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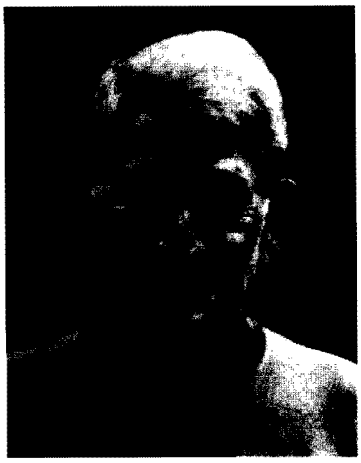
CONFERENCE DEDICATION

DEDICATION OF THE 64TH ANNUAL M.V.C.A.C. CONFERENCE TO ALLEN RALPH BARR

Bruce F. Eldridge

Director of Mosquito Research Program
University of California, Davis



Allen Ralph Barr
1926 - 1995

A. Ralph Barr, a scientist and teacher with a distinguished record of service to the State of California in the area of mosquito biology, and a long-time associate member of the California Mosquito and Vector Control Association, passed away July 2, 1995, after an extended illness. Ralph was a public health entomologist with an international reputation for his research on mosquito systematics and genetics. His scientific contributions were made at several U.S. universities, including the University of California at Los Angeles and at the Fresno field laboratory of the Bureau of Vector Control (BVC) of the California Department of Public Health. He also served on many national and international research boards and committees. Over the span of his 50-year career he wrote about 150 scientific articles and book chapters, and served as major professor for a number of graduate students.

Ralph was born August 13, 1926, in Fort Worth, Texas. He attended public schools in Fort Worth and Dallas, and after graduation from high school, volunteered for service in the U.S. Navy. During World War II he served aboard merchant ships and mine sweepers in the Pacific theater. After his discharge from the Navy in 1946, Ralph attended Southern Methodist University in Dallas where he received a B.Sc. Degree in biology.

In 1948, Ralph entered graduate school at the Johns Hopkins School of Public Health in Baltimore. He was guided in his study of medical entomology by Professor Lloyd Rozeboom. While at Johns Hopkins, Ralph met Slyvia Engel, who was a student in the Department of Art as Applied to Medicine. Ralph and Slyvia were married in 1952, and remained partners in life and in science for the next 43 years.

Ralph's first job after graduation was the University of Minnesota. During the three years he was there, Ralph published several very important papers, including the paper which resulted in the

resurrection of the name of the mosquito species *Aedes melanimon*, the description of *Culiseta minnesotae* Barr, and the publication of *The Mosquitoes of Minnesota*. This last work featured illustrations by Sylvia Barr, and is still regarded as one of the finest treatments of a state mosquito fauna ever produced

In 1955, Ralph moved on to the University of Kansas to become Assistant Professor. He completed several more important projects on mosquito biology here, and then, in 1958, Ralph was asked to come to California to become Supervisor of Vector Research for BVC and to direct the activities of the BVC field research laboratory in Fresno. Under Ralph's leadership, the Fresno laboratory established a fine reputation for conducting applied research, and Ralph and his staff produced reports of a number of significant studies on mosquito biology and control.

In 1965, as a result of a decree from the California Legislature that the Department "discontinue Department-staffed mosquito research activity" which led eventually to the transfer of BVC's research program to the University of California, Ralph and some of his staff became employees of the University, and Ralph was appointed Research Specialist II, then Associate Entomologist III (equivalent to Assistant and Associate Professor in the academic series). Then, in 1967, Ralph moved to the School of Public Health at UCLA as an Associate Professor. He served at the School for the next 24 years until his retirement as Professor in 1991. Perhaps his most famous scientific contribution while at UCLA was his discovery with J.H. Yen that "cytoplasmic incompatibility" in *Culex pipiens*, a phenomenon promoted by the World Health Organization as a means of controlling mosquito populations, was actually caused by a microorganism in the genus *Wohlbachia*.

During his years in California, Ralph was an active participant in the U.C. Mosquito Research Program, and made significant contributions to out understanding of the biology of anopheline vectors of malaria in southern California. He also contributed to the education of a number of graduate students in medical entomology at the School who went on to become prominent scientists in California and elsewhere.

