

# **PROCEEDINGS AND PAPERS**

**of the**

**Sixty-Seventh Annual Conference of the**

**Mosquito and Vector Control Association of California**

**January 24 through January 27, 1999**

Held at the West Coast Anaheim Hotel, Anaheim, California

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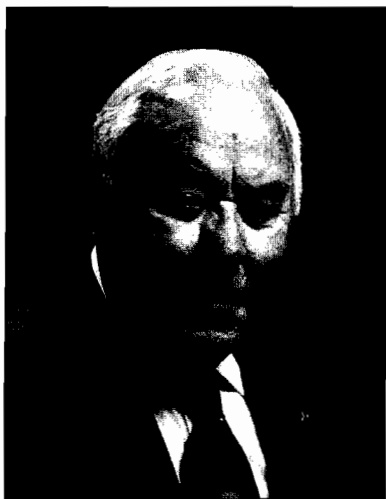
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## Conference Dedication In Memoriam - Howard R. Greenfield

Marvin C. Kramer

*Lake County Vector Control District  
Post Office Box 310, Lakeport, CA 95453*



This 67<sup>th</sup> conference of the Mosquito and Vector Control Association of California (MVCAC) is dedicated to Howard R. Greenfield in recognition of his thirty-five years of service to mosquito and vector control. Howard died of cancer at his home on August 31, 1998.

Howard was born in Santa Cruz on September 19, 1918, a third generation Californian. He was graduated from Palo Alto High School in 1937 and began his preparation for a career as an entomologist at San Jose State University. World War II interrupted further academic pursuits temporarily when he volunteered for duty in the U.S. Navy. His first leave of absence was to have begun on December 7, 1941, but that leave was canceled for obvious reasons. After his tour with the Navy he returned to San Jose State University and earned a B.S. degree in entomology in 1948. In 1949 with his service in the Navy and his degree behind him, he married Elizabeth (Betty) Johns. He worked briefly for the then Bureau of Vector Control, California State Department of Public Health (currently, California Department of Health Services, Vector Borne Disease Section), and as an entomologist for the Merced County M.A.D., and in 1950 he established the Northern Salinas Valley M.A.D., and he managed that with distinction until his retirement in 1982.

Colleagues, county officials, and state and national mosquito and vector control associations recognized his leadership qualities. He was a charter member and past president of the Salinas Golf and Country Club and served for two terms on the Salinas Elementary School Board. He was past president of the MVCAC and the Society for Vector Ecology, and was an honorary member of each. He served on the board of directors of the MVCAC for four terms. He was a member of the Ways and Means Committee for fourteen years and of the Publications Committee for eleven years. Other committee appointments were to Water Resources; Source Reduction; Weed Control; Membership; Budget; Operational Equipment and Procedures; Forms, Records and Statistics; By-Laws; Research; Insurance; Future; International Awareness; Finance and Policy; and Editorial. The grand total of service on committees was sixty-three committee-years, and as chairman for eighteen committee-years. He also served on several committees for the American Mosquito Control Association.

Howard made important contributions in the areas of source reduction and in machinations of government, whether internally with the district or state associations or externally in relations with county, regional, state, or federal agencies.

Rarely do you know a person of principle who is also warm and personable. Such a person was Howard Greenfield. Howard gave of himself completely and willingly, was self effacing, and no matter the degree of stress, he maintained a countenance of friendship and cheerfulness that was refreshing, whether working at the M.A.D. with state or national associations, or traveling to places like China.

You knew that his counsel was sincere and valuable. He was a true friend and a pleasure to know. Everyone with whom he came into contact has suffered a great loss at his passing. He will be missed.

*- thoughtfully submitted by Marvin C. Kramer,  
Retired, U. S. Public Health Service; California  
Department of Health Services, Bureau of Vector  
Control; MVCAC Honorary Member, Past Executive  
Director of MVCAC and currently a trustee at the  
Lake County Vector Control District.*

## Dedication Speech for Howard R. Greenfield

Earl W. Mortenson

*Contra Costa Mosquito and Vector Control District  
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Mr. President and members of the conference, I have the honor and privilege to participate in the dedication of this 67<sup>th</sup> conference of the MVCAC in memory of Howard Greenfield and in recognition of his 35 years of service to Mosquito and Vector Control.

Howard Greenfield passed away on August 31, 1998, after a yearlong struggle with cancer. I first had the opportunity to meet Howard in 1949 when he was an entomologist for the Merced Mosquito Abatement District. Deed Thurman who was a USPHS Entomologist assigned to the Bureau of Vector and the first project manager of the CMCA operational mosquito research program and I had been working on collecting soil samples for *Aedes* eggs from irrigated pastures. Howard was helping us to obtain soil samples from pastures in Merced County that were heavily infested with *Aedes nigromaculus*.

Bob Schoeppner and I, as members of the Archives Committee were fortunate to be involved in recording the oral history of Howard at his home in Salinas on June 6, 1996. I would like to share with you some of the interesting highlights of Howard's life.

Howard was born in 1918 in Santa Cruz, California, a third generation Californian. He spent a portion of his youth in Arizona where his father and grandparents operated a gold mine near the town of Prescott. It was in this kind of setting that he started to develop a curiosity and interest in insects and other animals that were a part of this high desert environment. He later moved back to California and lived in Los Altos.

Howard attended San Jose State College for two years before joining the US Navy in 1941. Most of his Navy experience was in the South Pacific, where he participated in the Leyte, Guadalcanal and other campaigns. His unit was awarded five battle stars. After his five year navy service he returned to San Jose State College to complete his B.S. degree.

Howard's interest in biology was soon channeled into courses in entomology. Dr. Carl Duncan, professor of Entomology and Dr. Ed Ross of the California Academy of Science served as his mentors. In his senior year Howard was awarded the Outstanding Science Student of the year.

His first professional position was with the Bureau of Vector Control assigned to work with Dr. Basil Markos and Harvey Magy to evaluate the effectiveness of DDT as pretreatment application on irrigated pastures in the Los Banos area of Merced County. It was during this fieldwork in Merced County that he met his wife-to-be, Betty, who was a nurse at the Los Banos Physician's Clinic. Ed Smith, Manager of the Merced Mosquito Abatement District hired Howard in 1948 as the District Entomologist.

In 1951 Howard was appointed the Manager-Entomologist of the newly formed North Salinas Valley Mosquito Abatement District. The legendary Harold Gray, Manager-Engineer of the Alameda Mosquito Abatement District was another mentor to Howard, providing consultation in designing the source reduction program that was required for the new district to help solve the drainage problems inherent in the northern part of Monterey County.

Howard was very active and made major contributions to the CMVCA (MVCAC), serving as President in 1957 and was on key committees. He was one of the original members of the 1948 Entomology Committee. He was elected Honorary Member in 1983.

Howard was a charter and honorary member of the Society for Vector Ecology and served as the third President of the Society in 1971. He received the AMCA Meritorious Service Award in 1973. Howard retired as manager of the North Salinas Mosquito Abatement district in 1982. He was active in his community, served as a school board member, past president of the Salinas country club (a very good golfer) and a member of many other community organizations.

For me, it was a rewarding experience to work with Howard. He maintained a countenance of friendship and cheerfulness that was refreshing. His counsel was sincere and valuable. Friends and colleagues will miss him.

*- thoughtfully submitted by Earl Mortenson, Assistant Chief, Retired, California Department of Health Services, Vector Surveillance and Control; MVCAC Honorary Member and currently a trustee at the Contra Costa Mosquito and Vector Control District.*

## In Memoriam - Robert H. Peters

Jack V. Fiori

*San Joaquin County Mosquito and Vector Control District  
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Robert "Bob" Henry Peters, of Woodbridge, died on November 13, 1998 in Pleasanton, California. He was born on December 19, 1915, in Oakland, California and lived in the Lodi area for 49 years.

Mr. Peters was employed as the manager of the Northern San Joaquin Mosquito Abatement District for 27 years, retiring in 1976. He was known for developing the foremost mosquito abatement program in the state.

Bob Mullens, of Lodi, said Mr. Peters is responsible for controlling the severe mosquito problem the county experienced.

"The mosquitoes were thick before Bob Peters came to the San Joaquin County and started his method of abatement," Mullens said. "He did a fantastic job of getting rid of the problem."

Bob Peters attended Oakland High School, Polytechnic Junior College and the University of California at Berkeley.

Bob served in the U.S. Marine Corps as a member of the Flying Golden Bear Squadron and later become a flight instructor during World War II.

He married Patricia Jane Harbinson on August 7, 1943 and after being stationed in Minneapolis, the couple set up house in Marysville while he served as Environmental Director for the Yuba/Sutter County Health Department.

The couple moved to Lodi in 1949 and Mr. Peters became involved in the community, serving as president of the Lions Club, the Chamber of Commerce, the Planning Commission, Lodi Art Club, Squares, Fortnightly Whist Club and volunteered his artistic ability to Lodi High School during the 1960's.

He had a passion for monitoring the Mokelumne River and East Bay Municipal Utility District, writing as many as 50 letters to the editor over a period of years.

He enjoyed traveling, playing bridge, spending time with this family and taking care of his home and acreage.

*The following comments are from Jack V. Fiori, assistant manager to Robert H. Peters from 1965 to his retirement in 1976:*

"Bob's approach to controlling mosquitoes was quite different than most managers in mosquito control districts in California.

His theory was if you get rid of the water that breeds mosquitoes, you don't have to apply pesticides. Land that breeds mosquitoes does not provide food for animals.

Bob's greatest accomplishment as the manager of the Northern San Joaquin County Mosquito Abatement District was the reclamation of land along the Mokelumne River. A distance of approximately 30 miles long the river ran through the center of the city of Lodi, thus causing major mosquito problems. The river bottom was a vast jungle that would flood in the spring when the snow melts. *Aedes vexans* and *Culex* mosquitoes were quite prevalent.

The district used its own equipment, a ditcher, scrapers, 2 - DT tractors, and 2 - draglines to clear and reclaim this land for farming. Today this stretch of river is virtually free of mosquito breeding."

*- thoughtfully submitted by Jack Fiori, Manager, Retired, San Joaquin County Mosquito Abatement District, MVCAC Past President, MVCAC Honorary Member and currently a trustee at the San Joaquin County Mosquito and Vector Control District.*



## Plague Activity in San Bernardino County During 1998

Lal S. Mian<sup>1/</sup>, Christian N. Nwadike, James C. Hitchcock<sup>2/</sup>, and Charles M. Myers<sup>2/</sup>

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**ABSTRACT:** In 1998, the San Bernardino County Vector Control Program carried out 23 plague surveys at various sites in San Bernardino County. Of the 227 collected animals, 96.0% were ground squirrels, 205 *Spermophilus beecheyi* and 13 *Spermophilus lateralis*. The remaining animals included 9 *Tamias merriami*. None of the 546 fleas collected from these animals tested positive for plague. Of the total sera drawn from 227 rodents, 7 tested positive for plague antibody from three different locations namely, Heart Bar campground, East Flat campground, and Serrano campground. Upon receipt of laboratory results on plague positive samples, the East Flat and Serrano campgrounds were closed to the public to implement ectoparasite control measures. Public educational information on plague was disseminated through the local press media

### INTRODUCTION

Plague is primarily a rodent disease caused by the bacterium, *Yersinia pestis*. It is transmitted to humans and animals through the bite of infected fleas. Human exposure to the disease can occur in plague infected rural areas, or in extension of human developments into previously wilderness areas.

Historically, plague is reported to have spread from Central Asia from to almost all continents of the world. It is evident from the three reported pandemics affecting Asia, Africa, Europe, South America, North America and Australia from 542 to 1900 (Twiggy 1978, Kettle, 1995). Since its introduction into North America, there have been four major urban epidemics in California: 1900-1903 and 1907-1909 in San Francisco; 1919 in Oakland; and 1924 in Los Angeles. Since that time, sporadic human cases in endemic areas have been traced to wild rodents and their fleas. Plague infection in wild rodents is widely distributed in many areas of southern California including the Tehachapi, San Gabriel, San Bernardino and San Jacinto Mountains. (Salmon and Gorenzel 1981, Anonymous 1983).

Enzootic plague foci are distributed throughout the mountains and foothill areas of San Bernardino County. During a 10 year-period (1988-97), plague activity was detected for eight years with a mean seropositivity rate of 5.3% (Mian and Hitchcock 1998). To safeguard public health and safety in these areas, the San Bernardino County Vector Control Program (SBCVCP) in collaboration with the California Department of Health Services-

Vector-Borne Disease Section (CDHS-VBDS) and the United States Department of Agriculture-Forest Service (USDA-FS), carries out routine surveillance during the season (April through November). The data generated in routine plague surveillance during 1998 are presented here.

### MATERIALS AND METHODS

In routine plague surveys, the general method described by Lang and Wills (1991) was used with some modifications as reported by Mian and Hitchcock (1998). Standard epizootic protocol and ectoparasite suppression measures were used according to Mian (1995).

### RESULTS AND DISCUSSION

During the 1998 season, the SBCVCP carried out routine plague surveys at various locations situated in plague enzootic mountain and foothill areas of San Bernardino County (Table 1). Of the total 227 rodents collected in live traps, 218 (96.0%) were ground squirrels, namely 205 (93M, 112F) California ground squirrels, *Spermophilus beecheyi*, and 13 (1M, 12F) golden mantled ground squirrels, *Spermophilus lateralis*. The remaining animals were 9 (5M, 4F) chipmunks, *Tamias merriami*.

All 546 fleas, 88.5% *Diamanus montanus* and 11.5% *Oropsyllus idahoensis*, combed from the animals tested negative for the plague bacterium.

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TABLE 1. Summary of plague surveys carried out in San Bernardino county during 1998.

Survey Date	Location (Altitude in feet)	Number of Sera		Number of Ectoparasites Tested	Flea Index
		Tested (M/F)	Tested Positive		
5/27	Dogwood Campground CG (5,800)	7 (4/3)	- <sup>d</sup>	4	0.60
6/24	Apple White CG <sup>d</sup> (3,300)	12 (1/11)	0	34	2.83
6/25	Yucaipa Regional Park (2,700)	15 (9/6)	0	4	0.27
7/22	Oak Glen (4,640)	2 (1/1)	0	2	1.00
7/22	Jurupa Regional Park (1,200)	10 (5/5)	0	14	1.40
8/3	Crab Flat CG (6,200)	14 (3/11)	0	25	1.78
8/4	Heart Bar CG (6,900)	26 (10/16)	3	26	1.00
8/12	Rancho Verde Golf Course (1,470)	9 (7/2)	-	6	0.70
8/17	East Flat CG (7,000)	10 (4/6)	2	21	2.10
8/18	Forest Falls CG (6,000)	16 (9/7)	0	12	0.75
8/24	Northshore CG (5,300)	13 (6/7)	0	15	1.15
8/31	Holcomb Valley CG (7,190)	5 (1/4)	0	2	0.40
9/2	Big Pine Flat Eq. C. <sup>b</sup> (6,490)	2 (0/2)	0	1	0.50
9/3	Devils Canyon Levee Road (1,630)	5 (3/2)	0	43	8.60
9/15	Hanna Flat CG (7,000)	21 (12/9)	0	52	2.40
9/15	Serrano CG (6,800)	16 (5/11)	2	23	1.44
9/16	Shady Cove CG (6,800)	4 (2/2)	-	2	0.50
9/21	San Antonio Park (1,200)	5 (2/3)	-	81	16.2
9/22	East Flat CG (7,000)	3 (3/0)	-	14	4.60
9/28	San Antonio Park (1,200)	4 (2/2)	-	11	2.75
9/29	Wildwood Canyon Park (3,300)	5 (5/0)	-	22	4.4
9/30	Littlefield-Shultis Community Park (1,100)	18 (6/12)	-	124	6.90
10/13	Meadow Edge CG (6,800)	5 (2/3)	-	30	6.00
<b>Total</b>		<b>227 (102/125)</b>	<b>7</b>	<b>546</b>	

<sup>d</sup> CG-Campground<sup>b</sup> Eq. C.-Equestrian Camp<sup>c</sup> Not tested—post-treatment survey on non-forest service site

Laboratory results on rodent sera revealed 7 seropositive samples, showing plague epizootics at three different sites, Heart Bar campground, East Flat campground, and Serrano campground (Table 1). At the Heart Bar campground, three ground squirrels, two *S. beecheyi* and one *S. lateralis* tested positive for the plague antibody (> 1: 16 titer). Due to the low flea index, the campground was not closed for flea suppression. This campground had shown plague activity the previous season and was then treated for flea control. At the East Flat campground there were two *S. beecheyi* seropositive with a flea index of 2.1. This site was closed and burrows were treated with 2% diazinon dust for flea control. Plague activity at the Serrano campground was detected late in the season when two *S. beecheyi* were found seropositive. As a result the campground was closed for public use about a week earlier than the normal closure time. Due to the onset of cold weather and low rodent activity, the campground remained closed until flea control next spring.

During the foregoing epizootics, public educational information was provided in a timely fashion through local newspapers. Plague warning signs were posted immediately upon receiving laboratory results and they were allowed to remain posted for the remainder of the season in affected areas.

Earlier in the season, Dogwood campground which was closed down due to plague activity the previous year, was treated for fleas prior to opening to the public.

Also, all private and National Forest Service campgrounds were mailed "Plague Caution" signs to be posted at various public access areas at these sites.

#### *Acknowledgements*

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## The Africanized Honey Bee Program in San Bernardino County

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**ABSTRACT:** Anticipating the arrival of the Africanized honey bees (AHBs), the San Bernardino County Vector Control Program developed the AHB program which became operational in 1997. The Program was divided into three phases namely, pre-arrival, arrival, and post-arrival. The pre-arrival phase (1993-97) focused on training, public education, revenue generation, response protocol, surveillance methods—both field and laboratory, procurement of equipment and supplies, and agency interaction involving county task force formation and AHB action plan. The arrival phase included surveillance and response—both emergency and non-emergency swarm and non-structural colony control, increased public education and continued personnel training and agency interaction. In the post-arrival phase, the emphasis was placed on a timely response, public education, data generation, and evaluation and interaction with other agencies. During the post-arrival phase, the program responded to three multiple stinging incidents. It also controlled 55 swarms/non-structural colonies in various service areas. Of these 42 (76%) were AHBs. The breakdown of these swarms/colonies by habitat was 42% on/in trees, 22% structure (outside), 9% utility boxes, 7% old bee boxes, and 20% other sources. During the three phases (1993-98), the Program spent \$18,800.00 on equipment and supplies and 1,938 man-hours (= \$100,776) in services.

### INTRODUCTION

The Africanized honey bees (AHBs), *Apis mellifera scutellata*, a cross between the African and European honey bees were developed in the mid-1950s in Brazil in an effort to improve the local bee industry. Unlike their European cousins, AHBs appeared to show more defensive behavior resulting in multiple stings and even fatal attacks on humans and animals, when provoked.

During the developmental stages in Brazil, some AHB colonies were distributed to bee keepers while others inadvertently escaped and started breeding with European honey bees. Since their escape in 1957, AHBs have been moving northwards at the rate of 100-300 miles per year (Bram et al. 1990). They arrived into the United States via Texas in 1990, spreading to Arizona and New Mexico in 1993. The bees were first reported in CA (Riverside County) in October, 1994 (Visscher et al. 1997). Their migration into Imperial and San Diego counties was confirmed in 1995 and 1997, respectively.

During the spring of 1998, AHBs were confirmed in San Bernardino County, and by December 3, 1998, the entire county was declared as colonized.

During their northward migration, AHBs caused many deaths of humans and animals in South and Central Americas. In the U.S., there were two AHB-related deaths in 1993 and 1994 in Texas and two in 1995 in Arizona. Since then there have been incidents of multiple stings of humans and animals. Since their arrival in southern California in 1994, AHBs caused multiple stings of about two dozen people, including eight victims in San Bernardino County.

Due to the anticipated arrival of AHBs and their impact on both human and animal lives in San Bernardino, the San Bernardino County Vector Control Program (SBCVCP) developed the AHB Program aimed at protecting the health and well-being of the county residents. This paper highlights the development, operation and cost of the Program over six years, 1993-98.

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## PROGRAM DESCRIPTION

The Africanized Honey Bee Program was developed with the following objectives:

- To respond to both emergency and non-emergency AHB-related calls.
- To provide training to first responders—fire, paramedics, and police and sheriff personnel.
- To educate the general public about AHBs and their behavior and how to avoid them.
- To coordinate efforts with other local, regional, and state agencies and exchange AHB-related information.

The Program was divided into three phases as follows:

### 1. Pre-Arrival Phase (1993-97):

#### 1993-96:

- County Task Force: SBCVCP played a major role in forming a task force with the San Bernardino County Agricultural Commissioner's Office. Under the auspices of the County Task Force an AHB Action Plan was prepared and got approved by the County Board of Supervisors in 1994.
- Training of First Responders: SBCVCP provided needed training to fire, paramedics, and police and/or sheriff department personnel, responding to AHB-related emergencies.
- Public Education: In order to provide some basic information on AHBs, SBCVCP utilized the news media to disseminate AHB information to the general public.
- Interaction with Other Agencies: SBCVCP kept close contact and exchanged AHB-related information with local member cities, adjoining counties, the Mosquito and Vector Control Association of California-Adhoc AHB Committee, and the statewide AHB-Steering Committee.

#### 1996:

- Funding and Response: In order to provide AHB-related services to the County residents, SBCVCP sought the County Board of Supervisors approval to revise the benefit assessment rates. The revision was approved via a Resolution by the County Board of Supervisors.

#### 1997:

- Surveillance: AHB surveillance was carried by placing surveillance traps along the frontal area, mostly along the southeastern boundaries of the County. The traps were monitored every other

week and any bee samples collected were identified under a binocular microscope, using the fast Africanized bee identification system (FABIS) in the laboratory. The AHB surveillance trap was made of a 5-gallon white plastic bucket lined and criss-crossed on the inside with a corrugated hard-board and charged with the queen bee pheromone (attractant) and a strip of natural bee wax.

- Personnel Training: Personnel handling bees were given periodic training on controlling bee swarms or established colonies with emphasis on safety precautions and the use of safety gear. Demonstrations on spray equipment using the AHB special local need pesticide, M-Pede, were included in the training.
- Equipment and Supplies: All needed equipment such as sprayers and AHB pesticides were purchased. Materials for making remnant bee traps including bee pheromone were also procured.
- Public Education: AHB information was periodically disseminated through the news media and AHB brochures were distributed to the general public.

### 2. Arrival Phase:

#### 1998:

- Surveillance: AHB surveillance was continued along with FABIS on bee samples collected in surveillance traps.
- AHB Response Directory: In consultation with the County taskforce an AHB Call Response Directory was prepared.
- AHB Response Plan:
  - a. Emergency: Upon completion of an emergency bee suppression operation by the first responders, remnant bee traps were used for the returning foraging bees.
  - b. Non-Emergency: SBCVCP responded to bee calls in AHB-colonized areas to suppress AHB swarms and non-structure established colonies.
  - c. SBCVCP provided names of AHB-certified pest control operators to control bee colonies inside structures on private properties.
  - d. FABIS was done on all bee samples taken after the swarm/colony was destroyed.
  - e. SBCVCP distributed free general information on AHB to the general public.
- Public Education: Enhanced public education was carried out using the local news media.

TABLE 1. Number of bee service requests (SRs) and bee samples identified in San Bernardino County during 1998.

Month	Feral bee samples identified			
	No. SRs	AHB	EHB	Total
January	2	0	0	0
February	1	0	0	0
March	3	0	0	0
April	10	0	4	4
May	30	0	3	3
June	51	2	0	2
July	23	3	0	3
August	23	2	0	2
September	40	1	1	2
October	42	4	1	5
November	66	10	0	10
December	60	20	4	24
<b>Total</b>	<b>351</b>	<b>42</b>	<b>13</b>	<b>55</b>
<b>%</b>		<b>76</b>	<b>24</b>	<b>100</b>

TABLE 2. Distribution of bees by region and habitat type in San Bernardino County during 1998.

Habitat	Number of bee samples identified								
	Desert			Valley			Total		
	AHBa/	EHBb/	Total	AHB	EHB	Total	AHB	EHB	Total
Tree	13	6	19	3	1	4	16	7	23
Structure	4	2	6	5	1	6	9	3	12
Utility Box	5	0	5	0	0	0	5	0	5
Old Bee Box	2	0	2	2	0	2	4	0	4
Fence Post	0	1	1	0	0	0	0	1	1
Lumber Pile	0	0	0	1	0	1	1	0	1
Old Car Fender	1	0	1	0	0	0	1	0	1
Old Drum	1	0	1	0	0	0	1	0	1
Old Piano Crate	0	0	0	1	0	1	1	0	1
Old Refrigerator	0	1	1	0	0	0	0	1	1
Rail Road Ties	0	0	0	1	0	1	1	0	1
Sandy Cliff	1	0	1	0	0	0	1	0	1
Telephone Pole	0	1	1	0	0	0	0	1	1
Truck Door	1	0	1	0	0	0	1	0	1
Waste Tires	1	0	1	0	0	0	1	0	1
<b>Total</b>	<b>29</b>	<b>11</b>	<b>40</b>	<b>13</b>	<b>2</b>	<b>15</b>	<b>42</b>	<b>13</b>	<b>55</b>
<b>%</b>	<b>73</b>	<b>27</b>	<b>100</b>	<b>87</b>	<b>13</b>	<b>100</b>	<b>76</b>	<b>24</b>	<b>100</b>

TABLE 3. Cost of equipment, supplies, and man-hours spent in developing the AHB Program by phase in 1993-98.

**a. Pre-Arrival Phase:**

<u>1993-96:</u>		
• County Task Force meetings .....		62 hours
• Training of first responders .....		109 hours
(Fire, Paramedics, Police/Sheriff)		
• Public Education		
• Newspaper interviews/articles (6) .....		5 hours
• Radio talk (1) .....		1 hour
• TV appearances (4) .....		8 hours
<u>1997:</u>		
• Surveillance		
• Trap monitoring .....		96 hours
• Laboratory .....		12 hours
• Equipment (Microscope micrometer) .....	\$200.00	
• Supplies		
• Material for surveillance traps .....	\$100.00	8 hours
• Material for remnant bee traps .....	\$50.00	2 hours
• Bee suits/glove/veil, etc. ....	\$3,000.00	1 hour
• Queen bee pheromone/Bee wax .....	\$150.00	1 hour
• Pesticides (M-Pede) .....	\$1,800.00	
• Pesticide sprayers		
• Vehicle mounted (3) plus trailer .....	\$12,000.00	
• Back-packs (5) .....	\$1,500.00	
• Staff training .....		16 hours
• Interagency Meetings(Task Force/Steering Committee/Ad hoc AHB Committee) .....		28 hours
• <u>Subtotal</u> .....	<u>\$18,800.00</u>	<u>349 hours</u>

**b. Arrival Phase:**

<u>1998:</u>		
• Surveillance		
• Trap monitoring .....		480 hours
• Lab testing .....		100 hours
• Response		
• Swarm/colony suppression .....		176 hours
• Remnant bee trap use .....		60 hours
• Training .....		16 hours
• Public Education		
• Newspaper interviews/articles (11) .....		11 hours
• Radio talk (1) .....		1 hour
• TV appearance s(2) .....		4 hours
• Interagency Meetings-Task Force/Steering/Ad hoc Committee .....		20 hours
• <u>Subtotal</u> .....		<u>868 hours</u>

**c. Post-Arrival Phase:**

<u>1998:</u>		
• Response		
• Swarm/colony suppression .....		544 hours
• Remnant bee traps .....		129 hours
Public Education (continued)		
• Newspaper articles (6) .....		4 hours
• Television appearances (2) .....		4 hours
• Interagency collaboration (continued) .....		20 hours
• Data compilation .....		20 hours
• <u>Subtotal</u> .....		<u>721 hours</u>

**Total (a+b+c)****\$18,800.00****1,938 hours**

### 3. Post-Arrival Phase:

#### 1998:

- Effective December 3, 1998, San Bernardino County was declared completely colonized by the AHB. That resulted in the continuation of a countywide full AHB Response Plan and Public Education.
- Collection of AHB data—distribution, nesting sites, and behavioral traits for future planning and statistical purposes, was continued.
- Interagency coordination and exchange of information at the local, regional, and state level were duly maintained.

## RESULTS AND DISCUSSION

The program developed over several years has been a success fulfilling its objectives. During the arrival and post-arrival phases the program staff handled 351 bee service requests including response to three emergency situations—multiple stinging (>15) at Newberry Springs on July 28 (1 person), Big River on October 22 (6 people), and San Bernardino on December 15, 1998 (1 person). In non-emergency yet potentially dangerous situations, the program controlled a total of 55 swarms or established colonies, 42 (76%) of which were AHBs (Table 1). Seasonally, 62% of the swarms/colonies were reported in November and December. Of the total swarms/colonies controlled in 1998, 40 (73%) were from the desert region and 42 (76%) of the desert and valley regions were AHBs (Table 2). The distribution of bees by habitat was 42% in/on trees, 22% in/on structures, 9% in utility boxes, 7% in old bee boxes, and 20% in miscellaneous habitats, i.e., fence post, lumber pile, old car fender, old drum, old piano crate, discarded refrigerator, railroad ties, sandy cliff, telephone pole, truck door, and waste tires.

In AHB training, the program provided 33 training sessions to approximately 710 first responders—fire, paramedic, and police/sheriff department personnel, and eight presentations to 110 employees of public works departments of various member cities.

In public education, the program gave 23 informational articles to newspapers, two radio talks, and eight television presentations on AHB, and distributed AHB brochures during all the phases. Last but not least, the program coordinated well with all local, regional, and state agencies in policy development and exchange of AHB-related information.

An analysis of the program cost (Table 3) shows that the biggest item was the staff time (hours), followed by equipment and supplies. During the pre-arrival phase, the time spent on AHB-related activities accounted for 18%, or \$18,148.00 (=349 hours x \$52.00/hour—current county rate). The time spent in the arrival phase was 44.8%, or \$45,136.00 (=868 hours) and post-arrival phase 37.2%, or \$37,492.00 (=721 hours). The expenditure on equipment and supplies was mostly incurred during the pre-arrival phase. The total program cost at the end of 1998 amounted to \$119,576.00.

With 416,988 assessed land parcels (both developed and undeveloped) in the SBCVCP service territory, the cost per parcel was \$0.28. Similarly, with a population of 1,057,055 in the territory, the cost per capita was \$0.11.

For the coming season, we anticipate an increase in staff time for the AHB response plan. The expenditure on equipment and supplies will be limited mostly to pesticides and equipment maintenance. It is hoped that with continued public awareness and education about AHBs over the next couple of years, the need for AHB-related services will slow down.

### *Acknowledgements*

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during the season. These samples were then mailed to the VBDR for detection of arboviral activity.

