crosscutting Concepts is a significant shift in the composition of the science standards.

Crosscutting Concepts
Patterns
Cause and effect: Mechanism and explanation
Scale, proportion, and quantity
Systems and system models
Energy and matter: Flows, cycles, and conservation
Structures and function
Stability and change

Table 1. The 7 Crosscutting Concepts from the NGSS.

Crosscutting Concepts are ideas consistently encountered throughout the natural world and "transcend disciplinary boundaries" (AAAS 1989). They discourage the compartmentalization of science disciplines in the classroom by creating conceptual links between the life, earth and physical sciences. For example, chemists, astronomers and marine biologists all used the Crosscutting Concept of Cause and Effect to develop rationale experiments and explanations for natural phenomena.

The Scientific and Engineering Practices teach students how to be scientists and engineers. They endeavor to "cultivate students' scientific habits of mind" and "help students become more critical consumers of scientific information" by internalizing a systematic process of evaluating information based on logic (NRC 2012) (Table 2).

Scientific and Engineering Practices
Asking questions (for science) and defining problems (for engineering)
Developing and using models
Planning and carrying out investigations
Analyzing and interpreting data
Using mathematics and computational thinking
Constructing explanations (for science) and designing solutions (for engineering)
Engaging in argument from evidence
Obtaining, evaluating, and communicating information

Table 2. The 8 Scientific and Engineering Practices from the NGSS.

In the information age, anyone can publish anything; it is crucial, therefore, for students to have the skills to be able to discern the credibility of the information, generate evidence-based decisions about the subject matter and communicate their conclusions effectively.

ASSESSMENT

Another area in which the standards have changed is assessment. Student assessment involves a greater number of word problems, and those problems involve multiple steps. In addition, students are expected to explain their process for arriving at the answer. For example, in the past a question on a statewide standardized test might ask a student to round a number to the nearest ten and provide four potential answer choices. This format allows for guessing and assesses the skill of rounding but does not evaluate the fundamental understanding of the concept. A new version of the question might require the student to read a table, evaluate the data present, round one or more numbers and then determine how the rounding affects any results (Figure 1).

Old Question:

Round the number 75 to the nearest ten.

a. 50 c. 70

b. 100 d. 80

New Question:

Which site needs the most larvicide? How would your answer change if the number of bags was rounded to the nearest ten? Explain:

Larvicide Treatment Quantities	
Site	Bags of Larvicide
Country Club	5
Lagoon	14
Creek	10

Answer: At first, the Lagoon needs the most. After you round the numbers, all require the same. 14 and 10 (ones column less than 5) and 5 (ones column greater than or equal to 5) all round to 10.

Figure 1. Sample assessment questions illustrating the difference between old and new standards.

VECTOR CONTROL AND THE NEW STANDARDS

Vector Control utilizes Crosscutting Concepts and Scientific and Engineering practices every day. When districts apply lessons learned about *Aedes aegypti* behavior in other parts of the world to inform the design of new surveillance protocols in California, they are recognizing and using the concept of patterns. The concept of cause and effect is the foundation for reducing and eliminating standing water to reduce mosquito populations. To protect the public from mosquitoes and other vectors, district and agency staff "practice" science and engineering. Laboratory staff analyzes and interprets surveillance data. Outreach staff develops and communicates evidence-based collateral and presentations to the public. Field staff uses mathematics and computational thinking to apply safe and effective quantities of pesticides.

As schools across California begin to implement the new standards, teachers and administrators are looking for curriculum that meets the expectations of the CCSS and NGSS. Vector Control is uniquely positioned to offer presentations and lessons that are highly engaging, fulfill content standard requirements and provide opportunities to apply knowledge and practices in real-world situations. Much of the existing school materials used by districts and agencies in California are in alignment with or can be easily modified to meet the new standards. A key element is to explicitly link the activities and collateral presented to specific core ideas (content), practices or concepts so that schools can confidently justify implementing a Vector Control curriculum.

When adapting or developing this curriculum, it is also important to consider the structure of a lesson plan. This will ensure that stand-alone vector lessons meet the needs of teachers and that presentations or activities will complement existing teacher-developed lessons. There are numerous lesson plan styles; however, all plans share the same basic components:

- Objective: the purpose of the lesson
- Standards: the state standard(s) addressed in the lesson
- Introduction: links to previous knowledge, teasers, questions
- Instruction and Activities: content and practices
- Assessment and Reflection: this includes evaluation of the lesson itself

By acknowledging these elements, Vector Control staff will communicate effectively with teachers and demonstrate the applicability of vector programs.

EXAMPLE OF A VECTOR CONTROL LESSON

The following example illustrates how vector control information and activities can be matched to the CCSS and NGSS for third grade classrooms. Activities can be scaled up or down in complexity or emphasis to meet the needs of different age groups.

The Mosquito Life Cycle:

1. **Objective:** The academic objective of this lesson is to meet the NGSS life cycle standard, and the vector objective is to encourage students and their families to remove standing water from their homes.

2. **Standards:**

   Core Content.
   a. NGSS: Develop models to describe that organisms have unique and diverse life cycles but all have in common birth, growth, reproduction, and death.
   b. CCSS ELA/Literacy: Plan and deliver an informative/explanatory presentation on a topic
   c. CCSSM: Develop understanding of fractions as numbers.

   NGSS Scientific and Engineering Practices.
   a. Asking questions
   b. Using models
   c. Constructing explanations
   d. Communicating information

   NGSS Crosscutting Concepts.
   a. Cause and effect
   b. Patterns

3. **Introduction:** Students are asked to access their prior knowledge of the butterfly life cycle to facilitate the introduction of the mosquito life cycle. Students are encouraged to create a class diagram (model) of the cycle using appropriate vocabulary.

4. **Instruction and Activities:** The teacher and students work together to label a mosquito life cycle diagram. Content retention is reinforced by comparing and contrasting the mosquito life cycle with that of the butterfly. The role of standing water in mosquito development is emphasized and the fraction of the life cycle completed in water is calculated as support. Next, students are given the opportunity to illustrate an empty cycle diagram using actual mosquito specimens. Finally, students are asked to respond to a challenge question: "Why is it important to get rid of standing water?" After the students discuss the answer using appropriate vocabulary and reference to the diagrams, they are encouraged to consider potential and existing sources of standing water around their homes.

5. **Assessment:** Students prepare presentations for their families that promote the removal of standing water from around their homes. The presentations are expected to include illustrations (Figure 2), an evidence based explanation of the link between mosquitoes and standing water, and a call to action. An evaluation form (which also serves as a requirement "cheat sheet") is sent home for the "audience" to complete and return.

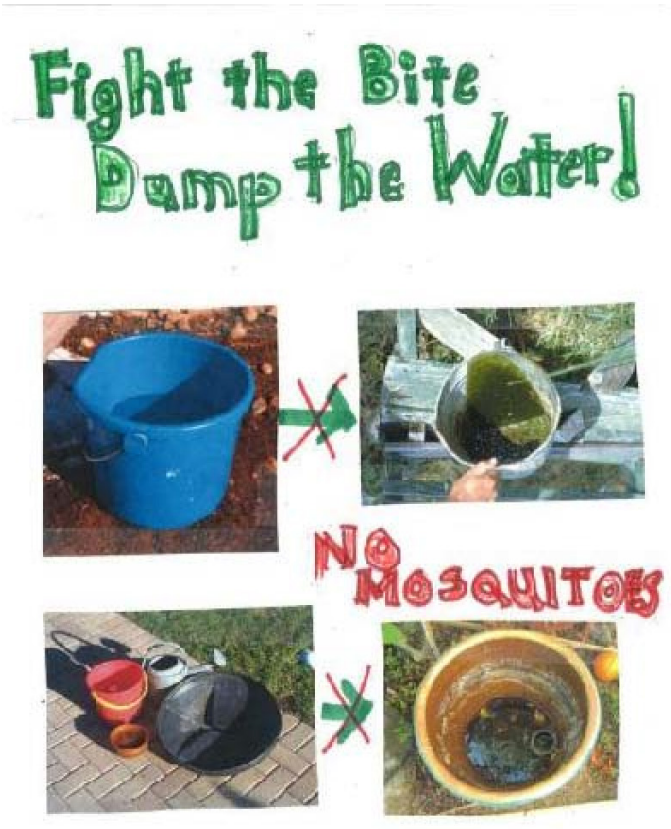

Figure 2. Example of a poster project.

Incorporating a basic lesson plan structure into curriculum materials allows vector staff to clearly demonstrate the value of a presentation to classroom teachers.

CONCLUSIONS

The implementation of the California Common Core Standards and California Next Generation Science Standards in schools present an amazing opportunity for Vector Control districts and agencies to strengthen their ties to the communities they serve through enhanced school outreach. By aligning with and clearly communicating the relevance of Vector Control lessons, presentations and demonstrations to the new standards, Vector Control districts and agencies support the efforts of teachers, enhance the learning experience of students, and protect the public health.

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Arbovirus Threats to California

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ABSTRACT: West Nile virus provides an excellent example of how an exotic virus can be introduced and become established in California to create major wildlife, veterinary and public health problems. Our brief review provides a listing of the 26 mosquito and arthropod-borne viruses previously or currently found in California as well as their primary vectors and hosts. With the exception of West Nile, western equine encephalomyelitis and St. Louis encephalitis viruses, the current status of these other viruses is virtually unknown because of the selection of mostly *Culex* vectors for virus testing and the specificity of the current molecular diagnostics for these three primary virus targets. An additional 13 mosquito-borne viruses with the potential to be introduced into California are presented along with their known endemic vectors and California ecological equivalents. Although the competence of some of these mosquito-virus combinations has been investigated, most are unknown. Research and development are needed to extend the surveillance program diagnostics to detect other endemic and exotic viruses as well as to determine the competence of California vectors for exotic viruses to understand the risk for their introduction and establishment.

INTRODUCTION

Changes in human population density and demography, increased global movement of people and commerce, and climate change have dramatically and perhaps permanently altered the ecology of our planet and facilitated the dispersal and transmission of wildlife, veterinary and human pathogens by arthropods (Reisen 2010). Many arboviruses (i.e., arthropod-borne viruses) have evolved and diversified in the tropics, but became major health problems after they invaded temperate latitudes (Coffey et al. 2013, Weaver and Reisen 2010). Perhaps the greatest health problems have arisen after viruses such as dengue transitioned from a zoonoses (i.e., virus maintained among animal hosts) to an anthroponoses (virus maintained among humans) and became urbanized. The global re-distribution and establishment of efficient vectors such as *Aedes aegypti* during the sailing vessel era and *Aedes albopictus* by the used tire trade serve as examples of how introductions of vectors precede the invasion of the pathogens they transmit. Epidemics in the New World caused historically by yellow fever virus (YFV) introduced from Africa, and now the on-going spread of dengue (DENV) and recently chikungunya (CHIKV) viruses also from Africa, serve notice on how zoonoses can emerge from sylvan settings to become urbanized and cause health problems of global proportions affecting millions of people.

California with its mild Mediterranean climate, landscape diversity ranging from arid deserts in the SE to rain forests in the NW, large human population, extensive irrigated agriculture, and far-reaching trade with the Pacific Rim and Mexico provides excellent opportunities for the emergence of endemic viruses and establishment of invasive viruses. Although vaccines exist for some arboviruses such as yellow fever and Japanese encephalitis viruses, most lack approval for commercial use from the USDA because of the expense and difficulty of conducting phase III trials. Therefore, the public health responses to an arbovirus invasion are limited mostly to vector control, public education or use of personal protection to prevent or interrupt transmission. Surveillance science and risk determination drive decision support models to focus, improve, evaluate and justify the application of insecticides necessary for intervention.

The purpose of this short review is to provide a listing of the arboviruses in California and those with potential for invasion. A brief synopsis of their vectors and health impacts are reviewed as well as the capability of our surveillance program to provide diagnostics to track them.

Endemic viruses. Currently, there at 13 mosquito-borne arboviruses endemic to California (Table 1).

Virus	Vector	Reservoir
Alphavirus		
Western equine encephalitis	*Cx. tarsalis, Ae. melanimon*	Birds
Flavivirus		
St. Louis encephalitis	*Cx. tarsalis, pipiens complex*	Birds
West Nile	*Cx. tarsalis. pipiens complex, stigmatosoma*	Birds
Culex flavivirus, Calbertado	*Cx. pipiens, Cx. tarsalis*	none
Bunyaviruses		
Cache Valley	*Cs. inomata, Aedes spp.*	Deer, rabbits?
California encephalitis	*Ae. dorsalis, melanimon*	Rabbits
Morro Bay	*Ae. squamiger*	Rabbits?
Jamestown canyon	*Cs. inomata, Aedes*	Rabbits, deer
[Jerry Slough]	*Cs. inomata*	Rabbits?
Northway-like	*Cs. inomata, Aedes, Anopheles?*	Rabbits
Turlock	*Cx. tarsalis*	Birds
Rhabdovirus		
Hart Park	*Cx. tarsalis*	Birds?
Gray Lodge	*Cx. tarsalis*	??
Orbivirus		
Llano Seco	*Cx. tarsalis*	??

Table 1. Mosquito-borne viruses endemic to California

Many were discovered during historic investigations into the epidemiology of Western equine encephalomyelitis (WEEV) and St. Louis encephalitis (SLEV) viruses (. et al. 1990) or by the state surveillance program (Emmons et al. 1991, Emmons

et al. 1992) when pools were screened by suckling mouse brain inoculation and viruses identified by cross neutralization assays. Although these procedures were slow and expensive, they were very sensitive and able to detect a broad range of viruses. WEEV was first isolated during a large epizootic of encephalitis in equines in the Central Valley and other parts of the western US (Meyer et al. 1931). Shortly after, SLEV was found to be the cause of summer encephalitis in humans in St. Louis (Muckenfuss et al. 1934) and later in the Central Valley of California (Howitt 1939). These viruses remained a health problem and the focus of California mosquito control through the 1990s. To provide rapid turn-around-time necessary for decision support for risk assessment, laboratory assays were modified to use cell culture (Graham et al. 1986) and then qRT-PCR (Husted et al. 2003) in 96 well format. These assay modifications and focus on testing *Culex* because of the West Nile virus invasion in 2003 (Reisen et al. 2004) precluded the detection of all but the three viruses of health importance listed in Table 1, namely WEEV, SLEV and WNV. Other viruses in Table 1 known to occasionally cause human illness include the Bunyaviruses Cache Valley, California encephalitis and Jamestown Canyon. These viruses are transmitted among mammals by *Culiseta* and *Aedes* mosquitoes and were found throughout California from Sierras to coastal marshes (Campbell et al. 1991a,b; Fulhorst et al. 1996). The UC Davis Center for Vectorborne Diseases (CVEC) can test for these latter viruses using either our new melt curve assays using family level primers or by Vero or mosquito cell culture. Novel molecular methods detected the presence of mosquito flaviviruses such as Calbertado and Culex flavivirus that have no known vertebrate hosts.

In addition to the mosquito-borne arboviruses in Table 1, there are 13 additional arboviruses known to occur in California (Table 2).

Virus	Vector	Reservoir
Flavivirus		
Powassan	*Ixodes*	Deer
Modoc	??	Rodents
Rio Bravo	??	Bats
Bunyavirus		
Lokern	*Culicoides*	Rabbits
Main Drain	*Culicoides*	Rabbits
Buttonwillow	*Culicoides*	Rabbits
Orbivirus		
Bluetongue	*Culicoides*	Ruminants
Colorado tick fever	*Dermacenter*	Rodents
Epizootic hemorrhagic disease	*Culicoides*	Deer
Mono Lake	*Argas*	Gulls
Rhabdovirus		
Kern Canyon	??	Bats
Klamath	??	Voles
Togaviridae		
Stone Lakes virus	swallow bugs	cliff swallows

Table 2. Arboviruses endemic to California that are transmitted by vectors other than mosquitoes.

Of these, the tick-borne viruses, Powassan and Colorado tick fever, have been documented to cause human disease (Bowen 1989, Ebel 2010, Smith et al. 1974), whereas the *Culicoides*-borne virus, Bluetongue, is a major veterinary health problem (MacLachlan 1994). Question marks in Table 2 indicate viruses that have been isolated from vertebrates for which there are no known vectors. Although CVEC has not routinely tested ticks, molecular and cell culture methods are available for screening pools if agencies submit specimens.

Exotic viruses. There are at least 13 mosquito-borne viruses that have the potential to invade California (Table 3) because they are currently causing widespread epidemics elsewhere, have invaded the US previously or are actively transmitted elsewhere within the continental US.

Family	Abbr.	Name	Endemic vectors	California equivalents
Bunyaviridae	RVFV*	Rift Valley Fever	*Aedes mcintoshi, Ae. vexans, Ae. juppi, Ae. caspius, Culex pipiens, Cx. univittatus, Cx. theileri, Cx. tritaeniorhynchus*	*Aedes vexans, Ae. dorsalis, Ae. melanimon, Cx. tarsalis, Cx. pipiens pipiens, Cx. p. quinquefasciatus*
Flaviviridae	DENV	dengue	*Aedes aegypti, Ae. albopictus*	*Ae. sierrensis, Ae. albopictus, Ae. aegypti*
Flaviviridae	JEV	Japanese encephalitis	*Culex vishnui* complex, *Cx. gelidus, Cx. bitaeniorhynchus*	*Cx. tarsalis, pipiens complex, stigmatosoma*
Flaviviridae	MVEV	Murray Valley encephalitis	*Culex annulirostris*	*Cx. tarsalis, pipiens complex, stigmatosoma*
Flaviviridae	YFV	Yellow fever	*Canopy Aedes such as africanus, Sabethes, Haemogogus, Aedes aegypti*	*Aedes aegypti, albopictus*
Flaviviridae	ZKV	Zika	*Canopy Aedes; Ae.hensilli*	*Aedes albopictus, Ae. aegypti*
Togaviridae	BFV	Barmah Forest	*Aedes vigilax*	*Ae. squamiger, dorsalis, melanimon, taeniorhynchus*
Togaviridae	CHIKV	chikungunya	*Aedes africanus, luteocephalus, aegypti, albopictus, Culex pipiens complex*	*Culex pipiens complex, Ae. sierrensis, Ae. albopictus, Ae. aegypti*
Togaviridae	EEEV*	Eastern equine encephalitis	*Culiseta melanura, Coquillettidia perturbans, Aedes sollicitans, Aedes vexans,*	*Cx. tarsalis, Ae. dorsalis, melanimon, Ae. vexans*
Togaviridae	GETV	Getah	*Culex vishnui complex, annulirostris, Aedes vexans*	*Cx. tarsalis, Ae. vexans*
Togaviridae	RRV	Ross River virus	*Culex annulirostris, Aedes vigilax, camptorhynchus, notoscriptus, aegypti*	*Ae. squamiger, dorsalis, melanimon, nigromaculis, Cx. tarsalis*
Togaviridae	SINV	Sindbis	*Culex pipiens complex, univittatus, vishnui complex, annulirostris,*	*Cx. pipiens complex, tarsalis, stigmatosoma*
Togaviridae	VEEV*	Venezuelan equine encephalitis	*Culex sbg melaniconion, Aedes taeniorhnynchus, Psorophora confinnis*	*Culex apicalis?, Ae. taeniorhynchus, dorsalis, Psorophora columbiae/toltectum*
	*Select agents or BSL4			

Table 3. Potentially invasive mosquito-borne viruses, their endemic and potential California vectors.

The invasion and establishment of *Aedes aegypti* and *Ae.albopictus* in California has created receptivity for the introduction of several important arboviruses. From a global health perspective, DENV is perhaps the most important and widespread of the arboviruses; it can be transmitted from human-mosquito-human by both *Ae. aegypti* and *Ae. albopictus* and is actively transmitted along the US-Mexico border and in southern Florida (Anez and Rios 2013). In addition, travelers infectious with DENV repeatedly enter the US, including entry into those states supporting suitable vector populations (Dick et al. 2012). Chikungunya virus is a similar anthroponosis that utilizes the same suite of vectors as DENV when transmitted outside of its zoonotic canopy cycle in western Africa involving non-human primates (Powers and Logue 2007, Townson and Nathan 2008). The current pandemic that began in Eastern Africa has spread globally to include India (Patil et al. 2013), Southeast Asia (Horwood et al. 2013), China (Wu et al. 2013), the South Pacific (Dupont-Rouzeyrol et al. 2012) and now the Caribbean (Van et al. 2014); virtually any place supporting populations of the *Aedes* vectors is at risk for local transmission of CHIKV, including California.