Ralph Barr was an accomplished scientist and teacher who left his mark in a number of areas. His most significant contributions were in the area of mosquito biology, especially in systematics and genetics, two areas in which he was regarded to be a national leader. His research in these areas was meticulously conducted and reported.

Ralph Barr will be remembered for his intellectual honesty, and for his scholarly approach to research. He will also be remembered for his love of classical writing, music, gourmet food, philately and fine wine. He will also be remembered as a person who hated neckties, and had the courage of his convictions to never wear one.

Ralph received the Meritorious Service Award in 1981 from the American Mosquito Control Association, and the Memorial Lectureship Award in 1986. Last year, shortly before his death, Ralph was awarded a Lifetime Achievement Award by MVCAC.

SURVEILLANCE FOR MOSQUITO-BORNE ENCEPHALITIS VIRUS ACTIVITY AND HUMAN DISEASE IN CALIFORNIA, 1995

Vicki L. Kramer, Michael S. Ascher, Barryett Enge, Lucia T. Hui, Sarah Ball, Franklin Ennik, Kevin Reilly, William K. Reisen[1,2], Donald A. Eliason[3], Jieyan Lin, Robert A. Murray, Marilyn M. Milby[1], James L. Hardy[1], Sally B. Presser[1], Robert E. Chiles[1,2], William C. Reeves[1]

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The California Arbovirus Surveillance program is a cooperative effort conducted by the California Department of Health Services' (CDHS) Division of Communicable Disease Control; the Arbovirus Research Unit at the University of California, Berkeley (recently moved to the Davis campus); the Mosquito and Vector Control Association of California; local mosquito and vector control agencies; local health departments, physicians and veterinarians; and other interested parties. The surveillance program is multifaceted and includes: 1) mosquito population monitoring and testing for arboviruses, 2) serological monitoring of sentinel chicken flocks in areas of California with historical evidence of encephalitis virus activity, 3) testing of domestic animal species that may show clinical illness with arbovirus infection, and 4) serological testing of patients suffering from fever, neurological symptoms, and other signs of viral meningitis or encephalitis. The 1995 surveillance program started in April with the first deliveries of sentinel chicken flocks to cooperating local agencies, and the beginning of light trapping and adult mosquito occurrence data collection. Twenty-seven weekly bulletins and adult mosquito abundance reports were faxed and mailed out on a weekly basis to all surveillance program participants starting on May 12. Positive serology and mosquito pool results were communicated immediately to the submitting agency.

Human Disease Surveillance

In 1995, 37 human sera and cerebral spinal fluid (CSF) specimens from patients suffering symptoms of viral meningitis or encephalitis were tested by the Department's Viral and Rickettsial Disease Laboratory (VRDL) for antibodies to St. Louis encephalitis (SLE) and Western equine encephalomyelitis (WEE). None of these sera showed IgM antibody of a four-fold rise in total antibody between paired sera to meet the diagnostic requirements to document a case of arboviral encephalitis.

In collaboration with the Health Departments of Imperial, Sacramento and Sutter Counties, the Arbovirus Research Unit tested the sera of 1,756 anonymous outpatients attending clinics from April through November 1995 for other unknown health problems to determine the prevalence of antibodies to WEE and SLE viruses. Sera were screened by enzyme immunoassay (EIA) and positives were confirmed by plaque reduction neutralization tests (PRNT). This survey describes the general infection experience of this patient population, but cannot determine accurately the time or place of infection and may detect antibodies to cross-reacting viruses. Overall, 0.8 and 4.8% sera were positive for WEE and SLE, respectively, by both EIA and PRNT. Of the SLE and WEE positives, 64 and 90%, respectively, were residents of Imperial County, agreeing well with the consistently elevated level of enzootic transmission to sentinel chickens in this area.