## RESULTS AND DISCUSSION

Of the total 14,259 mosquitoes collected at all sites during the season, 53.4% were trapped in the desert area, 3.5% in the mountain region, and 43.1% from the valley sites (Table 1). The most abundant mosquito in the desert region was *Culex tarsalis* Coquillet (59.7%), followed by *Culiseta inornata* Williston (31.2%), *Culex stigmatosoma* Dyar (3.3%), *Culex erythrothorax* Dyar (2.9%), *Culex quinquefasciatus* Say (1.6%), *Anopheles franciscanus* McCracken (<0.8%) and *Aedes vexans* Meigen (0.4%). In the mountain region, *Cs. inornata* (26.3%), *Culiseta incidens* (Thompson) (25.9%), *Cx. quinquefasciatus* (24.1%), *Cx. tarsalis* (19.3%) were the major species, followed by *Aedes sierrensis* (Ludlow) (3.0%), *Cx. stigmatosoma* (1.2%), and *An. franciscanus* (0.2%). Similarly, the most abundant species in the valley area was *Cx. quinquefasciatus* (46.6%), followed by *Cx. tarsalis* (26.1%), *Cs. incidens* (9.9%), *Cx. stigmatosoma* (6.7%), *Cs. inornata* (6.2%), *Cx. erythrothorax* (3.0%), *An. franciscanus* (0.7%), *Ae. sierrensis* (0.5%) *Ae. vexans* (0.2%), and *Anopheles freeborni* Atkin (<0.1%).

A total of 89 pools of culicine mosquitoes (*Cx. tarsalis*, *Cx. quinquefasciatus*, and *Cx. stigmatosoma*) collected in CO<sub>2</sub>-baited CDC traps in the desert and valley areas was sent to VBDR for testing. All pools tested negative for both SLE and WEE viruses.

The results on chicken serology showed three sero-conversions to WEE virus each in the Needles and Redlands flocks. The sero-conversion in the Needles flock was found early in July (bled July 13, 1998), whereas the sero-conversion in the Redlands flock was reported about two weeks later (bled July 27, 1998). There were no sero-conversions detected in the Yucaipa, San Bernardino, Fontana, and Ontario flocks.

Upon receipt of confirmation on sero-conversions in both the Needles and Redlands flocks, the areas were posted with "Encephalitis Warning" signs followed by press releases to local newspapers advising residents to take necessary precautions during outdoor activities especially at dusk and dawn in the affected areas. In the wake of virus activity, mosquito source reduction and control activities were intensified in both areas. During the 1998 EVS season there was no human case of mosquito-borne encephalitis in the SBCVCP territory.

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# An Inexpensive Collapsible Pyramidal Emergence Trap for the Assessment of Wetland Insect Populations

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**ABSTRACT:** A design for an inexpensive collapsible pyramidal emergence trap is discussed. Each emergence trap can be fabricated for less than \$12 in materials and in less than one hour. The trap presented here provides a cost effective and efficacious design for sampling mosquitoes, such as *Culex erythrothorax*, that are associated with emergent macrophytes and are difficult to sample as larvae.

## INTRODUCTION

Mosquito abundance and estimates of mosquito production from wetlands can be greatly underestimated by dip samples. *Culex erythrothorax* Dyar and other species that inhabit densely vegetated marshes and wetlands generally represent a low proportion of individuals in dip samples; whereas, they can constitute the majority of adults collected in carbon dioxide-baited suction trap samples (Walton and Workman 1998, Workman 1998). In addition to adult mosquitoes developing from hyponeustic immature stages, emergence traps also can be used to sample adult production from benthic insects, such as chironomids (Diptera: Chironomidae), and plant-dwelling insects, such as Odonata and Ephemeroptera.

In order to obtain representative samples from the different regions of large wetlands or from replicated experimental wetlands, it was necessary to use a large number of emergence traps. An inexpensive design which required minimal fabrication time was desirable. The present paper includes a description of a collapsible emergence trap.

## MATERIALS AND METHODS

Emergence traps were designed to sample an area equal to 0.25 m<sup>2</sup> (Figure 1A and B). Traps were constructed from pine furring strips (stock size: 0.025 × 0.05 × 2.4 m [1" × 2" × 8']) and pine board (stock size: 0.025 × 0.15 × 2.5 m [1" × 6" × 8']). Furring strips were cut to two lengths: 46 cm (vertical support: 18") and 58 cm (horizontal crosspiece: 22.75"). A radial arm saw was used to cut an angle of approximately 23° on one end of each 46 cm piece. In order to accommodate joining the sloping sides of the trap to a horizontal crosspiece, a right- or left-handed complex angle (23° and 25°) was cut on the opposite end of each vertical support. The

top assembly of the trap was fashioned by joining two 14 cm × 14 cm (5.5") cuts from the pine board after a 7.6 cm (3") hole was drilled through the bottom section and a 10.2 cm (4") hole was drilled through the upper section using hole saws. The two sections were fastened using four 3.2 cm (1.25") drywall screws.

In order to collapse the trap (Fig. 1C), each horizontal crosspiece was drilled at 1.9 cm (0.75") on center from both ends. Wooden dowels (0.64 cm diameter × 3.8 cm long [0.25" diameter × 1.5" long]) were glued into half of the horizontal crosspieces. Hinges (3.8 cm [1.5"]) were attached to the top of each vertical support and to the lower section of the top assembly, and then each horizontal crosspiece containing dowels was affixed to the vertical supports using drywall screws. The outside edge of each vertical support was offset 1.9 cm (0.75") from the dowel to allow placement of a horizontal crosspiece onto the dowel.

Collection jars were 0.5 L (16 oz) wide-mouth canning jars (Kerr® or Ball® jars: Alltrista Corp., Muncie, IN). A plastic funnel (80 mm diameter, #10-348-B; Fisher Scientific, Pittsburgh, PA) was inserted into each jar. The 80 mm funnel causes the screw top on the jar to be closed with difficulty; a newer model 79 mm funnel (#10-348-2B) may provide a better fit. The 10.2 cm (4") hole in the top assembly holds the collection jar. Additional support for the jar can be provided by inserting two cup hooks on either side of the jar and then rotating the hooks to come in contact with the exposed threads on the lip of the jar or with the crimped edge of the metal lid.

Rolls of fiberglass window screening (91 cm [36"] wide) were cut in half and then dimensions were marked using two templates. Duplicate screens of two sizes [(upper length × lower length × height) 14 cm × 53.5 cm × 46 cm or 14 cm × 61 cm × 46 cm] were affixed to the wooden frame of each trap using a staple gun.

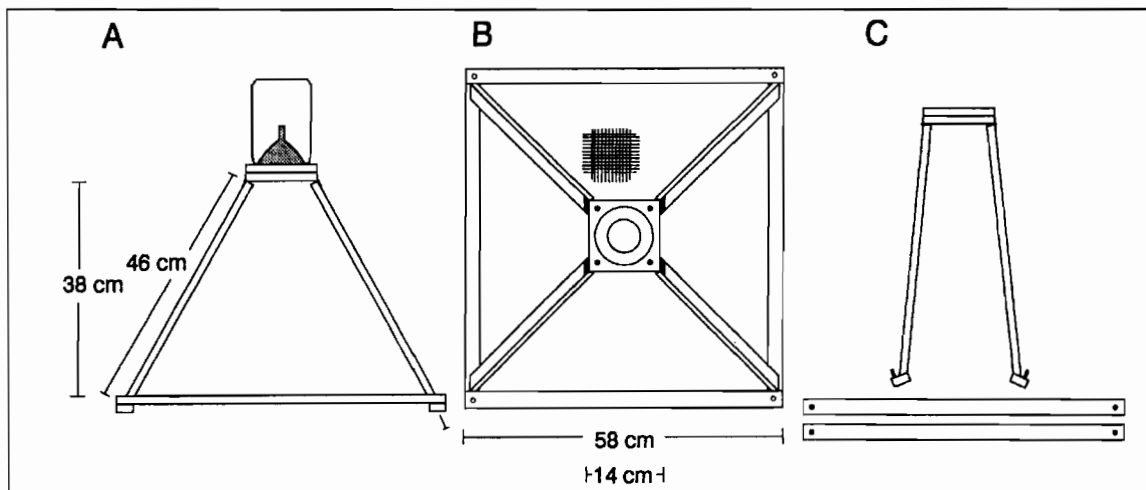


Figure 1. Different views of the Riverside emergence trap. Side (A) and overhead (B) view of an expanded trap. Side view (C) of a collapsed emergence trap.

## RESULTS AND DISCUSSION

Pyramidal emergence traps of several basic designs have been used to study mosquitoes emerging from a variety of permanently and intermittently inundated habitats (Service 1995). The trap described here combines the features of previous designs (Aubin et al. 1973, Slaff et al. 1984) to sample a potentially large number of emerging insects from treatment wetlands receiving enriched wastewater. The trap can be folded when space for transport to field sites is at a premium and set up easily in the field.

The Riverside emergence trap can be produced inexpensively and quickly within a modestly supplied wood shop. The cost of materials was \$11.60 per trap (1997 dollar values) and 45 minutes labor was required to fabricate each trap. The cost per trap can be reduced further by substituting plumber's tape for the hinges. The hinges were the most expensive component of the trap (\$1.10 per hinge). If space is not limited for transporting traps to field sites, then little advantage in space savings is gained by folding the traps as compared to stacking unfolded traps. Permanently affixing the corners of the horizontal crosspieces with drywall screws eliminated the need for a minimal expense (\$4.20) and labor (installation and maintenance/replacement) associated with the dowels.

We found that adding a 15.2 cm (6") screen skirt to the horizontal crosspieces appreciably reduced the likelihood that emerging insects could escape from the trap and prevented insects not derived from below the trap from entering when water depth fluctuated. The skirt

was attached to the horizontal crosspieces with staples and the free ends were sealed using a hot-glue gun. The skirt can be folded into the trap during storage and transport. Floats (e.g., styrofoam, FunNoodles®) can be attached using cable ties to the base of trap for use in comparatively open water habitats and a pair of cup hooks (\$0.11 each) can be added to secure the jar on top of the trap. Three persons can fabricate nearly 100 traps in approximately 3-4 days.

This emergence trap has been used to study the spatial and temporal patterns of wetland mosquito production (Walton et al. 1998), the efficacy of control measures against mosquitoes developing in dense vegetation (Walton et al. 1998) and the effects of vegetation management practices on mosquito production (Walton et al. 1999). The trap presented here provides a cost effective and efficacious design for sampling mosquitoes, such as *Culex erythrothorax*, that are associated with emergent macrophytes and are difficult to sample using a dipper.

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# Mosquito Production from Three Vegetation Management Practices for Constructed Treatment Wetlands in Southern California: Preliminary Findings

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**ABSTRACT:** The effects of three vegetation management practices on mosquito abundance and production were studied in 0.1 ha experimental wetland research cells in southern California. During the three months after flooding, wetland cells containing hummocks and wetland cells in which bulrush root densities were reduced produced significantly fewer adult *Culex tarsalis* as compared to wetlands that were only burned (controls). *Culex tarsalis* larvae reached undetectable levels first in the cells with hummocks and last in the wetlands assigned to the control treatment. Very few *Cx. erythrothorax* adults were produced from the sparsely vegetated research cells during the beginning of this multi-year study.

## INTRODUCTION

Mosquito production was directly related to vegetation coverage at the 10 ha Hemet/San Jacinto constructed treatment wetland in southern California. As the proportion of the water surface covered by bulrush (*Schoenoplectus californicus* (Meyer) Soják and *S. acutus* (Muhl. ex. Bigel.) Löve and Löve) increased from 2% to approximately 75% during the three years after beginning operation (Sartoris et al. 2000), host seeking mosquito abundance increased an order of magnitude per year (Walton, unpublished data). During mid-summer of the third year, nearly 40,000 host seeking mosquitoes were collected per night by carbon dioxide-baited suction traps (Walton et al. 1998).

Vegetation management strategies that are cost-effective and prevent extensive vegetation coverage of the water surface need to be developed for treatment wetlands that utilize emergent macrophytes for water treatment and wildlife habitat. Burning of emergent vegetation has received increased attention as a rapid means to eliminate above-ground plant biomass without incurring high costs associated with harvesting and disposal (Walton 1999). However, burning as the only means of reducing vegetation might not be a satisfactory mosquito control method because thick stands of vegetation would develop prior to burning. Thick vegetation enhances mosquito production and increases abatement efforts. Furthermore, inundation of burned material might significantly compromise water quality goals for treatment wetlands.

The objectives of this multi-year study are to evaluate the effects of three vegetation management strategies on mosquito populations and water quality parameters in replicated, experimental wetlands. In addition to burning of emergent vegetation, the impact of management strategies that also reduce bulrush root densities and create structures (hummocks) to encourage bulrush growth while retaining regions of open water are being evaluated. This is a joint project between the Department of Entomology at the University of California-Riverside, the United States Geological Survey, and the Eastern Municipal Water District. Here, we report the preliminary findings for mosquitoes during the initial three months of this study.

## MATERIALS AND METHODS

Three vegetation management strategies were carried out in eight 0.1 ha wetland research cells at the Wetlands Research Facility located at Eastern Municipal Water District's Hemet-San Jacinto Regional Wastewater Reclamation Facility in San Jacinto, CA. Each cell contained two shallow marshes (water depth 0.5 to 1 m) that were situated downstream from two deep ponds (water depth ~ 2.5 m). The emergent vegetation (bulrush, *Schoenoplectus californicus*) was dried and burned. Following burning, three of the research cells were not manipulated (controls) and a rock bucket attached to a backhoe was used to reduce bulrush root densities in the remaining five cells. Hummocks (approximate length x width x height: 4 m x 1.5 m x 0.4 m) were constructed in the shallow regions of two of the latter

cells. Hummocks in adjacent rows along the longitudinal axis of each cell were offset spatially so that either two or three hummocks occurred in each transverse row. The three vegetation management practices will be referred to as C (control: burning only), R (burning and rock bucket), and H (burning, rock bucket and hummocks).

Flooding of the research cells with secondary-treated effluent began on July 13, 1998. In order to examine the effects of burning on levels of metal contaminants and water quality of the first outflow event, water levels in the mesocosms were maintained to avoid overflow through the outlet weir for approximately one month. Thereafter, flow rates were maintained so that water residence times were approximately 13 days in the C and R cells, and 18 days in the H cells.

Temporal and spatial trends of mosquito production from the research cells were determined using 0.25 m<sup>2</sup> emergence traps. Eight emergence traps were placed into each cell on July 21 or 22; four traps were placed in each of the two marshes of each cell. Emergence traps were suspended from wooden stakes until sufficient vegetation developed to support the traps. Emergent vegetation beneath the traps was cut just above the water surface to maintain physical structure below the water surface, yet avoid tipping of the traps by rapid bulrush growth. Trap maintenance was carried out twice each week. Collection jars were retrieved weekly from July 29 through September 29. A group of samples also was collected on October 13.

Immature mosquitoes were sampled weekly by dipping at 16 stations positioned equidistantly along the research cell periphery and at 4 stations along each of two transects through the cell interior. At each peripheral station, three 350 ml dips were taken within a 2 m zone, combined in a concentrator cup and preserved. At each interior station, five dips were taken, combined and preserved. Developmental stage and abundance of immature mosquitoes were determined at 25X-50X magnification using a stereo dissecting microscope.

Differences in mosquito abundance or emergence among the three vegetation management practices were tested for statistical significance using a Friedman non-parametric ANOVA. *A posteriori* comparisons among treatment ranks were carried out using the Mann-Whitney U-test (Sokal and Rohlf 1995).

## RESULTS AND DISCUSSION

The hummocks showed considerable promise as a method for slowing the proliferation of bulrush and sig-

nificantly reducing mosquito production during the three months after vegetation management practices were carried out. The H mesocosms maintained comparatively more open water than did the other treatments. Bulrush grew along the marsh periphery and was sparse on the hummocks in the H cells. Whereas by October, bulrush coverage was more extensive in the marshes of the R and C cells, with the thickest growth occurring in the latter treatment. The open water in H cells will provide enhanced access to immature mosquitoes by predaceous invertebrates, reduce the abundance of favorable developmental sites for mosquitoes and deter oviposition. Maintenance of open water between small stands (i.e., surface area covered by emergent vegetation) of vegetation also will provide access for larvivorous fish and permit the application of pelletized or granular bacterial larvicides should such treatments be required for mosquito abatement.

The number of *Culex* spp. emerging per square meter of vegetated surface from the C mesocosms was 100- and 10-fold greater than from the H and R mesocosms, respectively, during the second week after flooding (Fig. 1). Thereafter, emerging mosquitoes were not collected from the H mesocosms. Adult production declined during the four to six weeks after flooding in the C and R treatments and a low level of emergence (< 2 individuals m<sup>-2</sup>) occurred until emergence traps were removed from the ponds in mid-October. *Culex tarsalis* Coquillett accounted for 71% of the emerged adults averaged across all treatments. Adult emergence differed significantly among treatments (Friedman's method:  $\chi^2_2 = 15.27$ ,  $P < 0.001$ ). Significantly more *Cx. tarsalis* emerged from C mesocosms than from H and R mesocosms (Mann-Whitney U-test,  $P < 0.05$ ). *Culex stigmatosoma* Dyar and *Cx. quinquefasciatus* Say accounted for 27% and 1%, respectively, of the adults collected in emergence traps.

The abundance of *Culex* larvae in dip samples from the three vegetation management treatments exhibited trends similar to those for adult emergence (Fig. 2). C mesocosms contained the largest larval populations which declined to low levels by six weeks after flooding. Larval populations in R and H mesocosms declined to low levels by four and two weeks after flooding, respectively. Larval abundance differed significantly among treatments (Friedman's method:  $\chi^2_2 = 11.56$ ,  $P < 0.003$ ). Significantly more *Cx. tarsalis* larvae were collected from C mesocosms than from H and R mesocosms (Mann-Whitney U-test,  $P < 0.05$ ).

*Culex erythrothorax* Dyar accounted for approximately 1% of the adults collected by emergence traps in the sparsely vegetated cells during the three months af-

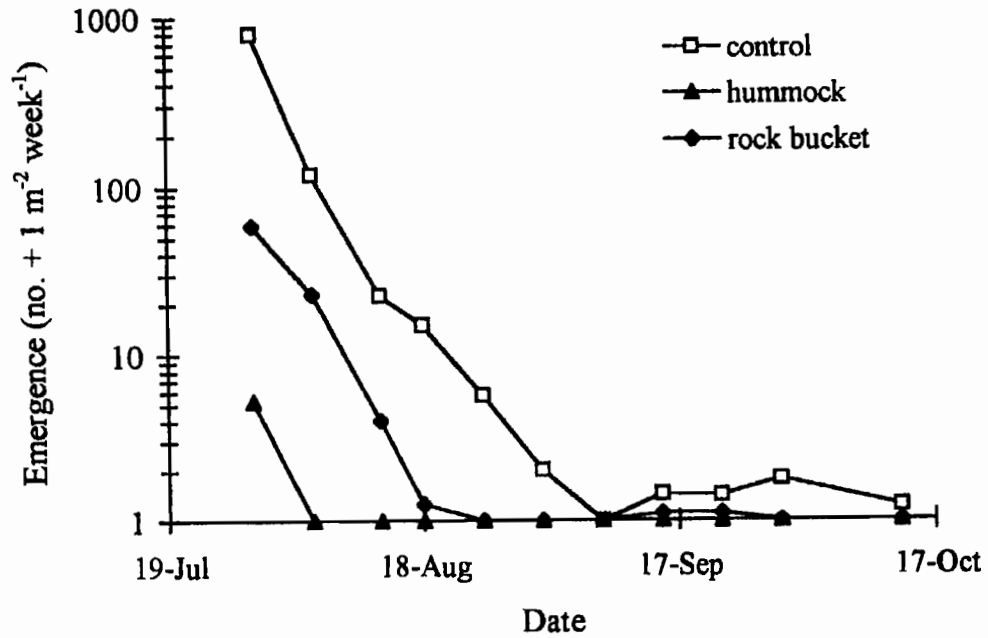


Figure 1. The average number of *Culex* spp. emerging each week from three vegetation management practices carried out in 0.1 ha research cells during July-October 1998.

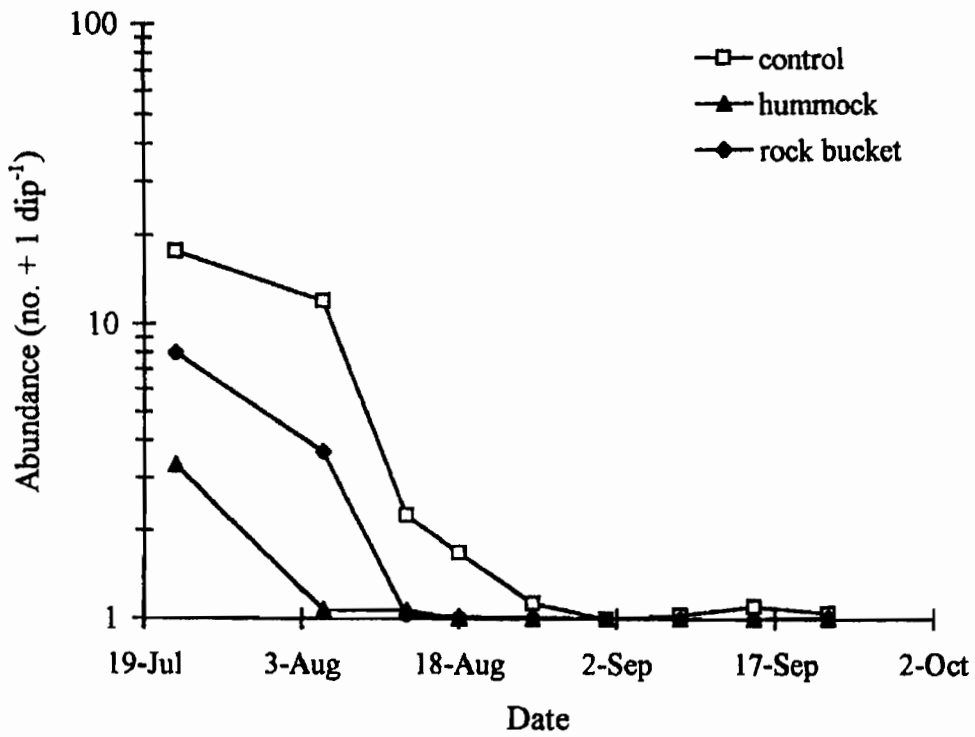


Figure 2. Abundance of *Culex* spp. larvae in dip samples from three vegetation management practices in the research cells during July-October 1998.

ter flooding. This contrasts sharply the findings of a previous study (Workman and Walton 2000) where the number of *Cx. erythrorhax* emerging weekly per unit area exceeded that of *Cx. tarsalis* by an order of magnitude or more when dense emergent vegetation was present in these wetlands. The developmental site preference of *Cx. erythrorhax* for thickly vegetated wetlands (Nielsen and Rees 1961, Chapman 1962, Bohart and Washino 1978), the association of immature stages with vertical surfaces such as bulrush culms (Workman 1998), and the direct relationship between adult emergence and vegetation density (Workman and Walton 2000) explain the differences in *Cx. erythrorhax* relative abundance in previous studies versus the current study. *Culex erythrorhax* production is expected to increase concomitantly with increases of vegetation densities and coverage.

Satisfying the goals of vector and nuisance insect reduction versus water quality improvement has proven to be difficult for constructed treatment wetlands utilizing emergent vegetation. We expect mosquito production to increase during the second year of operation as vegetation densities increase, especially in the comparatively more densely vegetated control wetlands. Whether the marshes containing hummocks provide the aforementioned benefits for vector control, continue to reduce bulrush coverage, and can concomitantly improve water quality are the foci of our continuing collaborative efforts.

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## A Model Surveillance Program for Vector-Borne Diseases in California, 1998-99

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**ABSTRACT:** This report summarizes the second year's research of a 5-year project to improve the efficiency of the California Vector-Borne Disease Surveillance Program. The project used California data on arboviruses as a model for the entire system. Results are discussed under 5 primary areas of study: (1) human viral infections, (2) enzootic viral activity, (3) mosquito abundance, (4) analysis, prediction and reporting and (5) weather and water. Complete results may be found in the various publications cited below based on this research. Enhanced surveillance for arboviral encephalitis began in 1998, and retrospectively detected a new case of California encephalitis. An analysis comparing sentinel chicken flocks and mist netting of birds for detection of arboviral antibodies suggested that in most instances, sentinel chicken flocks are more effective. Isolations of western equine encephalomyelitis virus from mosquitoes from Kern County provided 2-3 weeks of earlier warning than did seroconversions in sentinel chickens. Comparative studies of CDC-style mosquito traps showed that traps made by the Arbovirus Field Station caught more mosquitoes than any one of several commercially produced traps. The surveillance website (MosquitoNet) was further improved during 1998. A preliminary analysis in Kern County demonstrated that measurements of stream runoff were good predictors of mosquito abundance occurring one month later, and that both variables correlated well with snow pack at the headwaters of the Kern River.

### INTRODUCTION

On July 1, 1997, a cooperative program was begun to develop new approaches to arbovirus surveillance, eventually leading to their incorporation into the California Vector-Borne Disease Surveillance Program. The research was conducted under the direction of the UC Davis Center for Vector-Borne Disease Research (UCD-CVBDR) in cooperation with the California Department of Health Services (CDHS), the Mosquito and Vector Control Association of California (MVCAC), and 10 individual mosquito and vector control districts. Details of the organization of the project and its objectives were discussed by Eldridge et al. (1999).

### HUMAN VIRAL INFECTIONS

Ongoing studies continued to address the apparent disparity between the intensity of enzootic western equine encephalomyelitis virus (WEE) and St. Louis encephalitis virus (SLE) activity detected by

seroconversion among sentinel chickens and virus isolations from mosquitoes, and the low number of reported human infections. Enhanced surveillance for encephalitis was initiated in the San Francisco Bay counties in June of 1998; in January 1999 surveillance was expanded statewide. Health care providers were notified of the diagnostic service available and blood and spinal fluid specimens from individuals with encephalitis were tested on an ongoing basis.

Discharge diagnosis codes (ICD-9 codes) were analyzed over a 3-year period to examine trends (e.g., seasonality, geographic clustering, and gender) that might be indicative of arbovirus activity in California. Based on an analysis of ca. 1,800 cases, no patterns were identified that were consistent with infection by a mosquito-borne virus. There were higher incidences of encephalitis codes occurring in some small rural counties, but this relationship was inconclusive.

A retrospective serosurvey was done on sera submitted to the Viral and Rickettsial Disease Laboratory (VRDL), CDHS, from patients with undiagnosed CNS

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disease in preparation for the prospective encephalitis project. This retrospective serosurvey detected the fourth known human case of California encephalitis (Eldridge et al. 2000). A presumptive case of encephalitis attributed to SLE was investigated in Fresno County. The patient's diagnosis was based on test results of a commercial laboratory. Tests conducted by VRDL showed no evidence of recent infection. Other situations have occurred in which commercial laboratories report positive results on the basis of IgG screening, but subsequent testing failed to show evidence of recent infection. These studies illustrated the numerous problems in diagnosing and confirming human cases involving CNS impairment, i.e., incomplete patient data and lack of serum or spinal fluid samples for testing.

Future studies will focus detection efforts in south-eastern California where evidence of enzootic arboviral activity occurs annually, and will attempt to increase the number of human serum and spinal fluid samples available for laboratory analysis.

## ENZOOTIC VIRUS ACTIVITY

### Detection of enzootic viral activity in wild birds and sentinel chickens

Sentinel chicken flocks and wild birds captured in mist nets were studied in 3 mosquito abatement districts (Sacramento-Yolo, Kern, and Coachella) and the timing and detection of seropositive specimens were compared. A total of 20,200 wild birds was sampled. Nine sentinel chicken flocks were used to monitor virus activity in each district. Wild birds with antibodies against WEE were captured in all 3 districts, but only birds in the Coachella district had antibodies against SLE. As in the case of the wild birds, WEE activity was detected in sentinel chickens in all 3 districts and SLE seroconversions were detected only in the Coachella Valley. The timing of detection of antibodies in all 3 districts indicated that antibodies in wild bird blood do not necessarily indicate a recent infection. Unless specimens from wild birds that are recaptured by mist netting during a short time interval are negative during the 1st bleeding and positive during the 2nd, it is not possible to know with certainty when or where a wild bird was infected. Relatively few of the wild birds in our study were recaptured and none fit the scenario mentioned above. Sentinel chickens, which remain at the same site and can be bled at regular intervals, are not encumbered by uncertainty about their infection history.

An analysis of the relative costs associated with using mist netted wild birds, sentinel chickens or virus

isolation from mosquitoes for arbovirus surveillance in the Sacramento-Yolo district indicated that labor costs associated with capturing and processing wild birds are high. Estimated total and recurring costs associated with each system from May through October were: wild birds = \$28,489 & \$28,341; sentinel chickens = \$18,410 & \$10,724; and mosquitoes = \$9,355 & \$8,955.

Results support the conclusion that the use of sentinel chicken flocks are superior to mist netting wild birds for arbovirus surveillance in California. This conclusion does not necessarily apply to programs that focus on trapping common peridomestic birds in Australian crow traps for surveillance (Bennett et al. 1996, Bennett et al. 1997, Cummings et al. 1998). Future analyses should include this approach as well.

### Detection of enzootic activity in mosquitoes

**Isolation of WEE and SLE from known and potential vectors:** During 1998 the UC Davis Arbovirus Research Laboratory, UCD-CVBDR, tested 3,943 pools of mosquitoes submitted by mosquito control agencies as part of the Statewide Encephalitis Virus Surveillance program, of which 53 were positive for WEE and 1 for SLE viruses. Of these, 1,559 pools of *Cx. tarsalis* were collected from our study areas in Coachella Valley, Kern and Sacramento/Yolo MVEDs. Isolations of WEE from Coachella Valley and Kern provided 3 and 2 wks earlier warning of WEE enzootic activity, respectively, than did seroconversions of sentinel chickens. These data demonstrated the value of systematically testing mosquitoes for infection during years of moderate to high virus activity. In addition, 721 pools were tested from 7 mosquito species other than *Culex tarsalis*, with negative results. These negative results indicated that WEE activity in Kern County did not spread to the secondary lagomorph-*Aedes* cycle and reflected the low level of SLE activity in California during 1998.

**New mosquito-virus relationships:** To investigate novel vector-virus relationships, 347 pools of *Aedes sierrensis* and 55 pools of *Aedes bicristatus* were tested from Lake and Humboldt counties, with negative results. Both species were collected as immatures, reared to adults, and then tested to determine if they were infected vertically by an arbovirus. An additional, 37 pools of *An. freeborni* also were tested with negative results.

**Virus persistence:** As part of research to elucidate virus overwintering mechanisms, 1,877 mosquitoes (including 901 *Cx. tarsalis*) were collected resting in winter hibernacula in Kern from October 1998 through February 1999 and tested for virus infection by RT-PCR in 231 pools, with negative results. To detect early season virus amplification in Coachella Valley, an additional

3,614 *Cx. tarsalis* were tested for virus infection in 77 pools by RT-PCR with negative findings. Interestingly, a sentinel chicken housed in the same area as these winter collections seroconverted to WEE during the November 1998–March 1999 period. Both investigations agreed with previous in-depth studies that tested mosquitoes collected during winter for infectious virus using either suckling mice or cell culture indicated that WEE and SLE probably do not overwinter in infected vectors.