Northern Italy with a climate similar to California serves as a good example of how an outbreak vectored by *Ae. albopictus* can be initiated by a single traveler (Angelini et al. 2007, Rezza et al. 2007). Although recently less evident from a global health perspective, yellow fever virus has remained a problem in tropical Africa and South America with the potential to again emerge from its primate reservoir cycle and become a global problem due to its potential to be transmitted as an anthroponosis (Auguste et al. 2010). Zika virus (ZKV), another flavivirus that evolved in Africa among canopy primates, has appeared in Southeast Asia (Olson et al. 1981) and more recently Micronesia (Duffy et al. 2009). This pathogen could possibly could be transmitted as an anthroponosis by *Ae. albopictus*. In preparation for detecting these introduced arboviruses, CVEC developed a multiplex RT-PCR assay to test *Aedes* simultaneously for all four serotypes of DENV, CHIKV and WNV; the other viruses such as ZKV and YFV can be tracked by our melt curve assay, Vero cell culture or viral metagenomics (described below).

The remaining viruses listed in Table 3 are zoonoses and therefore are more difficult to disperse internationally because both vector and non-human hosts are required for establishment, and humans normally are 'dead-end' hosts and not likely to infect mosquitoes. However, the introduction, establishment and continuing outbreak of WNV in the New World has served notice that zoonoses also can be introduced (Reisen 2013) and once established are difficult to contain (Holloway 2000). The most important virus globally among the zoonoses in Table 3 is Japanese encephalitis virus (JEV). This virus evolved in Southeast Asia but did not become a significant health problem until invading the temperate rice growing areas of Japan, Korea and China (Weaver and Reisen 2010) and then India (Reuben and Gajanana 1997). The primary vector, *Culex tritaeniorhynchus,* is biologically very similar to *Culex tarsalis* (Reisen 1981) and circulates the JEV enzootically among herons and pigs (Buescher and Scherer 1959). JEV has invaded Australia (van den Hurk et al. 2009) and the Pacific Islands (Hammon et al. 1958), but has not become established. Previous studies have shown that California mosquitoes (Reeves and Hammon 1946) and wild birds (Hammon et al. 1951) are susceptible to infection, so it may not be necessary to establish a porcine amplification cycle (Erlanger et al. 2009) for this virus to become established. Ross River and Barmah Forest viruses are endemic to Australia where they cause annual outbreaks of severe arthralgia (Russell 2002), frequently at popular tourist destinations. Kangaroos and wallabies are considered natural hosts, but human-mosquito-human transmission has been suspected. Our unpublished studies have shown that *Aedes dorsalis* and *Ae. melanimon* are susceptible to infection with both viruses, although not highly competent vectors in the laboratory. Another arthralgia, Sindbis virus, is very widespread and transmitted among wild birds by *Culex* mosquitoes (Kurkela et al. 2008). In Europe SINV causes Pogosta and Ockelbo disease (Lvov et al. 1988, Sane et al. 2010). Getah virus is widespread and frequently isolated from mosquitoes. It has caused outbreaks in horses (Kamada et al. 1980) and abortion in pigs (Shibata et al. 1991), but has not been associated with human disease.

Eastern equine encephalitis virus (EEEV) is endemic to many areas east of the Mississippi River where it causes focal outbreaks of neuroinvasive disease among humans and horses with an exceptionally high fatality rate (Howard et al. 1996, Letson et al. 1993). Most transmission foci are permanent and associated with cypress swamps and elevated populations of *Culiseta melanura*. A horse infected with EEEV was detected in California, but the virus did not become established (Franklin et al. 2002). Venezuelan equine encephalitis virus (VEEV) is endemic to the Neotropics where it periodically emerges from its enzootic cycle and causes widespread disease among equines and humans, resulting in rolling epizootics that have moved northward through Mexico and into south Texas (Weaver and Barrett 2004). A final virus of veterinary and human health importance in Africa is Rift Valley Fever virus (Bird et al. 2009). There is considerable concern that this virus could be used as a biowarfare agent and intentionally released into the US to cripple our dairy, cattle and sheep industries (Hartley et al. 2011). EEEV, VEEV and RVFV are listed by the CDC as Select Agents and cannot be cultured at CVEC. However, molecular diagnostics are available and could be rapidly brought on-line by CVEC should these agents become a problem in California.

SUMMARY

As reviewed herein, there are a number of arboviruses with varying wildlife, veterinary and human health importance that are found within California or have a high risk of introduction. Although it is the primary responsibility of a surveillance program to provide specific, sensitive and rapid results to direct intervention operations, a complete program should also include diagnostics that will detect potential and emerging problems. Our current surveillance program carefully tracks WNV, WEEV and SLEV infection in mosquitoes and transmission to sentinel chickens in time and space, but neglects the remaining viruses. Detection of these other viruses would only come from passive case detection, if disease was recognized in humans by health care providers or in domestic animals by veterinarians. Our existing melt curve assays, complemented by viral metagenomic approaches newly available at CVEC, will enable identification of any virus in a sample without *a priori* knowledge of which are present. This is because viral metagenomics use a random amplification approach to characterize known and identify unknown viruses based on sequence similarities to any previously sequenced viral genome. The viral metagenomics approach has been validated by laboratory studies (Bishop-Lilly et al. 2010, Hall-Mendelin et al. 2013) and used in surveillance programs in Australia to detect known, introduced and novel arboviruses in field-collected mosquitoes (Coffey et al. 2014). Metagenomics can therefore be used at CVEC on selected homogenized mosquito samples to identify viruses that kill cultured cells but cannot be identified by through preliminary screening by qRT-PCR or melt curve assays. However, despite our increased capacity to identify known and novel viruses, detection is limited by sampling, testing paradigms and economics. Currently an outbreak of a novel

pathogen would not be recognized and diagnosed definitively until well after local transmission has occurred. This would be especially problematic for viruses such as DENV and CHIKV where humans are infectious to mosquitoes before becoming symptomatic. Especially neglected are zoonoses transmitted among wild mammals by *Aedes* and *Culiseta* that are known to intermittently cause veterinary or human health problems. These would not be found by testing *Culex* mosquitoes or sentinel chicken sera and mostly likely would not be recognized by clinicians. It is worth remembering here that most arboviruses are highly variable genetically and that small changes to the virus genome can increase virulence and pathology. CVEC presently has the capability to test for most of these arboviruses; however, the Mosquito and Vector Control Association of California and the California Department of Public Health will have to decide if the search for emerging viruses is worth funding.

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A 2013 Survey of Mosquitofish and Biological Control Programs in California: Updates of Mosquitofish Use, Production, and Facility Operations

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ABSTRACT: In 2013, the Orange County Vector Control District (OCVCD) prepared a survey to assess the current status of mosquitofish use, mosquitofish production, and other key operational features for all statewide mosquitofish and biological control programs (Programs). In August 2013, the OCVCD, with the assistance of the Mosquito and Vector Control Association of California (MVCAC) and their Integrated Vector Management Committee, distributed a 10-question survey to all 65 MVCAC member agencies using SurveyMonkey®. A total of 61 out of 65 agencies responded. Information from this questionnaire was used to: 1) report results and make these data available to all MVCAC membership; 2) compare annual statewide mosquitofish use (percent) by member regions; and 3) compare annual statewide mosquitofish use and production (number of fish) by member agencies. Data reported here depict an updated snapshot of major operational components inherent in these Programs.

RESULTS

Sixty-one out of 65 MVCAC member agencies completed the 10-question survey. About 93% of agencies that responded use mosquitofish, while over 8% use biological control organisms other than mosquitofish. Additional survey questions focused on key operational functions of Programs, including: mosquitofish and other biocontrol organism production, mosquitofish acquisition, facility structure and maintenance, staffing, policy and protocol, predator control, regulatory constraints, and current research. For a complete summary of all survey questions and responses, refer to the Appendix. The original 61 responses to this survey, including written comments, may be found on the OCVCD website (www.ocvcd.org).

Statewide annual mosquitofish use by MVCAC member regions (Sacramento Valley, Coastal, North San Joaquin Valley, South San Joaquin Valley, and Southern California) ranged from 10% (Coastal) to 33% (Sacramento Valley) [Figure 1]. Data displayed here include percentages of mosquitofish stocked in field sources only and do not include mosquitofish distributed to the public.

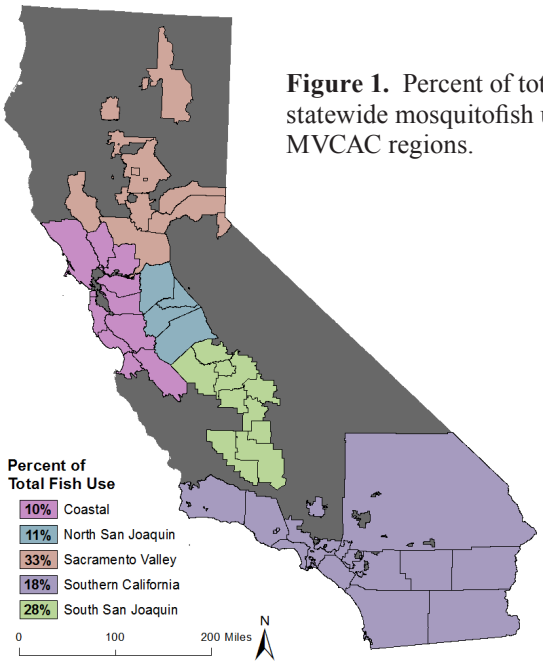

Figure 1. Percent of total annual statewide mosquitofish use by MVCAC regions.

Statewide annual mosquitofish use and production for each member agency ranged from 0 to >150,000 fish per year (Figure 2). Fish use was lowest for nine agencies (0 fish/agency/year) and production was lowest for 26 agencies (0 fish/agency/year). Both fish use and production were lowest for seven of the same agencies. Fish use was highest for nine agencies (>150,000 fish/agency/year) and production was highest for eight agencies (>150,000 fish/agency/year). Similarly, fish use and production were highest for six of the same agencies. Data displayed here refer to mosquitofish stocked in field sources (fish use) and mosquitofish reared by member agencies (fish production) and do not include mosquitofish distributed to the public or mosquitofish collected from field sources.

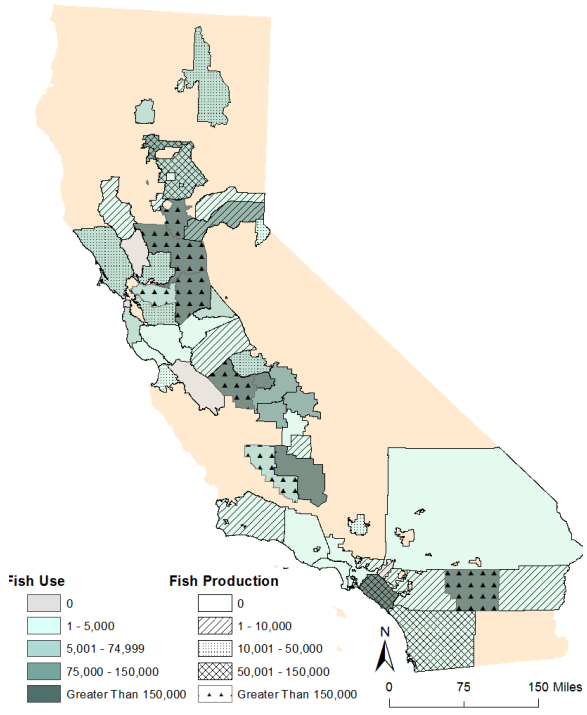

Figure 2. Total annual statewide mosquitofish use and production (number of fish) by MVCAC agencies.

CONCLUSIONS

Although mosquitofish are not endemic to California, their resilience, adaptability, hardiness, high rate of reproduction, and their ability to preferentially consume large numbers of immature forms of mosquitoes make these fish effective tools for use in statewide Integrated Mosquito Management programs. Survey data indicate that mosquitofish continue to be the predominant choice for the ongoing, statewide control of immature forms of mosquitoes. Thus, MVCAC membership are strongly encouraged to carefully review available survey data and use this information to best sustain and produce mosquitofish.

Differences in annual fish use observed among regions may be explained by several factors, including: 1) the size of a treatment source; 2) the type of treatment source; 3) program size; and 4) fish stocking restrictions regulated by state agencies such as the California Department of Fish and Wildlife. According to the California Department of Fish and Game Code, mosquitofish may be used for the purposes of mosquito control without obtaining a permit, except for Inyo, Mono, San Bernardino, Riverside, or Imperial counties, where mosquitofish may not be planted without written permission from the Department [Title 14, Section 238.5(f)]. Given the limited, temporal collection of data in this survey, further data collection and comparison are needed to more carefully deduce further explanations for differential, regional fish use.

Similarly, variations in annual fish use among agencies may be explained by the same factors that likely affect fish use among regions; however, differences in annual fish production may be attributed to the acquisition of fish by other means, such as field collection and purchase (as indicated by survey data). Programs producing the highest numbers of fish and meeting their annual demand likely possess at least one or more of the following: appropriate production facilities (including indoor), experienced and dedicated staff, high or sustained field collection success, and fish purchasing capability. To better understand other forces driving differences in annual mosquitofish use and production between agencies, further data collection and review is recommended, as noted above for regional fish use.

In addition to reviewing the data provided by this survey, all staff who manage Programs are also encouraged to become involved with the MVCAC's Mosquitofish and Biocontrol subcommittee, which formed recently in January 2014. Its mission is to provide a portal of salient information and open communication for all MVCAC members who manage Programs. This subcommittee was established to serve the needs of all mosquitofish and biocontrol Program staff on all levels, whether it be an agency who wants to know how to create and launch their first mosquitofish Program or a more established facility wishing to review and enhance their Program.

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APPENDIX

Survey questions and responses

Please provide your agency name, your name, title, and e-mail address.
Sixty-one out of 65 agencies, 61/65 (93.84%), provided complete responses to this request.

What organisms does your agency use for biocontrol of mosquitoes? (Check all that apply)
Mosquitofish: 57 (93.44%), Other fishes/organisms: 5 (8.20%), None: 4 (6.56%)

How many fish does your agency rear, collect, purchase, and distribute each year? (Assume 1,000 fish = 1 lb. of fish, unless your agency uses more specific data)
Fish Rearing: 35 combined (57.40%) >1,795,000 fish (>1,795 lb. fish) per year
Fish Collection: 46 combined (75.40%) >2,325,000 fish (>2,325 lb. fish) per year
Fish Purchase: 10 combined (16.40%) Approx. 160,000 fish (160 lb. fish) per year
Fish Distribution: 52 combined (85.25%) >2,130,000 fish (>2,130 lb. fish) per year
Fish Distribution to Public: 47 combined (77.05%) > 820,000 fish (>820 lb. fish) per year

What sources do your agency stock mosquitofish and/or other fishes/organisms in for mosquito control? (Check all that apply)
Ornamental and residential ponds: 55 (90.13%)
Livestock watering troughs: 51 (83.61%)
Manmade ponds: 49 (80.33%)
Neglected swimming pools or spas: 44 (72.13%)
Manmade storm channels or drainage ditches: 32 (52.46%)
Stormwater features or BMPs: 19 (31.15%)
Duck club ponds and Other sources: 12 each (19.67% each)
Rice fields: 11 (18.03%)

Is your mosquitofish program and/or biological control program managed by one or more dedicated employees?
No: 33 (54.10%), Yes: 18 (29.51%; ≥ One PT and/or FT staff), No program: 10 (16.40%)

Approximately how much total time per week (averaged over one year) do your employees spend performing the following program duties?
Other tasks: 197 total staff hours/week
Fish collection in the field: 41 total staff hours/week
Fish use/stocking: 30 total staff hours/week
Water quality/disease management: 6 total staff hours/week
Fish rearing/production: 5 total staff hours/week
Management of other biological control organisms: < 1 total staff hour/week

What does your agency use to hold and/or produce mosquitofish and/or other biological control organisms? (Select all that apply)
Recirculating outdoor fish tanks: 32 (52.46%)
Outdoor fish ponds: 28 (45.9%)
Recirculating indoor fish tanks: 13 (21.31%)
Indoor fish ponds: 2 (3.28%)
Other: 12 (19.67%)
None: 5 (8.20%)

Approximately how many mosquitofish does your agency lose to mortality/predation each year?
Unknown: 22 (36.07%)
Lose approx. 45,000 fish (45 lb. fish): 27 combined (44.27%)
Lose > 32,000 fish (32 lb. fish): 7 combined (11.48%)
Lose no fish: 5 (8.2%)

What methods does your agency use to reduce/prevent predation of your mosquitofish by birds or other wildlife?
None: 35 (57.38%)
Netting or mesh material and enclosed gates or fences: 12 each (19.67% each)
Other methods: 9 (14.75%)
Indoor facilities: 8 (13.11%)
Use some type or combination of prevention: 26 (42.62%)

Does your agency:
Have enough mosquitofish to meet annual demand?
Yes: 43 (70.49%), No: 16 (26.23%), Unknown: 2 (3.28%)

Have any local, state and/or federal constraints that prevent the stocking of mosquitofish in problematic mosquito sources?
Yes: 14 (22.95%), No: 39 (63.93%), Unknown: 8 (13.11%)

Allow the public to pick up fish?
Yes: 43 (70.49%), No: 18 (29.51%)

Have a written policy for the distribution of mosquitofish to the public?
Yes: 24 (39.34%), No: 36 (59.02%), Unknown: 1 (1.64%)

Have any written standard operating procedures (SOPs) for your fish and/or biological control programs?
Yes: 21 (34.43%), No: 38 (62.30%), Unknown: 2 (3.28%)

Have any current research with mosquitofish or other organisms used for mosquito control?
Yes: 9 (14.75%), No: 51 (83.61%), Unknown: 1 (1.64%)

Reflecting on the Murky Waters of the National Pollution Discharge Elimination System (NPDES): An Analysis of the Impact of NPDES Regulations on Mosquito Control Operations at San Joaquin County Mosquito and Vector Control District

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ABSTRACT: In November 2011 National Pollution Discharge Elimination System (NPDES) compliance became mandatory for all mosquito control agencies in California. For the 2013 mosquito season, NPDES compliance was put on hiatus pending further investigation and analysis of the data collected during the 2012 season. Compliance with NPDES regulations during the 2012 mosquito season placed an undue burden on mosquito districts and negatively impacted West Nile virus surveillance and mosquito control operations. Each district chose to direct their staff and budget in a unique way to manage this added work. This paper compares the impact of NPDES compliance efforts on operations at the San Joaquin County Mosquito and Vector Control District in 2012 to operations uninhibited by NPDES regulations in 2013, while giving an account of how the District managed resources to maintain NPDES compliance.

INTRODUCTION

San Joaquin County Mosquito and Vector Control District (SJCMVCD) is located in Stockton, California. Stockton and the surrounding area is home to hundreds of miles of California Delta waterways that provide habitat for wildlife, a mode of shipping and transportation and an important source for irrigation. While these extensive waterways are normally of benefit to the area, they can at times breed mosquitoes and require treatment for mosquito population control. Until 2011 SJCMVCD treated these waterways with larvicide as normal under the guidelines and regulations of the Federal Insecticide Fungicide and Rodenticide Act (FIFRA). In November 2011, pesticides applied in, near or around these waterways, called Waters of the United States (WOTUS), became considered pollutants as defined in court rulings. This meant the treating agency required a National Pollution Discharge Elimination System (NPDES) permit for discharge, even if the pesticide was approved for the application under FIFRA guidelines. Because of this, any vector control agency needing to treat for mosquitoes with a pesticide in or near a WOTUS, including SJCMVCD, was required to obtain and maintain a NPDES permit.

The NPDES permit requires three types of data collection: Visual, Physical and Chemical. Both the Visual and Physical categories require sampling of the water before application of the pesticide (Pre-Event), immediately after application (Event) and when the residual activity of the pesticide is completed (Post-Event). The Chemical category requires only a sampling before application and a sampling after application. Visual data collection involves visually inspecting the water, recording data such as clarity, color and presence of wildlife. Physical data collection consists of sampling the waterway for temperature, dissolved oxygen (DO), turbidity, electroconductivity (EC) and pH. Chemical data collection involves a laboratory analysis of a water sample for detectable levels of pesticide residue or breakdown products. Visual and Physical data collection were conducted in-house by SJCMVCD while Chemical data collection was conducted by URS and UC Davis. This paper will focus on the Visual and Physical data collection process and the steps SJCMVCD took to stay in compliance with the NPDES permit while treating for mosquitoes.