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(e.g., Reisen et al. 1992, Lothrop et al. 1994). The seropositivity rate for WEE among residents of Sacramento (0.3% of 977 tested) and Sutter (2% of 89) Counties was unexpectedly low, considering the widespread and elevated rates of enzootic transmission to sentinel chickens documented during 1993 and 1994 (Reisen et al. 1995a), and indicated that the risk of infection of the human population to WEE was minimal. Similar results from Coachella Valley where only 31% of 118 sera positive for antibodies to SLE by EIA were confirmed by PRNT (Reisen et al. 1995b), only 65% of 129 sera positive for SLE by EIA in the current survey were confirmed by PRNT (range, 20% in Sutter County to 82% in Imperial County). Of the EIA-positive but PRNT-negative sera, 26 were re-tested by the Centers for Disease Control and Prevention and 15 (58%) had neutralizing antibodies against at least one of the four serotypes of dengue virus. These infections most likely were acquired elsewhere, because dengue transmission has not been detected in California.

Equine Surveillance

A total of 13 sera and brain tissue specimens from horses displaying neurological signs were submitted by practicing California veterinarians for arboviral testing at VRDL in 1995. None of these specimens were positive on arbovirus serology or antigen testing.

Mosquito Testing

Mosquitoes captured with light traps and other methods conducted by 26 local mosquito control agencies around the state were pooled and tested for arboviruses at VRDL using standard methods. Of 2,147 mosquito pools (2,570 total mosquitoes) tested for both SLE and WEE (Table 1), only 4 pools were positive for WEE, and none were positive for SLE. Positive *Culex tarsalis* pools were collected from August 21 through September 20 in Lake, Sutter, Yolo, and Sacramento Counties.

Table 1. Mosquitoes tested by VRDL for WEE, SLE and CE viruses in 1995, by submitting agency and mosquito species.

		Cx. tarsalis		*Cx. pipiens/ quinquefasciatus*		*Cx. stigmatosoma*		*Ae. melanimon*		Total	
County	Agency	pool	mosq.	pool	mosq.	pool	mosq.	pool	mosq.	pool	mosq.
Alameda	ALAM	2	69	0	0	0	0	0	0	**2**	**69**
Contra Costa	CNTR	99	4822	0	0	0	0	0	0	**99**	**4822**
Fresno	FRNO	9	258	0	0	0	0	0	0	**9**	**258**
Glenn	GLEN	8	400	0	0	0	0	0	0	**8**	**400**
Inyo	INYO	10	370	0	0	0	0	6	300	**16**	**670**
Jackson/OR	JACK	78	2897	0	0	0	0	0	0	**78**	**2897**
Kern	KERN	192	9061	0	0	0	0	5	185	**197**	**9246**
Kings	KNGS	49	2118	0	0	0	0	0	0	**49**	**2118**
Lake	LAKE	213	10438	0	0	0	0	33	1496	**252**	**12167**
Los Angeles	LONG	53	2247	27	1030	0	0	0	0	**80**	**3277**
Los Angeles	LOSA	75	2989	16	800	1	21	0	0	**92**	**3810**
Los Angeles	SGVA	17	485	17	500	2	63	0	0	**36**	**1048**
Los Angeles	SOUE	74	2684	44	1485	6	81	0	0	**124**	**4250**
Madera	MADR	10	500	0	0	0	0	0	0	**10**	**500**
Orange	ORCO	5	106	76	2137	0	0	0	0	**81**	**2243**
Riverside	NWST	120	5997	82	4096	27	1340	0	0	**229**	**11433**
Sacramento/Yolo	SACR	185	8370	0	0	0	0	5	133	**190**	**8503**
San Bernardino	SANB	86	3789	23	800	9	275	0	0	**118**	**4864**
San Diego	SAND	7	313	0	0	0	0	0	0	**7**	**313**
San Mateo	SANM	21	995	0	0	0	0	1	48	**22**	**1043**
Santa Barbara	GLVY	9	225	12	524	0	0	0	0	**21**	**749**
Shasta	SHAS	24	1037	0	0	0	0	0	0	**24**	**1037**
Stanislaus	TRLK	22	1020	1	50	0	0	1	35	**24**	**1105**
Sutter/Yuba	SUYA	304	12672	0	0	0	0	24	898	**328**	**13570**
Tulare	DLTA	29	1078	0	0	0	0	1	35	**29**	**1078**
Ventura	VENT	21	1050	1	50	0	0	0	0	**22**	**1100**
SUM		**1722**	**75990**	**299**	**11472**	**51**	**2013**	**75**	**3095**	**2147**	**92570**