As part of ongoing studies to assess the possible role of birds as overwintering virus reservoirs, studies were completed on the effects of the method of inoculation (mosquito bite or needle) and source of virus strain on viremias and antibody development in house finches (Reisen et al. 2000a, Reisen et al. 2000b). This information is needed to plan additional experimental infection studies.

### MOSQUITO ABUNDANCE

During 1998, research to understand mosquito host-seeking behavior (Lothrop and Reisen 1999b) and to improve trap deployment strategies continued. New research on trap design and CO<sub>2</sub> delivery were completed and are described below. Research and development leading to guidelines for an integrated mosquito sampling and reporting program will be continued.

**Trap design:** The mean number of *Cx. tarsalis* collected by the Arbovirus Field Station (AFS) CDC-style trap was significantly greater than the mean number collected by traps manufactured by American Biophysics (ABC trap), Hock (CDC style) and Bioquip (EVS style made by Orange County VCD and commercially produced by Bioquip) companies when tested in suburban Orange and rural Kern Counties (Reisen et al. 2000c). Commercially produced traps were operated with their lights on and reflective rain shields in place as recommended by the manufacturer. The AFS trap was operated without lights or rain shield. All traps were baited with 3 lbs of dry-ice. Results indicated that light did not enhance catch size. Engineering analysis (Cummings and Meyer 2000) documented that increased catch by the AFS trap probably was related to the rainshield (American Biophysics and Hock) or battery-pack (Bioquip) that reduced airflow into the trap mouth. Differences in trap efficiency remained consistent despite marked changes in mosquito abundance over time and space.

**CO<sub>2</sub> presentation:** An experiment in Kern County compared the effects of CO<sub>2</sub> presentation on *Cx. tarsalis* catch in AFS traps. Treatments included CO<sub>2</sub> gas delivered at the constant rates of 0.5, 1.0 and 1.5 l/min, CO<sub>2</sub> gas delivered at 0.5 l/min as 15 pulses of 2 sec on: 2 sec off per min, and 3 lbs of dry-ice in a 1-gal plastic bucket or a Styrofoam Petri dish mailer. There was no significant difference among catch size in traps baited with CO<sub>2</sub> gas released in the 0.5 to 1.5 l/min range. However, catch size in traps baited with CO<sub>2</sub> gas released at a constant 0.5 l/min was significantly greater than catch in traps baited with pulsed CO<sub>2</sub> gas released at 0.5 l/min or dry-ice.

### ANALYSIS, PREDICTION AND REPORTING

Improvements were continued in 1998 in the electronic reporting of arbovirus surveillance information. Reporting of virus isolations from mosquito pools was added to the surveillance website located at <http://mosqnet.ucdavis.edu>, and a summary page was added that provides the same information as the summary table on the weekly surveillance bulletin. An entirely new type of data management system was initiated with emphasis on maintenance of open databases for use by mosquito abatement districts. Under the new plan, laboratories doing surveillance testing will have the responsibility for maintenance of databases containing data from those laboratories, and mosquito agencies will have full access to those databases using specially designed client software that will provide a full range of analysis options, including mapping, graphing and various types of reporting. Under the present system, analysis of historical data is difficult; under the new concept, users could select any time frame desired. The implementation of this concept is being coordinated with Advance Computer Resources, Inc. with a grant from the Centers for Disease Control and Prevention.

Details of a geographic information system developed to manage, analyze, and report surveillance and operational data of the Coachella Valley MVCD were published (Lothrop and Reisen 1999a).

### WEATHER AND WATER

Certain weather factors may affect the abundance of vector populations, and thus transmission of SLE and WEE. Increases in snow pack discharge from the Sierra Nevada, precipitation and/or alternative sources of water increase larval habitat for *Cx. tarsalis* resulting in the increased abundance of the host-seeking female

population. Higher ambient temperatures may affect abundance by shortening generation times and therefore increase the rate of population growth during the spring and early summer.

Relationships between weather and mosquito abundance were evaluated in an attempt to develop a model applicable to California in general (Wegbreit and Reisen 2000).

Research focused on data from the Kern MVCD surveillance program in the southern San Joaquin Valley, because a systematic dry-ice baited trapping program has been in place there since 1990, providing a sound and sensitive data set for analysis. Mosquito abundance was measured by 24 dry-ice baited traps that were operated biweekly from April through October. Temperature and precipitation data were from California Irrigation Management Information System stations and river discharge data were from the California Data Exchange Center. Discharge was measured at a site near Bakersfield closest to the mosquito trapping sites on the floor of the San Joaquin Valley.

Data were analyzed to determine time series relationships among weather parameters and mosquito abundance. No easily discernable relationships were detected between mosquito abundance and temperature or precipitation; however, there was a strong relationship between discharge of the Kern River and mosquito abundance. Regression analysis indicated that discharge from the Kern River explained 56% of the variability among measurements of host-seeking mosquito abundance during the following month. Deviation analysis confirmed the predictive value of discharge for mosquito abundance. Results of the deviation analysis indicated that low runoff is associated with low *Cx. tarsalis* abundance and for the most part, high runoff is associated with high mosquito abundance. Interestingly, WEE activity was not detected in Kern County between 1984 and 1995. However, after river discharge per month peaked above 150 thousand acre feet (TAF) and *Cx. tarsalis* maximal abundance exceeded 60 females per trap night per month, WEE activity was again detected in Kern County, mostly at sites on the west side of the valley. This trend continued through 1998.

Results from this preliminary analysis indicated that runoff, lagged forward one month, was a good predictor of mosquito abundance and perhaps WEE activity and that both variables were well correlated with snow pack at the headwaters of the Kern River. Other environmental parameters should be analyzed including vegetation and water management practices. Possibly, long range weather forecasts based on Pacific Ocean temperatures and El Niño events may be used to predict

snow pack on the western slopes of the Sierra Nevada, discharge of river systems, and mosquito abundance in Kern County.

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## Recreational Park Yellowjacket Control in Santa Clara County

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**ABSTRACT:** The efficacy of a baiting program for the ground-dwelling yellowjacket (Vespidae: Hymenoptera), *Vespula pensylvanica* was assessed in a high-use recreational park in Santa Clara County, California. Efficacy was measured by comparing yellowjacket abundance between control and treatment areas as well as between pre- and post-treatment intervals. Abundance of *V. pensylvanica* and *V. vulgaris* was assessed on a weekly basis using trifold sticky traps combined with a synthetic lure, heptyl butyrate. Preliminary attractancy tests suggested no significant ( $P > 0.05$ ) difference between heptyl butyrate, pentyl butyrate and Surefire<sup>®</sup>, a commercially available yellowjacket lure. Baiting consisted of hanging stations containing mackerel mixed with microencapsulated diazinon (Knoxout 2FM<sup>®</sup>). Due to low numbers, *V. vulgaris* was not included in assessments or statistical analyses. Analyses indicated a temporary, yet significant ( $P < 0.05$ ) reduction in yellowjacket abundance in the baited area during the treatment interval and to a greater degree, after the cessation of baiting from September through October.

Yellowjackets are serious pests in recreational parks due to their aggressive foraging behavior, omnivorous diet and ability to inflict painful stings. Each year numerous stinging incidents occur where recreationers, park staff and others are exposed to yellowjackets. Depending upon individual susceptibilities, medical treatment may be needed for stings received during accidental ingestion of a yellowjacket with food or beverage, or aggressive attacks near a colony entrance. Park staff are typically stung while emptying refuse containers or mowing fields. Yellowjackets, unlike honey bees, have the capacity to inflict repeated stings without losing their stinging apparatus due to smaller lancet barbs, stronger aculeus musculature, and sharper lancet and stylus (Edwards 1980).

Potential contacts between humans and ground-dwelling vespine wasps is high in Santa Clara County due to their propensity to colonize, abundantly available gopher and ground squirrel burrows. The latter are in fields and under loose vegetation such as the groundcover periwinkle, *Vinca major*. Contacts are usually with foragers, and Akre et al. (1975) found that 50% of the yellowjacket, *V. pensylvanica* foragers, traveled a distance of no more than 160 meters from the nest site. Foraging yellowjackets scavenge for either proteins and/or carbohydrates and can be quickly attracted in large numbers to these substances.

Chang (1988) baited foraging yellowjackets, *V. pensylvanica* in Oahu, Hawaii. He found that in order to be effective, low-density baiting with microencapsulated diazinon must be started early in the season and last

through October. High-density baiting yielded a 94% reduction in foragers after one week post-treatment (Chang 1988), although adjacent untreated areas also dropped by 59%. A dispenser density of 0.6 bait stations per ha was recommended by Ennik (1973) and Chang (1988).

The Santa Clara County Vector Control District (SCCVD) implemented a yellowjacket control program in five major county-maintained parks to reduce the stinging and nuisance potential of these insects. The purpose of this study was to determine the efficacy of such a yellowjacket management program using microencapsulated diazinon (Knoxout<sup>®</sup>) bait stations around picnic sites at a high-use recreational park.

### MATERIALS AND METHODS

To assess the efficacy of our yellowjacket baiting program, Vasona Lake County Park was selected as the study site, based on previous years' requests for service made by park staff. This park is composed of more than ten picnic and larger "group-picnic" areas, largely separated by mowed fields and hydrological features. The Los Gatos Creek and its riparian zone runs from the south side of the park to the center where it becomes Vasona Lake that encompasses roughly one third of the northern side of the park. The park's elongated shape lends itself to a north-south separation, where the south half was designated as the treatment area and the north part the control area.

The study included a preliminary test for attractancy of yellowjacket lures, followed by pre-treatment, treatment and post-treatment sampling intervals. Preliminary tests were conducted to compare the attractancy of heptyl butyrate (Penta Manufacturing, Livingston, NJ), pentyl butyrate (Penta Manufacturing, Livingston, NJ) and Surefire® (Alpine Pest Management Specialists, Bend, OR) to determine which should be used as a surveillance standard. From July 6 to 8, 1998, attractancy was assessed by running all three lures simultaneously at each of five distinct trap locations in the park, using trifold sticky traps (Catchmaster Insect Trap and Monitor) (Atlantic Paste and Glue Co. Inc., Brooklyn, NY) to collect yellowjackets for numerical comparison. Yellowjacket abundance was recorded after 1, 5, 23 and 52 hours of field exposure; taxonomic identifications were made after 52 hours. Sticky traps without lure (i.e., controls) were also assessed during the same time intervals. Results were analyzed using t-tests.

Treatment sites were baited with microencapsulated diazinon (Knoxout 2FM®) (Elf Atochem, Philadelphia, PA) hand mixed with canned Jack mackerel (*Trachurus murphyi*) (Orleans Food Co., Inc., New Orleans, LA) in water. The bait was mixed at a rate of one teaspoon Knoxout® per 15 oz mackerel and added to bait stations at about one ounce diazinon-laced mackerel per station. The stations were constructed from 60 dram amber plastic pill vials (Kerr Group Inc., Jackson, TN) with three, 0.75 inch holes drilled through the sides about one inch from the bottom. String was tethered to the lid of each vial to suspend the station on branches. A paperclip was used to fasten a dental wick to the unit for dispensing the attractant, heptyl butyrate. About 18 bait stations were deployed on the treatment side of the park and baited twice per week during the morning hours. The stations were hung around the periphery of picnic areas at 75 to 100 ft intervals and about five to six feet above the ground. The treatments were conducted from July 22 to September 14, 1998.

Yellowjacket abundance was monitored in the treatment and control sides of the park from July 8 to November 17, 1998. Each trifold sticky trap contained a dental wick laced with heptyl butyrate as an attractant. The wick was placed in a plastic bottle cap in the center of the sticky trap and about 1 ml heptyl butyrate was added just prior to or upon deployment in the field. Baling wire was used to hang the sticky traps in the branches of plants around picnic areas. Abundance of *V. pensylvanica* and *V. vulgaris* was assessed once each week during a 23 to 25 hour sampling period. Yellowjacket abundance was converted to a rate of

yellowjackets per hour and compared statistically using general linear ANOVA and Student-Newman-Keuls mean separation tests (SAS Institute Inc.) to determine if there was a significant difference between baited and control sites, as well as among-treatment differences between pre-treatment, treatment and post-treatment sampling intervals.

The SCCVCD routinely receives service requests from Vasona Lake County Park to remove known ground-dwelling yellowjacket nests. Such calls were responded to regardless of whether they were on the treatment or control-designated side of the park. Yellowjacket extirpation involved excavating the nest while wearing the proper protective gear, application of "wasp freeze" (permethrin/pyrethrin) spray into the nest entrance and nest proper, and burial of treated nest matter. It was found that an initial application of carbon dioxide into the nest entrance greatly reduced the number of aggressive individuals encountered.

## RESULTS AND DISCUSSION

Yellowjacket attractancy tests comparing heptyl butyrate, pentyl butyrate and Surefire® indicated that there was no significant difference ( $P > 0.05$ ) between these three compounds during each sampling interval (Table 1). Volatility of these compounds may have caused the declining recovery rate over time. Control sticky traps lacking attractant did not recover any yellowjackets.

Due to low *Vespula vulgaris* recovery rates on sticky traps, subsequent analyses will only be for *V. pensylvanica*. Yellowjacket abundance in both baited and control areas increased during the treatment interval (Fig. 1). Based on weekly comparisons, week seven of the treatment period had significantly ( $P < 0.05$ ) fewer yellowjackets in the baited area (Fig. 1). Baited and control yellowjacket abundance diverged to a greater extent during the post-treatment interval (Fig. 1 and Fig. 2). Overall, however, there was no significant ( $P > 0.05$ ) difference in yellowjacket abundance between baited and control areas during both pre-treatment and treatment intervals (Fig. 2); while post-treatment abundance was significantly greater in the control area. When intervals were compared, significant increases were detected between pre-treatment and treatment numbers for both baited and control areas (Fig. 3.) Only in the baited areas was there a significant decrease in yellowjacket abundance between the treatment and post-treatment intervals (Fig. 3).

Our post-treatment abundance of *V. pensylvanica* in the baited zone did not increase as found by Chang

Table 1. Average number ( $\pm$  1 standard error) of yellowjackets recovered per sampling interval in sticky traps deployed using three types of lures.

Sample time (hrs)	Surefire®	heptyl butyrate	pentyl butyrate
1	9.4 (1.6)	6.0 (1.7)	8.0 (2.1)
5	14.0 (3.4)	11.2 (2.3)	12.2 (2.5)
23	18.4 (2.9)	16.4 (2.4)	14.0 (3.0)
52	20.0 (4.2)	20.6 (2.2)	14.0 (3.0)

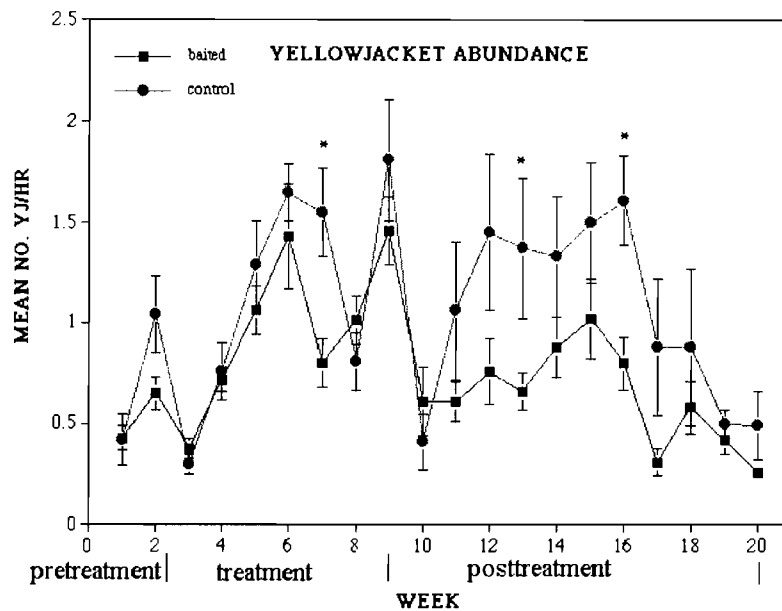


FIGURE 1. Yellowjacket abundance ( $\pm$  1 std error) in baited and unbaited areas of Vasona Lake County Park, Santa Clara County, California and during three study intervals. Asterisks indicate statistical significance ( $P < 0.05$ ).

(1988), perhaps due to our higher dispenser density and longer baiting interval. The contrary was evident on week 10 (Fig. 1) when both control and treatment abundances had abruptly declined. It is not clear what caused this area-wide decline, but it coincides with the seasonal production of new colony queens.

Using the sticky traps, this study suggested a "nuisance threshold" of about 36 yellowjackets per day or 1.5 per hour. Chang (1988) established a threshold of 50 per day using a "wet trap" consisting of 3.8 liter plastic bottles and heptyl butyrate lure. Earlier assessments by Grant et al. (1968) established a threshold of 50 per week. This somewhat arbitrary number may vary by sampling technique and by extrinsic factors such as air temperature, wind and rainfall.

During 1998, nine yellowjacket nests were extirpated from Vasona Lake County Park. This consisted of three within the baited area, three within the control

area and the remaining three in intermediate areas. These numbers were well below that of previous years which may in part be due to the unusually wet "el niño" type spring season of 1998.

This study found a delayed effect of the baiting program where yellowjacket abundance was reduced in the treatment area after the cessation of baiting. This delay may have been due to a high reproductive phase of the colonies during the first half of the study, where there were competing food sources available to foragers. Greater numbers of bait stations and improved park sanitation standards may be needed to improve baiting efficacy. The reduction in yellowjackets albeit delayed, was lauded as a success by park staff.

One factor that may have influenced the study was the replacement of numerous small trash receptacles with individual covered dumpsters in the baited area picnic sites. This change was due to back-injury complaints by



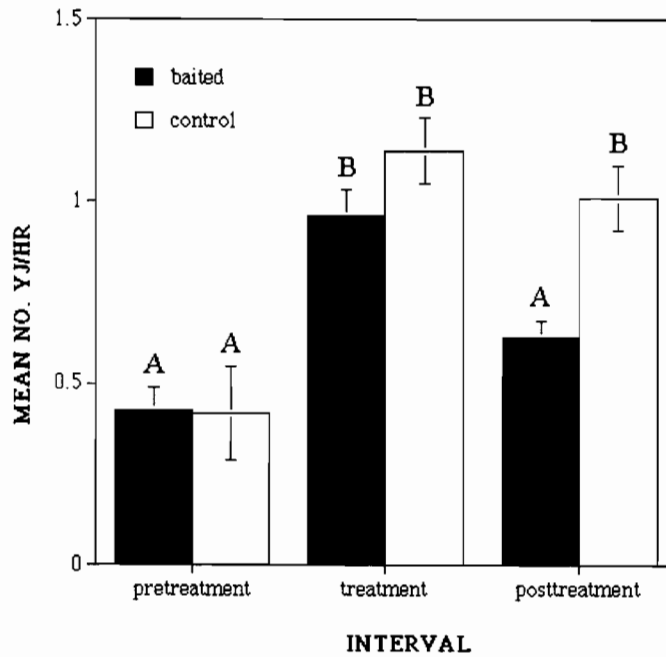


Figure 2. Yellowjacket abundance ( $\pm$  1 standard error) during separate study intervals. Different letters within an interval denotes significance ( $P < 0.05$ ).

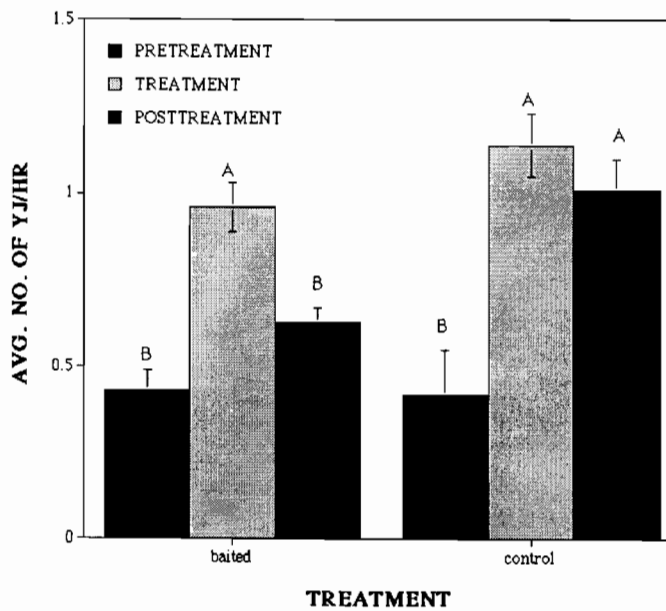


Figure 3. Yellowjacket abundance ( $\pm$  1 standard error) in baited and control areas. Different letters within treatment type denotes significance ( $P < 0.05$ ).

park staff. The dumpster lids effectively excluded yellowjackets as long as the containers were not over-filled. Replacing the trash receptacles may have also affected park sanitation due to greater inconvenience of using dumpsters. A second uncontrolled factor was the public's attempt to reduce yellowjackets using commercially available, hanging collection-type traps. Collection traps were removed when found within the sites, and probably were not greatly affecting the yellowjacket populations; however, in greater numbers perimeter trapout attempts have been deemed successful (Davis et al. 1978).

#### Acknowledgements

The authors thank Mike Philips, Mike Stephenson and Victor Romano of Santa Clara County Vector Control District and Raleigh Young and other staff of the Santa Clara County Parks and Recreation for their involvement in this study.

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## Surveillance for Mosquito-Borne Encephalitis Virus Activity and Human Disease in California, 1998

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The California Mosquito-Borne Encephalitis Surveillance Program is a cooperative effort of the California Department of Health Services (CDHS), the University of California at Davis (UCD), the Mosquito and Vector Control Association of California, local mosquito and vector control agencies, local health departments, physicians, veterinarians, and other interested parties. The multifaceted program includes: 1) mosquito population monitoring and testing for St. Louis encephalitis virus (SLE) and western equine encephalomyelitis virus (WEE) infection, 2) serological monitoring of sentinel chickens in areas of California with historical evidence of encephalitis virus activity, 3) testing of domestic animal species that show clinical signs of possible SLE or WEE infection, and 4) serological testing of patients presenting symptoms of viral meningitis or encephalitis. The 1998 surveillance program began in April with the deployment of sentinel chicken flocks and the beginning of mosquito collection data for the Adult Mosquito Occurrence Report. On May 1, the first of 28 weekly bulletins and adult mosquito abundance reports was distributed to all surveillance program participants. Positive serology and mosquito pool results were communicated immediately to submitting agencies.

### Human Disease Surveillance

In 1998, 140 human serum and/or cerebrospinal fluid specimens from patients presenting symptoms of viral meningitis or encephalitis were tested by the CDHS Viral and Rickettsial Disease Lab (VRDL) for antibodies to SLE and WEE viruses. Neither elevated IgM antibody nor a four-fold rise in total antibody between paired sera was observed in specimens from any of the suspect patients.

### Equine Surveillance

Serum and brain tissue specimens from five horses displaying neurological signs were submitted by practicing California veterinarians for arboviral testing at VRDL in 1998. None of these serum specimens was positive for arboviral antibody or antigen.

### Mosquito Testing

Twenty-five local mosquito control agencies submitted for testing a total of 154,463 mosquitoes (3,557 pools) in 1998 (Table 1). Mosquitoes were tested for arboviruses at the Arbovirus Research Unit of the Center for Vector-borne Disease Research, UCD, by an in situ enzyme immunoassay using Vero cell culture. Of these, 53 pools were positive for WEE and one for SLE. Positive *Culex tarsalis* pools were collected between June 5 and September 29 in Kern County (42 WEE) and Riverside County (11 WEE, 1 SLE).

### Chicken Serosurveillance

In 1998, 48 local mosquito and vector control agencies maintained a total of 134 sentinel chicken flocks in 36 counties. Fourteen of these flocks were involved in arbovirus research projects conducted by the Arbovirus Research Unit in Riverside and Imperial counties. Over 19,840 chicken sera were tested for WEE and SLE. Blood specimens were collected and tested biweekly from each flock. A total of 101 chickens seroconverted to WEE (Table 2) and 2 seroconverted to SLE (Table 3). The first seroconversions to SLE were detected in sera collected from Los Angeles and Orange Counties on April 17 and 21, respectively, and to WEE in sera collected from Imperial County on June 9. These SLE seroconversions in April are the earliest reported since

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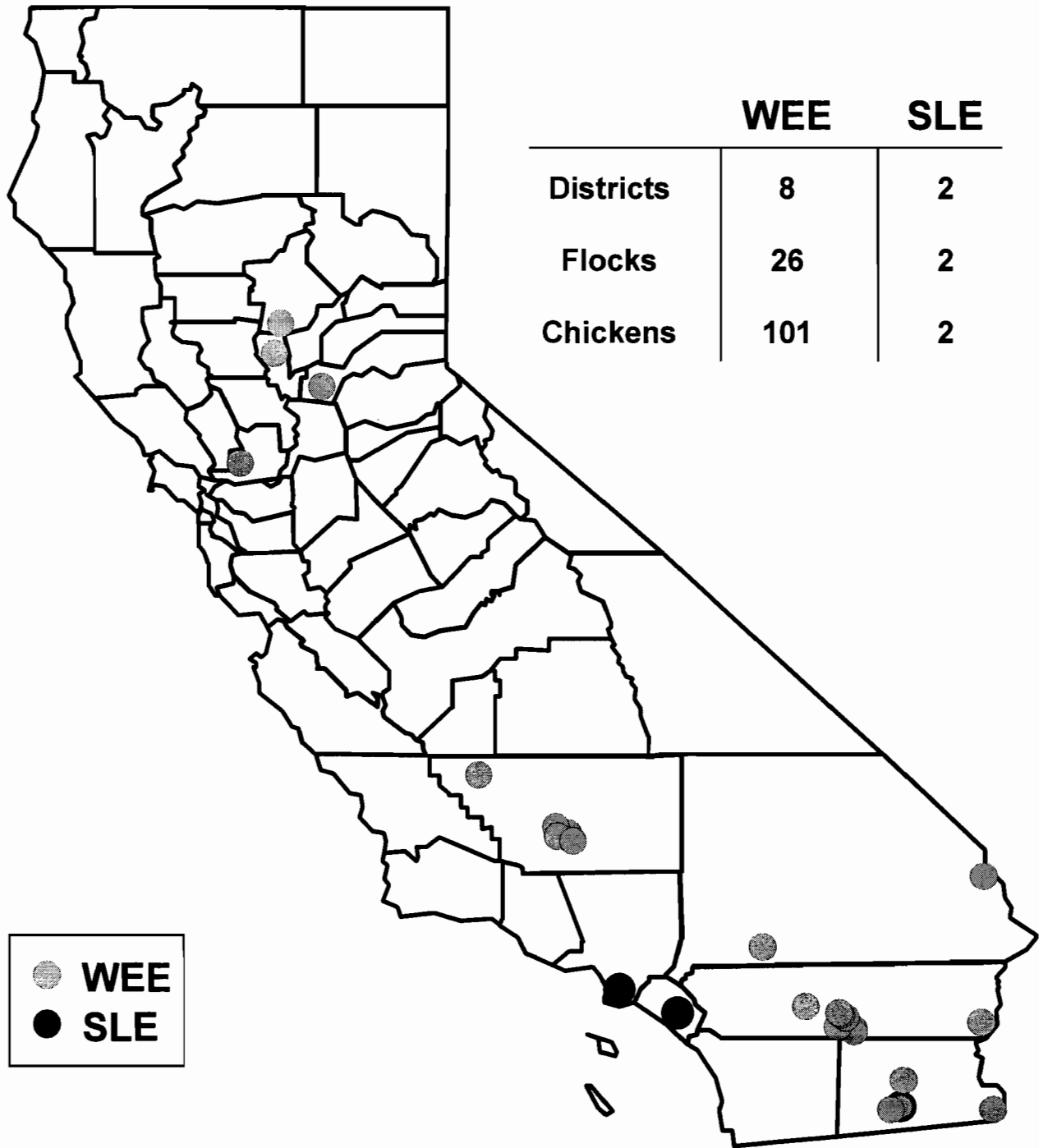


Figure 1. Sentinel chicken flocks with at least one seroconversion to St. Louis encephalitis (SLE) or western equine encephalomyelitis (WEE) virus, California, 1998.

TABLE 1. Mosquitoes tested for WEE and SLE viruses in 1998, by submitting agency.

County	Agency	<i>Cx. tarsalis</i>		<i>Cx. quinquefasciatus</i>		<i>Cx. pipiens</i> <sup>1</sup>		<i>Cx. stigmatosoma</i>		<i>An. freeborni</i>		<i>Ae. melanimon</i>		Total <sup>1</sup>	
		pool	mosq.	pool	mosq.	pool	mosq.	pool	mosq.	pool	mosq.	pool	mosq.	pool	mosq.
Contra Costa	CNTR	177	8841	0	0	0	0	0	0	0	0	0	0	177	8,841
Fresno	FRNO	13	471	0	0	0	0	0	0	0	0	0	0	13	471
Inyo	INYO	7	318	0	0	0	0	0	0	0	0	11	503	18	821
Kern <sup>2</sup>	KERN	684	29000	0	0	0	0	0	0	0	0	171	6988	855	35,988
Kings	KNGS	12	600	0	0	0	0	0	0	0	0	2	100	14	700
Lake	LAKE	157	7600	0	0	0	0	0	0	0	0	2	24	159	7,624
Los Angeles	GRLA	161	6953	189	7878	50	1582	0	0	0	0	0	0	400	16,413
Los Angeles	LACW	114	5700	0	0	0	0	0	0	0	0	0	0	114	5,700
Los Angeles	LONG	34	1334	9	299	0	0	0	0	0	0	0	0	43	1,633
Los Angeles	SGVA	11	488	3	123	0	0	0	0	0	0	0	0	14	611
Madera	MADR	4	200	4	200	0	0	0	0	0	0	0	0	8	400
Merced	TRLK	1	50	0	0	0	0	0	0	0	0	0	0	1	50
Orange	ORCO	5	97	65	1476	6	101	0	0	0	0	0	0	77	1,700
Placer	PLCR	75	3475	0	0	0	0	0	0	0	0	7	233	82	3,708
Placer	SUYA	1	32	0	0	0	0	1	10	0	2	0	0	2	42
Riverside <sup>3</sup>	COAV	717	34021	5	250	0	0	0	0	0	0	0	0	723	34,279
Riverside	NWST	78	2262	129	4927	38	1236	0	0	0	0	0	0	245	8,425
Sacramento	SAYO	66	2551	0	0	0	0	0	0	0	0	4	96	70	2,647
San Bernardino	SANB	62	2618	17	512	8	266	0	0	0	0	0	0	87	3,396
San Diego	SAND	76	3800	0	0	0	0	0	0	0	0	0	0	76	3,800
San Joaquin	SJCM	45	2197	0	0	0	0	0	0	0	0	0	0	45	2,197
Santa Barbara	GLVY	15	534	1	10	0	0	0	0	0	0	0	0	16	544
Santa Clara	STCL	1	30	0	0	0	0	0	0	0	0	0	0	1	30
Shasta	SHAS	3	91	0	0	0	0	0	0	0	0	0	0	3	91
Stanislaus	TRLK	25	1223	0	0	0	0	0	0	0	0	0	0	25	1,223
Sutter	SUYA	116	5197	0	0	0	0	20	768	0	0	0	0	136	5,965
Yolo	SAYO	110	5320	0	0	0	0	0	0	0	0	7	229	117	5,549
Yuba	SUYA	35	1597	0	0	0	0	1	18	0	0	0	0	36	1,615
<b>Total</b>		<b>2,805</b>	<b>126,600</b>	<b>422</b>	<b>15,675</b>	<b>102</b>	<b>3,185</b>	<b>22</b>	<b>796</b>	<b>204</b>	<b>8,173</b>	<b>3,557</b>	<b>154,463</b>		

<sup>1</sup> Totals include one pool (8 mosquitoes) of *Ae. sierrensis* and one pool (26 mosquitoes) of *Cx. erythrothorax* which were submitted for testing by Coachella Valley VCD (Riverside County) and Orange County VCD, respectively.