MATERIALS AND METHODS

In order to defray the costs of water sampling for NPDES compliance, SJCMVCD joined the coalition of California Mosquito Control Districts formed by the Mosquito and Vector Control Association of California (MVCAC). As a member of the coalition, SJCMVCD was responsible for conducting Visual data collection for ten percent of the mosquito treatments affecting WOTUS in San Joaquin County. Additionally, Physical data were collected six times per chemical from WOTUS treated with either a petroleum distillate or *Bacillus sphaericus* in agricultural environments.

To monitor the Physical data collection parameters, SJCMVCD purchased two pieces of equipment suggested by the MVCAC: the LaMotte 2020we Turbidimeter (LaMotte Company, Chestertown, Ma), and the YSI 556 Multimeter (YSI Inc., Yellow Springs, OH). The La Motte 2020we is a self-contained piece of equipment designed to allow for measurement of water turbidity in the field. The YSI 556 is a three probe system feeding into one piece of equipment that allows for the simultaneous monitoring of DO, EC, pH and temperature. It does not require a grab sample to be taken; the probe can be put directly into the waterway to collect data. Both of these pieces of equipment record data directly on internal memory drives for later downloading at the lab.

Data collection was initially conducted only by the senior author. By investing the responsibility of data collection in one person instead of sharing equipment and data collection duties with all technicians and staff, error could be minimized, and the precision and accuracy of the data collection could be increased. The author trained staff in the Visual data collection process, and eventually technicians collected Visual data on their own. However, Physical data collection along with all data management

and reporting duties remained the author's duties throughout the NPDES permit compliance process.

To streamline the data collection process and minimize the impact to mosquito control and lab operations, a six step process was devised involving the author and the mosquito control technicians. (1) If a technician detected mosquito breeding in a waterway they would contact their supervisor to determine if that water was considered WOTUS. (2) If the waterway was WOTUS, the supervisor would contact the author and let him know of the planned treatment, the location and the pesticide that was to be used. (3) The author would then schedule a time with the treating technician, typically that same day, to travel to the waterway and conduct Pre-Event and Event monitoring. (4) After all data collection was complete and the treatment was made, the author would contact the supervisor and schedule a future Post-Event data collection at the appropriate time for the chemical. (5) At a later date the author would return to the site and complete the Post-Event data collection. (6) All data collected would be kept in hardcopy form and also be entered into the State Water Resource Control Board (SWRCB) datasheet for future reporting.

In July of 2012 the Vector Control NPDES permit was put on hiatus sighting an increase in West Nile Virus (WNV) activity. This meant that Visual data collection would stop, but Physical data collection still needed to be completed. Because the hiatus continued for 2013, a comparison of a season with the NPDES permit (2012) and a season without the permit (2013) can be made to highlight the burden the permit placed on Vector Control Districts.

RESULTS AND DISCUSSION

Complying with the NPDES permit placed an undue strain on operations at SJCMVCD during the 2012 season, simply because of the huge amount of time that had to be dedicated to data collection and management. In 2012 there were 474 separate visual data collections taken. Of those, the author personally conducted 165 with the technicians in the field completing the balance after being trained in the data collection procedure. The author also personally conducted 48 Physical data collections.

Training and modifying technicians' schedules in the field proved to be one of the most difficult parts of the NPDES permit compliance process. In years prior a technician could treat after finding mosquito breeding regardless of WOTUS status. The NPDES permit introduced many hurdles that slowed the control process. In most cases data collection was completed the same day mosquito breeding was found. However, in some instances data collection could not be completed until the following day, meaning the mosquito source was treated later than it would have been if NPDES compliance was not required.

The primary effect of the NPDES permit requirements was to hamper mosquito control efforts in the field. Additionally, the need to conduct data collection for the NPDES permit slowed lab operations significantly. A conservative estimate of the time the author spent on NPDES related work for the time the permit was active is shown in Table 1. In total, the author spent approximately 251 hours, or 31.375 workdays conducting NPDES related data collection, data entry, equipment calibration and report writing.

Task	Time Per Task	Task Hours
Data Collection: Visual + Physical Background, Event, Post-Event	1 Hour/ Data Collection: 55 Treatments x 3 Data Collections	165 Hours
Data Management: Data Entry, Statistical Analysis, Management	20 min/Treatment: 158 Treatments x 20 min	53 Hours
Equipment Calibration: YSI 556, LaMotte 2020we Maintenance	1 Hour Week: 28 Weeks x 1 Hour	28 Hours
Report Development: Writing NPDES Annual Report	5 Hours/Year: 5 Hours x 1 Year	5 Hours
	Total NPDES Work Hours 2012	251 Hours

Table 1. Estimated work hours required to conduct NPDES related work.

The author's reduced presence in the lab meant that he was not always available to help with mosquito trap identification and other routine tasks. This increased the time it took the remaining lab staff to report mosquito trap and WNV testing results, therefore causing delayed pesticide applications in response to mosquito abundance and WNV activities. In 2013 with the author no longer conducting NPDES mandated data collection, the lab was able to maintain a same day turnaround time for the mosquito traps, meaning they could be collected, identified and tested all in the same day. This allowed the district to respond more quickly to high mosquito numbers and WNV activity the same night or next with ULV applications.

In addition to an increase in mosquito trap turn-around time, pesticide resistance testing of local mosquitoes suffered in 2012 because of the added burden of the NPDES permit requirements. The author normally assists with the testing of local mosquito populations for resistance to pesticides SJCMVCD uses for their control. However, in 2012 because the author was required to spend much of his time collecting data to comply with the NPDES permit, there was insufficient time to conduct resistance testing. By contrast, in 2013 the author was able to conduct extensive resistance testing of several local mosquito populations. The author's average daily breakdown of hours devoted to specific tasks is illustrated for 2012 and 2013 in Figure 1. On average, NPDES data collection took approximately thirty one percent, or two and a half hours of an eight-hour day to complete.

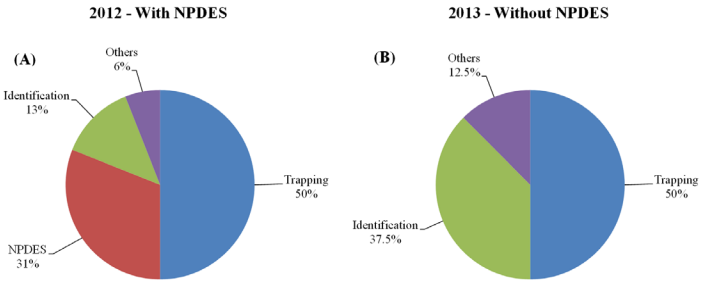

Figure 1. Breakdown of work hours allocated to different daily tasks. (A) Breakdown of work hours in 2012. (B) Breakdown of work hours in 2013. Hour breakdown is based on 8-hour working day during typical mosquito season from April to November in San Joaquin County. Task category "Other" typically includes mosquito trap reports, insectary maintenance, pesticide trials and resistance testing, dead bird collection and other vector surveillance activities.

CONCLUSIONS

The NPDES data collection requirements profoundly affected mosquito control operations at SJCMVCD. The need to sample regularly after certain treatments to WOTUS placed a strain on laboratory operations, which greatly reduced the number of mosquito trap identifications typically completed in a day. Other tasks, such as pesticide resistance testing of mosquitoes, were put on hold during the 2012 season and were not performed until the 2013 season when the NPDES permit requirements were on hiatus. NPDES permit requirements also delayed mosquito treatments directly and indirectly, greatly reducing response time to potential public health threats. Hopefully NPDES data collection requirements will not return to mosquito control districts in the future. When District time and money does not have to be diverted to NPDES permit compliance, the result is efficient mosquito control which facilitates preventing the spread of mosquito-borne viruses.

ACKNOWLEDGEMENTS

The authors wish to thank the Mosquito and Vector Control Association of California and the California Department of Public Health: Vector Borne Disease Section for their help with developing data collection strategies, equipment suggestions and report templates. The authors would also like to thank the SJCMVCD Board of Trustees for their approval of the necessary equipment expenditures to complete the required data collection under the NPDES permit and the staff of SJCMVCD for their diligence in their mosquito control efforts.

Optimization of Larviciding with Microbial Larvicides

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INTRODUCTION

Microbial mosquito larvicides have been playing important role in today's mosquito control operations. Various formulations customized for different habitats and residual efficacy have been developed and registered using ingredients derived from *Bacillus thuringiensis israelensis* de Bajac (*B.t.i.*), *B. sphaericus* Neide and *Saccharopolyspora spinosa* Mertz and Yao. Laboratory, semi-field and field trials were conducted to evaluate the formulations and method of application of various microbial larvicides to optimize the outcomes.

RESULTS

Optimization I - Synergism, Penetration and Coverage. Dairy wastewater lagoons are notorious breeding sources for *Culex quinquefasciatus* Say and *Cx. stigmatosoma* Dyar. Control efficacy in these habitats is often challenged by high organic content, vegetation coverage, high larval density and unpredictable flooding. Washed sand granules (Grade 30) coated with *B.t.i.* (AquaBac PP OSF) at 200 ITU/mg and *B. sphaericus* (VectoLex WDG) at 50 ITU/mg provided excellent control for up to 26 days as indicated by post-treatment counts of larvae and pupae per dip. The high control efficacy is attributable to the synergism of *B.t.i.* and *B. sphaericus*, better penetration (gravity 1.35 g/cm^3), as well as treatment coverage of the sand granules (6,000,000 grains/Lb, 1,377 grains/sq. ft. when applied at 10 Lb/ac.).

Optimization II: Coverage. Cryptic backyard mosquito sources such as small containers with water are significant habitats of *Culex* spp., *Culiseta* and *Aedes* spp. mosquitoes. Thoroughness of treatment coverage is critical for control efficacy. The granular formulations FourStar SBG (150 ITU/mg) and in-house made *B.t.i.* sand granules (200 ITU/mg) were used in the evaluation. FourStar SBG contains approximately 82,000 grains/lb, and theoretical coverage is 19 grains/sq. ft. when applied at 10 lb/ac. The in-house made *B.t.i.* sand granules provide about 6,000,000 grains/lb, and the theoretical coverage is 1,377 grains/sq. ft when applied at 10 Lb/ac. This coverage difference would be more profound when dealing with small treatment plots. The control efficacy in a simulated backyard studies rendered by in-house made *B.t.i.* sand granules was double that achieved by FourStar SBG at the same dosage.

Optimization III. Application in Tandem or in Combination. As a result of the control release feature of Natular G30, there was a three day lag time in the initial control efficacy in field trials when this formulation was applied alone to dairy wastewater lagoons. When FourStar SBG at 20 lb/acre and Natular G30 at 20 lb/acre were applied in tandem, or when Natular G30 coated with *B.t.i.* at 200 ITU/mg (AquaBac PP OSF, 7,000 ITU/mg) as applied at 20 lb/acre, there was no dealy in control and the emergence of adult mosquitoes was minimized. Furthermore, the synergistic effect of Natular G30 and *B.t.i.* provided longer residual efficacy than Natular G30 alone, extending >85% control from 28 days to 42 days. Application in tandem, or combining appropriate formulations, takes advantages of quick action of one formulation (FourStar SBG or AquaBac PP OSF), and longer residual activity of the second (Natular G30) to achieve maximum control of mosquito breeding under challenging field conditions.

Optimization IV: Avoidance of Sub-lethal Exposure. The mode of action of spinosad is to over-excite the postsynaptic nicotinic acetylcholine receptors in competition with acetylcholine. The recovery from treatment often occurs after an insect is exposed to sublethal concentrations. The late 3rd instar larvae of *Cx. quinquefasciatus* were exposed to Natular G30 at LC_{50} for 2, 4, 8, 24 or 48 h, then treatment was terminated by transferring all cadavers, moribound and surviving larvae to untreated water. The moribound and surviving larvae were given 48 h to recover from their previous exposure. The individuals that dove actively and exhibited normal larval behavior were considered recovered. The exposure time to allow recovery (ET_{50}) was 3.8 h, meaning greater than 50% of larvae recovered if exposure was less than 3.8 h. On the other hand, VectoMax CG contains endotoxins from *B.t.i.* and *B. sphaericus* that act on the gut epithelium of the mosquito larvae leading to mortality. When the late 3rd instar larvae of *Cx. quinquefasciatus* were treated at LC_{50} of VectoMax CG, more than 50% of larvae recovered if exposure was < 0.9 h (ET_{50} = 0.9 h). Obviously, larvae exposed to sub-lethal doses of Natular G30 are more likely to recover as compared to those exposed to VectoMax CG, due to different mode of actions.

Approximately 30% of 3rd instar larvae of *Cx. quinquefasciatus* recovered within 24 h after exposure to Natular G30 at LC_{50} for 2 h and then maintained in treatment water diluted with fresh water at the same temperature by 0% (no dilution), 25%, 50% and 100%. Following treatment with VectoMax CG, however, only a fraction (2-6%) of larvae recovered under the same treatment concentration and post-treatment dilutions.

It is imperative to avoid sublethal exposure whenever possible to ensure optimal efficacy and prevent resistance development. Sublethal exposures may occur due to unforeseen conditions such as inappropriate handling of products during storage and shipment, underestimation of treatment acreage and water depth or habitat dilution by precipitation or agricultural runoffs.

Optimization V: Method of Application. Subterranean storm water containment devices (or Best Management Practice, BMP) in urban areas have become significant sources of mosquito production. Natular T30 tablet scontaining 8.33% spinosad are among the few formulations designed to control immature mosquitoes breeding in these habitats. Because of the density of this formulation (approximately 1.9 g/cm^3), the tablets sink to the bottom of habitats immediately after application. No significant control was achieved with this product because the active ingredients were released far from the feeding zone of larvae. We modified the application method by attaching the Natular T30 tablets to wine bottle corks which kept the tablets floating close to the surface of water; hence the active ingredients of Natular T30 were released and remained in the feeding zone of mosquito larvae. This application methodology accomplished a high level of control (> 90%) which persisted for 44 days, well beyond the residual efficacy of 30 days stated on the label (Su et al. 2014).

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Profile of Resistance and Cross Resistance to Spinosad in Mosquitoes

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METHODS AND RESULTS

Resistance Development. A southern house mosquito *Culex quinquefasciatus* Say colony was established from surviving late instars and pupae from a semi-field evaluation of Natular® XRG (a granular formulation containing 2.5% spinosad). The initial lethal levels of Natular XRG against this colony were determined in the laboratory for the first generation progeny (F_1). Selection pressure was applied at LC_{70-90} levels of Natular XRG to 10,000-15,000 of late 3rd and early 4th instar larvae for each generation. Susceptibility changes in response to selection were determined every other generation. We found a gradual and steady decline in susceptibility to Natular XRG from generation F_1 to F_{35}, and for generations F_{37} to $F_{55,}$ the susceptibility decreased significantly. For reference purposes, the susceptibility of freshly collected wild populations as well as a laboratory reference colony of the same species was also determined concurrently. The susceptibility of freshly collected wild populations tended to fluctuate widely, whereas there was much less variability in susceptibility for the laboratory reference colony. When comparing the resistance ratio (RR) of wild populations to our selected laboratory reference colony, tolerance to spinosad was observed for all generations through F_9 in the selected population. Resistance ratios increased gradually from generation F_{11} to F_{35} and elevated significantly from generations F_{37} to F_{55} when the RR reached 13,607.8 – 16,760.8 fold at LC_{50} and 123,471.3 – 157,230.0 fold at LC_{90} in response to continuous selection. The exponential elevation of resistance levels among successive generations after selection indicated that a recessive mechanism might have been involved during development of resistance to spinosad (Su and Cheng 2012, 2014a).

Spectrum of Cross-resistance. While the spinosad-resistant *Cx. quinquefasciatus* colony exhibited varying levels of resistance, we did not detect any cross-resistance to *Bacillus thuringiensis israelensis* (*B.t.i.*) (RR = 0.5 - 2.9 fold), a combination of *B.t.i.* and *B. sphaericus* (RR = 1.8 - 2.3 fold), methoprene (RR = 1.4 - 2.9 fold), pyriproxyfen (RR = 0.5 - 1.5 fold), diflubenzuron (RR = 1.0 - 1.5 fold), novaluron (RR = 0.8 - 1.3 fold), temephos (RR = 1.9 - 2.0 fold), imidacloprid (RR = 1.4 - 2.1 fold) or indoxacarb (RR = 1.4 - 2.2 fold). However, results from our studies did show various levels of cross resistance to *B. sphaericus* (RR = 81 - 20,744 fold), spinetoram (RR = 59 - 108 fold), abamectin (RR = 26-83 fold) and fipronil (RR = 11 - 18 fold). On the other hand, a laboratory colony of *Cx. quinquefasciatus* that is highly resistant to *B. sphaericus* (RR = 24,840 - 510,851 fold) did not show cross-resistance to spinosad (RR = 1.2 fold) and spinetoram (RR = 0.6 - 0.8 fold). Field-collected and laboratory-selected *Cx. quinquefasciatus* that showed low to moderate resistance to methoprene (RR = 8 - 54 fold) did not show cross-resistance to spinosad (RR = 0.8-2.1 fold) or spinetoram (RR = 1.0 - 1.9 fold). The presence and absence of cross-resistance to other pesticides in spinosad-resistant mosquitoes seemed to be related to their modes of actions (Su and Cheng 2014b).

CONCLUSIONS

The information generated from this study is immediately applicable to mosquito control operations. Avoidance of sub-lethal exposure of mosquito populations to spinosad is a key factor for prevention of resistance development. The cross-resistance spectrum will guide the use of alternative larvicides to control mosquitoes that are resistant to spinosad. Other studies on spinosdad resistance stability, genetic mechanism, fitness cost of resistance, resistance prevention and susceptibility restoration are underway.

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Assessment of Native Fishes for Vector Control in Orange County, California

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ABSTRACT: Of the three native freshwater fish species (i.e., threespine stickleback, desert pupfish and arroyo chub) in southern California with a strong potential for consuming immature mosquitoes, the arroyo chub is the best candidate for integrated mosquito management programs in Orange County. At the present time, the California Department of Fish and Wildlife has considered approving two operational approaches for vector control using native fishes: (1) Translocation of native fishes within a watershed to sites below a physical impediment to upstream movement of translocated individuals, and (2) Translocation of fish into adjacent created, isolated aquatic habitat features (e.g., created wetlands). Within Orange County, the distribution of arroyo chub overlaps with source reduction activities carried out by the Orange County Vector Control District in the Arroyo Trabuco (Trabuco Creek and Tijeras Creek) within the San Juan Creek watershed. Numerous isolated water bodies exist within the watershed and in Orange County that could serve as supplemental conservation habitats for enhancing arroyo chub populations and for supplementing arroyo chub numbers for vector control. Discharge data for the Arroyo Trabuco, as well as for several other creeks, in coastal southern California indicate the late winter-early spring high-volume discharge events characteristic of streams in the region have the potential to wash a large proportion of the stocked fish downstream; thus, stocking should be delayed until after this period. It is recommended that between 1,000 and 2,000 arroyo chubs be translocated/stocked between mid-March through late May into Trabuco Creek and lower Tijeras Creek where vector control is currently required. Translocation of fish as early as possible in the spring will enhance the probability that arroyo chub reproduction will produce cohorts of young fish residing in the inundated vegetation of slow-moving sections of the water course where mosquito production is likely to be concentrated. Habitat characteristics favorable to the successful translocation of arroyo chubs and important gaps in our knowledge (e.g., persistence of stocked individuals in natural habitats, outcomes of interactions between native fishes with mosquitofish and introduced piscivores, temporal and spatial variations of important environmental variables in the target sites, estimates of arroyo chub population size, etc.) are discussed.

INTRODUCTION

One component of integrated mosquito management (IMM) programs for lacustrine and riverine wetlands is the use of larvivorous fish. Two species of the mosquitofish (*Gambusia affinis* (Baird and Girard) and *G. holbrooki* Girard) have been used worldwide for nearly a century as biological control agents for mosquitoes (Walton 2007, Walton et al. 2012). Both species are native to the southeastern United States and have native geographic distributions that are broad in comparison to all other North and Central American species in the genus (Moyle 2002). Few fish species can match the wide environmental tolerances and the favorable life history and morphological characteristics of *Gambusia* for mosquito control.