Chicken Serosurveillance

In 1995, a total of 181 sentinel chicken flocks were maintained by 54 local mosquito and vector control agencies. Twenty-eight of these flocks were involved in arbovirus research projects conducted by the Arbovirus Research Unit, University of California. Over 20,830 chicken sera were tested serologically for WEE and SLE by VRDL. Eighty sera tested positive for WEE (Table 2), and 113 were positive for SLE (Table 3). The first seroconversions to WEE occurred in sera collected from Imperial County on June 26, and to SLE in sera collected from Riverside County on July 10. Locations of chicken flocks that tested seropositive in 1995 are shown in Figures 1 and 2. SLE activity was confined to southern California, primarily in irrigated agricultural and salt marsh habitats in Imperial and Riverside Counties. WEE activity was found in that same area, and in the Sacramento Valley.

There was slightly more WEE activity detected by the surveillance program in 1995 than in 1994 (29 seroconverted flocks with 81 birds in 1995 versus 25 seroconverted flocks with 49 birds in 1994). SLE activity in 1995 was similar to activity in 1994. It had been anticipated that 1995 would have greater arbovirus activity because of the heavy winter and spring rains and flooding. Enhanced mosquito control efforts and relatively dry weather in late spring may account, in part, for the lack of extensive arbovirus activity.

Table 2. Chicken seroconversions to WEE by location and biweekly sampling date, 1995[1].

County	Location	City	6/30	7/15	7/31	8/15	8/30	9/15	9/30	10/15	10/30	Total
Butte	Grey Lodge	Gridley	0	0	0	0	0	0	3	2	0	**5**
Butte	Honcut Road	Honcut	0	0	0	0	0	0	0	2	0	**2**
Butte	M&T Ranch	Chico	0	0	0	0	0	1	0	0	0	**1**
Butte	Thebach Ranch	Biggs	0	0	0	0	0	0	1	0	0	**1**
Colusa	Grussenmeyer Ranch	Colusa	0	0	0	0	0	0	1	0	0	**1**
Fresno	Firebaugh High	Firebaugh	0	0	0	1	0	0	0	0	0	**1**
Glenn	Co. Rd. 20	Orland	0	0	0	0	0	1	0	1	0	**2**
Glenn	N.E. Willows	Willows	0	0	0	0	0	2	0	0	0	**2**
Imperial	Bard	Bard	0	3	3	2	0	0	0	0	0	**8**
Imperial	Cady Rd.	Brawley	0	0	1	3	0	0	0	0	0	**4**
Imperial	Christopher	El Centro	0	2	1	1	0	0	0	0	0	**4**
Imperial	Keefer	Holtville	0	0	0	2	0	0	0	0	0	**2**
Imperial	Rio Bend RV Park	Seeley	2	2	0	0	1	0	0	0	0	**5**
Riverside	Adhor Farms	Mecca	0	0	5	0	0	0	0	0	0	**5**
Riverside	Dex-O-Tex	Mecca	0	0	0	0	1	0	0	0	0	**1**
Riverside	El Rancho	N. Shore	0	0	0	0	1	0	0	0	0	**1**
Riverside	Gordon	N. Shore	0	1	4	0	2	0	0	0	0	**7**
Riverside	Hayes	Mecca	0	0	1	0	0	0	0	0	0	**1**
Riverside	Jessup	Valerie	0	1	2	0	0	0	0	0	0	**3**
Riverside	Mecca	Mecca	0	0	1	0	0	0	0	0	0	**1**
Riverside	Salton Sea St. Park	N. Shore	0	1	0	0	3	0	0	0	0	**4**
Sacramento	G. Whitney	Hood	0	0	0	0	0	1	0	0	0	**1**
Sacramento	N. Stone Lake Site 1	Hood	0	0	0	0	0	1	0	0	0	**1**
Sacramento	Natomas	Sacramento	0	0	0	0	0	1	0	0	0	**1**
Sacramento	S. Rigg	Hood	0	0	0	0	0	2	0	0	0	**2**
San Joaquin	Midsection Rd.	Thornton	0	0	0	0	0	1	0	0	0	**1**
Sutter	Barker	Rio Oso	0	0	0	0	0	3	0	0	0	**3**
Sutter	Dean Ranch	Sutter	0	0	0	0	1	3	0	2	0	**6**
Yolo	Merritt	Woodland	0	0	0	0	0	2	1	0	1	**4**
		WEE Totals	**2**	**10**	**18**	**9**	**9**	**18**	**6**	**7**	**1**	**80**