<sup>2</sup> 42 pools of *Cx. tarsalis* WEE positive

<sup>3</sup> 11 pools of *Cx. tarsalis* WEE positive, 1 pool of *Cx. tarsalis* SLE positive



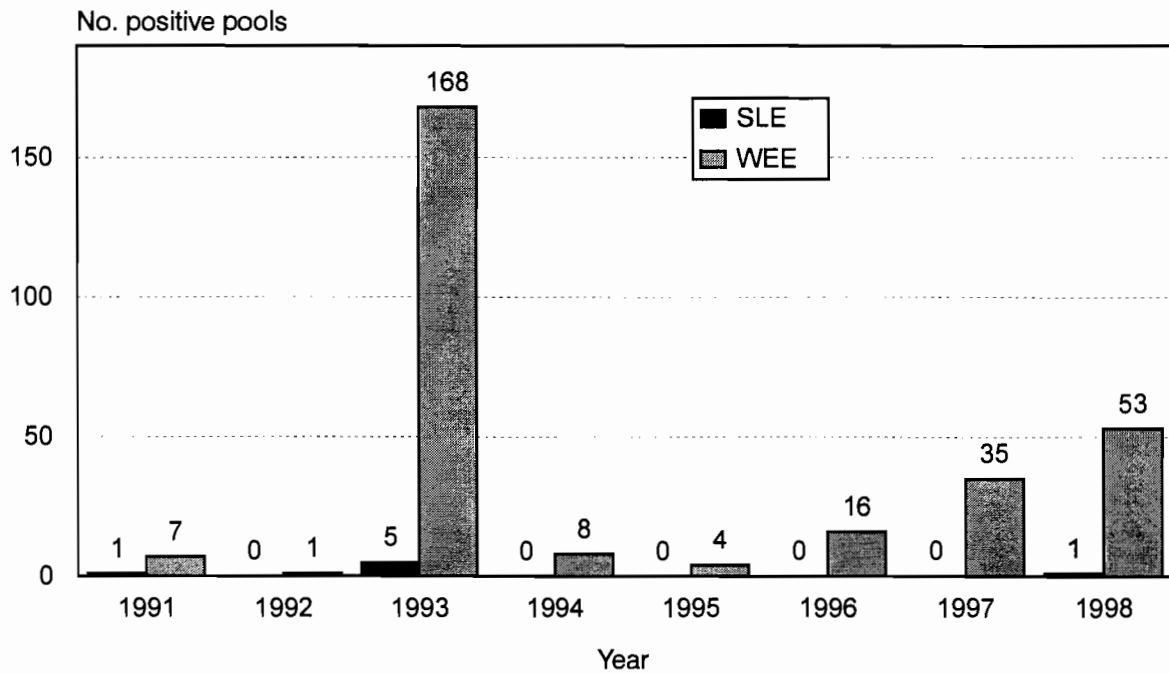


Figure 2. St. Louis encephalitis (SLE) or western equine encephalomyelitis (WEE) virus activity in pooled *Culex tarsalis*, 1991-1998.

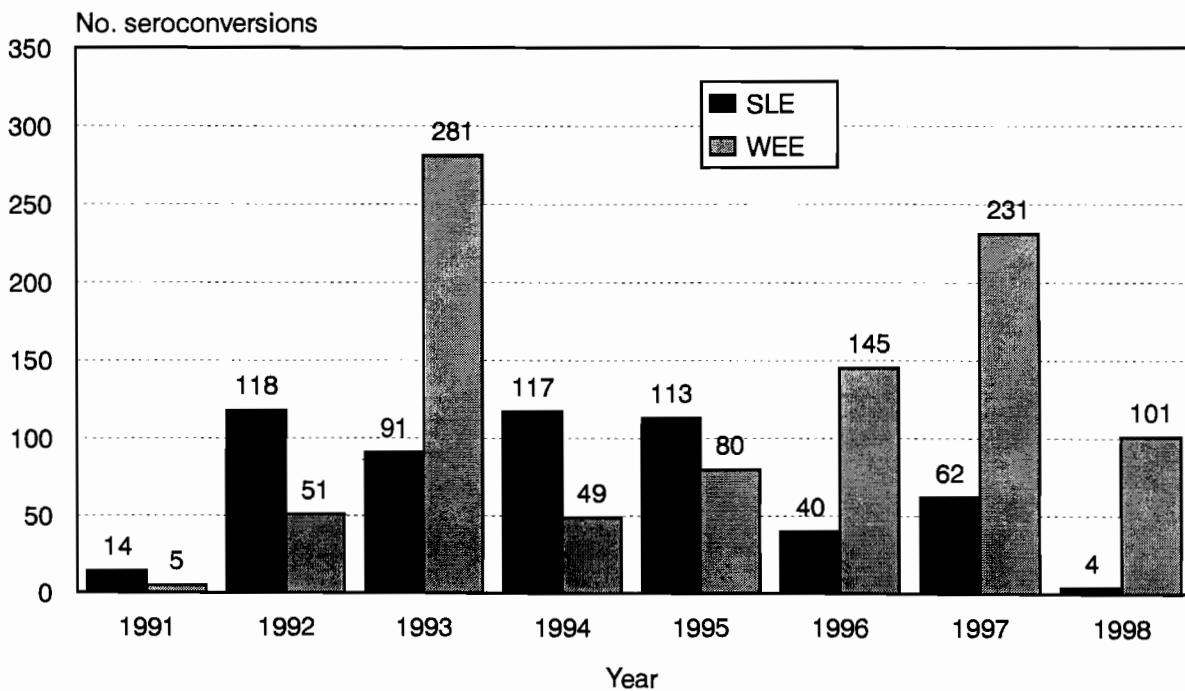


Figure 3. Seroconversions to St. Louis encephalitis (SLE) or western equine encephalomyelitis (WEE) virus in sentinel chickens, 1991-1998.

the statewide surveillance program was established in 1969. However, after these early seroconversions, no further SLE seroconversions were detected in California. Locations of chicken flocks that contained one or more positive chickens in 1998 are shown in Figure 1. WEE activity was found from Imperial County north to Kern County, and in the Sacramento Valley region.

Less WEE activity was detected in 1998 than in 1997; 28 flocks with 101 chickens seroconverted in 1998 versus 54 flocks with 231 chickens in 1997. SLE activity in 1998 was much less than in 1997 and any other year this decade. WEE and SLE activity detected by the encephalitis virus surveillance program from 1991-1998 is summarized in Figures 2 and 3.

Specimens from Washington, Oregon, Utah, and Nevada also were tested through the California Arbovirus Surveillance Program. Sentinel flocks located in Washoe County, Nevada, and Moab County, Utah, seroconverted to SLE in May and June, respectively. Washington and Oregon vector control agencies submitted 89 pools of *Cx. tarsalis* that were tested for virus infection; all were negative.

### *Acknowledgements*

We are grateful to the staff of the Viral and Rickettsial Disease Laboratory and the Disease Investigations and Surveillance Branch, California Department of Health Services; the Arbovirus Research Unit, University of California at Davis; participating local mosquito and vector control agencies; local health departments; the Department of Food and Agriculture, Animal Health Branch; and physicians and veterinarians who submitted specimens from suspect clinical cases.

Special thanks to the Mosquito and Vector Control Association of California and other participating agencies for financial support of laboratory testing.



# Comparison of the Physical Parameters of Four Types of Modified CDC-Style Traps in Reference to their Mosquito Collecting Efficiency

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**ABSTRACT:** An engineering analysis was performed on four types of miniature CO<sub>2</sub>-baited mosquito traps used by many mosquito control agencies in California to assess vector abundance. Airflow, motor rpm and electrical measurements were taken on traps designed by the following: University of California, Davis, Arbovirus Field Station (AFS), Orange County Vector Control District (OCVCD), J. W. Hock Company (Model 1012), and American Biophysics Corporation (ABC Trap). Performance data were compared to results obtained in separate field studies conducted concurrently at different habitats in Kern and Orange counties by the U.C.-AFS and OCVCD, respectively, during the summer of 1998. The AFS trap, with only modest comparative airflow and an unobstructed inlet, successfully collected more mosquitoes in field trials than the other three designs. The results of our engineering analysis and the accompanying field studies suggest that trap inlet configuration and direction of mosquito entry played a larger role in determining catch size than airflow.

## INTRODUCTION

Mosquito control agencies in California use a number of custom-designed and commercially produced versions of either the Centers for Disease Control (CDC) miniature light trap (Sudia and Chamberlain 1962) or the encephalitis virus surveillance (EVS) model (Rohe and Fall 1979, with modifications by Pfuntner 1979) to assess mosquito abundance. Each trap version employs a similar, battery-powered Mabuchi® motor/fan combination mounted in a plastic tube augmented with a carbon dioxide (CO<sub>2</sub>) source to attract and force host-seeking mosquitoes downward into a collecting cage. Although the functional concepts are similar, design variations in these miniature CO<sub>2</sub>-baited traps may produce comparative differences in mosquito numbers. Hence, trap design is one of several important factors that should be evaluated before a standardized, state-wide CO<sub>2</sub> trapping program is implemented.

Most trap efficiency evaluations have focused on field trials (e.g., Tikasingh and Davies 1972, Smith et al. 1979, Reisen and Pfuntner 1987, Milby and Reeves 1989) to determine which type collects the most mosquitoes. Relatively few researchers have included an engineering analysis of trap design with field data (Barr et al. 1963, Pfuntner 1979, Driggers et al. 1980 and Klein 1999). The objective of our study was to conduct air-

flow, motor rpm and electrical measurements on four different types of mosquito traps designed by the following: University of California, Davis, Arbovirus Field Station (AFS), Orange County Vector Control District (OCVCD), J.W. Hock® Company (JHC), and American Biophysics® Corporation (ABC). Results from the engineering analysis were compared with field performance data obtained from comparative trap studies conducted concurrently during the summer of 1998 by OCVCD in Orange County and by the AFS in Kern County (Reisen et al. 2000).

Some questions we attempted to answer in this analysis and the accompanying field trials were these:

- Do measurable differences in airflow correlate with catch size?
- What features of trap design are important?
- Do traps with light-sensitive photoswitches work better?
- Does the decrease in battery voltage at the end of the collection period adversely affect airflow?

MATERIALS AND METHODS

**Trap Components:**

Two traps of each design (8 total) were used in this engineering analysis. The CDC-style traps (AFS, JHC and ABC) were powered by rechargeable 6-volt, 10 ampere-hour, Eagle-Picher® (Model CFM6V10) gel cell batteries. They also used similar Mabuchi® motors with 4-bladed, Thorgen® fans mounted in comparably sized, 3-in. (76-mm) internal diameter plastic tubes. In contrast, the EVS-style, OCVCD trap used a Mabuchi® motor powered by three 1.5 volt, D-cell batteries (4.5 volts total) and employed a long, 2-bladed fan housed in a larger, 4.5-in. (115-mm) internal diameter plastic tube.

The AFS traps were constructed by AFS personnel and were made from 3.25-in. (83-mm) internal diameter, 5-in. (127-mm) lengths of Class 200 PVC pipe. A bracket mounted inside each trap supported a RF-510T motor with a 3-in. (76-mm) diameter, 4-bladed fan. The AFS design did not use a light as an attractant or have a rain shield placed over the top, unlike the other traps.

The J.W. Hock® Company Model 1012 trap contained a 2.875-in. (73-mm) diameter, 4-bladed fan on a RF-500TB motor mounted into a 3-in. (76-mm) internal diameter, 2.75-in. (70-mm) long, clear Plexiglas® cylinder. A light-sensitive photoswitch on the trap controlled both the motor and a bright light, activating them at dusk. The JHC trap had a large, 14-in. (356-mm) diameter rain shield mounted 1.25-in. (32-mm) above the inlet. It extended well over the trap periphery. The high-intensity light bulb was positioned directly over the motor in the center of the trap inlet area, underneath the rain shield.

The American Biophysics® Corporation ABC Trap consisted of a 2.875-in. (73-mm) diameter, 4-bladed fan on a RF-500TB motor fastened into a 3-in. (76-mm) internal diameter, 4-in. (102-mm) length of Schedule 40 PVC pipe. A night-activated photoswitch circuit controlled both the motor and light. A large, 12.75-in. (324-mm) diameter rain shield attached to the bottom of the dry ice container extended closely over the top of the trap. A light bulb was enclosed in a piece of PVC pipe positioned in the center of the inlet above the mo-

TABLE 1. Trap data for four types of mosquito traps run for 14-18 hours

Trap Model	Start Voltage	Decrease (Volts)	Light	Motor Current mA (% total)	Motor rpm	Inlet Velocity	Air Volume
ABC	+ 6.3	- 0.36	Yes	~ 92 mA (60%)	~ 2800	~ 8.3 ft/s (2.5 m/s)	~ 18.0 ft <sup>3</sup> /min (0.51 m <sup>3</sup> /min)
JHC	+ 6.3	- 0.37	Yes	~115 mA (40%)	~ 3300	~ 18.0 ft/s (5.5 m/s)	~ 28.0 ft <sup>3</sup> /min (0.79 m <sup>3</sup> /min)
OCVCD	+ 4.6	- 0.55	Yes	~ 46 mA (34%)	~ 1700	~ 5.5 ft/s (1.7 m/s)	~ 25.0 ft <sup>3</sup> /min (0.71 m <sup>3</sup> /min)
AFS	+ 6.3	- 0.17	No	~ 50 mA (100%)	~ 2600	~ 9.2 ft/s (2.8 m/s)	~ 21.0 ft <sup>3</sup> /min (0.59 m <sup>3</sup> /min)

TABLE 2. Comparative rankings of four types of mosquito traps, correlating air stream inlet velocity, discharge volume, light use and direction of trap access with field results

Trap Model	Relative Inlet Velocity	Relative Air Volume	Light	Direction of Mosquito Access to Trap Inlet	Relative Rank *
AFS	Medium	Medium	No	Top & Side (No Cover)	First
OCVCD	Low	Medium	Yes	Side of Small Cover	Second
JHC	High	High	Yes	Underneath Rain Cover	Second
ABC	Medium	Low	Yes	Underneath Rain Cover	Third

\* Rankings based on catch size in field tests; see Reisen et al. (2000) for data.

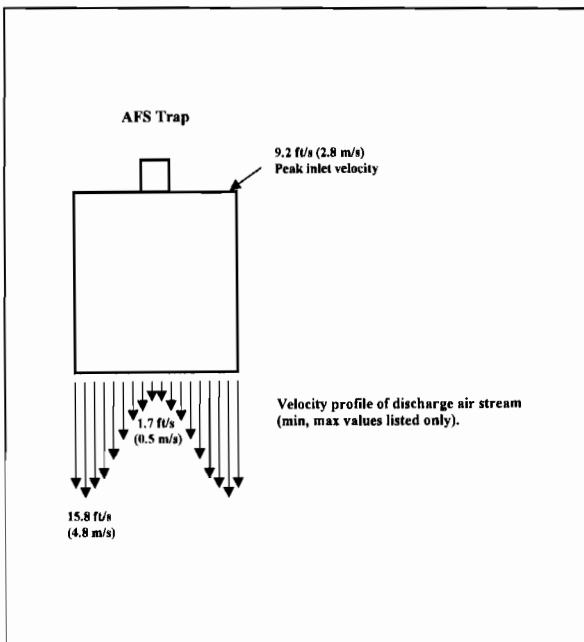


Figure 1. AFS trap and its discharge velocity profile (not to scale).

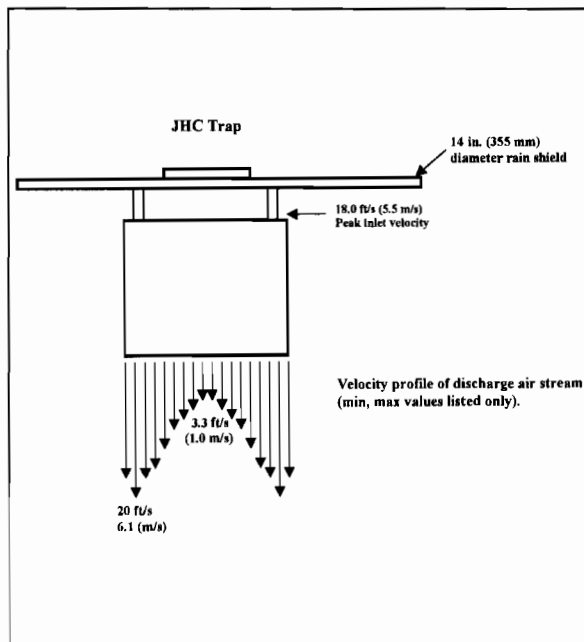


Figure 2. JHC trap and its discharge velocity profile (not to scale).

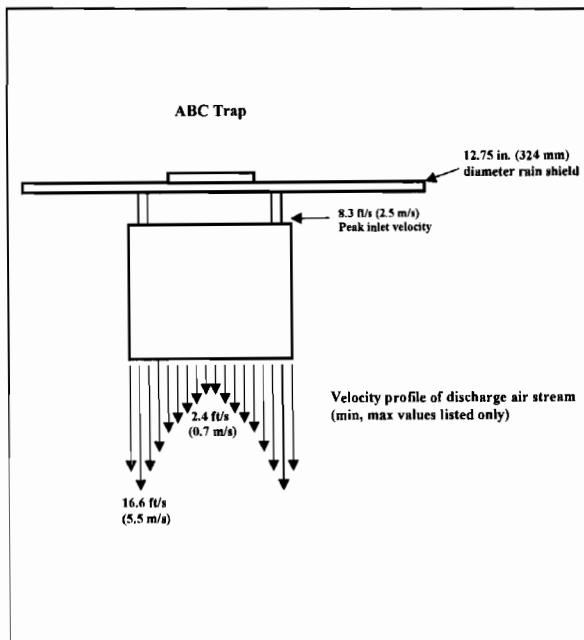


Figure 3. ABC trap and its discharge velocity profile (not to scale).

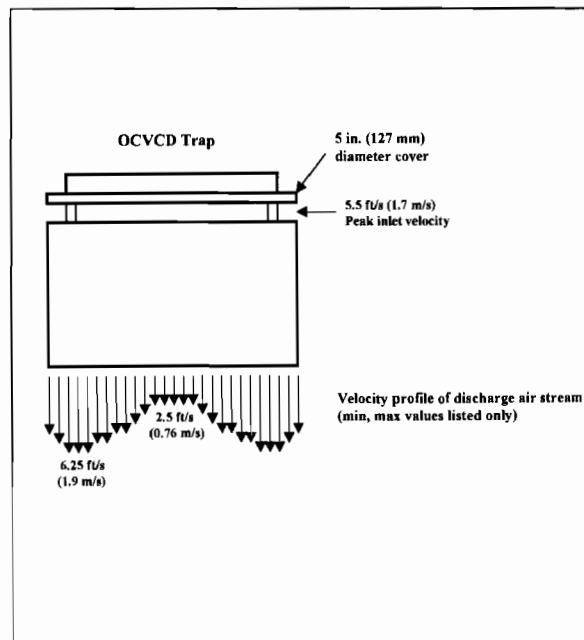


Figure 4. OCVCD trap and its discharge velocity profile (not to scale).

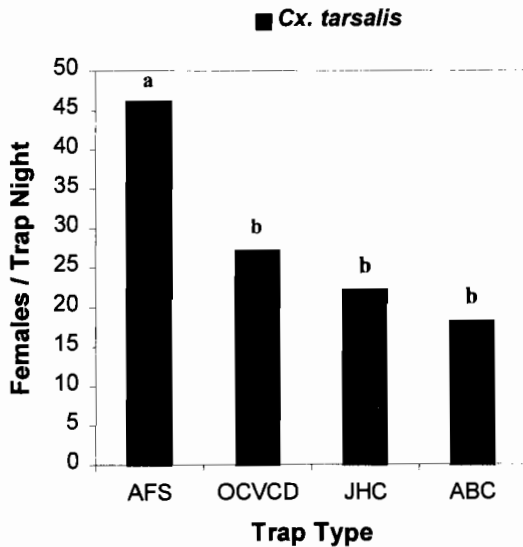


Figure 5. Effects of trap type on the catch of *Culex tarsalis* females at Tracy Ranch, Kern County, 1998. 64 trap nights total. Bars with the same letter were not significantly different when tested by a least significant range test ( $P > 0.05$ ). (Reisen et al. 2000).

tor, underneath the rain shield (similar to the JHC design). This model also had a perforated metal plate with 0.25-in. (6.4-mm) diameter holes in it for mosquitoes to pass through covering the inlet.

OCVCD personnel built the two EVS-style traps used in this study, incorporating several improvements from earlier models (J.P. Webb, M. B. Madon and others, unpublished). The OCVCD version used a RF-510T motor with a 4.25-in. (108-mm) long, 2-bladed fan housed in a 4.5-in. (115-mm) internal diameter, 5-in. (127-mm) long, ABS pipe-coupler hub. Both traps had a 5-in. (127-mm) diameter cover, mounted 0.75-in. (19-mm) above the inlet, which was used to support three 1.5 volt, D-cell batteries. Each trap also used a small, 1-Watt, high-intensity light. BioQuip® Products sells a commercial version of this design, copied by permission from OCVCD.

### Trap Testing

Traps were tested in a large, closed room with little air movement, located at an elevation slightly above sea level. Air temperature and humidity were fairly stable during the test period, ranging from 63°-66° F (17.2°-18.9° C) and 51-67% relative humidity. Because these conditions were close to standard air-table values measured at 59° F (15° C) at 1 atm. pressure, no correction was needed to account for mass balance changes in airflow from non-standard conditions for pressure, temperature and humidity.

All eight traps were run simultaneously for 14-18 hours, simulating an average night's operation (set up in the early afternoon with retrieval the following morning). The photoswitch-controlled traps, JHC and ABC models, were run for 14 continuous hours, because the light-sensitive photocells would not start these traps until the early evening, four hours after deployment of the AFS and OCVCD traps.

Measurements were taken every two hours on battery voltage, electrical current flow, fan blade rpm, and air stream inlet/discharge velocities for each trap. Two digital multimeters were used simultaneously to measure voltage and amperage. A Monarch Instruments® Nova Strobe (Model AA) stroboscopic light was used to gauge motor rpm. A Kurz Instruments® Model 491 mini-anemometer with a 3/32-in. (2.5-mm) wide probe was used to determine air stream velocity. A digital thermohygrometer was used to record air temperature and humidity.

The inlet velocity of the incoming air stream was measured by placing the anemometer probe at the outside edge of the trap inlet. Only the highest reading was recorded and used for comparative purposes.

The mean discharge velocity of the exiting air stream was determined by taking velocity measurements with the mini-anemometer probe at many set intervals in the discharge stream of each trap. With the motor running, each trap was inverted and held in place in a vise while the anemometer probe was moved along a diameter line from one side of the trap to the other. Measurements were taken every 3/32-in. (2.5-mm) in the discharge stream of each CDC-style trap for a total of 31 data points. Readings were taken every 1/4-in. (6-mm) in the discharge stream of the larger diameter OCVCD trap for a total of 19 data points. From these values, a discharge velocity profile was constructed for each trap design. The arithmetic mean then was calculated from this profile to determine the average air stream discharge velocity for each model. Next, the volume of airflow was determined by multiplying the mean discharge velocity with the cross-sectional area of each trap type. Overall, variations between two traps of the same model were very slight.

## RESULTS

The gel cell batteries of the CDC-style traps (AFS, JHC and ABC) started with a full charge greater than 6.3 volts and decreased less than 0.5 volt over the 14-18 hours of running. The highly efficient Mabuchi® motors and strength of the gel cell batteries provided a nearly unvarying set of numbers throughout the test period for

all three trap types. There were very slight reductions in motor rpm for each trap, and consequently, air stream velocities decreased little for each design throughout testing. Because changes from start to finish were small, only average values for the 14-18 hour test period are listed in Table 1. The JHC traps were the most powerful, generating relatively high air stream velocities and volume flows. Data for the AFS traps remained the most consistent over the duration of the test. The absence of a light enabled all the current supplied by the battery to go to the motor, resulting in only a slight decrease in voltage (-0.17 V) over 18 hours of operation. Lights on the other versions drew either 40% (ABC) or 60% (JHC) of the supply current.

OCVCD's EVS-style traps underwent the greatest voltage decrease (- 0.55 V) during the test period. Additionally, most of the current draw went to the light (66%), rather than the motor. This design also had the lowest motor rpm and air stream velocity values. Although these numbers were less, the volume of air moved was actually higher than the AFS and ABC models (Table 1), because of the larger cross-sectional area in the OCVCD version.

Graphical analysis of the parabolic-shaped velocity profiles provided an accurate method of determining the mean discharge velocity for each style of trap (Figs. 1-4). As shown in the drawings, the strongest cur-

rents came off the ends of the spinning fan blades along the trap periphery, whereas airflow near the center was much lower. It should be noted that if a large anemometer had been used to measure discharge velocity instead of one with a small probe, only the highest air stream values would have been detected. These distinct variations in velocity within a cross section of each trap's exiting air stream must be averaged together to obtain a reasonably accurate indication of airflow volume, as was done in this study.

## DISCUSSION

Performance changes from the beginning to end of the 14-18 hours of trap operation were relatively slight, implying that for each trap type, there should be little change in collection capability in the morning hours compared to the early evening. As long as batteries begin near peak voltage, motor rpm and airflow should remain relatively unchanged. Although many studies have emphasized using fresh batteries for each night's collection, our data indicate that battery strength above a reasonable minimum should not adversely affect catch size, agreeing with Pfunter (1979). Repeated use of one set of batteries for three to four nights would probably not lead to differences in mosquito counts when using traps with highly efficient motors.

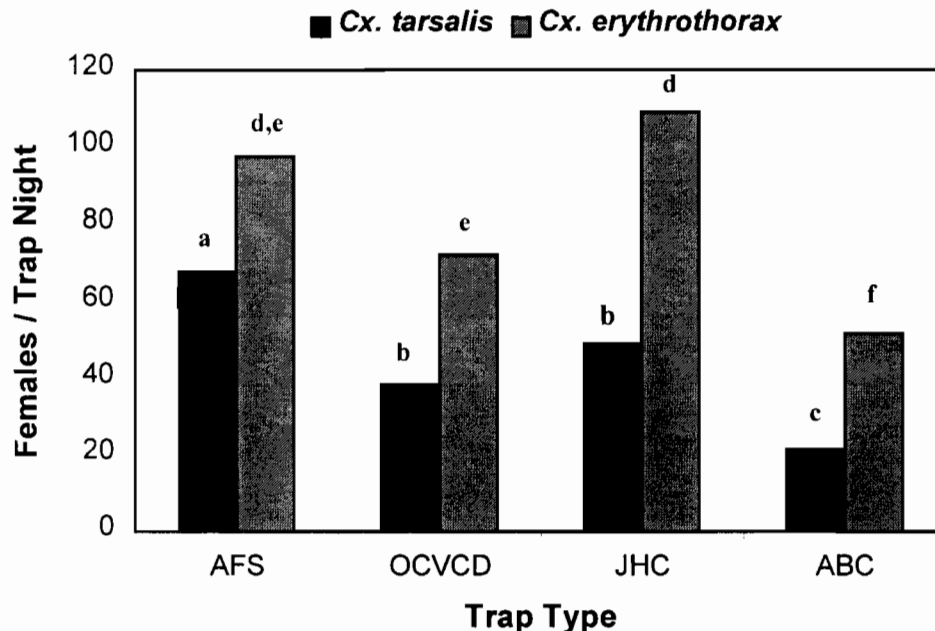


Figure 6. Effects of trap type on the catch of *Culex tarsalis* and *Culex erythrothorax* females at the San Joaquin Marsh, Orange County, 1998. 188 trap nights total. Bars with the same letter were not significantly different when tested by a least significant range test ( $P > 0.05$ ). (Reisen et al. 2000).

Comparative field data from concurrent studies in Kern and Orange counties in the summer of 1998 show that the AFS traps caught significantly more mosquitoes than the other three designs (Reisen et al. 2000). In terms of raw, unadjusted numbers, the AFS trap placed first, followed by the OCVCD, JHC and ABC traps (Reisen et al. 2000). The relative rankings are listed in Table 2 and comparative trap data for *Cx. tarsalis* collections in Kern and Orange Counties are shown in Figures 5 and 6, respectively.

Results for the OCVCD and JHC traps were similar statistically for collecting host-seeking *Cx. tarsalis* females at both habitats (Reisen et al. 2000). The JHC trap worked well at catching large numbers of *Cx. erythrothorax* adults (Figure 6) in the San Joaquin Marsh in Orange County, a prime habitat for this species. However, there was no significant difference between the AFS and JHC traps or between the AFS and OCVCD traps for *Cx. erythrothorax* at this site (Reisen et al. 2000). Finally, when considering data for all species from both trials, the ABC design was the least effective and placed third overall.

A distinguishing feature of the AFS trap was its lack of a rain shield over the trap inlet area (as well as no light). The OCVCD trap version had the smallest cover of the other three, extending only to the outside edge of the trap body. The JHC model caught surprisingly fewer mosquitoes than expected, considering its elaborate engineering and powerful motor. Even with the highest inlet velocities and airflow, the JHC traps proved to be less effective at collecting mosquitoes than the simpler AFS model and no better than the less forceful OCVCD traps. A possible explanation may be that its large rain shield reduced access of host-seeking adults by forcing mosquitoes to approach the trap inlet from the side and below. The relatively ineffective ABC Trap had several possible design flaws. This model also had a large rain shield, which, like the JHC traps, may have reduced mosquito access. Additionally, the perforated metal plate at the trap inlet proved to be very restrictive, resulting in the lowest airflow of the four designs (Note: American Biophysics® Corporation discontinued this early version several years ago).