Yet, the hardiness, high reproductive potential, adaptability, aggressiveness and other characteristics that make mosquitofish such a successful predator of mosquitoes in many different aquatic environments worldwide (Swanson et. al. 1996) also make mosquitofish ideal invasive species that have potentially serious negative effects on native fauna and ecosystems (Gratz et al. 1996, Schleier et al. 2008), especially in the southwestern U.S. (Courtenay and Meffe 1989, Mills et al. 2004) and Australia (Arthington and Lloyd 1989). Mosquitofish are purported to prey upon the eggs and immature stages of economically important fish species and competitively eliminate native fish around the world (Myers 1965). Mosquitofish also eat eggs and larvae of native stream-dwelling amphibians in southern California such as the Pacific Chorus Frog, *Pseudacris regilla* (Goodell and Kats 1999) and the California newt, *Taricha torosa* (Gamradt and Kats 1996). The World Health Organization, as well as governmental natural resources agencies and conservation organizations, have urged studies of native larvivorous fishes that can replace the mosquitofish for mosquito control. In many regions of the U.S. outside the native geographic range of the two *Gambusia* species, release of mosquitofish into waters of the United States is no longer permitted. Not surprisingly, there is great interest among agencies responsible for stewardship of wetlands in southern California, such as the U.S. Fish and Wildlife Service (USFWS), California Department of Fish and Wildlife (CDFW) and the Orange County Water District (OCWD), to find a native species to replace *Gambusia* for mosquito control and to extirpate populations of the exotic mosquitofish in sensitive watersheds and habitats. Although *Gambusia* provides effective reductions of mosquitoes in a variety of isolated water features, there is a need to assess the potential use and applicability of native fish populations for vector control in portions of their natural habitat and created aquatic habitats adjacent to their habitat.

The objectives of this study were: (1) To conduct a GIS-based assessment to identify and characterize sites of production of insect vectors within the waters of the United States in Orange

County, California where native fish populations could be used to supplement vector control activities, (2) To identify the native fish species amenable for vector control and estimate the number of fish of particular species likely to be stocked per annum, and (3) To review mass-rearing programs for native fishes and to estimate the costs for rearing native fishes. Here, we highlight the findings related to (1) and (2) of the report (Walton et al. 2013) filed with the Orange County Vector Control District (OCVCD).

MATERIALS AND METHODS

Three species [i.e., threespine stickleback (*Gasterosteus aculeatus* L.), desert pupfish (*Cyprinodon macularius* Baird and Girard) and the arroyo chub (*Gila orcutti* Eigenmann and Eigenmann)] of native fishes were chosen as potential candidates for IMM programs in Orange County based on biological and ecological characteristics such as life history (e.g., reproductive phenology and distribution of life cycle stages in the water column), known environmental tolerances, diets and potential for mass-rearing. ArcGIS (Esri, Redlands, CA) was used to map the distribution of the native fishes within Orange County and within 8 km of the county borders using distribution data contained within the California Natural Diversity Database (CNDDB; https://www.dfg.ca.gov/biogeodata/cnddb). Inspection and treatment (IT) activities carried out by the OCVCD were extracted from the District's database and mapped. The overlap between the distributions of the three native fish species and sites of problematic vector production were mapped and characterized (i.e., distance of vector control activities, discharge and environmental characteristics if known). In addition to vector control activities associated with waters of the U.S., bodies of standing water that might serve as potential conservation habitat were identified and categorized by surface area.

The interannual and seasonal trends for discharge were characterized at four gage stations in the USGS National Water Information System (http://waterdata.usgs.gov/nwis) in coastal Orange and San Diego counties. The Arroyo Trabuco (= Trabuco Creek plus Tijeras Creek; USGS station number: 11047300) and San Juan Creek (USGS station number: 11046530) stations are located near the terminus of water courses containing the arroyo chub and upstream of their confluence near San Juan Capistrano. The Bonita Canyon (USGS station number: 11048600) and San Mateo Creek (USGS station number: 11046399) stations are on water courses that do not contain the arroyo chub, but may have supported arroyo chub historically. Discharge at these two stations may be indicative of conditions to the north and south of the current arroyo chub habitats in coastal Orange County. The historical distribution of the arroyo chub ranged along the coast from San Luis Obispo County to northern San Diego County (http://ice.ucdavis.edu/aquadiv/fishcovs/ach.gif; also see Fig. 2 in Why 2012). The watershed area of the four streams differs by 20-fold (5.4-109 ha: Walton et al. 2013). Here, the trends for the Arroyo Trabuco are highlighted and discussed relative to the three other sites. The reader is referred to Walton et al. (2013) for a more in-depth presentation of the findings for the three sites.

RESULTS AND DISCUSSION

Candidate Native Fishes for Mosquito Biological Control in Southern California. Three species of native fishes are promising candidates to replace *G. affinis* for biological control of immature mosquitoes in waters of the U.S. in southern California. The threespine stickleback (*Gasterosteus aculeatus* L.), the desert pupfish (*Cyprinodon macularius* Baird and Girard) and the arroyo chub [*Gila orcutti* (Eigenmann and Eigenmann)] are found in watersheds in southern California (Figure 1). These species possess characteristics that are favorable for use as biological control agents for mosquitoes. They are planktivorous for at least a portion of their life cycle (i.e., likely to consume immature mosquitoes), reproduce across an extended period during the year when adult mosquitoes are actively reproducing and are potentially amenable to mass-rearing in tanks and earthen ponds.

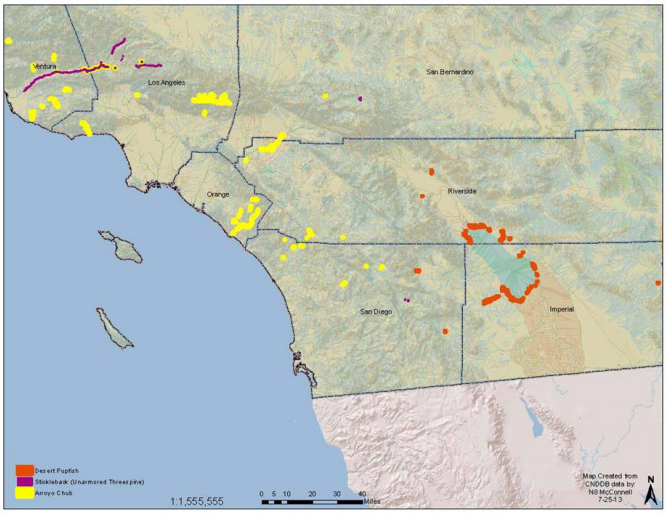

Figure 1. Distribution of three native fish species [desert pupfish: *Cyprinodon macularius* Baird and Girard; arroyo chub: *Gila orcutti* (Eigenmann and Eigenmann); threespine stickleback: *Gasterosteus aculeatus* L.)] which are potential replacements for the mosquitofish [*Gambusia affinis* (Baird and Girard)] as a biological control agent for immature mosquitoes in southern California.

Among these three fish species, the arroyo chub is the best candidate for IMM programs in Orange County. The desert pupfish is not native to Orange County and is listed as an endangered species by the USFWS. The threespine stickleback is broadly distributed across California, includes several morphological variants as well as anadromous populations and populations restricted to inland waters; there is also a federally listed subspecies in southern California. In addition to the inherent difficulties of working with a fish species listed under the Endangered Species Act, the stickleback also has some drawbacks for use in mosquito control such as comparatively narrow environmental tolerances and comparatively low reproductive potential (Offill and Walton 1999, Walton et al. 2007). The arroyo chub is endemic to several southern California watersheds and has been shown to be effective at controlling larval mosquito populations in earthen manmade

systems that were devoid of emergent vegetation (Van Dam and Walton 2007) and reducing immature mosquito abundance in experimental wetlands (Henke and Walton 2009).

The arroyo chub also possesses characteristics that enhance its survival and proliferation in vector control programs. In addition to broad environmental tolerances (such as tolerance of moderate hypoxia and temperature fluctuations, persistence in backwaters and lentic conditions as well as flowing water), the arroyo chub is a fractional spawner, breeding almost continuously from February to August, although most spawning takes place during peak breeding season in June and July (Moyle 2002). Captive populations held in earthen ponds may continue to reproduce as late as September or early October (W. Walton, personal observation). Adults typically spawn at one year of age (Tres 1992). Laboratory studies have shown the arroyo chub to be omnivorous, feeding on insects, algae and small crustaceans. Greenfield and Deckert (1973) found that 60-80% of the stomach contents consisted of algae. Arroyo chubs are also known to feed on nematodes infesting the roots of a floating water fern (*Azolla*) (Moyle 1976). While invertebrates are an important component of arroyo chub diets in spring, large arroyo chubs feed primarily on benthos (i.e., aquatic insects, snails).

Drawbacks to the widespread use of the arroyo chub for vector control include its listing as a "Species of Special Concern" by the CDFW, concerns of the CDFW that translocation of natural fish populations or stocking of hatchery-reared fish may promote the spread of potential pathogens and parasites, stocking fish may compromise the genetic distinctness of natural populations, and potential hybridization with other minnow species. Due to population declines and loss of habitat, the arroyo chub qualifies as a "Threatened Species" within its native range (Moyle et al. 1995, Veirs and Opler 1998). *Gila orcutti* is endemic to the Los Angeles, San Gabriel, San Luis Rey, Santa Margarita and Santa Ana river systems, as well as Malibu and San Juan creeks (Wells and Diana 1975, Swift et al. 1993; Figure 1). Chub have been introduced and have successfully established populations in the Santa Ynez, Santa Maria, Cuyama and Mojave river systems as well as smaller coastal streams such as Arroyo Grande Creek and Chorro Creek in San Luis Obispo County (Miller 1968, Moyle 1976, Moyle et al. 1995). Recently, arroyo chub were reintroduced into the Arroyo Seco as part of a restoration project carried out near Pasadena (Camm Swift, personal communication). However, as is observed for several minnow species in the region, hybridization occurs readily and poses a concern for conservation efforts of threatened and endangered native fishes. The arroyo chub hybridizes readily with two minnow species endemic to California: the Mohave tui chub (*Siphateles bicolor mohavensis* [Girard]) and the California roach (*Lavinia symmetricus* [Baird and Girard])(Hubbs and Miller 1943, Greenfield and Greenfield 1972, Greenfield and Deckert 1973).

Arroyo chubs have become scarce in their native range because the low-gradient streams, which are their preferred habitat, have largely disappeared due to urbanization (Swift et al. 1993). Arroyo chub populations, as well as those of other native fish, also have declined as a result of the introduction of several sport and non-native fishes to watersheds within southern California (Moyle et al. 1995). Specifically, green sunfish, *Lepomis cyanellus* Rafinesque, and largemouth bass, *Micropterus salmoides* (Lacèpéde), were introduced throughout the state for angling purposes and adults are piscivorous (Baltz and Moyle 1993). The statewide introduction of mosquitofish, *G. affinis*, for mosquito control has also contributed to the declines of native fish populations in California (Moyle et al. 1995). Arroyo chubs tend not to co-occur with red shiners [Lahontan redside, *Richardsonius egregious* (Girard)] that were introduced as forage fish for stocked trout or released by anglers using the species as a baitfish. Piscivory by centrarchids was likely the primary factor that contributed to the failure of stocked arroyo chubs to persist in a wetland within the Prado Basin in western Riverside County, CA (Why 2012).

Within Orange County (Figure 2), the arroyo chub is found in the San Juan Creek drainage in Bell Canyon, Hot Springs Canyon and the upper mainstem of San Juan Creek. Arroyo chub are also found in the Arroyo Trabuco (Trabuco Creek in Trabuco Canyon) and the lower Tijeras Creek. Contrary to the distribution data in the California Natural Diversity Database (CNDDB), the arroyo chub has been extirpated from Oso Creek where red shiners are predominant (John O'Brien, California Dept. Wildlife, personal communication).

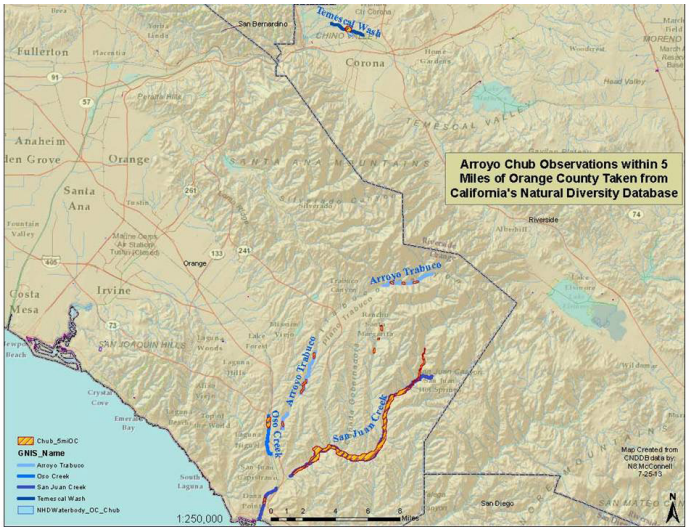

Figure 2. Distribution of the arroyo chub (*Gila orcutti*) in Orange County, California and within 8 km (5 mi) of the county borders. Data taken from California's Natural Diversity Database. Not shown is the presence of *G. orcutti* in the Santa Ana River between the City of Riverside and the Prado Basin.

It is difficult to ascertain the actual numbers of arroyo chubs within these watersheds. Quantitative estimates of population size are unavailable. The abundance of arroyo chubs presumably varies annually in relation to flooding and drying. The arroyo chub is adapted to warm, fluctuating streams that were characteristic of the southern California coastal plain (Moyle 2002). Stream discharge varies markedly seasonally, and some streams are intermittent in the lower reaches. Arroyo chubs attain greatest abundance in slow-moving or backwater sections of water courses where inundated vegetation provides important cover for young-of-the-year fish.

Overlap of Arroyo Chub Populations with Vector Control Activities. The distribution of arroyo chubs overlaps with IT activities carried out by the Orange County Vector Control District in the Arroyo Trabuco (Trabuco Creek and lower Tijeras Creek) within the San Juan Creek watershed (Figure 3). Trail maintenance activities carried out by the OCVCD along Aliso Creek, English Canyon, Dove Canyon, Laguna Canyon, Serrano Creek, within the San Clemente Coastal Streams watershed, at two locations within the upper Santa Ana River watershed, and at several locations within the Newport Bay watershed do not overlap with current arroyo chub distributions in the CNDDB (Figure 3).

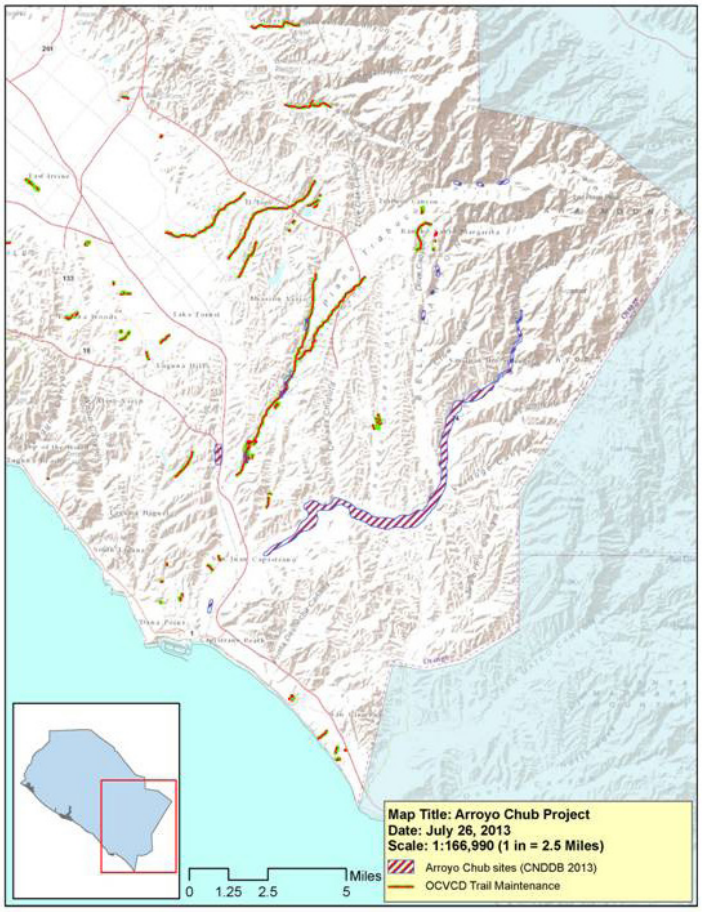

Figure 3. Overlap of the arroyo chub (*Gila orcutti*) and source reduction activities carried out by the Orange County Vector Control District.

At the present time, translocation of arroyo chubs within a watershed to sites below a physical impediment to upstream movement of translocated individuals is the one of the operational approaches for vector control using native fishes that might be considered for approval by the CDFW (Native Fishes Scoping Meeting, OCVCD, 10 July 2012). Trabuco Creek provides the best opportunity for enhancement of native fish populations in areas problematic for vector production. Potential source populations of fish occur upstream of, as well as within, the zone of vector control activities. Moreover, these source reduction activities are the most extensive (in terms of total distance) in southern Orange County. The lower Tijeras Creek is an adjacent site in the same watershed that appears favorable for the enhancement of arroyo chub populations. While impediments to upstream movement of fish are prevalent at the terminus of the water course, it is unknown whether any natural or man-made impediments to upstream movement exist above the trail maintenance activities in the Arroyo Trabuco.

Arroyo chub also are present in San Juan Creek which joins the Trabuco Creek near San Juan Capistrano. An ongoing study of the genetics of the arroyo chub is being carried out by the CDFW. This study should provide information on the relatedness of the *G. orcutti* populations in both water courses. Although trail maintenance activities are not carried out by the OCVCD along San Juan Creek (Figure 3), arroyo chub are found below the confluence of the two stream systems and may form one population within the San Juan Creek watershed.

IT activities also are carried out in numerous isolated habitats that might be amenable for stocking arroyo chub. For example, thirteen habitats near Trabuco Creek range in area from 0.03 to 40 ha (Figure 4). Other prospective sites within Orange County still need to be determined. Sites should maintain water throughout the year and should not contain piscivorous fish such as green sunfish and bass. Coexistence of the arroyo chub with mosquitofish is probably possible, but the outcome(s) of interactions between the two fish species require further study. Ownership of the water bodies needs to be determined, and the owners must be amenable to the stocking of arroyo chubs. The addition of native fish must not jeopardize the existence of endangered or threatened species already present in a site.

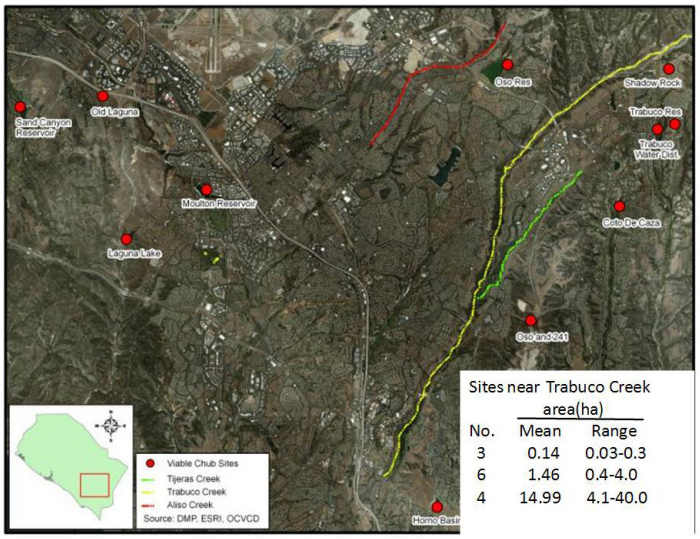

Figure 4. Viable stocking sites for arroyo chub in isolated standing-water habitats near Trabuco Creek. The distribution of sites based on surface area is provided in the lower right.

Translocation/stocking of Arroyo Chubs in Trabuco Creek and Tijeras Creek. Several factors should be considered in relation to the translocation/stocking of the arroyo chub within Trabuco and Tijeras Creeks. In addition to the concerns expressed by the CDFW related to the genetics, health and possible differences in the parasite burden of the fish among sites (i.e., stream systems within a watershed and among watersheds), the

fish should be relocated as early as possible during the period that adult mosquitoes are reproducing. This strategy should enhance the likelihood that robust numbers of native fish will occur within the sections of the stream system known to produce mosquitoes. The spawning period of the arroyo chub (late February to August: Moyle 2002) begins prior to the onset of host-seeking activity by the majority of mosquito species of concern in the region (in late March-early April) and may extend into October (W. Walton, personal observation) when host-seeking by the mosquitoes tends to decline markedly.