[1]Positive chickens from Riverside County replaced after confirmatory sample.

THE APPLICATION OF GEOGRAPHICAL INFORMATION SYSTEM TECHNOLOGY TO SPATIALLY ANALYZE ECOLOGICAL FACTORS AFFECTING THE TRANSMISSION OF WESTERN EQUINE ENCEPHALOMYELITIS AND ST. LOUIS ENCEPHALITIS IN THE COACHELLA VALLEY, CALIFORNIA

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ABSTRACT

We are developing a geographical information system (GIS) to analyze the spatial relationships among ecological factors affecting the transmission of Western equine encephalomyelitis (WEE) and Saint Louis encephalitis (SLE) viruses in the southern Coachella Valley. The study area encompasses approximately 80 mi^2 (207 km^2), and represents all habitats within the valley, except urban areas. The variables include adult mosquito abundance, larval presence, virus transmission to sentinel chickens, habitat (classified by plant association and other factors), soil type, elevation, and irrigation method. The 1994 and 1995 adult abundance and virus transmission, 1995 larval presence, habitat, and soil data have been entered.

WEE and SLE surveillance in the Coachella Valley were limited in scope and intensity, prior to 1991 (Reisen et al. 1992a), and attempted to describe virus movement over large areas of southeastern California. Results from this "best estimate" sampling indicated that, although virus activity was detected during most seasons, the intensity of transmission was variable from 1987 through 1990. In 1991, we expanded sampling to 15 flocks of sentinel chickens that were positioned in most of the habitats and geographic regions of the lower valley (Reisen et al. 1992b). In 1992, four sites were added in urban, desert and oasis habitats in the upper valley (Lothrop et al. 1993). Our hypothesis was that virus activity might be related to habitat as defined by plant community and proximity to the Salton Sea and Whitewater Channel. Results from 1991-1992 were inconclusive regarding habitats, but indicated a positive relationship between proximity to the Salton Sea and mosquito abundance and virus transmission to sentinel chickens. Data indicated that the onset of virus transmission was focal and usually restricted to areas near salt marshes along the Salton Sea. Further, we roughly delineated three geographic regions based upon adult mosquito seasonality and abundance. Patterns in these regions seemed to be related to the temporal occurrence of larval sites, but in the absence of larval sampling, this association was unsupported.

In April, 1994, in collaboration with the Coachella Valley Vector and Mosquito Control District, we began development of a geographical information system (GIS) to determine which subset of ecological factors may affect mosquito abundance and transmission of WEE and SLE along the northern margin of the Salton Sea. This area was chosen for study, because it contained all of the rural habitat types in the Coachella Valley and because our historical data indicated that virus is initiated here each year.

MATERIALS AND METHODS

The data categories were: 1) adult mosquito abundance, 2) presence or absence of larval breeding sites, 3) transmission rate to sentinel chicken flocks,

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4) plant communities, 5) water usage, 6) soil type, and 7) elevation. Surface features and base maps were derived from aerial photographs of 1 inch to 4000 feet scale (2.04 cm per kilometer) and scanned at 300 dots per inch (118 dots per centimeter). This provided the necessary resolution to define mosquito breeding sites as small as 10 feet (3.5 meters) in diameter.