Results from this engineering analysis, the accompanying field trials (Reisen et al. 2000), and Klein's (1999) recent study of American Biophysics'® counterflow trap configuration suggest that trap inlet design significantly affects catch size. In order to allow for easy access of host-seeking adults, the manner in which mosquitoes approach a trap must be considered

in the design process. Because California's weather (especially in Southern California) is relatively dry when most mosquito-borne disease and vector abundance surveillance is done, a protective cover over the trap inlet is unnecessary and may lower collection efficiency for our local species. Other additions, such as night-activated photoswitches and lights, also appear to be unnecessary. Photoswitch circuits can be expensive, and lights needlessly consume battery power without providing a notable increase in attractiveness for most *Culex* mosquitoes (Barr et al. 1960, Reisen et al. 2000). In conclusion, a relatively simple trap design incorporating an efficient motor and fan, long-life batteries, an unobstructed inlet, combined with proper trap placement in elevated vegetation (Schreiber et al. 1989, Reisen et al. 2000), should produce optimal results when sampling populations of host-seeking mosquitoes.

#### Acknowledgements

The authors thank Greg Pierson, Test Engineer, Ingersoll-Rand Pump Company, for supplying the Kurz mini-anemometer used in conducting airflow measurements. Gratitude is also extended to the San Gabriel Valley MVCD for use of the ABC traps.

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# Temporal Abundance of Mosquitoes at Microhabitats in the Coachella Valley, California

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**ABSTRACT:** At four of five sites during 1997 and three sites in 1998, *Culex tarsalis* host-seeking abundance, as measured by CO<sub>2</sub>-baited traps, closely paralleled the physical profile of vegetation. Four experiments done bimonthly from April through October 1998 indicated that this relationship did not change greatly throughout the virus surveillance season.

## INTRODUCTION

In California, mosquito and vector control agencies use CO<sub>2</sub>-baited traps to measure mosquito abundance and sample arbovirus infection rates in vector species. It has been generally observed that variation in habitat affects trap catch size. Therefore, trap placement is critical in optimizing collection efforts and accurately representing regional host-seeking mosquito abundance. To determine the magnitude of this variation and associate microhabitat with trap efficiency, we sampled five sites in 1997 (Lothrop and Reisen 1998) and three sites in 1998 around the northwestern margin of the Salton Sea (Fig. 1). To measure the behavior of host-seeking mosquitoes during the spring, summer, and fall, a comparison was conducted bimonthly at one site from April through October 1998. This experiment allowed us to evaluate the impact of temporal variables in experiments conducted at different sites and times in both years.

Site 6, a heron rookery, was sampled to determine if a large concentration of adult and nestling birds would attract host-seeking mosquitoes to offshore snags. Site 7, the agricultural environs and interior of the town of Mecca, was selected to determine the potential exposure of residents in towns within the zone of yearly virus activity. The results of these two experiments will be discussed in a future publication.

The exposure of bird species involved in enzootic transmission depends, in part, on how their nocturnal roosting and nesting habits interface with host-seeking *Culex tarsalis* Coquillett. To measure host-feeding patterns in these microhabitats, we made concurrent resting collections of engorged females at representative habitats within transects at all sites in 1997 and at site 1 in 1998.

## MATERIALS AND METHODS

Bi-monthly samples were taken at site 1 at 81<sup>st</sup> Street on the west shore of the Salton Sea. Six CO<sub>2</sub> traps were positioned within different microhabitats along each of three roughly parallel transects perpendicular to the shore of the Salton Sea. Two additional traps from our mosquito surveillance trap grid for the lower valley were run concurrently as controls. Traps were operated on three consecutive days during each experiment (54 trap-nights in each experiment, excluding controls). Habitats sampled were 1) sandbar, 2) shoreline tamarisk, 3) saltgrass/pickleweed, 4) mesquite clumps, 5) citrus orchard edge, and 6) citrus orchard interior. Artificial resting units [32-gallon plastic garbage cans] were set in habitats 2,3,4, and 6 to collect blood-engorged females for planned host identification studies.

## RESULTS AND DISCUSSION

Traps set in microhabitats with the tallest vegetation profile had the greatest mosquito host-seeking abundance. These results were consistent among three experiments in 1998 and in four of five experiments in 1997.

Overall, means of abundance for microhabitats in the temporal experiment ranked from lowest to highest were sandbar, saltgrass/pickleweed, shoreline tamarisk, mesquite, citrus interior, and citrus margin (Table 1). The edge of the habitat with the tallest physical profile had the greatest mosquito abundance. Differences among bimonthly sample periods were related largely to overall mosquito abundance. In April, catch at shoreline tamarisk was nearly as high as citrus, whereas in June, catch at shoreline tamarisk was lower than mesquite, citrus edge and citrus interior. The abundance profile



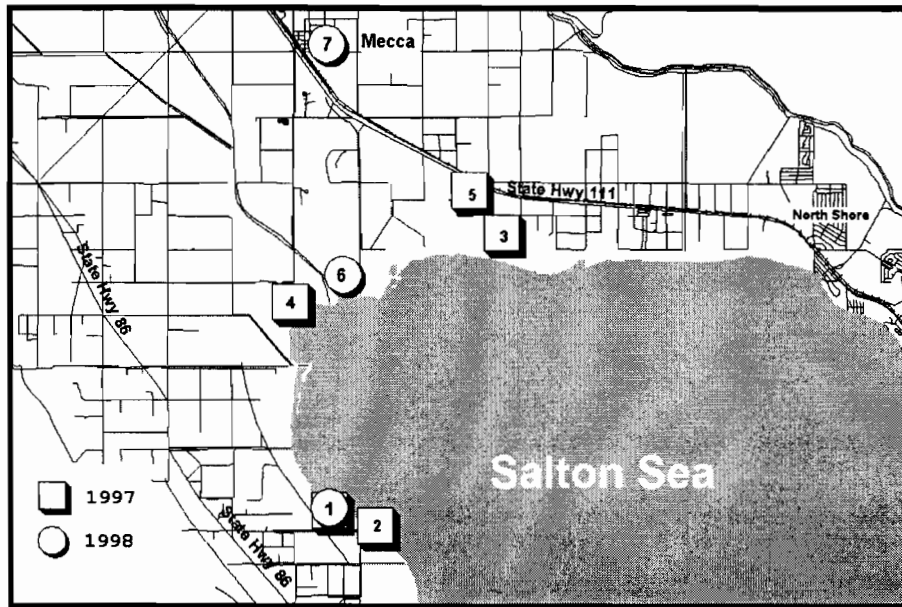


Figure 1. Map of study area showing sites for 1997 and 1998. Site 1 is temporal study site in 1998.

for August showed fewer differences among habitats, although catch at citrus remained highest. October was similar to April and June, except that citrus interior was lower than mesquite.

Most *Cx. tarsalis* were collected in resting units placed in citrus habitat. These results were consistent throughout the season. Feeding success seemed greatest in citrus, because the majority of blood-fed females were collected in this habitat.

In conclusion, *Cx. tarsalis* females appear to seek hosts roosting or nesting within taller profile vegetation because traps hung in these microhabitats consistently have the greatest catch. Seasonal changes in mosquito abundance, bird nesting/roosting behavior, temperature, and humidity did not greatly influence this pattern. These data strengthen the results of our previous blood meal host identification study where most avian and mammal meals came from passeriform birds that roost in elevated vegetation such as mesquite and citrus or rabbits that exploit these ecotones (Lothrop et al. 1997).

#### Acknowledgements

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TABLE 1. *Cx. tarsalis* geometric mean abundance [females per trap night] at each microhabitat during four bimonthly experiments.

Month	Sandbar	Shore/Tam	Saltgrass	Mesquite	Citrus Edge	Citrus Interior
April	27.0	745.2	352.0	554.0	795.6	920.1
June	1.7	79.7	21.1	107.0	100.0	94.2
August	1.7	8.9	7.0	5.7	13.2	14.0
October	1.0	22.8	7.4	39.9	48.9	27.3
Mean	3.5	60.3	25.9	62.7	85.9	77.1

## Distribution and Abundance of Culicidae and Chironomidae (Diptera) Following Storm Damage in a Southern California Constructed Wetlands

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**ABSTRACT:** A 122 ha constructed wetlands in the Prado Basin (Riverside Co., CA) was sampled during 1998 to determine factors influencing the distribution and abundance of mosquitoes (Culicidae) and non-biting midges (Chironomidae). Four CO<sub>2</sub>-baited traps in close proximity to the wetlands demonstrated that host-seeking populations were low, with 0-199 total adults (mostly *Culex quinquefasciatus*) taken on any one night. Emergence trapping and dipping were conducted from 24 July-9 October. Sixty-three sampling stations were placed in shallow 0.3 m (1.1 ft) and deep 0.6 m (2.2 ft) ponds at peripheral and central areas of stands of cattails (*Typha* sp.), California bulrush (*Schoenoplectus californicus*), and bulrush stands choked with duckweed (*Lemna minor*); additional stations were deployed over emergent buttercups (*Ranunculus flammula*) and submerged buttercups (*R. aquilis*). One emergence trap (0.25m<sup>2</sup>) was deployed and three dips executed on a weekly basis at each station. Mosquitoes were taken infrequently (40 adults and 16 immatures taken during entire sampling period). Emergence of *Culex* occurred 24 July-28 August while *Anopheles* emerged 14 August-9 October; however, no corresponding pattern in larval populations was detected. In contrast to the low numbers of immature mosquitoes, over 2,200 non-culicid aquatic macroinvertebrates were collected.

More than 35,000 adult midges were taken in emergence traps. Both species of *Ranunculus* produced low numbers of midges, while *Typha* and *Schoenoplectus* exhibited the largest emergent populations. Central areas of plant stands in deep ponds were on average the largest contributors to adult midge populations. No difference was apparent in the duckweed-choked and duckweed-free stands of *Schoenoplectus*. The large midge and non-mosquito macroinvertebrate populations demonstrate that these wetlands are productive, thus the low mosquito numbers are surprising. Recent disturbance by extreme vernal storms and subsequent flood events probably reverted the system to a successional stage ecologically unfavorable for mosquito production.

### INTRODUCTION

The Prado Constructed Wetlands (CA, Riverside Co.) is a series of interconnected marshes designed to polish tertiary-treated water diverted from the Santa Ana River. The system was put into operation in 1996 by the Orange County Water District. The close proximity of dense human populations makes these wetlands an abatement concern due to their potential as a developmental site for mosquitoes (Diptera: Culicidae). Other constructed wetlands in Riverside County have produced large numbers of host-seeking females (e.g., Walton et al. 1990, Walton and Workman 1998), and the Prado Basin has previously been shown to harbor pestiferous

and disease-transmitting species (Mian et al. 1990). However, unusually heavy vernal flooding in 1998 caused large volumes of water to move through the wetlands, which damaged water control structures and access berms (B. Baharie, OCWD, pers. comm.). This severe disturbance undoubtedly affected the biological communities there. Portions of the wetlands survived the spates relatively undamaged despite being temporarily inundated by flood waters, and the established vegetation remained intact in these areas.

Efforts to rebuild were conducted during the spring and summer months, and the wetlands were in operation by July. This situation provided an opportunity to study the recolonization patterns of mosquitoes and other

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aquatic insects by documenting adult emergence and the distribution of immature insects in the recently disturbed marsh. The surrounding natural areas and dairy farm drains provided potential sources of adults, and surviving vegetation constituted adequate habitat for mosquito colonization (e.g., Collins and Resh 1989, Walton and Mulla 1989). We attempted to determine if mosquito and midge (Diptera: Chironomidae) distribution and abundance were affected by wetland plant species and water depth variations within the constructed wetlands; the populations of other aquatic macroinvertebrates were also investigated. With these data available, sound abatement strategies can be applied to specific areas within large constructed wetlands, the size of which may prohibit application of control measures to the whole system. These data are also necessary for the development of vegetation management practices which enhance insect populations beneficial to maintaining biodiversity, waterfowl food production, and ecosystem function.

## MATERIALS AND METHODS

The 122 ha Prado Constructed Wetlands consist of 46 individual marshes (hereafter referred to as ponds) separated by earthen berms and interconnected by water control structures. The naturally-established vegetation included cattails (*Typha* sp.), California bulrush (*Schoenoplectus californicus* Meyer [Soják]), buttercups (*Ranunculus aquatilis* var. *capillaceus* [Thuill.] and *R. flammula* var. *ovalis* [Bigel.]), smartweeds (*Polygonum* sp.), pennywort (*Hydrocotyl ranunculoides* L.), pondweed (*Potamogeton* sp.), and duckweed (*Lemna minor* L.). Bulrush and cattails predominated, and buttercups lined the margins of many ponds. Repairs of storm damage were completed and normal wetlands operation (60-80 cfs) was resumed on 17 July 1998.

Sampling stations were set up in shallow 0.3 m (1.1 ft) and deep 0.6 m (2.2 ft) ponds within stands of cattails, bulrush, and bulrush stands choked with duckweed; an additional seven stations were positioned over emergent buttercups (*R. flammula*) and four over submerged buttercups (*R. aquilis*) in deep ponds only. One emergence trap (0.25m<sup>2</sup>) was deployed at each station, and vegetation cut to approximately 5-10 cm above the water level to permit trap placement without altering the physical structure below the water surface. The bottoms of traps remained below the water surface, and growing vegetation was cropped with shears weekly to prevent them from being tipped. Twenty-four traps were deployed in cattails (12 each in shallow and deep ponds), and 22 in bulrush (10 in shallow and 12 in deep ponds);

six traps were placed in duckweed-choked bulrush stands (deep ponds only) to determine if this floating macrophyte effects dipteran emergence when compared to duckweed-free bulrush sites. Traps in cattails and bulrush were divided equally between those positioned at peripheral and central locations of plant stands; those in central locations were approximately 3-5 m from the periphery of the stand. Funnels were inserted in 0.45 L screw cap jars, and the jars were secured to the top of the emergence traps to capture emergent insects. Jars were deployed during morning hours, recovered after approximately 95-97 hr, and returned to the laboratory where they were frozen to kill the specimens. Adult mosquitoes were identified to species, whereas midges were taken to family only. Traps were run weekly from 20 July through August 1998, and biweekly from September to 9 October 1998.

Larval mosquitoes and other aquatic macroinvertebrates were sampled with a standard 300 ml dipper. Three dips were taken at each station just prior to jar collection and combined in a concentrator cup. All specimens were preserved in 95% ethanol for sorting, identification, and enumeration.

Four dry ice (CO<sub>2</sub>)-baited traps were run for 24 hr on an approximately weekly basis at localities near the wetlands from 12 May - 24 November 1998 to monitor host-seeking activity. Two were placed at the perimeter of the wetlands; one at the diversion channel which supplies water to the wetlands (33° 55' 27" N 117° 36' 99" W) and the other on a suburban road (Bluff St.) bordering the wetlands (33° 54' 84" N, 117° 36' 84" W). The others were situated at a nearby rural park (Prado Park, 33° 55' 67" N, 117° 36' 11" W) and a dairy drain (McCarty Slough, 33° 55' 86" N, 117° 36' 92" W) for comparative purposes.

## RESULTS AND DISCUSSION

A surprising low number of adult mosquitoes (n=40) was obtained with emergence traps. *Culex tarsalis* Coquillett, *Culex quinquefasciatus* Say, *Culex erythrothorax* Dyar, *Culex stigmatosoma* Dyar, *Anopheles hermsi* Barr and Guptavanji, and *Anopheles franciscanus* McCracken were represented in the emergence data. Mian et al. (1990) obtained three species of *Culiseta* in addition to the taxa listed above during their sampling in the Prado Basin, but did not report *An. franciscanus* from their collections. Their work was conducted during 1985-1986, approximately 10 years prior to the construction of the present wetlands system. This suggests that construction efforts, flooding, eco-

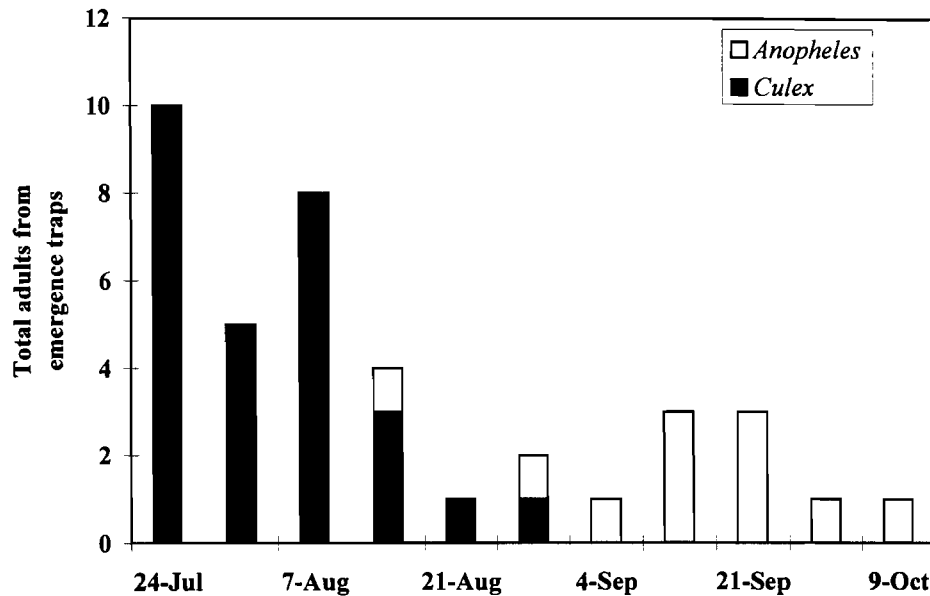


Figure 1. Total *Anopheles* and *Culex* adults taken in emergence traps at Prado Wetlands, 1998.

logical succession, or any combination of these factors may have caused the composition of local mosquito species to change during this period.

A disjunct emergence pattern between *Culex* spp. and *Anopheles* spp. is evident (Fig. 1). During the early sampling efforts (24 July - 7 August), only *Culex* were obtained, and during the later periods (4 September - 9 October) *Anopheles* represented the only adults taken. Only during the short period of 14-28 August did the two genera overlap. The fact that *Culex* spp. numbers dropped off as the summer progressed is not surprising. *Anopheles* spp. populations are known to thrive in the presence of algal blooms, and we noted that the appearance of *Anopheles* coincided with the late summer development of extensive masses of the filamentous green alga *Spirogyra* (Chlorophyta). This potential food source was probably beneficial to the small *Anopheles* sp. populations present, and may explain the disjunct temporal distributions of the two genera. Algicides are periodically applied to ponds to counter blooms of the green alga "water-net" (Chlorophyta: *Hydrodictyon*) which are known to entrap and cause high mortality among mosquito fish (*Gambusia affinis*). Due to the trophic association of *Anopheles* and algae-laden fresh waters (Meyer and Durso 1998), the Prado wetlands should be considered an excellent potential breeding site for this vector of human malaria, and applications of algicides targeted at ponds exhibiting *Spirogyra* blooms may represent a colonization deterrent or an alternative abatement strategy here. A recent human malaria outbreak

in southern California was attributed to undocumented workers who acquired their infections before entering the US (Maldonado et al. 1990).

The CO<sub>2</sub> traps on the wetlands periphery indicated a low population of host-seeking mosquitoes in the general area, despite the close proximity of marshes, duck ponds, and dairy farm sewage lagoons. The number of host-seeking adults obtained in one trap during any 24 hr period never exceeded 200, and a total of only 2,817 adults were taken (Figs. 2 a-d). *Culex quinquefasciatus* represented 84.3% of the collection, while *Cx. tarsalis*, *Cx. stigmatosoma*, and *Cx. erythrothorax* comprised 9.3%, 3.6%, and 2.8% respectively. While collections of *Cx. quinquefasciatus* were low (8.8-41.2 per trap night), the other three *Culex* sp. were comparatively insignificant (0.1-8.0 adults per trap night). Twenty-two adults of the genera *Culiseta*, *Anopheles*, and *Aedes* were collected, representing <1% of the total catch (0.3 adults per trap night) (Table 1).

*Culex quinquefasciatus* adults were obtained mostly at the dairy drain (35.2%) and Prado Park (36.4%); only 672 specimens (28.3%) were taken at the other two sites adjacent to the wetlands. Mian et al. (1990) reported taking nearly 300 *Culex* sp. adults per trap night from the Prado Basin during the 1985-86 breeding season, and obtained over 200 adults per trap night regularly.

Dip samples also produced sparse numbers of mosquitoes, with only 15 larvae and 1 pupa being collected in nearly 1,900 separate 300 ml dippers. Larval *Culex* and *Anopheles* did not produce disjunct temporal distri-

TABLE 1. Total number of adult mosquitoes taken at four CO<sub>2</sub> trap sites near Prado Constructed Wetlands, 1998.

SITE	<i>Cx. tarsalis</i>	<i>Cx. quinquefasciatus</i>	<i>Cx. stigmatosoma</i>	<i>Cx. erythrothorax</i>	others	TOTAL
Bluff St.	43	488	41	3	4	579
Dairy Drain	168	836	37	32	10	1,083
Diversion	20	184	0	28	6	238
Prado Park	31	866	23	17	2	939
TOTAL	262	2,374	101	80	22	2,839

butions as seen in the adult emergence data. However studies in other habitats have demonstrated this (e.g., Kramer et al. 1987). Conversely, over 2,200 non-mosquito macroinvertebrates were obtained, and were dominated by Odonata, chironomid midge larvae, and amphipod crustaceans (Table 2). Numerous crustacean zooplankters were also taken. Of the culicid larvae encountered, eight were *Culex* spp. and seven were *Anopheles* spp.

TABLE 2. Total number of non-mosquito aquatic macroinvertebrates obtained in 300 ml dip samples at the Prado constructed wetlands, July-October 1998.

TAXA	TOTAL OBTAINED
Odonata	598
Chironomidae (Diptera)	573
Amphipoda (Crustacea)	460
Corixidae (Hemiptera)	306
Ephemeroptera	209
Notonectidae (Hemiptera)	44
Coleoptera	27
Ceratopogonidae (Diptera)	7
Ephydriidae (Diptera)	3

In contrast to mosquito emergence, over 35,000 chironomid midge adults were obtained in emergence traps (Figs. 3a-c). Adult emergence was highest in central areas of bulrush and cattails in deep ponds than other sampling areas during the first seven to eight weeks (Fig. 3a-b). Midge production in emergent buttercups was consistently low (roughly 20-40 per week on average), whereas emergence from submerged buttercups produced an initial surge of almost 150 per trap on 7 August; these numbers dropped quickly to <20 by 21 August, and reached zero during the last four weeks of sampling (Fig. 3c). The dramatic decline in midge production in the vicinity of submerged buttercups coincided with its eventual senescence.

Duckweed-choked bulrush stands produced comparable numbers to those from locations free of duckweed; however, central areas produced a notable peak on 4 September when an average number of 200 midges per trap was taken (Fig. 3c). All emergence trap sites produced low numbers of midges as the sampling season came to an end (average <50 adults per trap, Figs. 3a-c).

The results obtained suggest that emergence patterns are altered by vegetation species. Midge emergence was heaviest from central areas of cattail and bulrush stands in deeper ponds; the presence of duckweed appeared to affect emergence patterns minimally and probably does not represent a physical barrier to newly-emerged adults escaping the water surface. Emergent buttercups provides a high degree of structural heterogeneity under the water surface compared to the simple structure provided by bulrush and cattail. However, the increased structural complexity did not provide improved habitat for midges relative to the other wetland plants examined. One explanation may be that larvivorous fish or invertebrate predators such as odonate larvae may find refuge from their predators, and therefore decimate larval midge populations.

Natural disturbances can have both positive and negative effects on invertebrate communities depending on the severity and predictability of the cause (Reice 1994). The intermediate disturbance hypothesis suggests that disturbance that is both unpredictable and moderate may increase the species richness of a community by keeping the populations of dominant competitors low (Connell 1978). Flowing water habitats frequently are disturbed by spates, whereas standing water environments such as lakes, ponds, and wetlands are not impacted with such regularity. Nevertheless, the vernal flooding, subsequent reconstruction, and flushing of sediments at Prado constituted an unpredicted and severe disturbance which appeared to be catastrophic to the mosquito populations which may have been present prior to these events. This probably brought the marsh

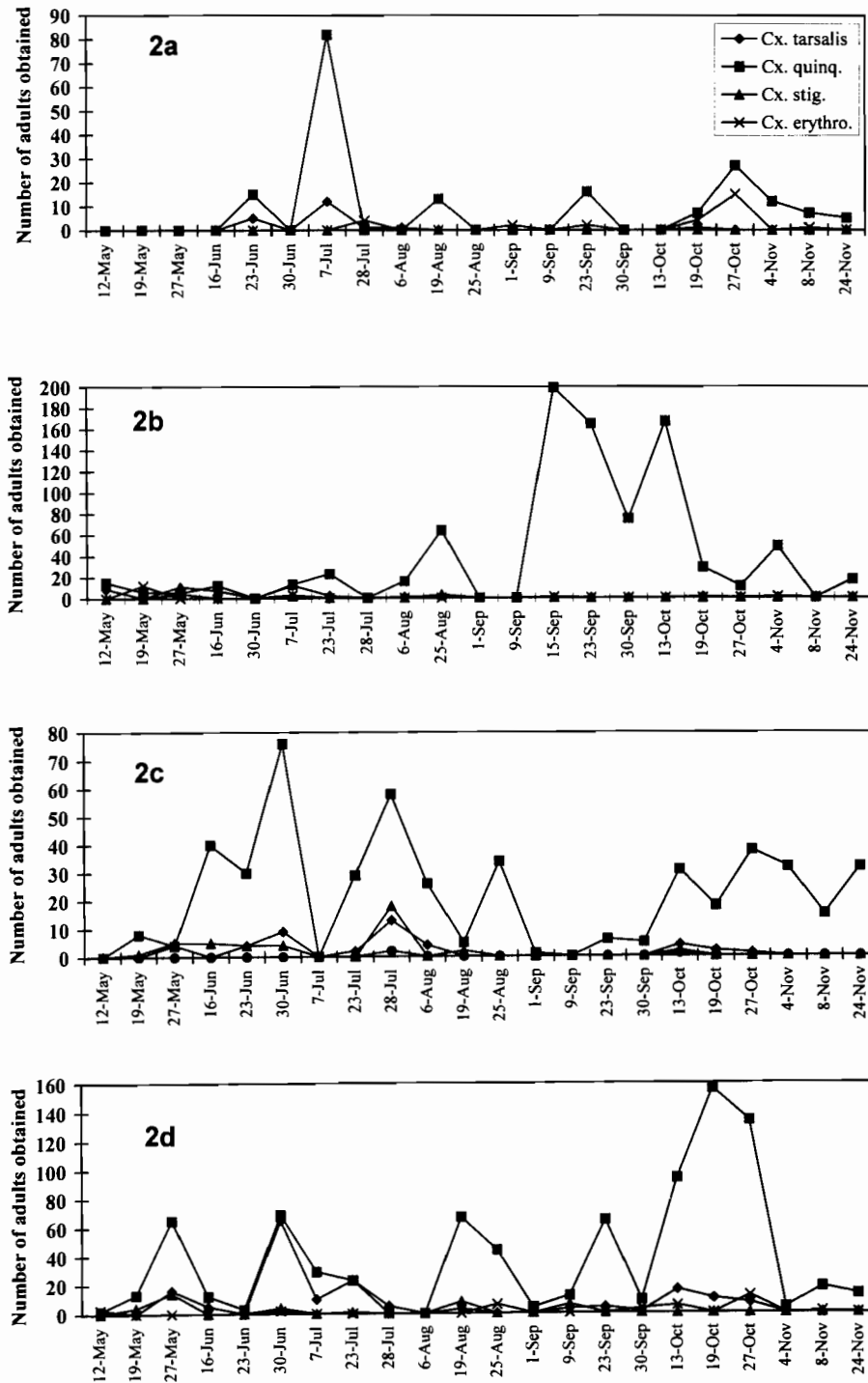


Figure 2. Number of *Culex* spp. host-seeking females taken in CO<sub>2</sub>-baited suction traps at a) Prado Wetlands diversion channel, b) Prado Park, c) Bluff Street bordering wetlands, and d) nearby dairy, 1998.

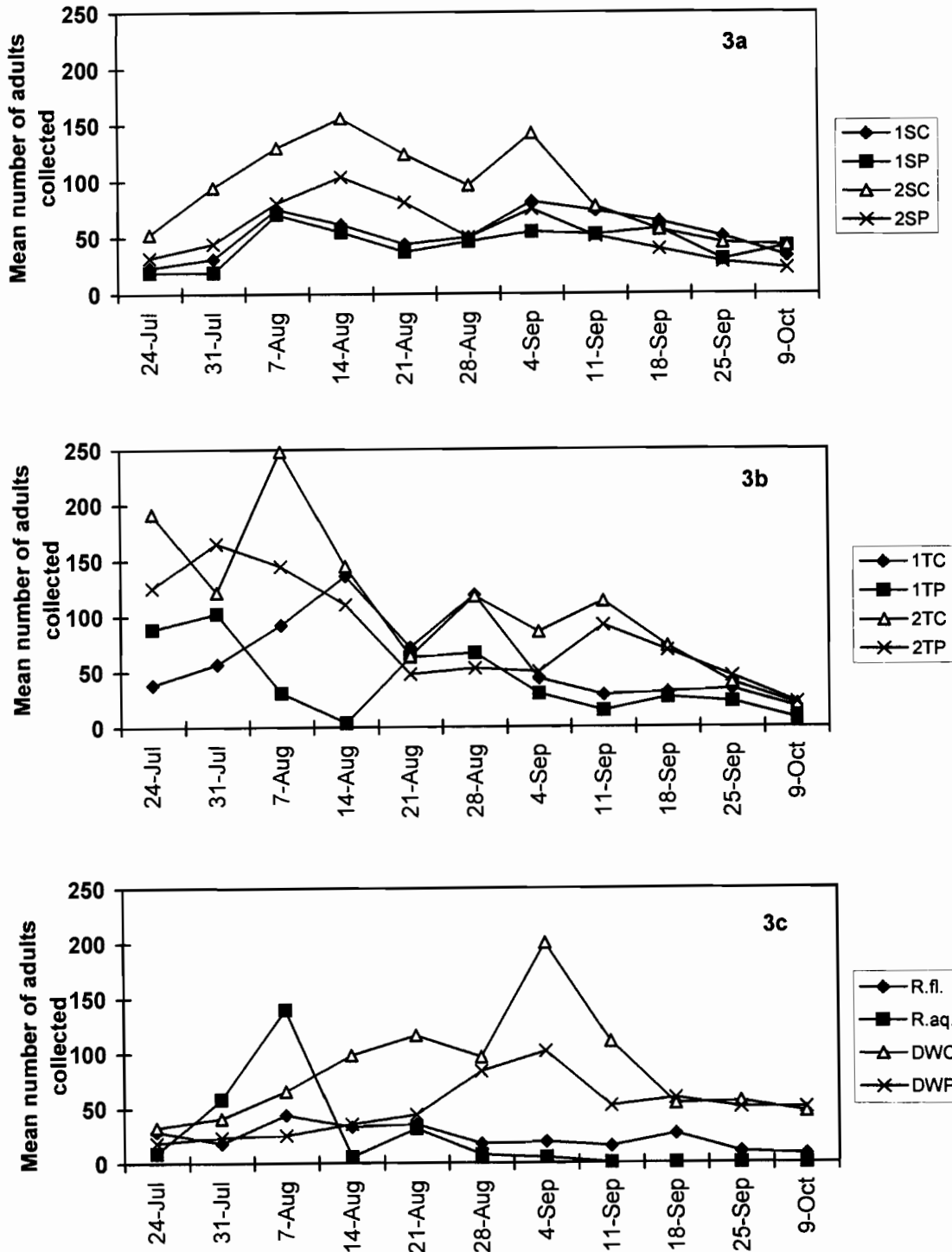


Figure 3a-c. Mean number of Chironomidae taken in emergence traps at Prado Wetlands. 1 = shallow pond; 2 = deep pond; S = *Schoenoplectus*; T = *Typha*; R. aq. = *Ranunculus aquatilis*; R. fl. = *Ranunculus flammula*; DW = duckweed-choked *Schoenoplectus*; C = central area; P = peripheral area.

to an earlier successional stage not conducive to culicid production. Conversely, the large midge populations demonstrated that the disturbance was less destructive to these insects, and that secondary production within the Prado wetlands was high. Many midge larvae were observed to reside within tubes constructed of detrital and algal particles attached to wetland plants (J.B. Keiper, unpublished data). This epiphytic existence probably made the midge populations more resistant to the flooding and flushing of the marsh. Central areas of plant stands are probably more protected than peripheral areas, and this may explain why traps placed away from the periphery of cattail and bulrush stands produced relatively high numbers of midge adults. Alternatively, the deeper water provided more submerged surface area on the inundated vegetation, therefore, the larger numbers taken in deeper ponds may be a result simply of increased substrate for larvae to reside on. However, if increased surface area on macrophytes increases midge populations, one would expect buttercups (a structurally complex plant) to produce larger numbers of emergent adults.