The late winter-early spring high-volume discharge events characteristic of streams in the region have the potential to wash a large proportion of the stocked fish downstream; stocking should be delayed until after this period. Translocation of fish as early as possible in the spring will enhance the probability that arroyo chub reproduction will produce cohorts of young fish residing in the inundated vegetation of slow-moving sections of the water course where mosquito production is likely to be concentrated. Based on the differences in the diets and spatial distribution of fish within native habitats (Moyle 2002) related to age (and size), the young-of-the-year presumably are more important in the consumption of immature mosquitoes than are adult arroyo chubs. Nevertheless, immature mosquito abundance was reduced in the presence of adult arroyo chub as compared to lentic vegetated habitats without fishes (Henke and Walton 2009), but this effect might reflect oviposition deterrence of the mosquitoes (Why 2012) rather than predation of mosquito larvae by the adult arroyo chubs. Moreover, the establishment of fish prior to the peak reproductive period in June and July (Moyle 2002) will ensure that reproductive adults have the greatest chance to acclimate to new surroundings after translocation.

Other factors that should be considered to enhance the production of a new cohort of arroyo chub in environments where fish have been stocked are the characteristics of the habitat related to the survival and reproduction of adults as well as the survival of fry. Arroyo chub prefer slow-moving or backwater sections of warm to cool streams (10 - 24 °C) with muddy or sandy bottoms (Moyle 2002). Whereas arroyo chubs can be found in shallow fast-moving sections of streams with coarse (i.e., rocky, scoured substrate) bottoms, they prefer depositional habitats with depths > 40 cm (Moyle 2002). Habitats with cover (vegetation, root masses, etc.), overhanging banks, deep areas (> 40 cm depth) and/or boulders are favorable for stocking arroyo chub (C. Swift, personal communication). Survival of young fish will be enhanced in habitats containing inundated vegetation. The fish should be stocked into sites where these types of habitats are likely to be present well into the summer.

An additional consideration related to the translocation and stocking of the arroyo chub is the timing and extent of source reduction. Thinning or elimination of inundated vegetation along stream channels reduces mosquito production but also has the potential to reduce the favorability for the survival of young arroyo chub. Presumably the timing of vegetation management is influenced by considerations of the nesting activities of birds. Generally, vegetation management is only permitted after the nesting season; such activities are restricted to autumn and winter when arroyo chub reproduction ceases annually. From the perspective of arroyo chub life history, late-season vegetation management is preferable to late spring-early summer activities.

The interannual pattern of daily discharge is, not surprisingly, similar among the four gage stations for the period between 2001 and 2013 when data are available for all stations. A discontinuous record of daily discharge from autumn 1972 through August 2013 is available for Arroyo Trabuco (Figure 5). A continuous 27-year record of daily discharge is available for San Juan Creek and a continuous 13-year (autumn 2001 through August 2013) dataset is available for Bonita Canyon (Walton et al. 2013). The discharge record for San Mateo Creek is discontinuous, including a period from autumn 1952 through 1968 and a second period beginning in 1994 until August 2013 (Walton et al. 2013). Although the magnitude of the daily discharge is indicative of watershed area and diversions/restrictions of flow within each watershed, the pattern of discharge events reflects the interannual variation in rainfall caused by short-term variation in climate such as El Niño-Southern Oscillation (ENSO) events. Maximum daily discharge events were comparatively greater in 2005 and 2011 than in other years. The ENSO events of 1995 and 1998 are also evident in the daily discharge data at sites.

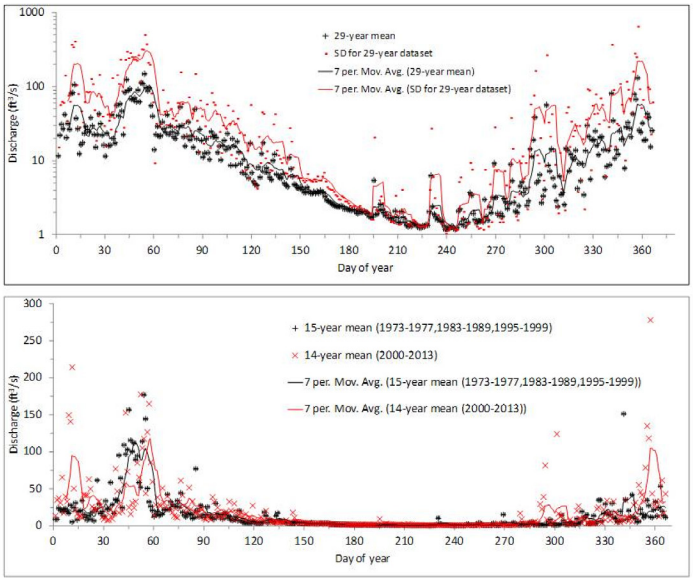

Figure 5. Mean and variation (SD) for daily discharge (ft^3/s) for 29 years during 1973-2013 (upper panel) and mean daily discharge for periods before or after 2000 (lower panel) at the Arroyo Trabuco gage station in the USGS National Water Information System database. Seven-day running averages depicted.

For each day of the year across years, the mean and the variation of daily discharge is comparatively higher in January through March and from mid-October through December in both Arroyo Trabuco (Figure 5) and San Juan Creek (Walton et al. 2013). Variation in daily discharge declines appreciably around day 150 (May 29 based on a 366-day year to include data for leap years) in Arroyo Trabuco. The monthly mean discharge for April decreases to 14.4 ft^3/s from 25.5 ft^3/s for March and drops to 8 ft^3/s in July (U.S. Geological Survey 2013b). Water flow is unaffected by upstream diversions. In contrast to the Arroyo Trabuco, there is no regulation upstream from the gage station on San Juan Creek, but the Capistrano Water Company diverts water

3.2 km (2.0 mi) upstream of the gage station, and various amounts of diverted water reach the station as irrigation return flow (U.S. Geological Survey 2013a). No discharge was recorded from early May through September 2012. A similar decline in the mean daily discharge through about day 250 (Sept. 6) is observed in both water courses. However, sporadic flooding events are evident in the variation of daily discharge for Arroyo Trabuco (Figure 5). The mean and variation for daily discharge increase in both water courses beginning in early October (~ day 280).

Similar annual trends for daily discharge are evident in the two water courses not currently supporting the arroyo chub (Walton et al. 2013). Low summer flows (essentially no measurable flow) are evident in Bonita Canyon. The variation in daily discharge is lower than the long-term daily mean. The decline in the mean daily discharge in San Mateo Creek across spring and summer is similar to that observed in the two water courses containing the arroyo chub (Walton et al. 2013). Interestingly, San Mateo Creek exhibits a marked increase in the mean and variation of daily discharge around day 300 and again during December.

It is possible that recent development within watersheds has altered the patterns of daily discharge. For example, increases in the proportion of area covered by impervious substrate might increase the mean and variation in daily discharge. Installation of structures to capture stormwater runoff might show an opposite effect on daily discharge. The mean daily discharge for the period 2000 - 2013 was greater during December and January than for the discontinuous record from 1972 through 1999 in Arroyo Trabuco (Figure 5). The mean daily discharge for February was equivalently variable for both periods.

In San Juan Creek, mean daily discharge during December increased for the period 2000-2013 relative to the period from 1987 through 1999 (Walton et al. 2013). Surprisingly, the mean daily discharge in January and February during the late 1980s and throughout the 1990s was larger and more variable than during the most recent decade. However, it is difficult to discount differences in interannual variation of precipitation between the two time periods.

Discharge data are the most complete environmental variable dataset available for these sites. Water temperature and water quality data are limited to only a couple of years, if at all, in the USGS datasets. These variables are not recorded in conjunction with source reduction activities. Although we intended to assess the environmental characteristics of habitats producing vectors such as habitat size, water depth, vector abundance, general site conditions, etc., these variables are not routinely recorded along the stream sites where source reduction is being carried out.

The overwinter survival rates of minnows often depend on the interaction between summertime temperatures and food supply. Prevention of starvation during winter is linked to the development of lipid stores during the summer (Meffe and Snelson 1993a, b). These factors are particularly important for fish populations that must survive low water temperatures that accompany freezing conditions during winter. Clearly these conditions are not occurring in Orange County, but poor overwintering survival of larvivorous fishes can affect IMM programs in southern California (Walton et al. 2012). Stocking fish prior to summer would therefore be preferable to stocking fish in late summer and autumn.

An additional consideration when stocking native fish is the abundance of non-native fishes, especially mosquitofish and piscivorous centrarchids in the stream reach to be stocked. The western mosquitofish (*G. affinis*) has negative impacts on the least chub [*Iotichthys phlegethontis* (Cope)] directly through predation and indirectly via exclusion from suitable rearing habitats (Mills et al. 2004, Wagner et al. 2005). In laboratory studies, age-0 least chub sought refuge in the presence of western mosquitofish and spent less time feeding; this resulted in reduced growth and a longer period of time in which least chub were vulnerable to predation (Mills et al. 2004). In natural habitats, western mosquitofish can also reduce the growth of least chub by forcing age-0 least chub to seek refuge from predation by utilizing the cooler pool habitats. Slower growth rate and reduced accumulation of lipid stores which enhance overwinter survival would result from such habitat shifts. Aggressive interactions with western mosquitofish adults (Schoenherr 1981) may cause minnows to reproduce in less favorable habitats (i.e., pools). Young-of-the-year least chub were readily consumed by western mosquitofish, and those not eaten, including adults, experienced reduced growth as a result of competition with western mosquitofish (Mills et al. 2004). Whereas the larger arroyo chub should be less susceptible to the detrimental effects of competition and predation than the least chub, taken together these factors are expected to reduce the size and robustness of chub age classes stocked into habitats where mosquitofish are abundant and predacious centrarchids are prevalent.

How many fish need to be transplanted? Based on previous stocking efforts for the arroyo chub, a minimum of several hundred fish and a maximum of around 2,000 should be transplanted in the region of vector control activities in Trabuco Canyon; however, at present, a more definitive estimate of the number of fish needed cannot be made. The number of arroyo chubs to be transplanted will be a function of suitable habitat for the arroyo chubs, barriers to dispersal upstream, the presence of non-native fishes and other considerations, especially the availability of extant fish to be transplanted. The extent of source reduction activities on Trabuco Creek equals about 10.7 km (6.67 mi) plus an additional 4.0 km (2.5 mi) on Tijeras Creek, a southerly branch (Figure 3). Arroyo chubs are present in both streams (J. O'Brien and C. Swift, personal communication), so suitable habitat is presumably present. Habitats with cover, overhanging banks, deep areas (> 40 cm depth) and/or boulders are favorable for stocking arroyo chub (C. Swift, personal communication). For sites with a high proportion of canopy cover, aquatic vegetation is not very prevalent, but root masses and overhanging banks may provide suitable cover for the fish.

In summer 2008, approximately 300 chubs were translocated from Big Tujunga Wash near the crossing of Oro Vista Avenue (approximately 1 km north of the 210 freeway) and placed in a 4.5 km (2.8 mi) stretch of the Arroyo Seco between the 134 and 210 freeways near Pasadena (C. Swift, personal communication). The introduced fish were placed into two small naturalized areas with cement-lined channels or impassable barriers closely limiting the areas upstream and downstream.

Assuming a transplantation rate of 70 adult fish/km, then 1,050 fish would be needed in Trabuco and Tijeras Creeks to provide a stocking rate comparable to that of the Arroyo Seco

reintroduction study. Large numbers of native arroyo chub can be found in San Juan Creek within a 1.6 km or so up and downstream of the mouth of Arroyo Trabuco, and farther up Trabuco Creek near the junction of Oso Creek. Although arroyo chub were present in substantial numbers near O'Neill Park area (C. Swift, personal communication), this population may no longer be present (J. O'Brien, CDFW, personal communication) because of large numbers of red shiners. In 2010, large numbers of arroyo chub were observed in Tijeras Creek upstream of its confluence with Trabuco Creek (C. Swift, personal communication). It seems unlikely that native populations in Trabuco Canyon could sustain the level of harvesting of the aforementioned range of individuals, and hatchery-raised fishes would be needed to supplement the stocked population. There is, however, currently no scientific evidence supporting the aforementioned stocking rate.

Stocking rates of arroyo chub in lentic habitats seem unrealistically high for the lotic habitats of Trabuco Canyon. Van Dam and Walton (2007) used stocking rates of 4.5 kg/ha (mean = 12.5 g/pond as 4 reproductive and 35 larval chubs) and 13.2 kg/ha (mean = 37 g/pond as 31 adult fish) in earthen ponds. Assuming an average stream width of 3 m for 15 km of vector control activities, approximately 17,000 and 50,000 adult arroyo chubs would be needed to provide comparable stocking rates. The number of arroyo chubs present after nearly two years in experimental wetlands was more similar to that of the lower stocking rate than the higher stocking rate (J. A. Henke, unpublished data). Moreover, arroyo chubs and mosquito production are concentrated at the periphery of streams and rivers. Arroyo chubs prefer more marginal, slow or standing flows on the periphery of streams (Feeney and Swift 2008). If mosquito production is problematic, these backwater habitats containing inundated vegetation are likely to be the sites producing mosquitoes. The slow moving regions also can support exotic predators of arroyo chubs, such as sunfish and bass (C. Swift, personal communication), which presumably limit the distribution of arroyo chubs in river systems (i.e., the Santa Ana River: Feeney and Swift 2008) and likely extirpated arroyo chubs from a wetland in the Prado Basin (Why 2012). If about one-tenth of the surface area of Trabuco Creek and Tijeras Creek is conducive for supporting the arroyo chub, then ≤ 1,700 adult arroyo chub would be needed.

Whereas, an estimate of arroyo chub population size and more reliable estimates of the surface area of the two creeks, of suitable chub habitat, and of the regions of problematic mosquito production would be beneficial, as well as other factors (Table 1), this preliminary assessment finds that supplementation of native arroyo chub populations for vector control seems feasible. A characterization of sites that differ in mosquito production, especially the presence and abundance of native fishes in these sites, is suggested. An assessment of the barriers to upstream movement and the amount of suitable habitat for the arroyo chub is needed in the places where source reduction is carried out before the translocation of fish is made. Also, the efficacy of supplementation of native fishes for the reduction in mosquito production and persistence of the stocked fish should be evaluated.

• Habitat characteristics favorable for successful translocation
• Stocking density that facilitates persistence of fish and provides effective mosquito control
• Persistence of stocked individuals in natural habitats
• Temporal and spatial variation of important environmental variables influencing the success of translocations
• Estimates of arroyo chub population size
• The abundance of non-native fishes, especially mosquitofish and piscivorous centrarchids, in the habitats to be stocked.
• Outcome of interactions between arroyo chub and mosquitofish
• A characterization of sites that differ in mosquito production, especially the presence and abundance of native fishes
• An assessment of the barriers to upstream movement and the amount of suitable habitat for the arroyo chub in the places where trail maintenance is carried out.
• Efficacy of supplementation of native fishes for the reduction in mosquito production: direct vs. indirect effects on mosquito abundance
• Hatchery management plans

Table 1. Some of the additional information needed to evaluate the success and to assist decision-making for the use of the arroyo chub as a component of IMM programs in southern California.

Based on the aforementioned considerations, it is recommended that the arroyo chub are translocated/stocked between mid-March through late May (Figure 6). Stocking from June through August is possible, but it is expected that annual recruitment for fish stocked in summer will be lower than for fish stocked earlier in the year. The persistence of stocked individuals in natural habitats should be studied.

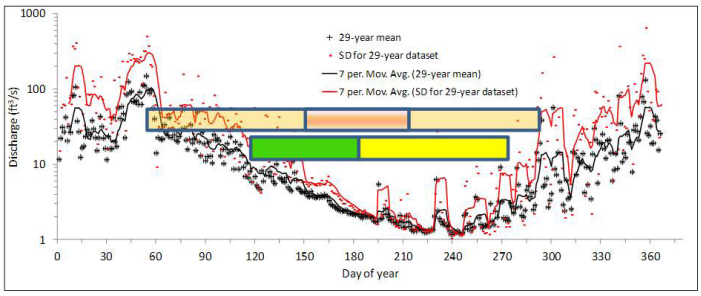

Figure 6. Mean and variation of daily discharge (ft^3/s) for the Arroyo Trabuco, annual period of reproduction for the arroyo chub (*G. orcutti*) (upper bar: peak period of reproduction is highlighted in the center of the histogram) and recommended timing of translocation of arroyo chub (lower bar). The green histogram in the lower bar represents the better time for translocation based on reproduction of the arroyo chub and annual discharge patterns in the Arroyo Trabuco.

Mass Rearing of Native Fishes. While native fishes that could potentially serve as alternatives to the mosquitofish as biological control agents for immature mosquitoes, these fishes have been raised by only a few vector control districts in California. Moreover, most of the rearing studies have not been at a scale large enough to be practical for stocking fish into mosquito developmental sites. The greatest success to date has been with the California roach [*Lavinia* (*Hesperoleucus*) *symmetricus* (Baird and Girard); Cyprinidae]. Approximately 4700 and 7300 fish were produced in 2010 and 2011, respectively, but production declined precipitously in 2012 to only 1500 fish (Chris Miller, Contra Costa MVCD, personal communication). The factor(s) contributing to the decline in production is/are unknown; however, brood stock age and food levels are the most likely reasons.

This minnow species was reared in 200-gallon tanks with a recirculating system. Flowing water was required for the fish to initiate spawning, and the primary reproductive period was from April until the end of June. Spawning material for egg deposition was added to tanks containing reproductive adults and then removed so the eggs could be incubated separately from the adult fish. The larvae readily consumed commercial fish food.

California roach has been stocked by the Contra Costa MVCD into abandoned swimming pools at a rate of between 40 and 100 fish/pool. Large adults (~ 2 years old) and 3-month-old young-of-the-year were included in the stocking populations. Approximately 500 individuals also were added to a large wetland; but the success (i.e., persistence of the fish) of that introduction has not been determined.

Efforts by the Contra Costa MVCD to mass-rear the Sacramento perch (*Archoplites interruptus* (Girard); Centrarchidae); Sacramento blackfish (*Orthodon microlepidotus* (Ayres); Cyprinidae) and hitch (*Lavinia exilicauda* Baird and Girard; Cyprinidae) have been comparatively less successful than for the California roach (León et al. 2008; C. Miller, CCMVCD, personal communication). Successful rearing of the Sacramento perch through the larval stage requires a diet rich in rotifers. Piscivory by adult fish is potentially problematic for mass rearing. Consumption of young-of-the-year by adults also limited the successful rearing of the Sacramento blackfish (C. Miller, CCMVCD, personal communication).

The threespine stickleback has been used successfully for larval mosquito control in backyard ponds and abandoned swimming pools in the Central Valley (Sacramento-Yolo MVCD 1999; Woody Schoen, personal communication). Twenty to twenty-five adult fish/habitat were stocked into habitats with depths > 46 cm. However, a poor tolerance for high water temperature and hypoxia, complex mating behavior (e.g., high levels of aggression between males and complex nest construction) and a low reproductive rate (Offill and Walton 1999, Walton et al. 2007) limit the usefulness of this species for mosquito control and in mass rearing programs. The use of this species for vector control is further complicated in southern California because a federally-listed unarmored subspecies, *Gasterosteus aculeatus williamsoni* (Girard) is associated with the Santa Ana River. Although this subspecies has not been collected/observed for nearly fifty years in the Santa Ana River, and has probably been extirpated from the Santa Ana watershed, three relict populations [upper Santa Clara River (Los Angeles County), San Antonio Creek (Santa Barbara County) and Sweetwater River (San Diego County)], and two transplanted populations, exist in southern California (Moyle 2002).

The desert pupfish has been raised successfully in earthen ponds (Walters and Legner 1980; M. Saba, personal observation). Listing of the species under the Endangered Species Act and controversy related to the genetic relatedness of extant populations severely restrict future use of this species for vector control.

The arroyo chub has been raised successfully in an artificial stream at the Riverside-Corona Resource Conservation District (Riverside, CA) and in earthen ponds at U.C. Riverside. The production of arroyo chub in the artificial stream is over 1,000 individuals/annum and cohorts of fish have been used for reintroductions and will be used for a future study in constructed treatment wetlands adjacent to Cucamonga-Mill Creeks (Kerwin Russell, Riverside-Corona Resource Conservation District, Riverside, CA). The wet mass of arroyo chub populations in earthen ponds approximately doubled during 6-week studies spanning the peak reproductive period, and the population growth rate on natural prey assemblages was circa 0.04 individuals/individual/day (Van Dam and Walton 2007).