Culex tarsalis Say was the dominant mosquito in the study area and is considered to be the primary vector of both viruses (Reisen et al. 1992a). Therefore, although abundance of all species was recorded, the current presentation was limited to that species. Adult abundance was monitored using 63 CO_2-baited CDC style traps, located at each section boundary intersect and operated biweekly from April through November 1994 and March through November 1995. Mosquito breeding sites were sampled by mosquito control technicians in the course of their normal duties, and a subset was rechecked by supplemental staff under our direction. Because of the inherent difficulty in estimating abundance, larval samples were used to indicate species presence or absence. Plant communities were mapped from aerial photographs and "ground truthed". Agricultural land was classified by crop type or cropping method. Wetlands were divided into duck ponds (managed) and salt marsh (unmanaged). Soil types and distribution were entered from a soil survey published in 1980 by the USDA Soil Conservation Service. Water usage was gathered by ground survey, and elevation gradients were taken from 7.5' series topographical maps of the U.S. Geological Survey. Virus transmission activity was monitored using ten flocks of ten chickens each that were positioned approximately three miles apart and bled biweekly from April through November.

RESULTS

The accompanying figures are printed output from our GIS program to a HP LaserJet printer, and are the gray scale reproductions of the color images on the monitor.

The habitat types with the largest area were citrus, grape, undeveloped desert, and row crops (Figure 1). Crop type was related to soil type and elevation, with citrus and grapes in well-drained upland sites, row crops in intermediate sites, and duck ponds, fish ponds, and salt marshes near the level of the Salton Sea in alkali clay soils.

Irrigation practices and seasonal levels of the Salton Sea influenced mosquito abundance disproportionately to habitat area, therefore salt marsh, duck ponds, and some citrus produced most of the positive *Cx. tarsalis* larval samples, whereas row crops, dates, and grapes contributed few or none. Larval data for 1995 was more complete than 1994 due to delays in training seasonal field technicians in larval sampling protocols. For this reason our presentation will focus on 1995 data. In the adult data figures, abundance is represented by a dot density halo in natural log with approx. 1 km radius. Adult *Cx. tarsalis* abundance during spring began with peaks along the northeastern and western margins of the Salton Sea (Figure 2). Larval presence generally coincided with this distribution as indicated by hollow circles representing positive larval breeding sites. Areas along the western shore of the sea were not sampled due to a lack of access to Indian lands; however, from sampling done in 1994, we consider salt marsh to be the most important source for *Cx. tarsalis* in this area. Abundance generally declined during summer throughout the study area (Figure 3). In fall, abundance increased at areas along the Whitewater Channel and adjacent duck clubs (Figure 4), in the center of the study area. Late fall brought a general decline as the population entered diapause.

In the following description of virus transmission to sentinel chickens, it should be noted that seroconversion rates at the time of sampling represent infection history starting from up to ten days before the last sample taken two weeks earlier. As in previous years, transmission of WEE and SLE did not coincide with the spring peak of *Cx. tarsalis* abundance. WEE was first detected on July 10 at sites along the northern and western shores of the Salton Sea (Figure 5). The diameter of the hash marked circles represents percentage seroconversion for each flock, with the smallest mark representing $\geq$ 10%. On July 24, WEE transmission levels increased in the central region of the study area, dispersing to two new flocks and disappearing from one previously positive flock (Figure 6). WEE was no longer detected on the west shore after August 7 (Figure 7), but was detected at low levels in the northern shore area until August 21, after which it was no longer detected throughout the lower valley

Figure 1. Habitat types and distribution, undeveloped desert not shaded.