Too few mosquitoes were collected in 1998 to discuss the effects of vegetation type, location within plant stands, and pond depth on their distribution and abundance. However, chironomid midges exhibited distinct spatial emergence patterns within the plant stands examined. Midge larvae are important for maintaining biodiversity and supporting ecosystem functions such as energy transfer and nutrient recycling (Merritt and Cummins 1996). However, they have the potential to emerge in such high numbers in eutrophic situations that they can become a nuisance to people dwelling near these areas, and potentially causing a dilemma for constructed wetlands managers. The preliminary results reported here suggest that breaking extensive stands of *Typha* and *Schoenoplectus* into insular habitats with proportionally larger ecotone areas by harvesting or herbicide application may reduce chironomid populations. These actions should allow mosquitofish to access central areas of plant stands (Collins and Resh 1989). However, we recommend such actions only if 1) local business owners or residents complain of nuisance swarms of midges or 2) mosquito breeding is detected via dip or emergence trap data at interior areas of these plant stands. Furthermore, shallow areas at the edge of ponds seeded with emergent buttercups may keep midge emergence low, however we cannot yet draw conclusions regarding the ability of this plant to promote or deter mosquito colonization. Continued monitoring of dipteran emergence at the Prado constructed wetlands is needed to

follow these populations of potential economic concern as the system ages and vegetation proliferates; dense vegetation has been shown to promote high mosquito production in constructed wetlands in Phoenix (AZ), Tucson (AZ), Albuquerque (NM), and Hemet (CA). Furthermore, we will document the eventual colonization by mosquitoes and the spatial dynamics of adult emergence from plant stands.

### Acknowledgements

This work was supported by the Northwest Mosquito and Vector Control District and the Orange County Water District, and we thank Brian Baharie (OCWD) and Major S. Dhillon (NWMVCD) for their cooperation. Michelle Sanford, Karrie Chan, Louie Randall, R. Trudy Pachon, and L. Hannah Gould assisted greatly in the laboratory and field. We benefited from insightful discussions with Parker D. Workman and Margaret C. Wirth.

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## Evaluation of Mosquito and Arbovirus Activity in Orange County During 1998

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**ABSTRACT:** The Orange County Vector Control District continued its surveillance of mosquito and arbovirus activity throughout 1998 by collecting blood samples from wild birds and sentinel chickens, as well as collecting adult mosquitoes from ovipositional and CDC/CO<sub>2</sub>-baited traps. There were no positive mosquito pools or human cases in Orange County during 1998. However, one sentinel chicken seroconverted for SLE antibodies in April, and SLE-positive wild birds were found in most months (9) of 1998. Overall, 0.83% of the 2,294 sampled house finches tested positive for SLE antibodies. *Culex quinquefasciatus* was the most commonly trapped mosquito throughout Orange County, except for a freshwater wetland area of Irvine, where *Cx. tarsalis* was predominant.

### MOSQUITO SURVEILLANCE

The Orange County Vector Control District (OCVCD) continued its adult mosquito surveillance program throughout 1998 by collecting mosquitoes from a variety of suburban trapping sites. Mosquito collections were made at twelve permanent sites in the county, using six gravid female ovipositional traps (Cummings 1992) and nineteen CDC/CO<sub>2</sub>-baited traps (Sudia and Chamberlain 1962). In addition, blood-fed female mosquitoes were collected from one modified Australian crow trap (McClure 1984) used to capture wild birds and the mosquitoes that entered.

A total of 81 pools from 1,865 mosquitoes was sent to the University of California-Davis, Center for Vector-borne Disease Research (UCD-CVDR), for testing (Table 1). Mosquito pools were obtained primarily from ovipositional traps (67 pools), and of these, *Culex quinquefasciatus* Say composed the majority of mosquitoes pooled (66 pools). Nine pools of *Culex tarsalis* Coquillett and six pools of *Culex stigmatosoma* Dyar were also submitted for testing. No pools tested positive for either St. Louis encephalitis (SLE) virus or western equine encephalomyelitis (WEE) virus.

Gravid *Cx. quinquefasciatus* were collected most abundantly from the Seal Beach and Garden Grove trap sites (Figs. 1 and 2). The highest collection (100 gravid female mosquitoes) occurred in early July (28th week of 1998) at Hellman Ranch in Seal Beach (Fig. 2). Suburban mosquito collections by CDC/CO<sub>2</sub>-baited traps in 1998 showed an overall increase over 1997, with the

highest number of mosquitoes collected per trap night in June (Fig. 3).

Mosquito collections from the San Joaquin Marsh (Fig. 4) showed a seasonal shift in 1998 compared to 1997, with the peak occurring in August, three months later than in 1997. This shift may have been due to the increased precipitation experienced in the 1997-98-rainfall season, which extended into May. The 1996-97 season in Orange County had a total of 13.6 inches (Annoy. NOAA 1997) of rainfall, and the 1997-98 season had more than twice as much with 29.5 inches (Annoy. NOAA 1998). *Culex tarsalis* mosquitoes, for which the numbers collected in the San Joaquin Marsh were much higher in 1998 than in 1997, were the major contributors to this pattern seen in 1998 (Fig. 5).

### ARBOVIRUS SEROSURVEILLANCE

Arbovirus serosurveillance consisted of biweekly testing of one flock of ten sentinel chickens and weekly testing of wild birds, mostly house finches (*Carpodacus mexicanus*) and house sparrows (*Passer domesticus*), captured in nine modified Australian crow traps dispersed throughout Orange County. Sentinel chickens were tested for SLE and WEE antibodies by the State laboratory from April to October and the OCVCD laboratory every two weeks throughout the year. One chicken in OCVD's sentinel flock at the San Joaquin Marsh seroconverted for SLE antibodies in April (Fig. 6). A sentinel chicken also seroconverted in the same month in Los Angeles County (Fig. 6). These were the

TABLE 1. Number of mosquitoes and mosquito pools submitted for SLE and WEE virus testing by species and trap type from Orange County during 1998.

Species	Number of mosquitoes	Oviposition Traps (pools)	Modified Crow Trap (pools)	CDC Traps (pools)	Total Number of pools
<i>Culex quinquefasciatus</i>	1,498	61	5	0	66
<i>Culex tarsalis</i>	262	0	5	4	9
<i>Culex stigmatosoma</i>	105	6	0	0	6
TOTALS	1,865	67	10	4	81

TABLE 2. Small bird seroconversions for SLE and WEE antibodies in Orange County during 1998.

Species	SLE	WEE	No. Blood Samples	% SLE	% WEE
House finch	19	0	2,294	0.83	0.00
House sparrow	0	0	987	0.00	0.00
Other	0	0	141	0.00	0.00
TOTALS	19	0	3,422	0.56	0.00

only SLE-positive sentinel chickens for the Los Angeles basin during 1998.

A total of 3,422 wild bird blood samples was tested by hemagglutination inhibition (HAI) assay (Gruwell et al. 1988) at the OCVCD laboratory, resulting in 19 positive birds; all were house finches (Table 2). The highest percent of positive birds occurred in January (1.3%), April (1.3%), and May (1.2%) of 1998 (Fig. 6). The sentinel chicken seroconversions paralleled the wild bird seropositives in April (Fig. 6).

Overall, 1998 was a year marked by low arboviral activity compared to previous years (Fig. 7). Less than 1% of wild birds tested seropositive for SLE, and there were no birds positive for WEE. Additionally, no human cases of SLE or WEE were reported in Orange County during 1998.

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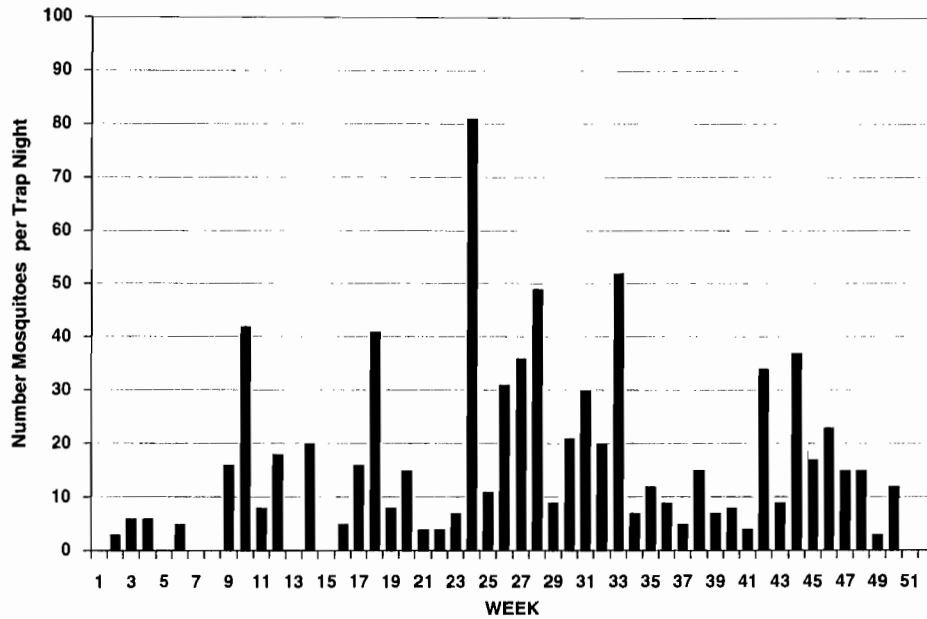


Figure 1. Weekly counts of gravid mosquitoes collected from ovipositional traps, OCVCD, Garden Grove, Calif. during 1998.

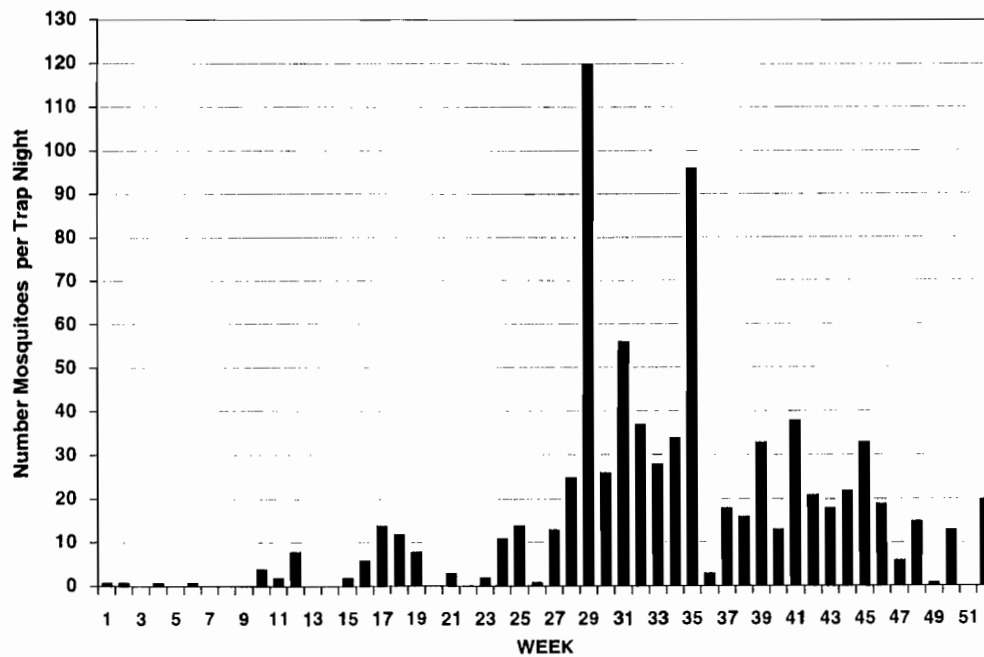


Figure 2. Weekly counts of gravid mosquitoes collected from ovipositional traps, Hellman Ranch, Seal Beach, Calif., during 1998.

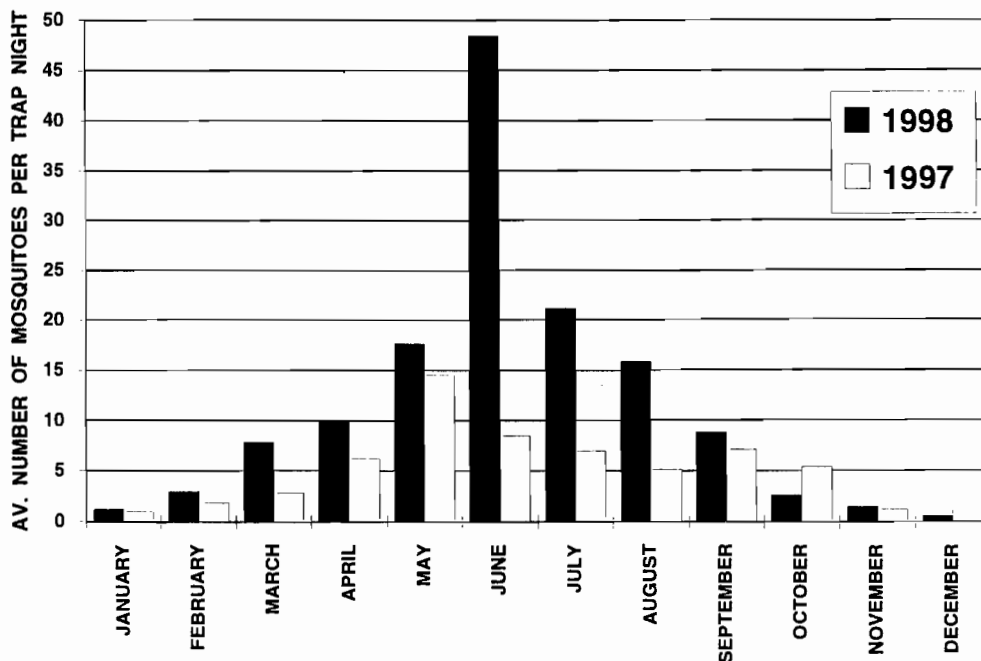


Figure 3. Host-seeking mosquito activity at urban mosquito collecting sites, Orange County, Calif., for 1997 and 1998 (10 CDC traps).

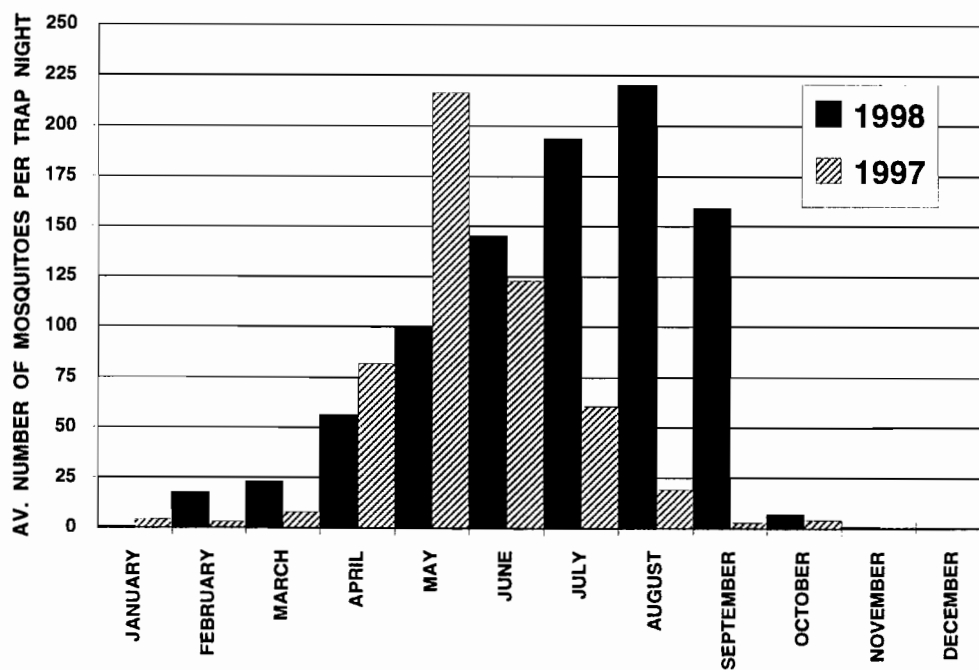


Figure 4. Host-seeking mosquito activity at the San Joaquin Marsh, Irvine, Calif. for 1997 and 1998 (5 CDC traps).

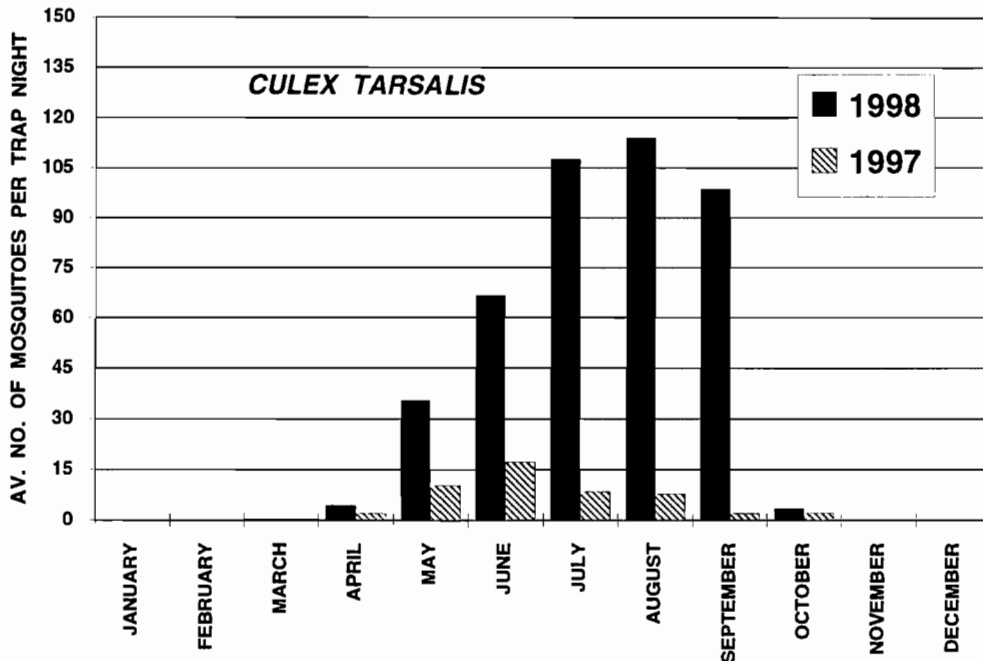


Figure 5. Host-seeking *Culex tarsalis* activity at the San Joaquin Marsh, Irvine, Calif. during 1997 and 1998 (5 CDC traps).

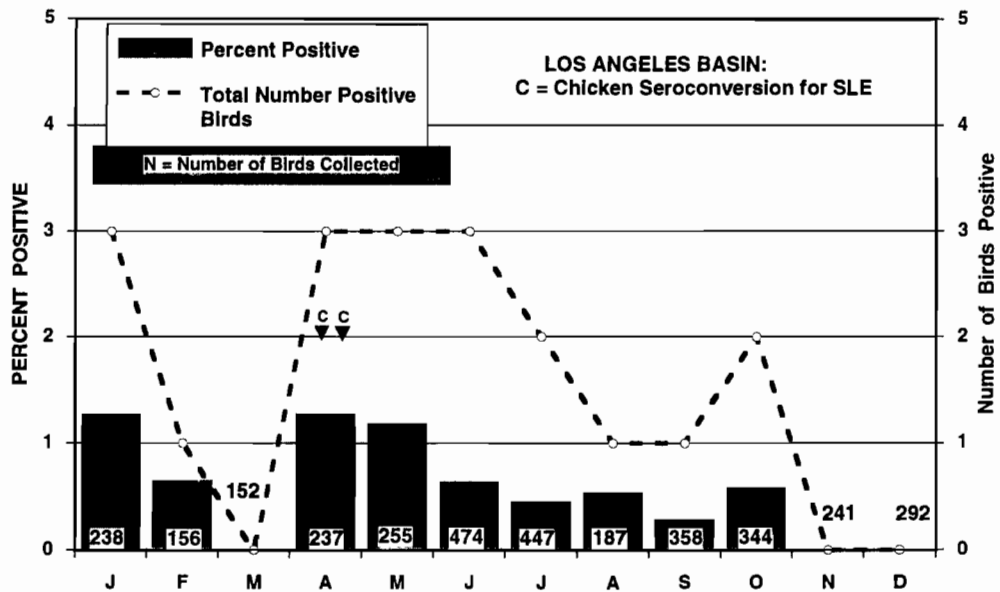


Figure 6. Arbovirus activity and seroprevalence in wild birds (house finches and house sparrows) from Orange County, Calif. during 1998.

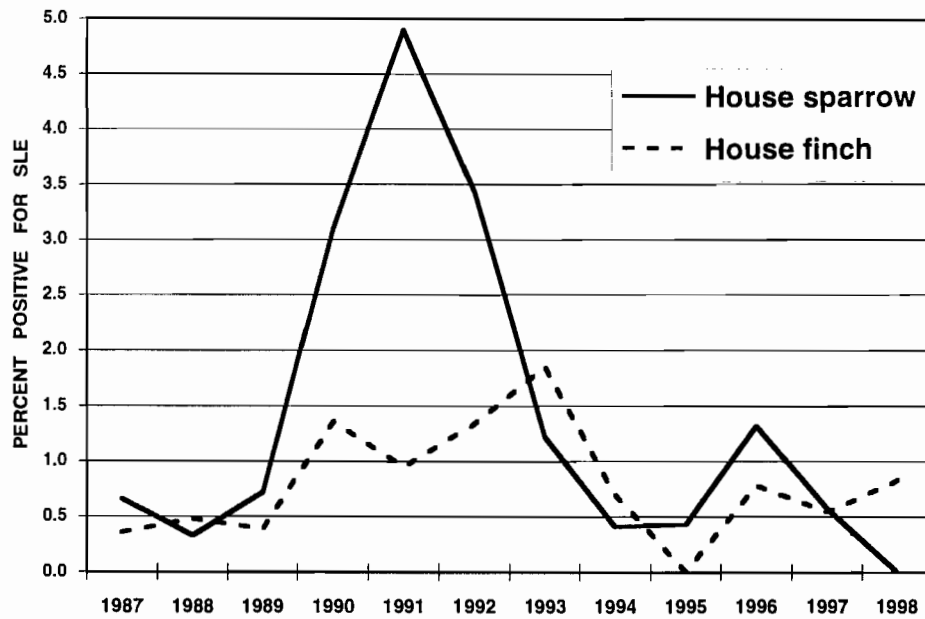


Figure 7. Seroprevalence of SLE antibodies in wild birds in Orange County, Calif. for 1987-1998.

## Summary of Reported St. Louis Encephalitis and Western Equine Encephalomyelitis Virus Activity in California from 1969-1997

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**ABSTRACT:** This resource document is in tabular form to summarize St. Louis encephalitis and western equine encephalomyelitis virus surveillance results in California from 1969-1997. Human and equine cases, sentinel chicken seroconversions, and positive mosquito pools are listed by county and year. This summary of California Department of Health Services and University of California activities supplements the comprehensive work of Reeves et al. (1990) which covers 1943-1987.

Western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses are endemic zoonoses in California that caused widespread epizootics among equines and epidemics among residents of the Central Valley prior to the mid-1950s (Lennette et al. 1961). Initial field research was centered in Kern County and the southern San Joaquin Valley (Reeves and Hammon 1962). The unprecedented epidemic of WEE and SLE in 1952 (Longshore et al. 1956) prompted the California Department of Public Health [currently: California Department of Health Services (CDHS)] to organize a surveillance program to provide an early warning of the risk of human infection (Longshore 1960). The program included a search for human cases of WEE and SLE; detection of equine cases of WEE; detection of arbovirus infection in mosquitoes; and monitoring of larval and adult mosquito populations (Emmons and Grodhaus 1976). Subsequently, a concerted effort was made among the CDHS; local mosquito control agencies; and the University of California, School of Public Health, Berkeley to develop and implement a statewide program. Early surveillance [1969-1978] was based on mosquito infection rates only (Longshore et al. 1960), but later (1979) the program was expanded to include flocks of sentinel chickens originally deployed by the University of California, Berkeley (Reeves et al. 1990). The findings have been published annually since 1971. This surveillance program has been augmented through the years into a cooperative system, which includes state-wide

weekly adult mosquito occurrence report, weekly bulletin of laboratory isolations of arboviruses from mosquito populations, serological detection of virus activity in sentinel chickens and epidemiological investigation of suspect human and equine cases (Walsh 1987). The current Encephalitis Virus Surveillance Program monitors enzootic activity by testing blood samples from flocks of sentinel chickens and viruses isolated from pools of mosquitoes, emphasizing the vectors, *Culex tarsalis*, *Aedes melanimon*, *Cx. pipiens*, and *Cx. stigmatosoma*. Specimens from suspected human and equine cases are diagnosed by passive case detection systems and confirmed by the Viral and Rickettsial Diseases Laboratory (VRDL), CDHS in Berkeley.

Certain events influenced the sampling effort summarized in this report. Initial program expansion emphasized known affected areas within the Central Valley (Longshore 1960). The threat of Venezuelan equine encephalomyelitis virus introduction into California during the mid-1970s expanded sampling into San Diego and Imperial counties near the Mexican border (Workman et al. 1976) and stimulated the initiation of an arbovirus research program by the University of California at Los Angeles (Work et al. 1985) (Meylan et al. 1989). Subsequent studies by the University of California, Berkeley and CDHS extended these studies to include the Colorado River and Coachella Valley (Reisen et al. 1992). In 1984, southern California experienced the first major outbreak of SLE in the metropolitan area

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of Los Angeles and Orange counties (Murray et al. 1985). Twenty-six SLE cases were confirmed during the summer (the largest number of SLE human cases since 1959). All major outbreaks of WEE and SLE had previously occurred in rural and suburban areas in the Sacramento and San Joaquin Valley, and along the Colorado River. This outbreak, not only had expanded surveillance in Los Angeles and Orange counties, but also facilitated publishing of first statewide guideline of California's Mosquito-borne Encephalitis Virus Surveillance and Control Program (Walsh 1987).

Changes in field and laboratory methods have affected the data set. Although mosquito pool size has remained constant at 50 females per pool, assay procedures changed from a suckling mouse system in 1988 (Emmons et al 1989) to an *in situ* enzyme immunoassay (EIA) based on Vero cell culture. This change in testing protocol focused detection on WEE and SLE, and voided information on other concurrently circulating viruses such as Turlock and Hart Park. In the early 1990s, the number of sentinel chickens per flock was progressively reduced from 25 to 20 and to 10 hens and the number of flocks increased to expand geographical coverage. In the mid-1980s testing shifted from a complement fixation test to an indirect immunoassay, and subsequently bleeding methods were changed from jugular puncture to a lancet prick of the comb (Reisen et al. 1994). Reduction in flock size and improvements in bleeding and testing methods allowed bleeding frequency to be increased from every 4 to every 2 weeks, improving information flow and forecasting. Comprehensive guidelines targeting the standardization of sampling methods, interpretation of results, and interagency information exchange were developed by the Mosquito and Vector Control Association of California (Reisen 1995).

This publication summarizes historical arbovirus surveillance results for easy reference by health departments, universities, and local mosquito and vector control agencies, and to supplement the monograph, Epidemiology and Control of Arboviruses in California, 1943-1987, by Reeves et al (1990). Unless otherwise stated, the surveillance testing was done by VRDL.

Special research on arbovirus occurrence was conducted in Imperial and Riverside counties (Madon et al. 1974) (Workman et al. 1976), Kern county (Reisen 1984) (Reisen et al. 1990), Los Angeles Basin (Reisen et al. 1992), San Bernardino County (Reisen et al. 1990), and southeastern California (Reisen et al. 1992, 1995, 1997). Each study had its special focus on surveying the landscape ecology, vector population dynamics and virus activities at designated sites. The detection of SLE and

WEE activities of mosquitoes or sentinel chickens from the above studies were not included in this summary.

Reported virus activity for SLE and WEE is summarized in Table 1 by county and year. The summary data is from the CDHS annual arbovirus presentations published in the Proceedings of the California Mosquito and Vector Control Association (currently: MVCAC) 1971-1998 [Emmons, et al. 1972-1994, Reilly et al. 1995, Kramer, et al. 1996-98]. The table also includes information from Reeves et al. (1990), Reeves (1970), Sudia (1971), Reeves and Milby (1980), and Meylan (1988). When there was a conflict between publications on the reported cases, the numbers listed in the CDHS annual surveillance reports were used.

Table 1 indicates the total number of SLE and WEE human cases, mosquito pool viral isolations, chicken seroconversions, and equine cases along with the total number of mosquito pools and sentinel chicken flocks tested for each year by county.

In addition to Table 1 by county and year, the information has been placed in an Access 97 database and PowerPoint software available to CDHS and requesting agencies for maps or other tables delineating specific information. Figures 1-4 are included to indicate statewide statistics by year.