A very successful program using native fishes for vector control has been implemented in Utah. The Utah Division of Wildlife Resources (UDWR) has developed a partnership with vector control districts in Utah to use the least chub as a substitute for mosquitofish as a biological control agent for mosquitoes. A Memorandum of Agreement (MOA) among the Utah Division of Wildlife Resources, Utah Department of Agriculture and Food and Utah Vector Control Districts established administrative policies and procedures for collecting, holding, distributing, transporting, rearing and releasing mosquitofish (see Appendix A in Walton et al. 2013). Whereas the MOA restricted the use of *Gambusia* by the signatory districts, the use and possession of mosquitofish by districts not signing the MOA is prohibited. The UDWR further developed procedures and policies allowing the vector control districts to rear and distribute the least chub. Under the joint program of the UDWR and the vector control districts, the least chub has been stocked into about 240 backyard ponds near Salt Lake City as part of ongoing research. Last, the UDWR and university researchers conducted a study to understand better the requirements for raising the least chub, the interactions between mosquitofish and least chub and the environmental factors that influence (i.e., potentially limit) the distribution of the invasive mosquitofish. Many of the lessons learned in these research endeavors focused on the least chub are relevant to prospective future efforts for mass rearing the related arroyo chub. Further discussion of the factors to be considered for successful propagation and costs associated with the culture of native fishes can be found in Walton et al. (2013).

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Three Years (2011- 2013) of *Aedes albopictus* in the City of El Monte, San Gabriel Valley, Los Angeles County, California

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ABSTRACT: The Asian tiger mosquito, *Aedes albopictus* (Skuse), is a daytime-biting nuisance and an efficient vector of several arboviruses and filarial worms. Over the past three decades, this mosquito has spread rapidly around the world. In 2011, a population of *Ae. albopictus* was discovered in the City of El Monte, Los Angeles County, California (the City). Since the discovery of *Ae. albopictus* in the City, the San Gabriel Valley Mosquito and Vector Control District (SGVMVCD) has been conducting intensive surveillance to determine the intensity and extent of the infestation. Three years of surveillance for *Ae. albopictus* has shown a steady expansion of the infested area in the northeasterly direction of the City (as predicted by Fujioka *et al*, 2012) covering approximately 1787 acres in 2011, 2780 acres in 2012 and 5266 acres in 2013. This expansion seems to have occurred in a geometric rather than exponential fashion. The temporal distribution shows peak months for *Ae. albopictus* activity to be August through October. Surveillance efforts to determine the intensity of infestation within properties and neighborhoods and to identify efficient control strategies are ongoing and to be reported elsewhere.

INTRODUCTION

Aedes albopictus (Skuse) is one of the most successful species of invasive mosquitoes in the world (Mitchell 1995, Benedict *et al*. 2007). Over the past three decades, *Ae*. *albopictus*, also known as the Asian tiger mosquito (ATM), has spread from Southeast Asia to every continent with the exception of Antarctica (Benedict *et al*. 2007, Enscrink 2008). Although *Ae*. *albopictus* is a less competent vector of arboviruses and other diseases compared to its congeneric species *Aedes aegypti* (L.), its importance in transmitting dengue in rural Asia, dengue and chikungunya in parts of Africa and Europe, and the first local endogenous transmission of chikungunya in the Caribbean Americas is well documented (Carrier *et al*. 2011, Delatte *et al*. 2013, PAHO 2014).

Over the past three decades, *Ae*. *albopictus* has been introduced into most parts of southeastern United States through transport of dormant eggs in imported tires from Asia (Moore *et al*. 1999 and references therein). In 2001, small populations of *Ae*. *albopictus* were "unsuccessfully" introduced into California via trans-oceanic shipments of "lucky bamboo" (*Dracaena* spp.) packaged in standing water (Kluh *et al*. 2002, Linthicum et al. 2003, Madon *et al*. 2002). On September 2, 2011, *Ae albopictus* was discovered in the City of El Monte (the City), Los Angeles County, California (Fujioka *et al*. 2012), and subsequent investigations found the mosquito had infested many parts of the City as well as portions of the neighboring City of South El Monte (Metzger and Hu, 2012). The San Gabriel Valley Mosquito and Vector Control District embarked on a campaign to eradicate *Ae*. *albopictus*; however, the population survived the winter and reemerged throughout the infested area in May 2012 and expanded into additional parts of El Monte in 2013. Current populations of *Ae*. *albopictus* in San Gabriel Valley are genetically similar to those introduced into Los Angeles County in 2001 through importation of *Dracaena* from Asia (Zhong *et al*. 2013). These populations are less likely to have originated from the southeast of the United States. Here we describe the spatial distribution and population dynamics of *Ae*. *albopictus* for the past three years within San Gabriel Valley and speculate on its spread in the near future.

MATERIALS AND METHODS

Site Description. Locations inspected for infestations with *Ae*. *albopictus* in the City of El Monte and neighboring cities are typical of the large Los Angeles metropolitan area (i.e., old, settled, residential neighborhoods with north/south and east/west road network systems). El Monte is a residential, industrial, and commercial city in the San Gabriel Valley, east of the City of Los Angeles, Los Angeles County. It is bound by the cities of South El Monte to the south, Arcadia to the north and between the San Gabriel and Rio Hondo rivers to the east and west, respectively. The City is about seven miles long and four miles wide, lying in a northeasterly stretch.

Most residential neighborhoods in the City are of mixed sizes; properties may have single family unit, duplexes, or multiple single family residences on the same property – an important feature posing major challenges to effective truck-mounted applications of pesticides. The south side of the City has large properties, the majority being more than 200 feet deep; the depth of property then decreasing northwards to between 40 -100 feet in the northern portion of the City.

Sampling. The discovery of *Ae*. *albopictus* on September 2, 2011 at a mobile home community in the south portion of the City sparked intense door-to-door inspections for the rest of the year. These inspections identified additional properties with *Ae*. *albopictus*. To establish which neighborhoods and properties were infested with *Ae*. *albopictus*, several techniques were used. The door-to-door inspections identified properties with different containers or receptacles in their yards that could hold water for *Ae*. *albopictus* to lay eggs, for larvae and pupae to develop, and for adults to emerge. In addition, evidence of *Ae*. *albopictus* breeding was determined by use of ovicups (ovitraps) placed on properties (with homeowner consent) within the City to detect egg-laying *Ae*. *albopictus*. Finding adults and/or exuvia of different stages of *Ae*. *albopictus* larvae confirmed their presence. Adult *Ae*. *albopictus* were collected using Carbon dioxide-baited traps and Biogent's (BG-Sentinel) traps (Farajollahi *et al*. 2009). The discovery of eggs, larvae, pupa, or adult *Ae*. *albopictus* indicated a positive property and monthly totals were tabulated for yearly comparisons.

RESULTS AND DISCUSSION

Spatial Distribution of *Ae. albopictus*. The initial discovery of *Ae. albopictus* on September 2, 2011 occurred in the southern portion of the City (Figure 1). A total of 61 positive properties were identified by December 31, 2011, with the majority of positive properties being in the southern portion of the City. However, one positive property was found in the unincorporated Los Angeles County area south of the City of Duarte. In 2012, a total of 5,246 inspections were conducted and 227 properties were found positive for *Ae. albopictus*. The infestation was mostly in the south and central portions of the City of El Monte, and none in the northern portion. One *Ae. albopictus*-positive property was found in the southern portion of the City of Arcadia. In 2013, a total of 7,775 inspections were conducted and 236 properties were positive for *Ae. albopictus* (Figure 1). Three years of surveillance for *Ae. albopictus* in the City of El Monte showed steady expansion of the infested area in the northeasterly direction (as predicted by Fujioka *et al*, 2012) covering approximately 1,787 acres in 2011, 2,780 acres in 2012, and 5,266 acres in 2013. This expansion seemed to have occurred in a geometric rather than exponential manner.

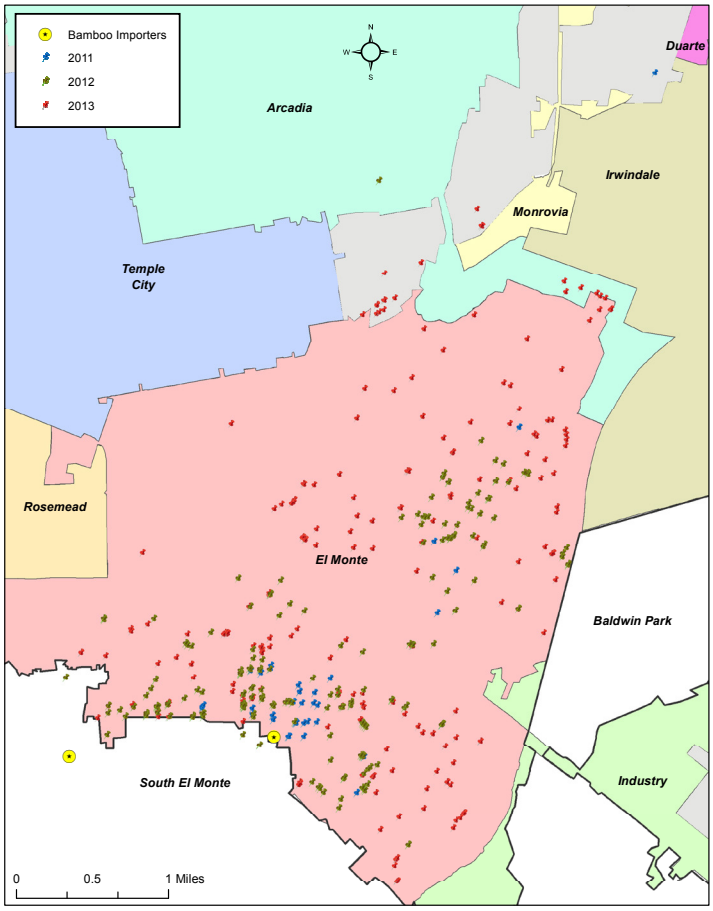

Figure 1. Spatial distribution of *Aedes albopictus* in San Gabriel Valley, Los Angeles County from 2011 to 2013.

In 2001, among other locations, *Ae. albopictus* was found in El Monte at a "Lucky Bamboo" wholesaler located in the southern edge of the City, with an additional address in the western part of City of South El Monte (Figure 1) (Linthicum *et al*. 2003, Madon *et al*. 2003). The distribution and proximity of 2011 *Ae. albopictus*-positive properties to the 2001 El Monte "lucky bamboo" importer (see Figure 1) does indicate an association and plausible source of the current populations in El Monte and South El Monte. This supports recently published data by Zhong and others (2013) which showed that *Ae. albopictus* collected in 2001 and 2011 from Los Angeles County are genetically related and similar to those from Asia and distinct from those found in the eastern and southern United States.

Seasonal Distribution of *Ae. albopictus*. The request for service by a resident of El Monte on August 31, 2011 led to the subsequent discovery of *Ae. albopictus* and occurred during peak activity time for adult mosquitoes. After the initial discovery of *Ae. albopictus* on September 2, 2011, a total of 24 properties were found positive during the remainder of the month. Twenty-two additional properties were found positive for *Ae. albopictus* in October 2011. Fifteen more properties in November and finally, one property in December 2011 also were positive for *Ae. albopictus*. In 2012, *Ae. albopictus* was found first on May 9th, and by the end of the month, five positive properties were identified. In the months after May 2012, there was a steady increase of positive properties in the City with the months of highest activity being August through October (Figure 2).

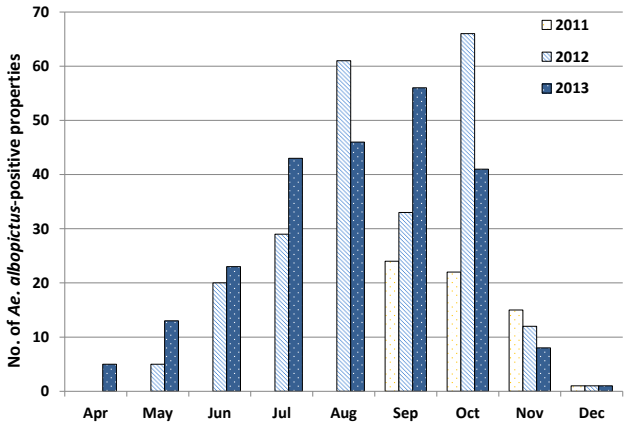

Figure 2. Monthly cumulative number of *Aedes albopictus*-infested properties in San Gabriel Valley, Los Angeles County in 2011 through 2013.

In 2013, the District dedicated staff dubbed the "Albo Crew" comprised of eight seasonal employees for surveillance and control. The "Albo Crew" began surveillance and control of *Ae. albopictus* in April of 2013. The first *Ae. albopictus* of 2013 was found on April 10th with continued increase of *Ae. albopictus*-positive properties that peaked in September 2013 (Figure 2). Over the three-year period of study, each year populations of *Ae. albopictus* declined dramatically in November and were almost non-existent in December.

Based on the temporal distribution of *Ae. albopictus* observed between 2011 and 2013, peak activity occurred from August through October (Figure 2). The request for service by an El Monte resident received August 31, 2011 and the discovery of *Ae. albopictus* on September 2, 2011 by the San Gabriel Valley MVCD technician (Fujioka *et al*. 2012) were not coincidences but seasonal phenomena for this mosquito in Los Angeles County. The requester's mention of bites from the same kind of mosquito for a few years indicates *Ae. albopictus* was present in the neighborhood for a longer period. As reported previously (Barker *et al*. 2013), the introduction and establishment of *Ae. albopictus* may have occurred from a batch of unhatched eggs in a container. However, the spread of adult individuals from property onto similar neighboring properties is dependent on individual adult mosquitoes immigrating into new properties covering a specific distance over short periods of time (C. Barker, personal communication). The adult populations in 2011 may have reached a threshold by becoming a sufficient biting nuisance to illicit the resident's reaction to seek help. This implies that *Ae. albopictus* could have been active in low numbers over the previous 10 years. The population of *Ae. albopictus* may have increased and spread slowly to the point where it successfully adapted to Los Angeles County neighborhoods, perhaps confirming previous predictions of overall ecological conditions in California not being conducive for establishment of this mosquito but once here it may prove difficult to eradicate (Washburn and Hartman 1992, Linthicum *et al*. 2003).

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Suitability of the Arroyo Chub (*Gila orcutti*) for the Biological Control of Mosquitoes in a Southern California Treatment Wetland

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ABSTRACT: The construction of multipurpose constructed treatment wetlands for treating municipal wastewater, and providing much needed habitat for riparian and wetland species, has increased over the last few decades. The production of mosquitoes which can transmit pathogens of humans and companion animals is a potential drawback to utilizing these treatment wetlands. We evaluated the efficacy of the arroyo chub, *Gila orcutti*, as a biological control agent for larval mosquitoes in the Prado Wetlands, Riverside County, CA. The arroyo chub is native to southern California watersheds and has been designated by the California Dept. of Fish and Wildlife as a Threatened Species within its native range. Local vector control districts are in need of an alternative to stocking the invasive western mosquitofish, *Gambusia affinis*, for larval mosquito control in sensitive watersheds. Twelve enclosures were installed in the wetland and three stocking treatments used: control, 0 kg/ha (No fish); low stocking density, 1.5 kg/ha (2 fish); and high stocking density, 6 kg/ha (8 fish). Our results indicate that arroyo chub did not adversely affect the diversity or abundance of macroinvertebrate and microinvertebrate taxa collected in the wetland over the course of the 5-week trial.

INTRODUCTION

The construction and use of multipurpose constructed treatment wetlands has proliferated over the past several decades (Walton 2002, Kadlec and Wallace 2008, Vymazal 2010). As well as water quality improvement, the projected benefits of multipurpose constructed treatment wetlands are numerous and varied; they include amenities for nearby housing developments, crucial wetland habitat for a variety of species, wildlife conservation and recreation (Cole 1998). The production of mosquitoes which can transmit pathogens to humans and companion animals is a potential drawback to utilizing multipurpose constructed treatment wetlands to treat municipal wastewater (Walton et al. 1998, CH2M Hill 1999, Russell 1999, Knight et al. 2003). In the southwestern United States, and particularly southern California, a major cause for concern is the spread of West Nile Virus by mosquitoes near human populations (Reisen et al. 2006). This issue is becoming more pronounced as continued human development encroaches on what was previously isolated wetland habitat, bringing humans in ever increasing contact with mosquitoes (Walton 2002).

Larvivorous fish can be an important component of mosquito abatement strategies in wetlands (Meisch 1985, Kramer et al. 1988, Walton and Mulla 1991, Walton 2007). The use of various fish species for the biological control of mosquito larvae began worldwide in the early 1800s (Walton et al. 2011). The western mosquitofish, *Gambusia affinis* (Baird and Girard), has been introduced widely for mosquito control and has subsequently caused significant impacts to the natural ecology of the river systems where it has been introduced outside its native geographic range (Moyle 2002). Negative effects attributed to *Gambusia* include consumption of non-target fauna (Sokolov and Chvaliova 1936, Washino 1968, Harrington and Harrington 1982) and competition with native fishes (Moyle 1995, 2002).

Moreover, studies in different habitats provide conflicting results as to whether *Gambusia* is truly effective at controlling mosquito larvae (Gratz et al. 1996). Mosquitofish seem to be very effective in habitats such as manmade pools, cattle troughs and areas with poor water quality and low oxygen levels, but their effectiveness at controlling mosquitoes in more natural conditions and habitats is less clear (Pyke 2008). Some studies have shown that in more heavily vegetated areas, *Gambusia* is not effective at maintaining low levels of mosquito production (Harrington and Harrington 1961, Pyke 2008, Walton et al. 2011).

Vector control districts tasked with keeping mosquito populations at low levels that prevent the spread of mosquito-transmitted diseases (OCVCD 2011) are left with few viable alternatives to the use of *G. affinis* for biological control of mosquitoes in ponds, lakes and streams. The arroyo chub, *Gila orcutti* (Eigenmann and Eigenmann) is native to southern California coastal watersheds (Moyle et al. 1995, Veirs and Opler 1998) and has been shown to be a potential alternative to the use of *Gambusia affinis* in habitats connected to the waters of U.S. (Van Dam and Walton 2007). Arroyo chubs typically inhabit pools and runs of headwater creeks and small to medium-sized rivers (Fishbase 2011). This fish have been maintained successfully in rearing ponds (Van Dam and Walton 2007), but its ability to proliferate in riverine wetlands is unknown. Currently, due to population declines and loss of habitat (Moyle et al. 1995, Veirs and Opler 1998), the arroyo chub is listed as a "Species of Special Concern" by the California Department of Fish and Wildlife and qualifies as a "Threatened Species" within its native range.

The objectives of this study were to evaluate: (1) Invertebrate community structure across a range of arroyo chub stocking densites in cage mesocosms, and (2) The suitability of a riverine constructed wetland as a habitat for conservation of the arroyo chub in the lower Santa Ana River.

METHODS AND MATERIALS

Study Site. The experiment was carried out at the Prado Wetlands in Riverside County, California. The 186-ha wetlands are located 7 km northwest of Corona (33.9°N, 117.9°W) and

consist of 47 interconnected marshes/ponds managed by the Orange County Water District (OCWD). The wetland complex receives approximately one-half of the flow (1.7 - 2.3 $m^3 \cdot s^{-1}$) of the Santa Ana River. A 0.9-ha wetland was used for this experiment (Figure 1). A channel approximately 0.5 m deep x 3 m wide x 15 m long was cut into the bottom of the wetland adjacent to the outlet weir using a backhoe to facilitate the collection of fish at the end of the experiment.

Figure 1. An aerial view of the 0.9-ha test wetland in the Prado Wetlands, Corona, CA.

Inflow and outflow drop boxes (inflow: 1.2 m wide; outflow: 0.6 m wide) were located at the east and west sides of the wetland, respectively (Figure 1). Exclusion screens were placed in the drop boxes to prohibit invasive species [e.g., mosquitofish (*Gambusia affinis)*; green sunfish (*Lepomis cyanellus*)] from entering the wetland and to prevent arroyo chub from leaving during the experiment. The exclusion screens were composed of fiberglass window screen (mesh aperture = 1.5 mm) attached to a wooden frame. The fine mesh screening was supported on one side with 1.5 mm gauge metal wire fencing to prevent debris from puncturing holes in the fine mesh and to facilitate removal of debris from the screen. The screens were installed in the inflow and outflow weirs prior to inundation of the wetland.

Initial flooding of the wetland occurred in May 2009. Wetland vegetation (California bulrush, *Schoenoplectus californicus*, and cattail, *Typha latifolia*) was allowed to colonize the wetland naturally. Aquatic macroinvertebrates were also allowed to recolonize the system naturally.

Impact of *G. orcutti* on Invertebrate Community Structure

Cages. Twelve 0.9 m x 0.8 m x 3.7 m cages were installed in the wetland on 6 October 2009 (Figure 1). Lumite screen (mesh aperture = 0.53 mm; BioQuip Corp., Rancho Dominguez, CA) was stapled onto four sides of the wood frame (1 in. x 2 in. pine furring strips, mounted to 2 in. x 2 in. wooden vertical posts). Fiberglass window screen (Model # 3003947; Phifer, Tuscaloosa, AL) was stapled across the bottom to prevent fish from entering the cages at deployment.