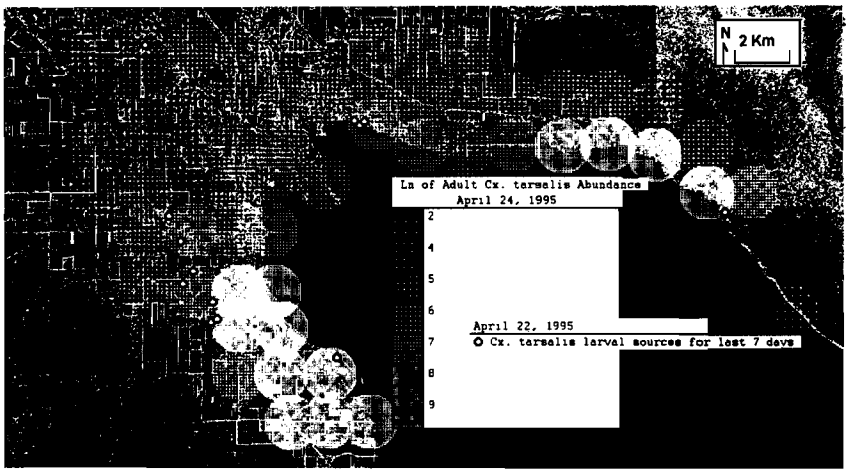

Figure 2. Larval presence and female *Cx. tarsalis* abundance data for April, 1995.

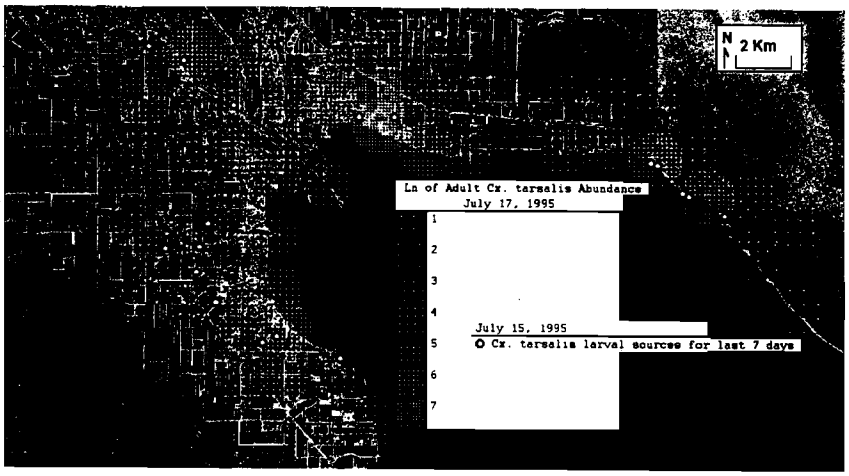

Figure 3. Larval presence and female *Cx. tarsalis* abundance data for July, 1995.

(Figure 8). WEE was not detected at two of three flocks located north of our study area.

SLE was first detected at low levels at two flocks along the northern shore (Figure 5), increasing in extent and intensity on July 24 (Figure 6). On August 7, SLE shifted westward and increased in intensity (Figure 7). Transmission was detected at all flocks and at high levels at three sites along the shore on August 21 (Figure 8). On September 4, transmission levels decreased, and SLE was detected at only four flocks (Figure 9). SLE transmission again increased in intensity and distribution by September 18 (Figure 10), followed by a decline by October 2 where it was detected at low levels at five flocks (Figure 11). Thereafter, SLE was no longer detected at high levels at two of three flocks located, to the north, in the towns of Mecca and Thermal.

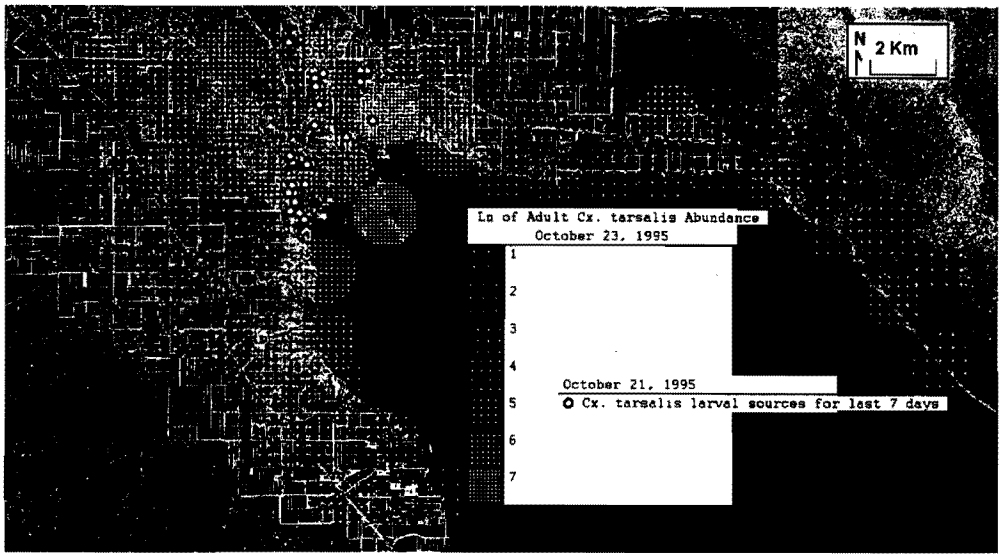

Figure 4. Larval presence and female *Cx. tarsalis* abundance data for October, 1995.