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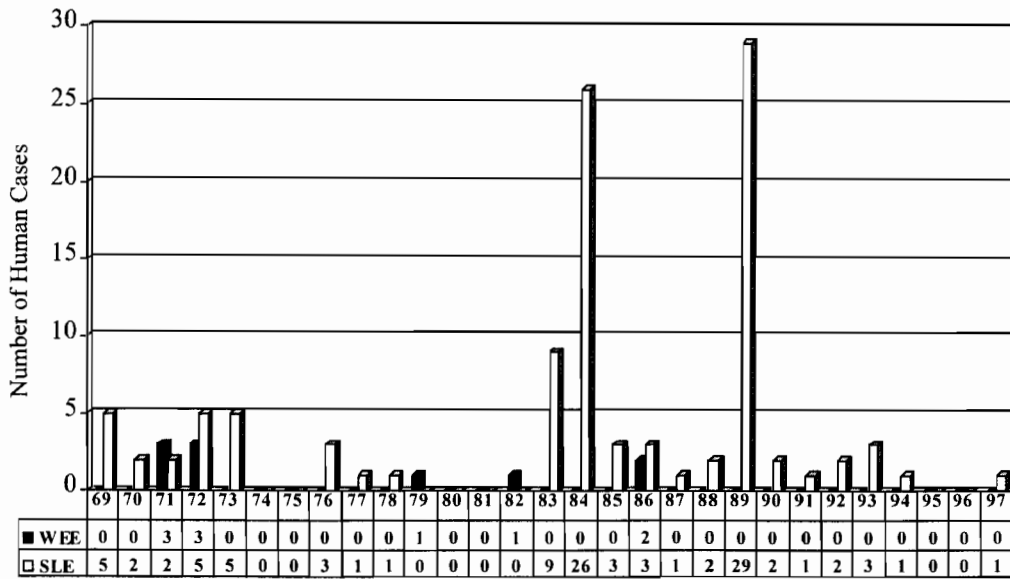


Figure 1. Human Cases of WEE and SLE in California from 1969 to 1997.

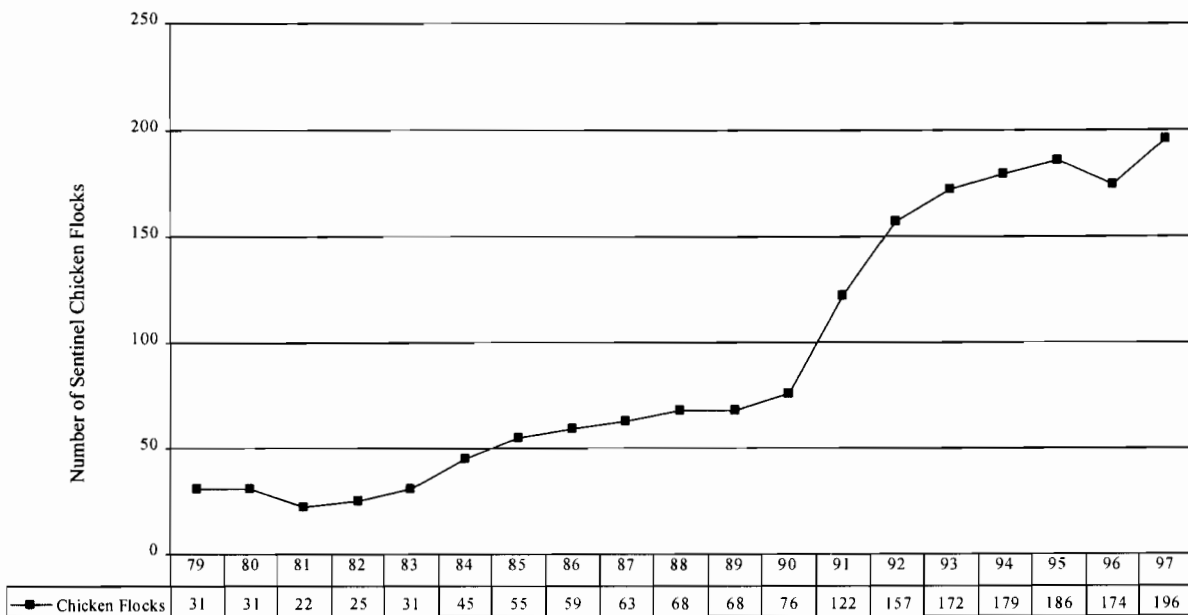


Figure 2. Number of Sentinel Chicken Flocks from 1979 to 1997.

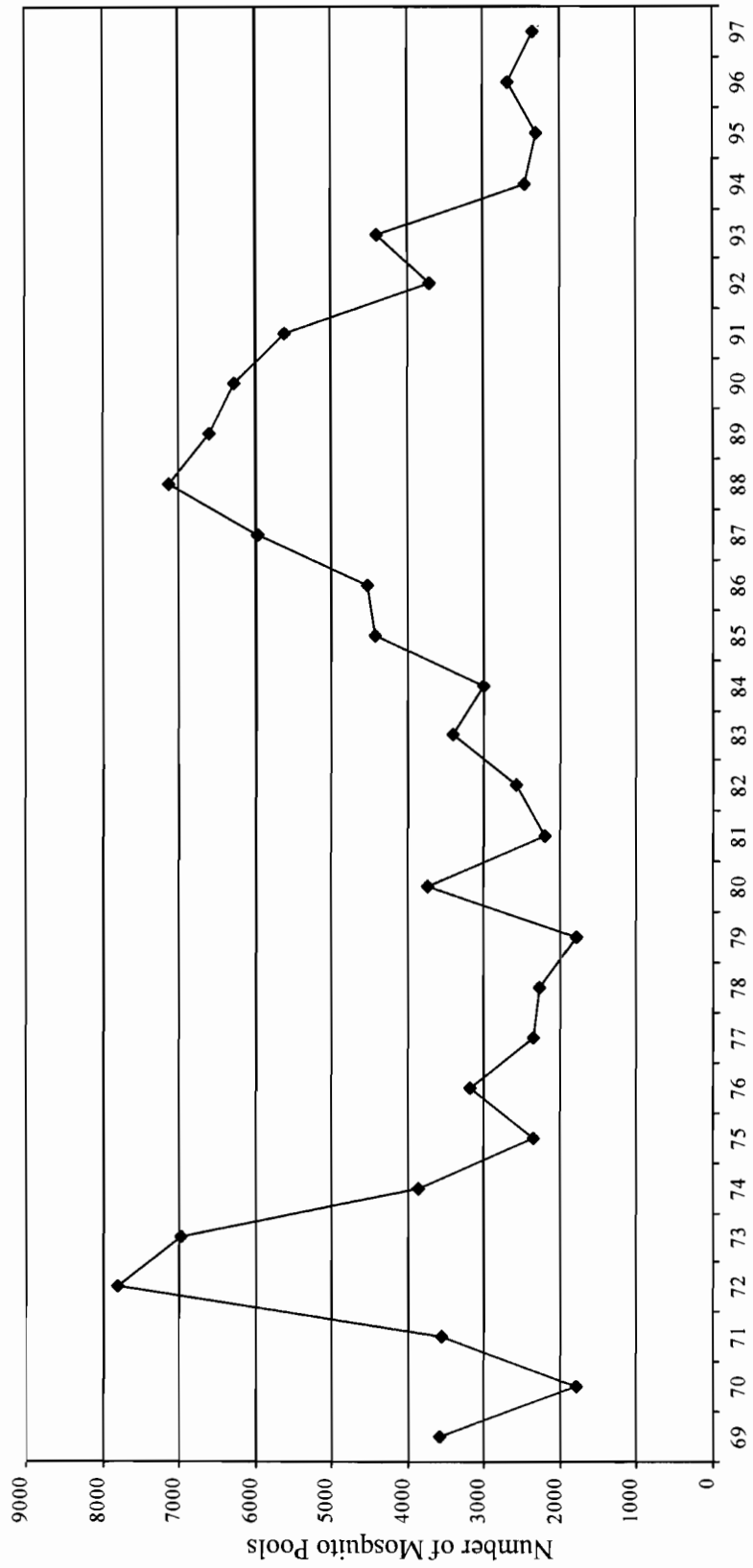


Figure 3. Number of Mosquito Pools Tested from 1969 to 1997.

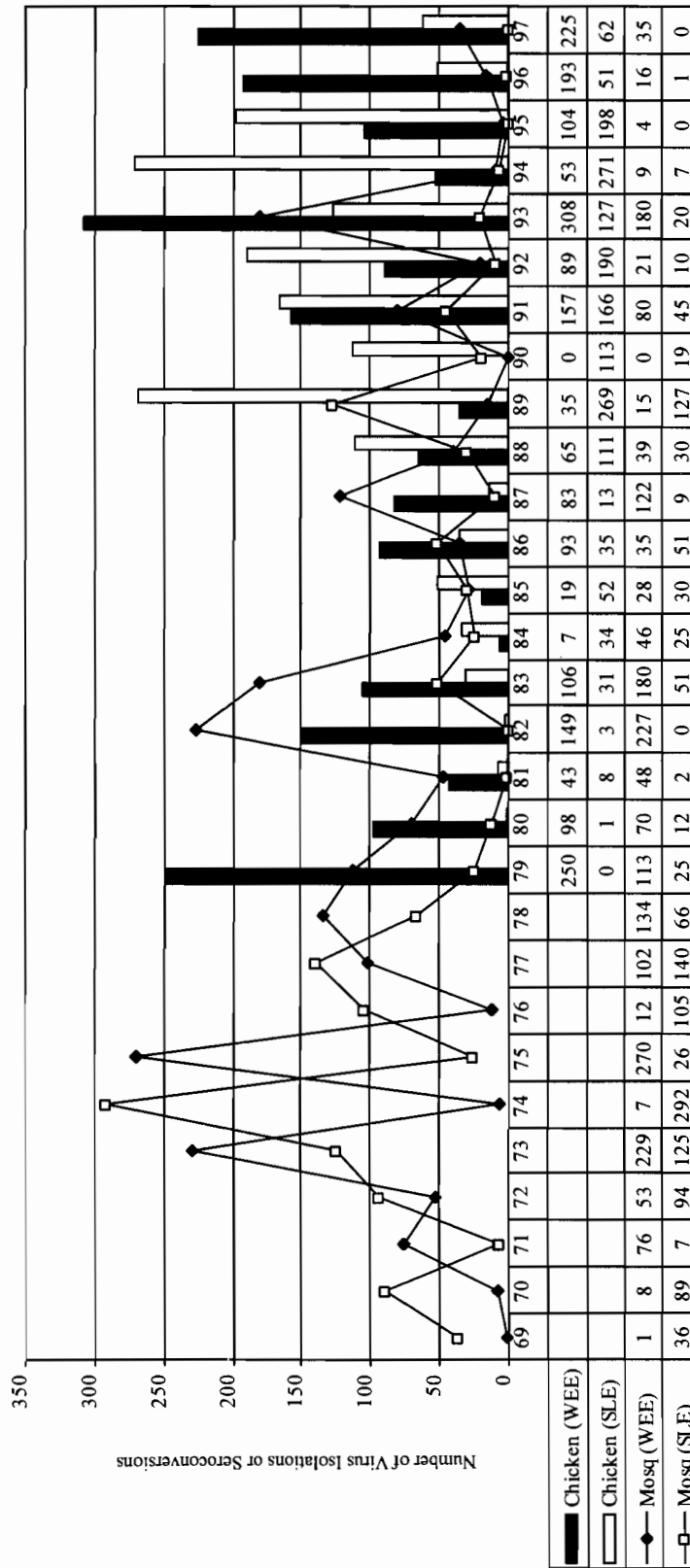


Figure 4. WEE and SLE Virus Isolations from Mosquitoes and Seroconversions in Sentinel Chickens in California 1969 to 1997.

TABLE 1. Summary of WEE and SLE in California From 1969 to 1997.

## COUNTIES:

BU=BUTTE

IM=IMPERIAL

LS=LASSEN

OR=ORANGE

SD=SAN DIEGO

SN=SONOMA

VE=VENTURA

CC=CONTRA COSTA

KE=KERN

MA=MADERA

PL=PLACER

SJ=SAN JOAQUIN

ST=STANISLAUS

YL=YOLO

CO=COLUSA

KI=KINGS

ME=MERCED

RI=RIVERSIDE

SH=SHASTA

SU=SUTTER

YB=YUBA

FR=FRESNO

LA=LOS ANGELES

MD=MODOC

SA=SACRAMENTO

SI=SISKIYOU

TE=TEHAMA

GL=GLENN

LK=LAKE

MR=MARIN

SB=SAN BERNARDINO

SL=SOLANO

TU=TULARE

<sup>1</sup> University of California, Los Angeles<sup>2</sup> Arbovirus Research Laboratory, University of California, Berkeley<sup>3</sup> Data included all the positive isolations tested by Arbovirus Research Laboratory, University of California, Berkeley<sup>4</sup> Positive isolations were tested by Animal and Plant Health Inspection Service, United States Department of Agriculture, Ames, Iowa

YEAR	HUMAN CASES		MOSQUITO POSITIVES		CHICKEN SERA		HORSE CASES
	WEE	SLE	WEE	SLE	WEE	SLE	WEE
1969	0	5 GL-2 SA-2 SU-1	0 + 1 <sup>1</sup> IM-1 <sup>1</sup>	35 + 1 <sup>1</sup> BU-4, IM-1 <sup>1</sup> SH-2, SL-1 SU-5, TE-23	Testing not done until 1979		2 SJ-1, TE-1
			(3487 POOLS TESTED) + 104 POOLS <sup>1</sup> =3591				
1970	0	2	8 <sup>1</sup> IM-8	89 <sup>1</sup> IM-89			
			1801 POOLS <sup>1</sup>				
1971	3 BU-1 SA-1 SU-1	2 SA-1 SN-1	16 + 60 <sup>1</sup> CO-5, IM-8 +60 <sup>1</sup> SI-1, TE-2	6 + 1 <sup>1</sup> CO-4, IM-1 <sup>1</sup> SD-1, SU-1			16 BU-2, FR-1 GL-5, IM-1 SA-1, SH-2 SJ-1, ST-1 TE-1, SU-1
			(1759 POOLS TESTED) + 1801 POOLS <sup>1</sup> =3560				
1972	3 FR-2 MA-1	5 KE-1 SD-1 TE-1 TU-1 YL-1	41 + 12 <sup>1</sup> CO-1, FR-3 KE-2, KI-1 IM-14 +12 <sup>1</sup> MA-15, RI-1 SU-1, TU-3	61 + 33 <sup>1</sup> CO-9 IM-29 +33 <sup>1</sup> MA-2, RI-1 SA-1, SD-1 SH-3, TE-10 TU-3, YL-2			1 KE-1
			(6180 POOLS TESTED) + 1638 POOLS <sup>1</sup> =7818				
1973	0	5 IM-1 KE-1 RI-1 SD-1 SJ-1	81 + 148 <sup>1</sup> CO-1 IM-72 +148 <sup>1</sup> RI-4, SB-2 ST-1, SU-1	64 + 61 <sup>1</sup> KE-2, IM-48 +61 <sup>1</sup> LS-1, RI-2 SB-3, SD-7 TU-1			2 SH-1 YL-1
			(4326 POOLS TESTED) + 2655 POOLS <sup>1</sup> =6981				
1974	0	0	4 + 3 <sup>1</sup> IM-3 <sup>1</sup> , RI-4	2 + 290 <sup>1</sup> IM-2 + 290 <sup>1</sup>			2 KE-1 RI-1
			(1320 POOLS TESTED) + 2556 POOLS <sup>1</sup> =3876				
1975	0	0	0 + 270 <sup>1</sup> IM-270 <sup>1</sup>	0 + 26 <sup>1</sup> IM-26 <sup>1</sup>			0
			(891 POOLS TESTED) + 1460 POOLS <sup>1</sup> =2351				
1976	0	3 MA-1 RI-1 SD-1	0 + 12 <sup>1</sup> IM-12 <sup>1</sup>	9 + 96 <sup>1</sup> IM-5 + 96 <sup>1</sup> RI-2, SB-2			0
			(1174 POOLS TESTED) + 2024 POOLS <sup>1</sup> =3198				
1977	0	1 SB-1	10 + 92 <sup>1</sup> IM-3 + 92 <sup>1</sup> SB-7	3 + 137 <sup>1</sup> IM-1 + 137 <sup>1</sup> RI-1, SB-1			0
			(727 POOLS TESTED) + 1619 POOLS <sup>1</sup> =2346				

1978	0	1 SD-1	87 + 47 <sup>1</sup> BU-8 IM-9 + 47 <sup>1</sup> KE-1, KI-2 MA-2, ME-1 RI-40, SA-2 SB-13, ST-6 TU-3	37 + 29 <sup>1</sup> IM-4 + 29 <sup>1</sup> RI-32, TU-1			12 BU-1, FR-1 RI-1, SA-1 SJ-1, ST-1 SU-1, TE-1 YL-3, YB-1
			(1690 POOLS TESTED) + 573 POOLS <sup>1</sup> =2263				
1979	1 SA-1	0	113 BU-2, CO-2 IM-28, KE-26 PL-1, RI-13 SA-2, SB-4 SH-7, SJ-1 ST-1, SU-8 TE-2, TU-14 YB-1, YL-1	25 IM-11 RI-7 SB-7	250 Sac Valley-142 (BU, GL, SU, YB) San Joaq Valley- 100 KE) So. CA-8	0	18 BU-1, FR-1 IM-1, KE-1 MA-1, MD-1 SA-4, SJ-3 SH-1, SL-1 ST-2, YL-1
			(1803 POOLS TESTED)		(31 FLOCKS)		
1980	0	0	70 BU-1, CO-5 GL-1, IM-8 KE-19, RI-15 SA-1, SB-10 ST-1, SU-3 TU-5, YB-1	12 IM-1 RI-8 SB-3	98 BU-2, GL-4 IM-11, KE-59 PL-1, RI-1 SH-3, SU-2 TE-10, YB-5	1 RI-1	2 KE-1 PL-1
			(3745 POOLS TESTED)		(31 FLOCKS)		
1981	0	0	48 BU-2, IM-1 KE-18, PL-1 RI-17, SA-1 SU-2, TU-4 YB-2	2 SB-2	43 BU-2, CO-2 IM-4, KE-24 RI-6, TE-2 YB-3	8 RI-3 IM-5	1 SA-1
			(2197 POOLS TESTED)		(22 FLOCKS)		
1982	1 SB-1	0	227 IM-34, KE-100 KI-4, RI-7 SB-1, TU-81	0	149 FR-13, IM-37 KE-79, KI-19 TE-1	3 IM-3	3 FR-1, KE-1 RI-1
			(2590 POOLS TESTED)		(25 FLOCKS)		
1983	0	9 IM-3 LA-1 RI-3 SB-1 SD-1	180 IM-20, KE-126 RI-9, SA-1 SB-15, TU-8 YL-1	51 IM-19 KE-3 RI-23 SB-6	106 FR-10, GL-1 IM-16, KE-68 KI-4, RI-1 TU-5, YB-1	31 IM-28 RI-3	2 RI-1 LA-1
			(3403 POOLS TESTED)		(31 FLOCKS)		
1984	0	26 LA-16 OR-5 RI-4 SD-1	46 IM-36 SB-9	25 IM-20 LA-3 SB-2	7 IM-2, KE-1 OR-1, RI-2 SB-1	34 LA-10, IM-9 OR-3, RI-5 SB-7	1 FR-1
			(3004 POOLS TESTED)		(45 FLOCKS)		
1985	0	3 LA-1 RI-1 SB-1	28 IM-21 RI-3 SB-4	30 LA-1, IM-15 RI-8, SB-6	19 IM-12, SB-7	52 LA-1, IM-42 RI-1, SB-8	0
			(4417 POOLS TESTED)		(55 FLOCKS)		
1986	2 SH-1 YL-1	3 LA-3	35 IM-15, LA-2 RI-2, SB-3 SA-2, SU-1 YL-10	51 IM-7, LA-32 KE-3, OR-5 RI-4	93 BU-12, FR-1 IM-23, RI-21 SB-18, SL-11 ST-1, SU-6	35 IM-12, KE-2 LA-7, OR-2 RI-11, SA-1	1 TE-1
			(4530 POOLS TESTED)		(59 FLOCKS)		

1987	0	1 SB-1	122 BU-6, IM-14 ME-1, RI-46 SA-15, SH-2 SU-19, YB-3 YL-16	2 + 7 <sup>1</sup> IM-1 + 7 <sup>1</sup> LA-1	83 BU-7, FR-1 IM-10, RI-28 SA-2, SD-1 SH-2, SJ-1 SL-1, SN-1 SU-12, TE-15 YB-1, YL-1	13 IM-4 LA-9	0
			(5840 POOLS TESTED) + 117 POOLS <sup>1</sup> =5957	(63 FLOCKS)			
1988	0	2 IM-1 SB-1	39 IM-13 RI-23 SB-3	9 + 21 <sup>2</sup> IM-1 + 21 <sup>2</sup> LA-6, RI-1 SB-1	65 IM-63 RI-1 SB-1	111 IM-71, KE-3 LA-27, OR-5 RI-1, SB-4	0
			(5711 POOLS TESTED) + 1425 POOLS <sup>2</sup> =7136	(68 FLOCKS)			
1989	0	29 LA-1 KE-15 KI-8 TU-5	15 <sup>3</sup> IM-15	127 <sup>1</sup> IM-10, LA-4 KE-85, RI-28	35 <sup>3</sup> IM-34 SB-1	269 <sup>3</sup> FR-7, IM-21 KE-177 KI-18, LA-5 OR-3, RI-24 TU-14	0
			(3845 POOLS TESTED) + 2748 POOLS <sup>2</sup> =6593	(68 FLOCKS)			
1990	0	2 LA-1 TU-1	0	18 + 1 <sup>4</sup> IM-6, LA-1 RI-7 + 1 <sup>2</sup> SB-1, TU-3	0	90 + 23 <sup>2</sup> FR-8, IM-34 KE-11, LA-2 OR-1 RI-31 + 23 <sup>2</sup> TU-3	0
			(5262 POOLS TESTED) + 1005 POOLS <sup>2</sup> =6267	(71 FLOCKS) + 5 FLOCKS <sup>2</sup> =76			
1991	0	1 LA-1	7 + 73 <sup>2</sup> IM-58 <sup>2</sup> , LA-7 RI-15 <sup>2</sup>	1 + 44 <sup>4</sup> IM-40 <sup>2</sup> , LA-1 RI-4 <sup>2</sup>	157 <sup>2</sup> IM-70 <sup>2</sup> RI-87 <sup>2</sup>	16 + 150 <sup>2</sup> IM-73 <sup>2</sup> LA-15 RI-77 <sup>2</sup> SB-1	0
			(4589 POOLS TESTED) + 1027 POOL <sup>2</sup> =5616	(100 FLOCKS) + 22 FLOCKS <sup>2</sup> =122			
1992	0	2 LA-1 VE-1	1 + 20 <sup>2</sup> IM-14 <sup>2</sup> , RI-6 <sup>2</sup> SB-1	0 + 10 <sup>2</sup> IM-4 <sup>2</sup> , RI-6 <sup>2</sup>	50 + 39 <sup>2</sup> FR-1 IM-23 + 23 <sup>2</sup> LA-3 RI-18 + 16 <sup>2</sup> SB-4, TU-1	117 + 73 <sup>2</sup> FR-1 IM-22 + 24 <sup>2</sup> KE-3, LA-36 MA-4, OR-5 RI-43 + 49 <sup>2</sup> TU-1, VE-2	1 IM-1
			(2329 POOLS TESTED) + 1393 POOLS <sup>2</sup> =3722	(131 FLOCKS) + 26 FLOCKS <sup>2</sup> =157			
1993	0	3 SB-1 SD-1 OR-1	168 + 12 <sup>2</sup> BU-2, CC-11 CO-15, GL-3 IM-1 + 12 <sup>2</sup> LK-8, MA-1 PL-2, SA-45 SJ-1, SB-2 SH-7, ST-2 SU-27, TE-1 YL-36, YB-4	5 + 15 <sup>2</sup> IM-15 <sup>2</sup> LA-1 SB-4	277 + 31 <sup>2</sup> BU-47, CC-6 CO-6, FR-2 GL-11 IM-26 + 28 <sup>2</sup> LA-1, LK-6 MA-2, ME-3 RI-1 + 3 <sup>2</sup> SA-62, SJ-12 SB-5, SH-1 SL-17, SN-3 ST-8, SU-28 TE-7, TU-1 YL-7, YB-15	90 + 37 <sup>2</sup> IM-47 + 36 <sup>2</sup> LA-23 RI-4 + 1 <sup>2</sup> SB-16	1 TE-1
			(3800 POOLS TESTED) + 595 POOLS <sup>2</sup> =4395	(152 FLOCKS) + 20 FLOCKS <sup>2</sup> =172			

1994	0	1 RI-1	8 + 1 <sup>1</sup> IM-1 <sup>2</sup> LK-4 SA-4	0 + 7 <sup>2</sup> IM-4 <sup>2</sup> RI-3 <sup>2</sup>	47 + 6 <sup>2</sup> BU-5, CO-3 GL-4 IM-8 + 6 <sup>2</sup> LK-3, MR-1 SA-7, SJ-2 SL-3, TE-6 YL-2, YB-3	117 + 154 <sup>2</sup> IM-70 + 69 <sup>2</sup> LA-1 RI-42 + 85 <sup>2</sup> SB-4	0
			(1859 POOLS TESTED) + 585 POOLS <sup>2</sup> = 2444	(156 FLOCKS) + 23 FLOCKS <sup>2</sup> = 179			
1995	0	0	4 LK-1 SA-1 SU-1 YL-1	0	80 + 24 <sup>2</sup> BU-9, CO-1 FR-1, GL-4 IM-23 IR-23 + 24 <sup>2</sup> SA-5, SJ-1 SU-9, YL-4	113 + 85 <sup>2</sup> IM-28 LA-1 RI-83 + 85 <sup>2</sup> SB-1	0
			(2069 POOLS TESTED) + 237 POOLS <sup>2</sup> = 2306	(173 FLOCKS) + 13 FLOCKS <sup>2</sup> = 186			
1996	0	0	16 BU-1 KE-3 LK-1 SA-3 SU-2 YL-6	0 + 1 <sup>2</sup> RI-1 <sup>2</sup>	193 BU-42, CC-1 FR-4, GL-17 IM-6, KE-46 LK-5, ME-4 NA-1, SA-12 SJ-5, SL-6 ST-1, SU-20 TE-9, TU-1 YL-8, YB-5	40 + 11 <sup>2</sup> IM-15 LA-11 RI-11 + 11 <sup>2</sup> SB-3	0
			(2236 POOLS TESTED) + 444 POOLS <sup>2</sup> = 2680	(164 FLOCKS) + 10 FLOCKS <sup>2</sup> = 174			
1997	0	1 LA-1	35 <sup>1</sup> GL-2 LK-4 PL-3 SA-2 ST-2 SU-14 YL-8	0	225 <sup>1</sup> BU-45, CO-2 CC-2, FR-6 GL-13, IM-26 KE-4, KI-3 LK-7, MA-3 ME-10, RI-10 SA-14, SJ-2 SH-4, SL-8 SN-3, ST-11 SU-30, TE-7 YL-4, YB-11	62 <sup>1</sup> IM-24 LA-4 RI-32 SB-2	4 KE-1 <sup>4</sup> ME-1 MD-1 <sup>4</sup> SH-1 <sup>4</sup>
			(2360 POOLS TESTED) <sup>1</sup>	(196 FLOCKS) <sup>1</sup>			
<b>Total</b>	<b>10</b>	<b>107</b>	<b>2160</b>	<b>1444</b>	<b>2077</b>	<b>1735</b>	<b>69</b>



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## Comparison of the Efficiency of Four Types of Carbon Dioxide-Baited Traps for Sampling *Culex* Mosquitoes in Orange County, California

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### INTRODUCTION

Mosquito surveillance is continuously being refined as science discovers new and more efficient methodologies that increase our ability to effectively sample field populations. This inevitable trend in "modernization" also introduces an opportunity to develop uniformity in sampling by applying trap design, operation, and placement standards intended to provide continuity in data acquisition. Continuity subsequently allows for near direct comparison of population data obtained at either the local agency, regional or statewide level.

Mosquito populations statewide currently are being quantified somewhat "helter-skelter" by a variety of adult traps that employ either light (e.g., standard New Jersey Light Trap or NJLT) or carbon dioxide (e.g., various CDC style host-seeking traps supplemented with dry ice) as an attractant. Data obtained by this "hybrid" surveillance program are difficult to correlate tempered by the fact that NJLTs are declining in sensitivity.

Since being introduced as the standard mosquito trap in California, NJLT efficiency has declined noticeably in urban environments where both security and convenience lighting significantly diminish the photo attractancy of the trap. The consequences of this diminished attractancy means that "light prone" NJLTs substantially underestimate mosquito relative abundance and introduce confounding data that compromise sampling accuracy essential to evaluating 1) risk to human exposure to mosquito-borne disease and 2) retrospective analyses of control program successes and failures.

Technical discussions between the Mosquito and Vector Control Association of California (MVCAC), California Department of Health Services Vector-Borne Disease Section (CDHS-VBDS), and the University of California (UC) concluded that current application of

the NJLT may not be providing "accurate" estimates of mosquito populations in "light prone" areas of the state. Therefore, studies were initiated, and just recently concluded, to resolve this dilemma and offer both realistic and affordable sampling/trapping alternatives. Comparative trap studies conducted in southern, central, and northern California over the past several years have revealed that substituting historical NJLTs with the CO<sub>2</sub> trap (operated without light) alternative is not only practical, but preferred.

Switching to CO<sub>2</sub> trap based surveillance will involve considerable commitment from all mosquito control based agencies statewide. At the same time, it is realized that standard NJLTs will have to be replaced with a standardized CO<sub>2</sub> trap. There are a number of vector control agencies in California that currently operate one of several either "home built" or commercially available versions/modifications of the standard Sudia & Chamberlain CDC-style miniature light trap. Among the multitude of CDC-style models, four similar and unique configurations were considered for standard deployment in California. Included are the UC Arbovirus Field Station trap (UC), the Hock model 1012 trap (Hock), the Orange County Vector Control District Encephalitis Virus Surveillance trap (OC), and American Biophysics Corporation trap (AB). Each trap configuration features a suction fan housed in a cylindrical body, portable operation provided by either dry or gel cell battery DC power source, screened collection carton/bag, external/attached receptacle for holding dry-ice. Technical information on these traps is provided by Cummings and Meyer (1999) elsewhere in this issue.

Studies evaluating the efficiency of these traps were concluded last summer. A detailed statistical analysis of the results are being published by Reisen et al. (2000). This paper reports on the findings of trap evaluations

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conducted in Orange County (San Joaquin Marsh) by staff of the Orange County Vector Control District (OCVCD).