A stand of California bulrush 0.3 - 0.6 m in diameter (15 – 25 culms per stand) was placed into each cage to maintain a source of natural wetland vegetation for macroinvertebrate and microinvertebrate colonization and to provide refugia for the fish. One week after placing the live bulrush into the enclosures, bundles of dried bulrush (mean ± SD: 66.65 ± 7.68 g, n = 12) were placed into the cages to provide an oviposition attractant for female mosquitoes.

The experiment was conducted for five weeks until above-normal rains in southern California caused massive flooding on 8 December 2009. Debris associated with the flooding clogged the outflow weir box, causing the water level in the experimental wetland to rise. Cages were either lifted out of the sediment and tipped or completely submerged.

Fish. Arroyo chubs were stocked into the cages on 27 October 2009. Three stocking treatments were used: control, 0 kg/ha (No fish); low stocking density, 1.5 kg/ha (2 fish); and high stocking density, 6 kg/ha (8 fish). A completely randomized experimental design was used, and each treatment was replicated four times. The mean (± SD) wet weight and mean standard length of the 40 *G. orcutti* stocked into the cages were 4.28 ± 1.3 g per fish and 58.8 ± 6.9 mm, respectively. After stocking, the fish were monitored throughout the duration of the experiment using minnow traps lined with window screen (mesh opening = 1.5 mm) and baited with dog food. Despite the impact of the flooding event on the cages, all of the chubs that were stocked into the cages were removed at the end of the experiment and returned to the stock population maintained by the Orange County Water District.

Invertebrates. Immature mosquitoes, macroinvertebrate and microinvertebrate fauna were sampled weekly beginning 2 November until 1 December. Samples were taken using a standard 350-ml dip cup (Bioquip, Rancho Dominguez, CA). Three dips per cage were taken, combined in a concentrator cup (mesh opening = 153 μm) and preserved in 80% ethanol. Dip samples (3 dips per location) were also taken at six locations within the wetland (Figure 1).

Funnel activity traps were used to sample macroinvertebrate and microinvertebrate fauna leaving the benthos. Funnel traps were constructed by inserting and affixing the top one-third of a 2-liter clear plastic soda bottle into a second 2-liter clear soda bottle from which the bottom had been removed. One funnel trap was vertically attached to one corner within each cage with flagging tape, approximately 0.3 m below the surface of the water. The location of the funnel trap within each cage was rotated weekly among the corners of each cage. Funnel traps were deployed for at least 24 h. The organisms collected from each funnel trap were concentrated using a concentrator cup and preserved in 80% ethanol. Funnel traps were also deployed in the wetland at the same locations in which dip samples were collected. Funnel traps were attached to emergent vegetation using flagging tape approximately 0.3 m below the water surface.

Macroinvertebrate and microinvertebrate faunal composition and abundance were determined at 25 - 50X magnification using a stereo dissecting microscope. Macroinvertebrates were identified to at least the family level according to the taxonomic classification of Merritt et al. (2008). Additional aquatic taxa (non-insects) were identified according to the taxonomic classification of Pennak (1989). If a high density of microinvertebrate taxa (cladocerans, ostracods and copepods) was encountered in a sample, collections

were sub-sampled using a fixed-area count. In that case, a 19-cm Petri dish (Fisher Scientific, Pittsburgh, PA) was divided into 16 equally sized units. Four of the sections were randomly chosen, and all microinvertebrates located within the boundaries of those sections were enumerated and taxonomically identified to at least the class or order level. The remaining sections of the Petri dish were then scanned for macroinvertebrate and non-planktonic taxa (e.g., Mollusca), which were counted and taxonomically identified to the family or order level. A list of all taxa identified can be found in Table 1.

Invertebrate group	Taxa Collected in Dip/Funnel Trap Samples	Common/Rare*
Macroinvertebrate	Aeshnidae	Rare
	Amphipod	Common
	Anopheles	Rare
	Callibaetis	Common
	Coenagrionidae	Common
	Ceratopogonidae	Common
	Chironomidae	Common
	Culex	Rare
	Ephydridae	Rare
	Gastrostricha	Common
	Hebridae	Rare
	Hymenoptera	Rare
	Leech	Common
	Libellulidae	Rare
	Mollusca	Common
	Muscidae	Common
	Oligochaeta	Common
	Platyhelminthes	Common
	Ram's Horn snail	Rare
	Veliidae	Rare
Microinvertebrate	Cladocera	Very Common
	Copepoda	Very Common
	Ostracoda	Common

*Rare is < 20 individuals; Common is 20 to < 10,000 individuals; Very Common is >10,000 individuals

Table 1. Macroinvertebrate and microinvertebrate taxa collected in the cage mesocosms in the Prado Wetlands during 2009 and 2010.

Statistical Analyses. Repeated-measures MANOVA (SAS Version 9.2; SAS Institute Inc., Cary, NC) was used to determine if fish stocking density significantly affected invertebrate communities. Arroyo chub stocking density was the between-subject variable, while sampling date and taxon were the within-subject dependent variables. Rare taxa, defined as less than 20 individuals of a given taxon, were removed from the analysis. Abundances of the invertebrate taxa were log-transformed (x + 1) prior to analysis.

The impact of arroyo chub stocking density and other factors affecting invertebrate community structure were analyzed using ordination (CANOCO for Windows 4.5, ter Braak and Šmilauer 2002, Lepš and Šmilauer 2003). A detrended correspondence analysis (DCA) performed on the log-transformed abundance of taxa in the invertebrate community indicated that the lengths of axis 1 and axis 2 of the ordination was <2 standard deviations. Based on this result, linear ordination methods (principal components analysis: PCA) were used to examine the macroinvertebrate and microinvertebrate community structure across arroyo chub stocking densities. The species included in the ordination analyses are listed in Table 1. Rare taxa, which we defined as being less than 5 individuals of a given taxon, were removed from the analysis.

Forward stepwise regression was used to assess the proportion of the variation of the invertebrate community in the PCA ordination explained by arroyo chub stocking density, sampling date and physico-chemical variables. The conditional effect of adding a particular variable to the regression model was tested using a partial Monte Carlo permutation test (499 permutations/test) using CANOCO.

Suitability of a Riverine Constructed Wetland for *G. orcutti*:

Fish. *Gila orcutti* used in the experiment were obtained from a stock population maintained by the Orange County Water District. The parental-stocks were wild-caught fish that had been collected from the Santa Ana River within the city of Riverside, CA (Van Dam and Walton 2007). At the time of the experiment, the fish had been in aquaculture for no more than four generations.

The mean (± SD) wet weight and mean standard length of the 209 *G. orcutti* stocked in early summer 2009 were 4.04 ± 2.00 g per fish (Figure 2A), and 58.59 ± 8.77 mm (Figure 2B), respectively. The exponent for relationship between length and weight of the stocked fish exceeds 3 (Figure 2C), which indicates that the chub were healthy when stocked into the wetland.

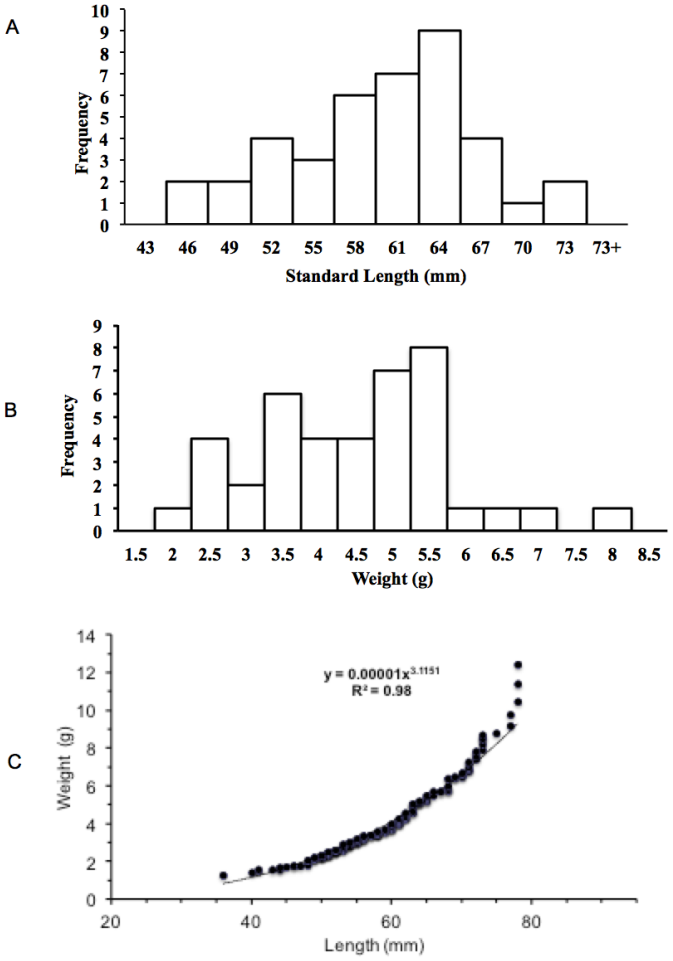

Figure 2. Relative abundance of (A) wet weight, (B) standard length classes and (C) the relationship between wet weight and standard length of arroyo chub (Gila orcutti) stocked into the test wetland on 24 June 2009.

Monitoring of fish populations. After stocking, the fish were monitored throughout the duration of the experiment using minnow traps lined with window screen (mesh opening = 1.5 mm) and baited with dog food. Minnow traps were deployed for 24 h and tied to emergent vegetation. Floats were placed within the traps to maintain a position just below the surface of the water, and visual inspections within the wetland were also carried out to monitor for distressed or dead fish.

The wetland was drained during a one-week period in late August and early September 2010 (16 months after stocking) and was searched for isolated standing water that might have contained fishes. Fish retained within the channel were collected by seine and hand net on 2 September 2010. The individuals collected were identified to species and the wet weight and standard length were determined for all specimens except for mosquitofish. More than 3,100 mosquitofish were collected; the length and weight of a representative sample (n = 39) of the fish collected was measured.

Water quality. Water quality variables were measured bi-weekly in the channel near the cages and adjacent to the outflow weir of the wetland using a potentiometric sensor array (YSI model 6920 sonde; YSI Incorporated, Yellow Springs, OH). Dissolved oxygen concentration (sensor #6562), turbidity (sensor #6136), temperature and specific conductance (sensor #6560) and pH (sensor #6361) were stored on a YSI 650 MDS data logger (YSI Incorporated, Yellow Springs, OH).

Nutrient concentrations in the wetland were measured by taking a 1-liter composite water sample. Three samples were collected near the outflow weir of the wetland and combined. The composite sample was placed on ice in the field and brought back to the laboratory. Ammonium nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N) and phosphate (PO_4) concentrations were determined colorimetrically (Hach DR 5000 spectrophotometer; Hach Company, Loveland CO) using TNT test kits (NH_3 = TNT 830, NO_3 = TNT 835, NO_2 = TNT 839, PO_4 = TNT 844; Hach Company, Loveland, CO).

RESULTS

Impact of *G. orcutti* on Invertebrate Community Structure:

Minnow traps. Arroyo chubs were collected in the minnow traps during the 5-week cage experiment in order to monitor the overall health of the stocked population. However, fewer than five fish were collected across the eight cages containing fish on each sample date, except for the last collection date. The number of fish collected by minnow traps on each sampling date was therefore not representative of the differences in the two stocking treatments. Nevertheless, the initial stocking densities were maintained throughout the experiment; all of the stocked fish were collected from the cages at the end of the experiment. Minnow trap collections indicated that no additional fish species entered the cages and that the arroyo chub did not reproduce during the five-week study.

Dip Samples. Arroyo chub did not affect the abundances of taxa present, even at the highest stocking level of 8 fish per cage (Wilks' Lambda: $F_{8,12}$ = 1.07, P = 0.444). The interaction between arroyo chub stocking density and the taxa collected (stocking density x taxon interaction: $F_{11,22}$ = 1.99, P = 0.08) and between sample date and fish stocking density level ($F_{4,8}$ = 0.73, P = 0.66) were not statistically significant, indicating that the invertebrate community in the three stocking levels of fish did not respond differently across sample dates. However, sample date had a significant effect on the taxa present in the cages ($F_{44, 88}$ = 3.18, P < 0.0054). This finding indicates that variables within the wetland, other than arroyo chub stocking density, were the major determinant of taxon abundance and diversity (Table 2).

Source	df	MS	F	Pr>F
Between Subject Effects				
Stocking Density	2	65020.36	1.21	0.34
Error	9	53547.90		
Within Subject Effects				
Taxon	11	12699996.4	395.94	0.001
Taxon*Stocking Density	22	63214.7	1.99	0.08
Error (Taxon)	99	31823.5		
Week	4	155694.96	1.88	0.14
Week*Stocking Density	8	60537.79	0.73	0.66
Error (Week)	36	82800.16		
Taxon*Week	44	156200.52	3.18	0.0054
Taxon*Week*Stocking Density	88	34516.38	0.70	0.98
Error (Taxon*Week)	396	49125.08		

Table 2. MANOVA results for invertebrate abundance in dip samples

Taxa were spilt into macroinvertebrates (aquatic insects, annelids and mollusks) and microinvertebrates (crustacean zooplankton) to determine if arroyo chub stocking density affected abundances based on prey size. No significant difference in the abundance of either prey category in dip samples was found (macroinvertebrates: Wilks' Lambda: $F_{4,6}$ = 1.42, P = 0.51; microinvertebrates: Wilks' Lambda: $F_{4,6}$ = 4.84, P = 0.24; Figures 3A and 3B) across the three fish stocking densities.

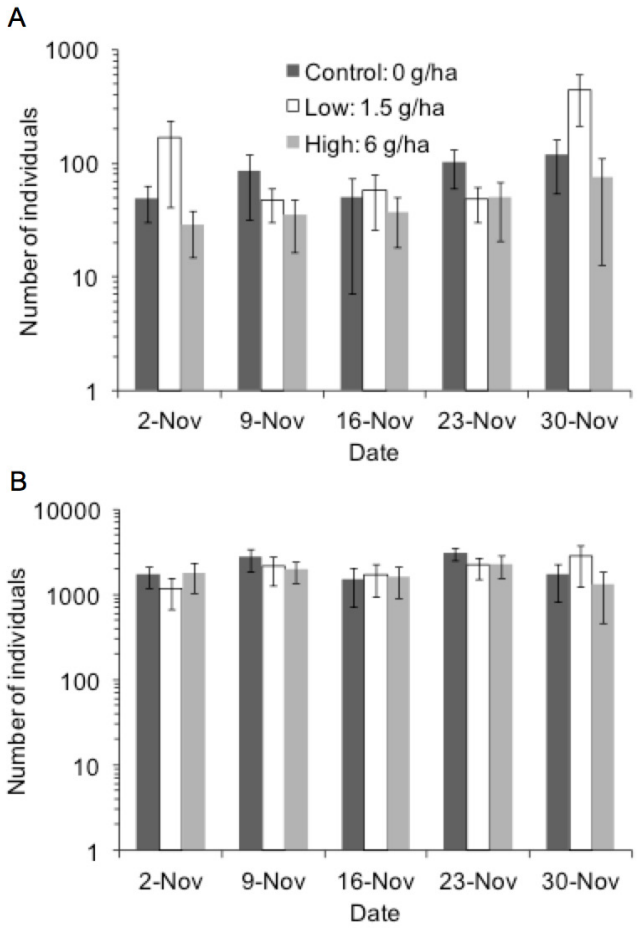

Figure 3. Mean (±SE) abundance of (A) macroinvertebrates and (B) microinvertebrates collected in dip samples from three fish stocking densities.

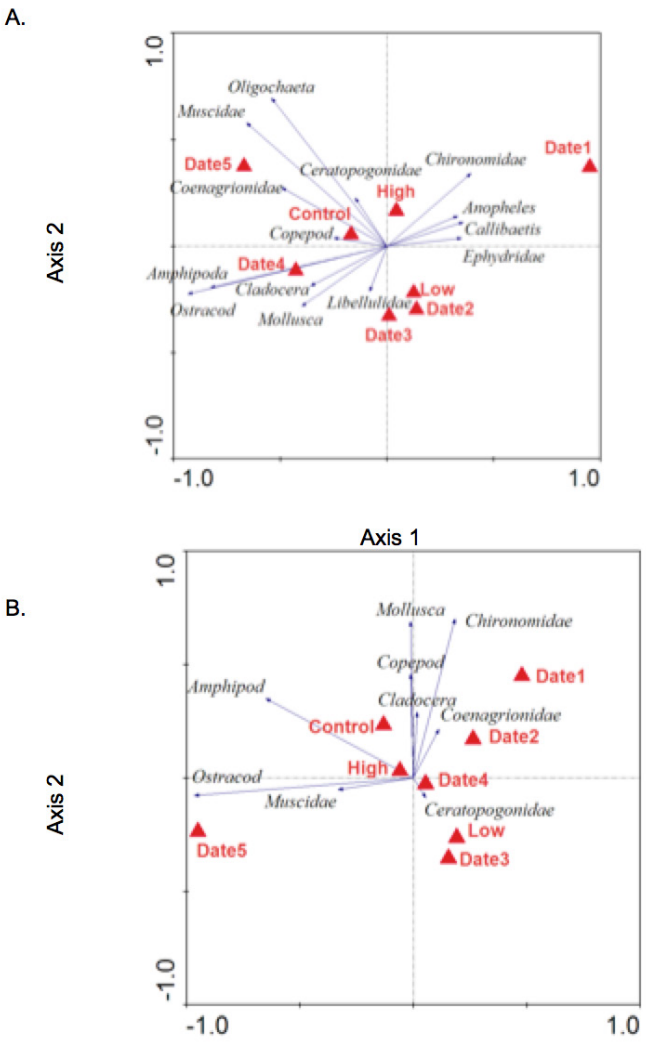

Figure 4. Ordination (PCA) diagrams illustrating the variation in the abundances of invertebrate taxa in (A) dip samples and (B) funnel traps from three arroyo chub stocking densities. The centroids for the arroyo chub treatments (High, Low and Control) and the sampling dates (Date 1 – 5) are indicated by triangles.

The first principal component was associated with changes in taxon abundance across the experiment. Chironomidae, *Callibaetis,* Ephydridae and *Anopheles* decreased in abundance from Date 1 compared to the last week of the experiment, Date 5. The abundance of taxa in dip samples collected on the first date was positively associated with axis 1; dip samples collected on the last date were negatively associated with axis 1 (Date1: $r = 0.649$; Date 5: $r = -0.458$; Figure 4A). Muscidae, Oligochaeta, Amphipoda and Ostracoda showed the greatest increase in abundance during the 5-week experiment, with abundances peaking at the end of the 5-week trial. Mollusca, Cladocera, Coenagrionidae, Ceratopogonidae, Copepoda and Libellulidae increased in abundance to varying degrees over the course of the experiment (Figure 4A).

The second PCA axis was associated with differences in the invertebrate communities on first and last sample dates (Date 1 and Date 5) versus the intermediate sample dates (Date 2 and Date 3) as well as the Low stocking level of arroyo chubs. Date 2, Date 3, and the Low stocking level of chubs were associated with increases of abundances of Mollusca, Cladocera, amphipods, ostracods and Libellulidae.

The first PCA axis accounted for 39.8% of the total variability in the species data, and together with the second axis explained 52.7% of the total variability in species data present in the model. Sample date explained 24% of the variability in the species data (Monte Carlo permutation test: $F_{1,499} = 18.32$, $P < 0.002$), while the fish-stocking level only explained an additional 2% of the variability in the invertebrate community ($F_{1,499} = 1.42$, $P > 0.192$).

Funnel Traps. The abundance of taxa collected in the funnel trap samples over the 5-week trial did not differ among arroyo

chub stocking treatments (Wilks' Lambda: $F_{8,12} = 1.97$, $P > 0.14$). There was no interaction found between the levels of chub stocked into the cages and taxa collected ($F_{5,10} = 0.14$, $P = 0.99$). There was also no interaction between sample date and fish stocking treatment ($F_{4,8} = 0.87$, $P > 0.52$), indicating that the invertebrate communities responded similarly across time to each of the three fish stocking treatments . Sample date had a marginally significant effect on the invertebrate community present in the cages ($F_{20,40} = 3.15$, $P = 0.054$; Table 3). No significant difference among stocking treatments was detected when taxa were grouped into macroinvertebrates or microinvertebrates (macroinvertebrate: Wilks' Lambda: $F_{4,6} = 3.17$, $P = 0.32$; microinvertebrate: Wilks' Lambda: $F_{4,6} = 5.37$, $P = 0.22$; Figs. 5A and 5B).