## MATERIALS AND METHODS

**Study Site:** Trap evaluations (June through October) were conducted at San Joaquin Marsh located in central Orange County aside the campus of the University of California Irvine (UCI) campus. The site is known for its long history on mosquito research and encephalitis virus surveillance. During the summer months, the marsh produces exceptional numbers of *Culex erythrothorax* Dyar, and subordinate numbers of *Culex tarsalis* Coquillett, *Culex quinquefasciatus* Say, *Anopheles hermsi* Barr & Guptavanji, *Culiseta incidens* (Thompson), and rarely *Culex stigmatosoma* Dyar and *Culiseta inornata* (Williston). All of these species were represented in collections during trap evaluations, however, only the first two species, *Cx. erythrothorax* and *Cx. tarsalis* were sampled in adequate numbers to support detailed statistical interpretations linked to evaluation parameters.

**Mosquito Control Activities:** As part of OCVCD's commitment (contract with UCI) to controlling mosquitoes on the San Joaquin Marsh, the site is treated Tuesday through Friday (each week @ 4 nights/week, May-Oct) with Scourge R (resmethrin) applied via vehicle-mounted fogger. Mosquito populations sampled on Monday night of each week were represented by a mix of those that 1) survived previous resmethrin treatments and 2) newly emerged/recruited individuals from Saturday through Monday. Mosquitoes sampled on Tuesday night of each week were subjected to an application of resmethrin. This treatment regimen provided an excellent opportunity to determine trap efficiencies measured against high versus low mosquito abundances.

**Trap Operation:** Traps were operated (N=48 trap nights) each week of the study on Monday (no resmethrin application) and Tuesday (+ resmethrin application) nights from sunset to sunrise. The American Biophysics trap was equipped with a photocell switch that automatically engaged the motor/fan and mini-light at sunset. Each trap was supplied with approximately 2.5 kg of dry ice as the carbon dioxide attractant. Collections were picked up the following morning and returned to the laboratory for processing. The number and sex of each species collected by each trap was recorded.

**Trap Rotation and Placement Transects:** Traps were arranged in two parallel transects with each trap placement site (4/transect) separated by 25m. All traps

were hung from metal standards at a distance of 1 to 1.5m above ground level. To avoid site bias, traps representing each of the four models evaluated were rotated each night (two weeks for one complete rotation series- model/trap at each site within each transect) independent of resmethrin treatments.

## RESULTS

Throughout the period of trap operation, nightly weather and related environmental parameters remained relatively uniform with no noticeable disruptions created by spates in temperature, wind, and precipitation. Similarly, population tracking provided by the traps (average collection sizes) reflected stable environmental conditions with population numbers remaining relatively stable until the last two weeks of sampling at which time both *Cx. tarsalis* and *Cx. erythrothorax* abundance declined to well below the preceding tracking average.

The results of the CDC-style CO<sub>2</sub> trap evaluation studies at San Joaquin Marsh indicated noticeable differences in trap efficiencies that were surprisingly inversely related to model/unit cost, but positively associated with low cost coupled with engineering superiority. All models effectively sampled host-seeking female *Cx. tarsalis* and *Cx. erythrothorax* with the latter species in greatest abundance in both pre- and post-treatment collections. Rotation of trap positions intended to compensate for location effects did provide some indication that position did influence to a lesser extent trap efficiency. However, the UC trap in transect 1 consistently (> 90%) outperformed all other traps regardless of where it was placed and replicated night-to-night.

Overall, the UC trap performed the best, followed by the OC, Hock, and AB. As of this writing, the AB trap has been discontinued and replaced by a more efficient, but costly model that operates using updraft versus down draft technology.

### Evaluations- Pretreatment:

***Culex tarsalis*:** Pretreatment trapping data indicates that both the UC and OC traps were about equally superior for sampling host-seeking *Cx. tarsalis*. The only exception is demonstrated by the overwhelming efficiency demonstrated of the UC trap operated in transect 1 (Fig. 1A). The remaining two models yielded catches that were at/or well below either the UC or OC models with the Hock comparable to OC, but 2-fold more efficient than the overall least effective AB trap.

Comparing trapping efficiencies between transects 1 and 2, there were no distinct indications in the data that sampling results were spatially different. Traps op-

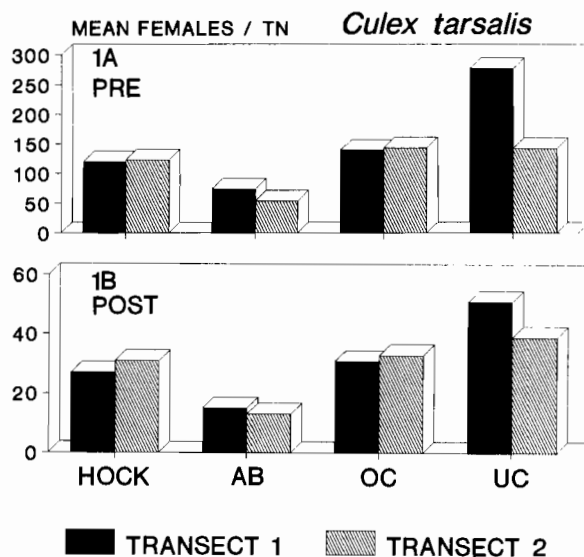


Figure 1. A-Mean number of female *Culex tarsalis* collected per trap night by each trap model evaluated in transects 1 and 2 on pretreatment nights. B-Mean number collected per trap night in transects 1 and 2 on posttreatment nights.

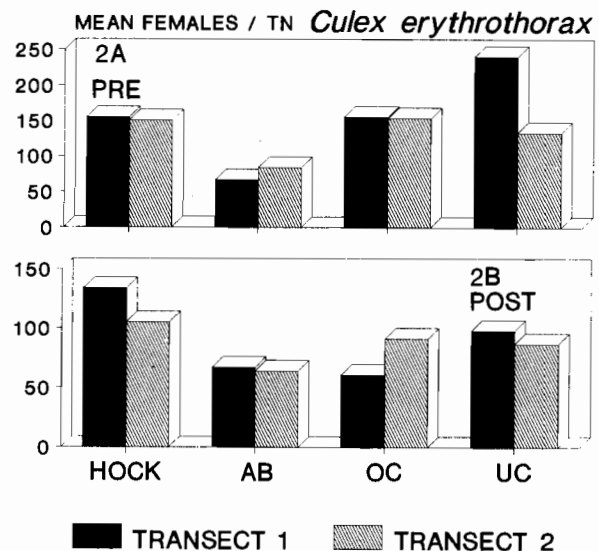


Figure 2. A-Mean number of female *Culex erythrothorax* collected per trap night by each trap model evaluated in transects 1 and 2 on pretreatment nights. B-Mean number collected per trap night in transects 1 and 2 on posttreatment nights.

erated in transect 1 did not consistently sample more *Cx. tarsalis* than traps operated simultaneously in transect 2, and visa versa.

***Culex erythrothorax*:** Trapping data from pretreatment collections paralleled those obtained for *Cx. tarsalis* (Fig. 2A). However, results for the UC trap were mixed with that model performing less efficiently than the OC and Hock operated in transect 2. Both the OC and Hock were equally effective within and between transects, with both models consistently yielding larger (ca. 2-fold) collections than the AB trap in either transect 1 or 2.

Between and within transect results were also inconsistent and similar to collection size patterns observed for *Cx. tarsalis*.

#### Evaluations- Posttreatment:

***Culex tarsalis*:** Posttreatment trapping indicated that treatments with ULV resmethrin significantly reduced (ca. 5-fold) overall mosquito abundance on San Joaquin Marsh. Though mosquitoes abundances were reduced, trapping results and comparative efficiencies remained relatively unchanged (Fig. 1B). The UC model still performed most effectively in transect 1, but less effectively in transect 2 where both the OC and Hock yielded comparable collections of host-seeking females. Collections obtained by the AB trap were still consistently 2-fold or less than the other models evaluated. Individual transect

results were inconsistent with no appearance of a transect bias.

***Culex erythrothorax*:** The pattern of UC trap superiority remained consistent with the exception of pretreatment sampling of *Cx. erythrothorax* where the Hock model trap operated in both transects 1 and 2 were superior to both the UC and OC traps. Contrary to diminished trapping success, the AB actually performed nearly equal to both the UC and OC traps with both of the latter models yielding nearly identical average collection sizes. The OC trap operated in transect 1 actually collected fewer *Cx. erythrothorax* than either the AB or UC traps. No differences were evident in the data to indicate a sampling bias between transects.

## CONCLUSIONS

The results of this study have demonstrated that significant differences in trap efficiency exist between the 4 models of CDC-type traps evaluated at San Joaquin Marsh. Further analysis of the engineering profiles (refer to Cummings and Meyer, 2000) also indicates that trap configurations incorporating simple designs and superior dynamics in suction capacity are "preferred" for sampling both *Cx. tarsalis* and *Cx. erythrothorax*.

The obvious application of highly efficient and economical traps in statewide surveillance will be a major selling point for agencies having limited resources, but ample manpower to shuttle/deploy traps on a routine weekly or biweekly basis. The additional pluses include having traps that are 1) largely unaffected by competitive light sources, 2) portable and easy to deploy, 3) capable of capturing statistically valid numbers of mosquitoes (not fractional as with NJLT data), 4) operationally comparable over time and space, and 5) maintained effectively with limited resources.

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## **WILLIAM C. REEVES NEW INVESTIGATOR AWARD**

The William C. Reeves New Investigator Award is given annually by the Mosquito and Vector Control Association of California in honor of the long and productive scientific career of Dr. William C. Reeves, Professor Emeritus, School of Public Health, University of California at Berkeley.

The award is presented to the outstanding research paper delivered by a new investigator based on quality of the study, the written report, and presentation at the annual conference.

Parker D. Workman was the recipient of the 1999 award at the 67<sup>th</sup> Annual Conference held in Anaheim, California. The other finalist was Jeny Wegbreit. The two finalists' papers are printed on pages 78-84.

Previous William C. Reeves New Investigator Award Winners:

- 1999 - Parker D. Workman**
- 1998 - Yvonne Ann Offill
- 1997 - John Gimnig
- 1996 - (none)
- 1995 - Margaret C. Wirth
- 1994 - Merry L. Holliday-Hanson
- 1993 - Jeffrey W. Beehler
- 1992 - Darold P. Batzer
- 1991 - David R. Mercer
- 1990 - Gary N. Fritz
- 1989 - Truls Jensen
- 1988 - Vicki L. Kramer



## Adult Spatial Emergence Patterns and Larval Behavior of the "Tule Mosquito," *Culex erythrothorax*

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**ABSTRACT:** Previous studies found a disparity in the relative abundance of *Cx. erythrothorax* in larval dipping versus carbon dioxide-baited trap samples. Whether this disparity was caused primarily by spatial emergence patterns or larval behavior, was the subject of this study. Studies on the spatial emergence patterns of adult mosquitoes were carried out in a 14 x 69 m experimental wetland mesocosm in southern California using 0.25 m<sup>2</sup> emergence traps during the summer of 1996. *Culex erythrothorax* appeared to be the dominant species collected and the number of emerging individuals exhibited a strong positive correlation with increasing density of emergent vegetation. Larval behavior of this mosquito in a laboratory aquarium was recorded by videotaping and analyzed by image analysis to determine larval location and aggregation. Larval behavior was measured under three regimens: low food, high food, and after a light/shadow stimulus. *Culex erythrothorax* larvae aggregated strongly and were distributed predominantly in the corners of the aquarium. The larvae also responded to a small (11%) change in light intensity. The strong association of larvae of this species with vertical structures and its propensity to dive when a change in light intensity occurs probably contribute to the difficulty of sampling populations of *Cx. erythrothorax* larvae. These phenomena may lead to an underestimation of the population densities of *Cx. erythrothorax* larvae using sampling techniques such as dipping. Additionally, the strong association of this species with emergent vegetation may confound control strategies aimed at larval populations.

key words: *Culex erythrothorax*, adult emergence, larval behavior, *Schoenoplectus*, mosquito control.

### INTRODUCTION

Constructed treatment wetlands are becoming an increasingly important aspect of water treatment throughout the world (McCarthy 1997). Potential applications of such technology include the treatment of industrial effluent, the mitigation of gray water, the treatment of stormwater runoff from freeways and the treatment of secondary and tertiary sewage effluent. Constructed wetlands can be built in nearly any area, are typically a low cost alternative to physical or chemical treatment, and generally do not require elaborate technology. They also provide habitat for indigenous and migrating wildlife, and serve as a site for public education about water related issues.

Application of wetlands technology is of particular interest in arid regions of the world including the southwestern United States. Currently, there are 50 constructed wetlands in this region (Brown and Reed 1994). The Multipurpose Wetlands Research and Demonstration Project in San Jacinto, California is a multiagency effort to develop design and operational criteria for large

wetlands intended to process wastewater. The project is evaluating wastewater processing in a large (10 ha) demonstration wetland and eight smaller (0.1 ha) experimental wetlands (research cells). The wetlands are planted primarily with the California bulrush [*Schoenoplectus californicus* (Meyer) Soják], which is used to remove nutrients, particularly nitrogen, from secondary treated sewage effluent and to provide habitat for wildlife. Unfortunately, the bulrush which serves a vital role in the functioning of the wetland, also provides harborage for mosquitoes.

Previous studies have shown that the constructed wetlands in the Multipurpose Wetlands Research and Demonstration Project are capable of producing large numbers of host-seeking mosquitoes (Walton et al. 1996, Walton and Workman 1998). As vegetation stands became increasingly dense and extensive, host-seeking female populations of *Cx. erythrothorax* Dyar reached 33,000 females/trap night. However, concurrent dip sampling for larval populations at both sites yielded few *Cx. erythrothorax* larvae (Walton and Workman 1998). The total number of *Culex erythrothorax* larvae rarely exceeded 1 larva per dip, with a one-time maximum of 7

larvae per dip. Further, several capture-mark-recapture studies illustrated that adult *Cx. erythrothorax* remained near the wetlands complex and rarely dispersed further than 0.5 km outside of the confines of the marsh systems (Walton et al. 1999).

The purpose of this research was to address the discrepancy in host-seeking female versus larval abundance for *Cx. erythrothorax*. Our studies focused on two aspects of *Cx. erythrothorax* biology: the spatial emergence patterns of adults from a research cell mesocosm and the behavior of larvae under laboratory conditions.

## MATERIALS AND METHODS

**Adult Emergence.** Adult collections were carried out from August until October 1996 using a 0.1-ha (14 x 69 m) research cell mesocosm. California bulrush grew throughout the wetland which was supplied with secondary-treated sewage effluent (average flow of 32 liters  $\text{min}^{-1}$ ) from a nearby treatment plant.

The relationship between adult mosquito emergence and vegetation density was examined by placing nine 0.25  $\text{m}^2$  emergence traps over stands of *S. californicus* with varying culm densities. The vegetation in each quadrat was cut to ~ 3 cm above the water surface to allow for trap placement while not disturbing the integrity of the vegetation at or below the surface of the water. The number of culms in each quadrat was determined during initial trap placement for each vegetation stand. To prevent traps from being tipped over by growing vegetation, the bulrush height was maintained by clipping twice weekly. A tenth trap was placed over open water. All traps were placed to ensure that the bottom margin of the trap remained below the water surface.

Sample jars at the apex of the emergence traps were collected and replaced weekly, the contents were killed by freezing and counted under 25x magnification. Adults were identified to species using (Bohart and Washino 1978) and sexed. The relationship between tulle culm density and the number of emerged mosquitoes was analyzed using a least squares linear regression. Prior to analysis, emergence and culm density data were  $\log_e(x+1)$  transformed.

**Larval Behavior.** *Culex erythrothorax* larvae used for behavioral observations were the first generation progeny of wild adults collected with  $\text{CO}_2$ -baited traps at the constructed wetlands research complex. Adults were offered blood meals from both mice and 1 day-old chicks in the laboratory. Egg rafts were separated into 6 ounce (200 ml) Sweetheart waxed ice-cream cups for rearing. Larvae were reared at  $31 \pm 2^\circ\text{C}$ , a light:dark cycle

of 14:10 hours, and fed a mixture of mouse chow and brewer's yeast (3:1).

For behavioral observations, five larvae from a single egg raft in the first or second day of the fourth instar were chosen at random for each videotaping session. Eight 45-minute videotape sessions were conducted, which were comprised of three 15 minute episodes: diving behavior under low food conditions, diving behavior associated with a light/shadow stimulus and diving behavior under high food conditions. Larval behavior was videotaped using a Sharp Hi8 Viewcam (Model VL-H410V).

During the course of the study, one of two acrylic aquaria were used: an aquarium (14.0 x 0.9 x 11 cm) with a colorless front and back and blackened sides and bottom that was placed against a white backdrop; the other, (14.0 x 2.6 x 12.2 cm) with a colorless front, white sides and back and a black bottom. The latter allowed better resolution of the larvae during tape analysis and was used in approximately half of the taping sessions. The aquaria were filled to a depth of 10 cm with aged (2 weeks of aeration), 0.22 mm filtered (Super 200 membrane filter) tap water which was changed between sessions. The aquarium was illuminated using a combination of a 20W full spectrum fluorescent light bulb (Fluor Laboratories #RS1-24-20W) suspended 20 cm above the aquarium, a 75W incandescent light bulb (Phillips) suspended 30 cm above the top of the aquarium, and six 40W overhead fluorescent lights (Sylvania Super Saver Cool White). Ambient light levels at the surface of the water during taping were 786  $\text{lm m}^{-2}$ .

Larvae were allowed ten minutes to acclimate in the aquarium before recording their behavior. Behavior under low food conditions was recorded first in all sessions.

Diving behavior associated with the light/shadow stimulus was elicited by switching the overhead fluorescent lights off then back on. The change in light level was approximately 89  $\text{lm m}^{-2}$ , representing a decrease in light level of 11% from ambient levels in the room. The light stimulus episodes were taped immediately following exposure of larvae to low food conditions.

Larval behavior in the high food conditions was recorded after the addition of 0.5 ml of an activated yeast solution [0.026  $\text{g ml}^{-1}$  (small aquarium) or 0.075  $\text{g ml}^{-1}$  (large aquarium) Fleishman's Yeast]. After addition of the yeast suspension, the media in the aquarium was thoroughly mixed by pumping with the pipette approximately 15 times. Larvae were acclimated for 10 minutes before taping commenced.

Larval mosquito behavior was analyzed using a Sony EVS-5000 Hi8 videocassette recorder and a personal computer equipped with Image Pro® image analysis software. During diving analyses, larval position was tracked by following the larval head capsule.

In order to determine whether the larvae preferred particular regions of the aquarium (e.g., corners) and whether larvae were aggregated, the horizontal position of larvae at the surface was measured under low and high food conditions. A single frame was captured every thirty seconds within each episode (low/high food treatments only) and the horizontal position of each larva at the water surface was measured relative to the left wall of the aquarium. The minimum distance between larvae was measured for each frame.

Analysis of diving behavior associated with light stimulus considered the number of larvae responding to the change in light level. Additionally, the duration of each dive was measured.

Statistical analysis of the degree of aggregation of larvae was complicated due to the left-skewed distribution of distances. Therefore, the minimum distances between larvae were log-transformed and compared using a Kolmogorov-Smirnov test to determine whether the distribution of the transformed minimum distances between larvae for a food level combination was normal. Distributions were then compared within a particular food level by the Kolmogorov-Smirnov test. A Kruskal-Wallis nonparametric one-way ANOVA on ranked medians for the distance between larvae for each session was used to determine whether significant differences in aggregation existed between *Cx. erythrothorax* and a random distribution.

Horizontal distribution of the larvae was analyzed by dividing the aquarium into four horizontal zones. Data were analyzed using a Chi-squared test where the null hypothesis assumed a random distribution over the area would result in 10% of the larvae occurring in Zone A and 30% of the larvae occurring in each of the Zones B, C and D.

## RESULTS AND DISCUSSION

**Adult Emergence.** Emergence of adult *Cx. erythrothorax* showed a strong positive correlation with vegetation density ( $r = 0.93$ ). As stem density increased above 800 culms  $m^{-2}$ , adult emergence exceeded 300 adults  $week^{-1}$ .

**Larval Behavior.** In both low and high food conditions, *Cx. erythrothorax* was highly aggregated compared to a random larval distribution (Table 1). The

median minimum distance between larvae under low food conditions (0.58 cm) was significantly less than the median minimum distance (1.46 cm) expected under a random distribution (Tukey's test,  $P < 0.05$ ). Under high food conditions, the degree of aggregation of *Cx. erythrothorax* (median distance 0.36 cm) was significantly different (Tukey's test,  $P < 0.05$ ) than the random model (Table 1).

*Culex erythrothorax* was distributed non-randomly in the aquarium. Larvae occurred most frequently near the sides of the aquarium in both food levels (Zone A: 51% of the time). This is significantly more frequently than the 10% expectation for the larvae randomly distributing themselves ( $\chi^2_3 = 44.362$ ,  $P < 0.001$ , and  $\chi^2_3 = 49.393$ ,  $P < 0.001$ , low food and high food, respectively).

*Culex erythrothorax* was sensitive to the low level light stimulus (Table 2). An average of 69% of the larvae responded to any given stimulus. In contrast *Cx. tarsalis* larvae were found to be unresponsive to such a stimulus (Workman 1998). When responding to a light stimulus, the average dive time for *Cx. erythrothorax*, was  $58 \pm 4$  seconds (Table 2).

TABLE 1. Degree of aggregation as measured by median distance to nearest neighbor for a hypothetical random distribution of larvae and for *Culex erythrothorax* under low and high food condition.

	Low Food	High Food
Random Distribution	1.46	1.35
<i>Cx. erythrothorax</i>	0.58	0.36

TABLE 2. Proportional response of *Culex erythrothorax* larvae to 11% change in ambient light levels and average dive time for those larvae responding.

	Proportional Response	Dive Time (s)
Mean	0.611	58.2
S.E.	0.061	4.24

The aggregation behavior and horizontal distribution of *Cx. erythrothorax* larvae in the laboratory is congruent with that observed for adult emergence in the field. The propensity of larvae to spend the majority of their time in the corners of the aquaria used in the behavioral studies indicates that these larvae are drawn to vertical structures in the water column. This situation appears to be analogous to the association between vegetation density and *Cx. erythrothorax* emergence in the

field. Additionally, larvae were observed to scrape the vertical surfaces of the aquarium before taping commenced (personal observation), further suggesting that *Cx. erythrothorax* larvae utilize vertical surfaces for feeding. Interestingly, a high degree of aggregation existed in both low and high food environments. Even though low food environments are qualitatively very different than the turbid, organically enriched habitat in which these larvae are found at the study site, under low food conditions, the larvae continued searching for food on vertical surfaces.

The strong association of *Cx. erythrothorax* with vertical surfaces in the laboratory and with vegetation in the field contribute to making this species difficult to monitor in the pre-adult stages using conventional dipping techniques. When vegetation becomes very dense as seen in the emergence experiment, it is very difficult to sample larvae by dipping (personal observation). This problem is compounded by the habit of this species to dive when exposed to a disturbance in light level and remain submerged for approximately one minute. Clearly, different techniques are needed to assess these populations prior to adult emergence.

The behavior of *Cx. erythrothorax* also may confound control strategies aimed at the larval populations. The dense vegetation inhabited by these larvae may hinder current control technology. Fish and macroinvertebrate predators might also have difficulty penetrating the dense vegetation.

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# Incorporating Weather Variables in Disease Models: A Retrospective Longitudinal Study of Dengue Fever in Trinidad and Tobago

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**ABSTRACT:** Historical dengue fever morbidity and weather data from Trinidad and Tobago were compiled and analyzed to assess possible relationships between weather variables and dengue fever incidence. This study evaluated retrospectively the relationship between morbidity incidence attributed to dengue fever in Trinidad and Tobago and local variations in temperature and precipitation. Weekly dengue morbidity data from the country of Trinidad and Tobago and monthly precipitation and temperature data from the Port of Spain, Trinidad were analyzed for a nine-year period (1982-1990).

## INTRODUCTION

Dengue fever is caused by a virus from the family Flaviviridae; four serotypes are recognized, dengue-1, dengue-2, dengue-3, and dengue-4. All four serotypes have been found in the Americas. However, only serotypes 1, 2, and 4 circulated during 1982-1990.

The virus is transmitted primarily by female *Aedes aegypti* which imbibe human blood to nourish their developing eggs. *Aedes aegypti* is a highly domesticated mosquito, breeding in freshwater containers stored for drinking and bathing and in unused tires outside homes. Because severe frost and cold weather kills adult *Ae. aegypti* mosquitoes and eggs (Knipling and Sullivan 1957), dengue is currently restricted to tropical and subtropical regions.

The clinical manifestations of dengue fever can range from a minor fever, often with flu-like symptoms, to severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) that may result in death. Between 200,000 and 500,000 cases of the severe DHF/DSS form occur yearly throughout the world and case fatalities can reach forty to fifty percent if not treated with fluid replacement therapy (Benenson 1990).

The relationship between weather and dengue fever incidence is multifaceted, involving both the mosquito life cycle and viral replication requirements. It has been determined that warmer temperatures reduce larval size of *Ae. aegypti* (Rueda et al. 1990), resulting in smaller adults. Smaller adult female mosquitoes have been found to feed more frequently to nourish their developing eggs (MacDonald 1956), increasing the probability of obtaining and transmitting the virus.

A second consideration in viral transmission is the extrinsic incubation period (EIP), defined as the time from the uptake of an infectious bloodmeal until a mosquito is capable of transmitting a virus. A study by Watts et al. (1987) determined that the EIP is heavily contingent upon temperature; the EIP for *Ae. aegypti* decreased from twelve to seven days when mosquitoes were kept at 32-35°C instead of 30°C. The reduced EIP observed by Watts et al. may result in a greater number of dengue fever cases because it will take less time before the vector can transmit the virus.

Higher precipitation may either increase or decrease dengue incidence rates. In the first scenario, high precipitation may enhance human contact with *Ae. aegypti* because both the vector and humans are more likely to spend time indoors. In the second scenario, an inverse relationship between precipitation and transmission of the dengue virus exists. In a study in Brazil, it was found that low rainfall in results in more water storage containers in homes and therefore more *Ae. aegypti* in residential areas (Soper 1938). This relationship results in a negative correlation between rainfall and dengue fever incidence.

Climatologists have predicted a two-degree increase in global temperatures by the end of the next century caused by an expected doubling in atmospheric carbon dioxide (Houghton et al. 1996). If global warming occurs, increased temperatures may have tremendous implications for viral diseases whose vectors are sensitive to climate changes. Dengue fever is the arboviral disease thought to pose the greatest threat in North America, should global warming occur (Shope 1991). It has been predicted that warming may shift the distribution and

frequency of dengue, with dire consequences to public health in the areas affected (Patz et al. 1996).

The implications of global warming on dengue transmission has been modeled by Martens et al. (1987) in a study that indicated that the transmission potential of dengue may be highly sensitive to climate changes. They predicted that transmission should be particularly sensitive to warming in higher altitudes and in areas that are currently at the periphery of endemic transmission.

Jetten and Focks (1997) have also developed a model demonstrating the influence of warming on the intensity and distribution of dengue throughout the world. Using a simulation model projection, their results indicate that the current warming prediction of two degrees by 2100 may result in an increase in both the latitude and altitude range of dengue fever. They also concluded that the duration of the transmission season could increase in temperate locations.

As yet, there has been no study published of actual case data that seeks a retrospective statistical association between weather variables and dengue incidence. Studies of dengue in the past have not looked at weather factors independently, nor have they looked longitudinally at how these factors may affect morbidity rates. This study is intended to evaluate the relative importance of temperature and precipitation to transmission using long-term case data from the islands of Trinidad and Tobago.

## OBJECTIVES

The main objective of this study was to determine retrospectively, whether there was a temporal pattern of association between weather variables and dengue fever morbidity rates. Because mosquito density, activity, and survival are related to various weather conditions, there may be a correlation between dengue fever incidence and weather patterns. However, it is uncertain whether temperature or precipitation will be the strongest predictor of dengue incidence, or whether a more predictive relationship exists when both are taken into account.

The time lag between weather phenomenon and changes in dengue fever incidence is also uncertain. The components of the probable time lag include: the period of embryonic development of the mosquito, hatching time, larval and pupal development, the adult and sexual development period, the time before the first blood meal, the EIP, and the time before the appearance of clinical manifestations of dengue fever. It has been found that the EIP for *Ae. aegypti* will range from seven to twelve

days depending on ambient temperature (Watts 1987). It has also been determined that the period between infection and clinical manifestations of disease in humans ranges from four to six days (Pan American Health Organization 1994). It is therefore assumed that a time lag of at least 11 and perhaps as much as 18 days will be found. However, this may be a very conservative estimate as it does not take into account the life cycle and development of the mosquito which varies according to ambient weather conditions, the availability of food, and larval density in the container that serves as the mosquito's breeding site.

## MATERIALS AND METHODS

Monthly mean precipitation and temperature data from the Piarco International Airport in Trinidad was downloaded from the National Climatic Data Center (<http://www.ncdc.noaa.gov/cgi-bin/res40.pl>).

Weekly incidence rates of dengue fever in Trinidad and Tobago were obtained from Dr. Jose Campione at the Caribbean Epidemiology Center, who gathered and entered the morbidity data.

The data were analyzed both qualitatively and quantitatively. The weather parameters were plotted over time with respect to the case data, and quantitative models were developed to determine which had the best fit.

Both linear and exponential univariate and bivariate regressions were conducted. Models were developed expressing no time lag between the weather variables and dengue fever incidence, a lag of up to 12 months, with both temperature and precipitation as independent variables, and with each alone as the independent variable, removing outliers, and testing precipitation and temperature with different time lags.

## RESULTS AND DISCUSSION

Two models demonstrated a statistically significant relationship between weather variables and morbidity due to dengue fever. Both of the models occur at a time lag of six months. The first model expresses a relationship in which temperature is the independent variable and morbidity, lagged back six months is the dependant variable. The second model expresses a relationship in which both temperature and precipitation are independent variables and morbidity, lagged back six months is the dependent variable. Both models were statistically significant, but neither was robust, thus their utility should be questioned.

Despite the fact that neither model was robust, both indicate interesting relationships that are worthy of comment. In both models, a positive correlation exists between temperature and dengue fever morbidity. It is possible that temperature influences the life cycle of the mosquito or the viral replication rate, resulting in more cases of dengue fever at higher temperatures.

Another interesting relationship indicated by the second model is the negative correlation between precipitation and morbidity. This may be due to either more water storage during low rainfall or the flushing of outdoor larval breeding sites when rainfall is high.

The last significant relationship is the six-month lag observed in both models. The results indicate that it takes six months for weather to affect dengue fever morbidity rates. The reason for this length of time is unknown. A time length of one to at most two months was expected given the timing of the mosquito life cycle. However, both models appear to follow natural trends.

To improve upon the model, a larger data set should be used, incorporating a longer time-span. Or possibly, other weather factors including humidity and wind speed should be analyzed.

In conclusion, the present effort is a demonstration of model development for arboviral diseases. Similar models can be used by public health practitioners and vector control specialists to develop control measures before epidemic situations arise.

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