Source	df	MS	F	Pr>F
Between Subject Effects				
Stocking Density	2	5489448.8	0.17	0.8462
Error	9	3226845.9		
Within Subject Effects				
Taxon	5	11344618.8	3.22	<0.0001
Taxon*Stocking Density	10	4562108	0.14	0.99
Error (Taxon)	45	31667168		
Week	4	11344618.8	3.22	0.05
Week*Stocking Density	8	3061203.8	0.87	0.52
Error (Week)	36	3526305.5		
Taxon*Week	20	11272377.3	3.15	0.054
Taxon*Week* Stocking Density	40	3012815.3	0.84	0.53
Error (Taxon*Week)	180	3574277.0		

Table 3. MANOVA results for invertebrate abundance in funnel trap samples.

The first principal component was associated with changes in taxon abundance from Date 1 to Date 5 (Date 1: $r = 0.649$; Date 5: $r = -0.458$). The second axis was weakly associated with invertebrate taxa present at the start of the experiment and inversely associated with invertebrate community on Date 3 and in the Low fish stocking treatment. Mollusks, chironomids and copepods were abundant at the start of the study, and the abundances of ostracods and amphipods increased towards the end of the experiment (Figure 4B). Higher abundances of Cladocera, Copepoda, Mollusca, Chironomidae and Coenagrionidae were associated with the Control treatment as compared to the low fish stocking density. The first PCA axis accounted for 35.8% of the total variability in the species data, with the second axis explaining an additional 18.2% of the total variability present in the model.

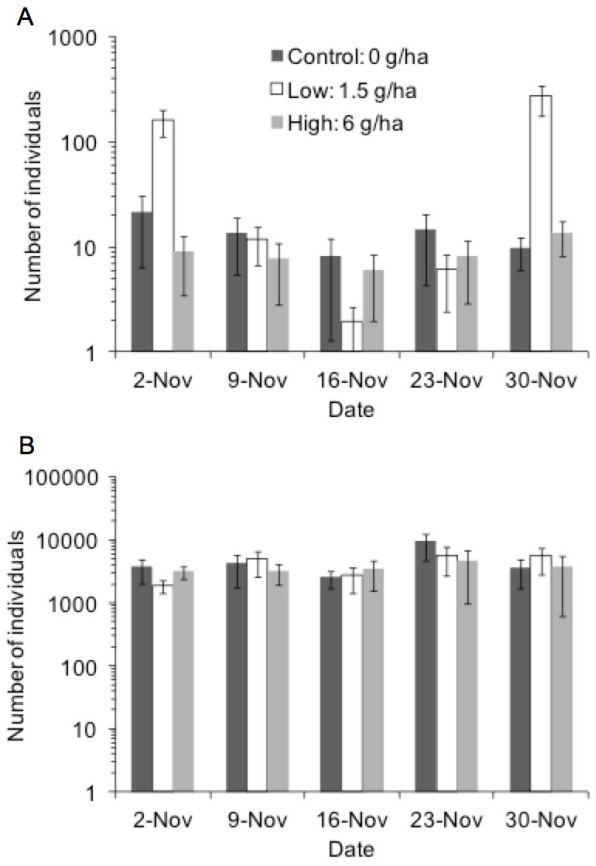

Figure 5. Mean (±SE) abundance of (A) macroinvertebrates and (B) microinvertebrates collected in funnel trap samples from three fish stocking densities.

Sample date explained 19% of the variability in the invertebrate community (Monte Carlo permutation test: $F_{1, 499} = 13.31$; $P < 0.002$), and treatment level only explained an additional 1% of the variability in the data ($F_{1, 499} = 0.88$; $P > 0.506$) in funnel trap collections (Figure 5).

Wetland. Minnow traps that were deployed in the wetland collected a diverse range of taxa, most notable were *G. affinis, L. cyanellus* and *Xenopus*. Arroyo chubs were not collected in the wetland over the course of the 5-week cage experiment. The numbers of invertebrates collected increased from week 2 through week 4 and showed a similar pattern to the collections of taxa from within the cages (see Why 2012). Mollusca and amphipods exhibited the highest overall abundances during the experiment. As was observed in the collections within the cages, cladocerans were collected in much larger numbers (thousands) compared to less than a hundred individuals per taxa of all other groups collected.

Suitability of a Riverine Constructed Wetland for *G. orcutti*:

Fish and other aquatic vertebrates. Approximately 16 months after stocking *G. orcutti* into the wetland, 3,689 fish were collected; however, no arroyo chubs were recovered. All of the fish collected were invasive species in the Santa Ana River system (Table 4). *Gambusia affinis* and *L. cyanellus* were predominant among the collections, making up about 86% and 12%, respectively, of the individuals collected. By wet mass, *C. carpio*, *M. salmoides* and *L. cyanellus* were the dominant species in the fish community.

Species	Total no. collected	Length (mm) mean ± S.D. (minimum, maximum)	Weight (g) mean ± S.D. (min.,max)
Cyprinus carpio	23	360.4 ± 94.9 (105, 480)	4.51 ± 938.94 (35.6, 3175.15)
Ameiurus melas	40	54 ± 9.43 (34, 74)	4.1 ± 2.03 (0.7, 9.2)
Gambusia affinis	3184	23.46 ± 6.10* (7, 35)	0.49 ± 0.56* (0.1, 2.9)
Lepomis cyanellus	432	69 ± 32.48 (21, 160)	18.58 ± 23.92 (0.4, 127.3)
Micropterus salmoides	8	214.63 ± 62.8 (72, 275)	311.61 ± 198.4 (9.0, 680.39)
Menidia beryllina	1	61	1.8
Ameriurus natalis	1	171	90.9
Total Weight of Fish Collected			52,079.69 (114 lbs.)

* $N = 39$

Table 4. Fish collected from the test wetland at the end of the experiment in late summer 2010.

The abundance and total mass of fish present in the wetland at the end of the experiment was most likely underestimated because birds, predominantly ardeids, were observed consuming fish as the wetland was being drawn down prior to seining (A. Why pers. observation). In addition to the fish collected, African clawed frogs [*Xenopus laevis* (Duadin)], American bullfrog tadpoles (*Lithobates catesbeianus* Shaw) and red swamp crayfish [*Procambarus* (*Scapulicambarus*) *clarkia* (Girard)] were collected.

Water quality. Water quality remained consistently high in the wetland throughout the duration of the experiment (Table 5) and therefore should not have affected the overall health of the *G. orcutti* population.

Water quality variable	Mean ± SD ($N = 18$)
Dissolved oxygen [DO]	4.3 ± 1.91 mg L^{-1}*
Specific conductance	1.03 ± 0.11 mS/cm^{-1}
Temperature	19.3 ± 4.45 °C
pH	7.2 ± 0.25
NO_3-N	1.4 ± 2.0 mg L^{-1}
NO_2-N	0.4 ± 0.7 mg L^{-1}
NH_4-N	0.2 ± 0.3 mg L^{-1}
PO_4^{-3}	2.6 ± 1.5 mg L^{-1}

Table 5. Water quality in the study wetland from June through November 2009.

DISCUSSION

Arroyo chub did not adversely affect the diversity or abundance of macroinvertebrate and microinvertebrate taxa collected in enclosures in the wetland during the 5-week experiment. Even at the highest stocking level of 6 kg/ha (8 fish) per cage, arroyo chub had no discernable impact on abundances and composition of animal taxa in lower trophic levels.

Cladoceran abundance in the cages was high (> 100 individuals/liter) and, even if *G. orcutti* was consuming cladocerans, a small change in cladoceran abundance might not have been detectable. Greenfield and Deckert (1973) found that cladocerans comprised a small proportion of the arroyo chub's overall diet, even when cladocerans were dominant in the system. Van Dam and Walton (2007) showed that arroyo chub had no effect on microinvertebrate abundances during two 6-week studies conducted in earthen ponds. The abundance of microinvertebrates in ponds containing arroyo chub was 14 times higher than in ponds containing mosquitofish, *G. affinis* (Van Dam and Walton 2007).

A decrease in abundance of ephydrid larvae in cages containing *G. orcutti* was observed over the course of the experiment. It is possible that consumption of brine fly larvae might have been incidental when the chubs were consuming plant material. Brine fly larvae generally inhabit the littoral areas of lentic habitats but can be benthic algivores and also are associated with vascular hydrophytes (Merritt et al. 2008). Ephydrid pupae were found within the thallus of duckweed which was ubiquitous on the surface of the cages and the wetland. Greenfield and Deckert (1973) showed that 60 - 80% of the stomach contents of adult arroyo chub consisted of algae. They also found that arroyo chubs are opportunistic feeders and the composition of their diet changes seasonally and with availability of insect and other aquatic fauna.

Arroyo chub adults tend to occur low in the water column (A. Why pers. observation), and this may be related to the decline seen in Chironomidae abundance over the course of the experiment. Chironomid larvae are typically benthic in nature, feeding on detritus at the bottom of a lake or stream. Chironomid larvae also feed on a variety of other organic substances (Merritt et al. 2008). It is probable that the chub were more likely to consume chironomid larvae as they remained lower in the water column, as well as incidental consumption as the fish consumed plant material such as algae.

Arroyo chub fry tend to stay at the surface of the water column where they can provide effective control of mosquito larval populations in some aquatic habitats. Henke and Walton (2009) found that immature mosquito abundance in mesocosms containing bulrush (*Schoenoplectus californicus*) and arroyo chubs was lower than in vegetated mesocosms lacking *G. orcutti*; however, the effectiveness of mosquito control provided by *G. orcutti* appeared to differ seasonally (Jennifer Henke pers. comm.). Van Dam and Walton (2007) found that mosquitofish populations grew at a much higher rate than arroyo chub populations, after initially being stocked at equivalent levels, but that greater reproduction of *Gambusia* did not translate into significantly better control of larval mosquito populations when compared with the smaller population of arroyo chubs.

We were not able to assess the effect of predation by immature arroyo chubs on the invertebrate community because reproduction did not occur during the study. The cage experiment in the Prado Wetlands was performed after the peak period of reproduction for *G. orcutti,* which occurs in late spring and early summer (Tres 1992). Adult *G. orcutti* were not caught in the floating minnow traps deployed in the wetland during the 5-week cage experiment; this was most likely due to the fact that the fish tended to remain close to the benthos (A. Why pers, observation). It is therefore unlikely that adult *G. orcutti* would have a strong negative, direct effect on necktonic invertebrates and on invertebrates residing near the water surface. The changes detected in the composition of the invertebrate community can be attributed to the physicochemical changes in the wetland during the 5-week cage study, rather than to *G. orcutti*.

Although one of the initial goals of this experiment was to evaluate whether *G. orcutti* could be an effective biological control agent of mosquitoes in a surface-flow treatment wetland, few mosquito larvae were collected during the five-week trial. The extremely low abundance of mosquito larvae in the system can be attributed to the treatment of the test wetland with Bti, *Bacillus thuringiensis israelensis*, a few weeks prior to the start of the experiment by the local vector control district. Mosquito abundance had increased dramatically and reached unacceptably high levels following vegetation management in which cuttings from the macrophytes remained in the wetland. Only five *Anopheles hermsi* larvae were collected during the experiment.

Even though fine-mesh screens were deployed to inhibit colonization of the test wetland by non-native fishes, non-native fishes were observed in the wetland prior to the start of the cage experiment. Mosquitofish, green sunfish and carp were visually confirmed or caught in minnow traps deployed within the wetland to monitor the chub population. Unforeseen difficulties maintaining water level in the test wetland were caused in part by backflow from the downstream wetland perhaps due to unauthorized manipulation of the boards in the weir boxes. Even though the exclusion screens remained intact, it is unknown whether backflow into the test wetland, movement of juvenile fish through the window screen mesh or some other factor(s) accounted for colonization of the test wetland by non-native competitors and piscivores.

Over 3600 fish were seined from the wetland at the end of the experiment, with 86% of the individuals being mosquitofish and another 12% comprised of green sunfish. While only small numbers of American bullfrog, African clawed frog and crayfish were seined out at the end of the experiment, over 40 bullfrog tadpoles had been seen previously in a single day in the wetland during the course of the experiment (A. Why pers. observation).

The extirpation of the arroyo chubs and the overwhelming abundance of invasive species recovered from the wetland at the end of the experiment raise the obvious issue of how to reintroduce native species to their historical ranges while mitigating for their survival. Although we cannot ascribe the disappearance of *G. orcutti* directly to piscivory or competition with the invasive species present in the test wetland, we feel these factors were likely important. The water quality in the wetland should have been conducive for the survival of *G. orcutti*. A massive die-off or dead individual *G. orcutti* was never observed in visual surveys of the wetland.

The persistence of *G. orcutti* in pond or wetland studies (Van Dam and Walton 2007, Henke and Walton 2009) that lacked invasive fishes, but permitted predation by avian predators such as ardeids, provides evidence that lentic ecosystems can be conducive for survival of arroyo chubs. If invasive fishes have a negative impact on *G. orcutti* in certain types of aquatic ecosystems associated with rivers within their native geographic range, and if vector control districts in southern California anticipate using arroyo chub as an alternative biological control agent to mosquitofish, then they will need to work in concert with agencies such as the California Department of Fish and Wildlife to remove invasive species, especially piscivorous fish, such as largemouth bass and green sunfish, from areas in which they hope to release chub. This will not be easy and periodic monitoring of the system will be needed to prevent both the reintroduction of invasive species and extirpation of the arroyo chub.

Additional studies need to be conducted investigating competition between mosquitofish and arroyo chub to see if *G. orcutii* can survive and reproduce in sufficient numbers within the

same system. *Gambusia affinis* can currently be found throughout almost all of the watersheds in southern California, and the cost of trying to remove them would be astronomically prohibitive (Moyle et al.1995, Walton et al. 2011). Therefore studies need to be conducted to see if chub populations can adequately compete with mosquitofish given that arroyo chub have a much slower reproductive rate and require habitat conducive to egg laying.

Riverine and wetland systems within southern California that lack a high abundance of invasive species appear to provide the best habitat for using arroyo chub as an alternative biological control agent to *G. affinis*. However if measures are undertaken to reduce the abundance of large predatory fish, more habitat would become suitable, not only for the arroyo chub, but for other imperiled native fish species. Arroyo chubs are capable of withstanding seasonal temperature fluctuations and changes in flow rate, which makes them well suited to survive in a managed wetland habitat. Though their effectiveness at controlling larval mosquito populations could not be directly tested in this experiment, results of previous studies indicate that arroyo chub are a viable alternative to the use of mosquitofish for the biological control of mosquitoes in sensitive watersheds. However, additional studies looking at larval mosquito control by arroyo chub in natural systems and their interactions with other native species need to be conducted.

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Sugar Baits Incorporated with Boric Acid and Pyriproxyfen against *Aedes abbopictus* Skuse

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Aedes albopictus Skuse is an important vector of dengue fever and Chikungunya viruses and a frequently encountered domestic/peridomestic pest species. Efforts to control this species with attractive toxic sugar baits (ATSB) and bait stations, or spraying the ATSB on vegetation around residential areas, are based on sugar and plant feeding behavior of the adult mosquitoes (Xue et al. 2013). This project started in 2002 after the discovering that boric acid can kill adult *Ae. albopictus* through sugar feeding (stomach poison) (Xue & Barnard 2003). In the past 12 years, there have been more than 20 publications on this topic, and the ATSB has been documented as a novel and effective control method in the integrated mosquito management program (Xue et al. 2013). There were four phases to our study involving many scientists, technicians and intern students from several organizations. The first phase involved laboratory bioassays for acute toxicity against male and female adult mosquitoes. The application rate of 1% boric acid mixed with 3-5% sugar resulted in 100% mortality at 48 h post treatment. The second phase included a semi-field experiment, conducted in screened buildings with bait stations and boric acid-sprayed plants against released mosquito populations. These treatments resulted in an 80-100% reduction of released population of *Ae. Albopictus*, and the effectiveness lasted for about two weeks (Xue et al 2006, 2011). The third phase was conducted with attractive ATSB material (i.e., fruit juices and other natural plant substances with attractive and killing properties) that was sprayed on vegetation around residential areas. This treatment resulted in an 80-100% reduction in human landing rate counts, numbers of mosquitoes caught by BG sentinel traps and numbers of eggs monitored by ovitraps (Naranjo et al 2013). The fourth phase involved incorporating an insect growth regulator (pyriproxyfen) to the ATSB material in order to control adult and larval *Ae. albopictus* in the laboratory, semi-field and residential areas by ULV spraying. The treatment with the IGR provided effective control of adult and larval mosquitoes by reducing populations by 80 - 100% (Scott et al 2013, Fulcher et al 2014).

Attractive toxic sugar bait (active ingredient 1% boric acid) was evaluated against *Ae. albopictus* populations in the laboratory, semi-field trials and field trials in residential communities in St. Augustine, Florida. Laboratory evaluations of boric acid sugar baits applied to the plant *Penta lanceolata* (Rubiaceae) demonstrated 100% and 92% mortality of *Ae. albopictus* at day 7 and 14 post treatment, respectively. A semi-field study comparing the efficacy of placing the bait on the bottom or top portion of plants resulted in no significant difference in mortality. Overall, combined top and bottom boric acid sugar bait applications resulted in 95% mortality based on leaf bioassays on day 7 post treatment. Field application of the boric acid sugar baits also reduced oviposition.

Three semi-field experiments were conducted in St. Augustine, Florida on the efficacy of NyGuard® Concentrate, an insect growth regulator with 10% pyriproxyfen active ingredient, dispensed via a ultra-low volume (ULV) cold aerosol spray, to evaluate control of *Ae. albopictus*. The experiments measured the effects of both direct and indirect contact of pyriproxyfen on the emergence of 3rd - 4th instar *Ae. albopictus* larvae. Direct contact was analyzed by direct exposure of pyriproxyfen to larvae in reverse osmosis water. Effects of indirect contact of ULV-sprayed pyriproxyfen was analyzed by removing vegetation from the experimental areas and soaking the leaves in reverse osmosis water for 24 hours before adding larvae. Both direct and indirect experiments indicated that pyriproxyfen exhibited emergence inhibition greater than 85% for *Ae. albopictus*. These results suggest that ULV-sprayed 10% pyriproxyfen application would suitably inhibit adult emergence of *Ae. albopictus*. We recommend that further testing be conducted so that this insect growth regulator can be relabeled for large area ULV applications for mosquito control. Also, further test about the IGRs mixed with ATSB sprayed on plants against adult and larval mosquitoes have been conducted.

The effect of spraying a mixture of the insect growth regulator (IGR) pyriproxyfen (1 mg/liter) and 1% boric acid sugar bait or eugenol sugar bait on croton plants (*Codiaeum variegatum* L.) was evaluated against *Ae. albopictus*. Treatments were applied to plants and evaluated against adult and larval *Ae. albopictus* in the laboratory through contact and wash off experiments, respectively. The control treatment lacked any active ingredient and were treated only with an Attractive Sugar Bait (ASB). The plants treated with Attractive Toxic Sugar Baits (ATSB) plus the IGR resulted in 60 - 100% mortality of laboratory-reared adult *Ae. albopictus*. The pyriproxyfen solutions collected from the plant wash experiment resulted in 80-100% emergence inhibition to the exposed 3rd and 4th instar larvae, compared with the untreated control. ATSBs mixed with the IGR not only provided effective control of adult mosquitoes, but also provide additional control of larval mosquitoes after being washed off from the treated plants. This effectiveness lasted for 4 - 5 weeks.

The application of ATSB (active ingredient: eugenol) made to non-flowering vegetation in St. Augustine, Florida (Revay et al 2014) resulted in a more significant reduction of *Aedes albopictus* populations compared to the application of ATSB presented at a bait station. Over 5.5% of the non-targets were stained in the flowering vegetation application site. However, when Attractive

Sugar Bait (ASB) application was made to non-flowering vegetation or presented in bait stations, the impact on non-target insects was very low for all non-target insect orders; only 0.6% of the individual insects were stained with the dye from the sugar solutions. There were no significant differences between the staining of mosquitoes collected in flowering vegetation or non-flowering vegetation sites during the non-target evaluation.

The ATSB techniques for control of adult mosquitoes have been patented in the USA. A couple of new active ingredients such as garlic oil and eugenol have been discovered. These substances are extracted from plants and exempt from EPA registration exemption. The research will benefit from cooperation between development and commercialization companies, and we hope the ATSB products for bait stations, insect spray and barrier spray will be adopted as a part of the integrated mosquito control program in the near future.

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