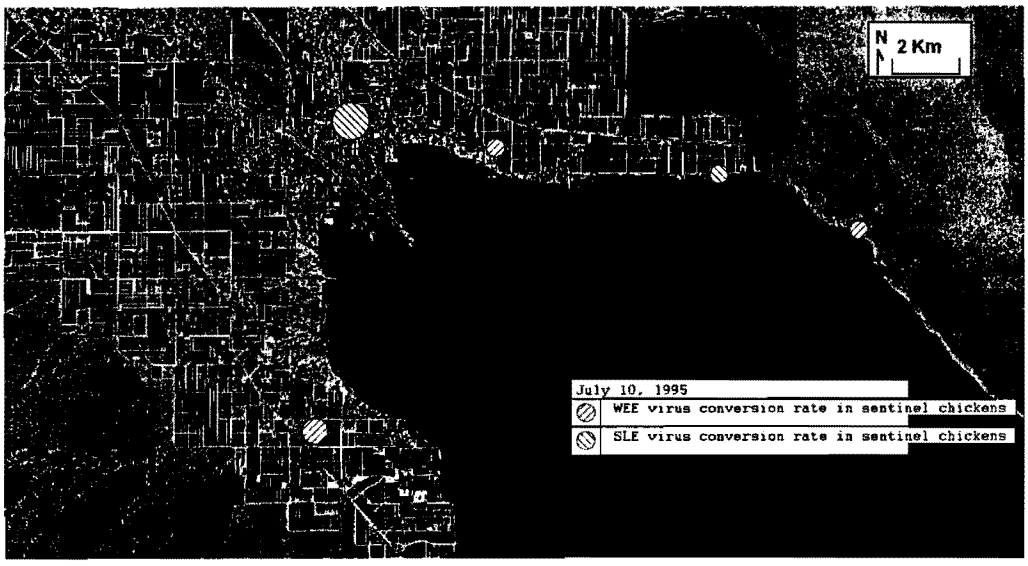

Figure 5. Distribution of SLE and WEE data as indicated by seroconversions among sentinel chickens, July 10, 1995.

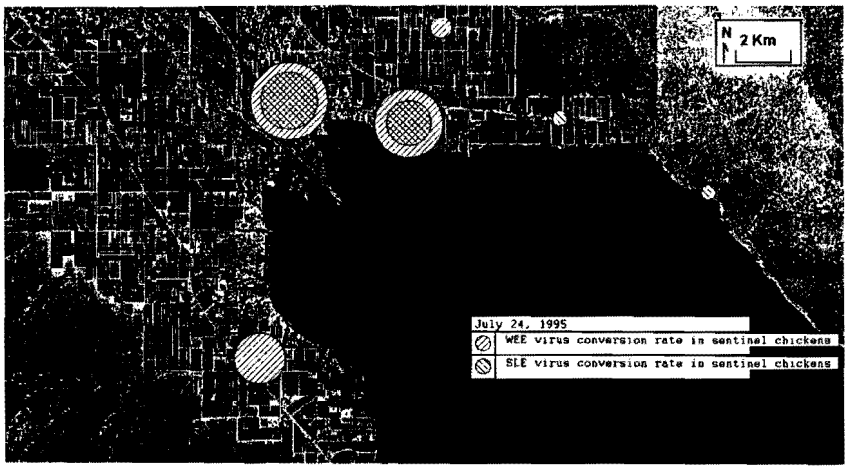

Figure 6. Distribution of SLE and WEE data as indicated by seroconversions among sentinel chickens, July 24, 1995.

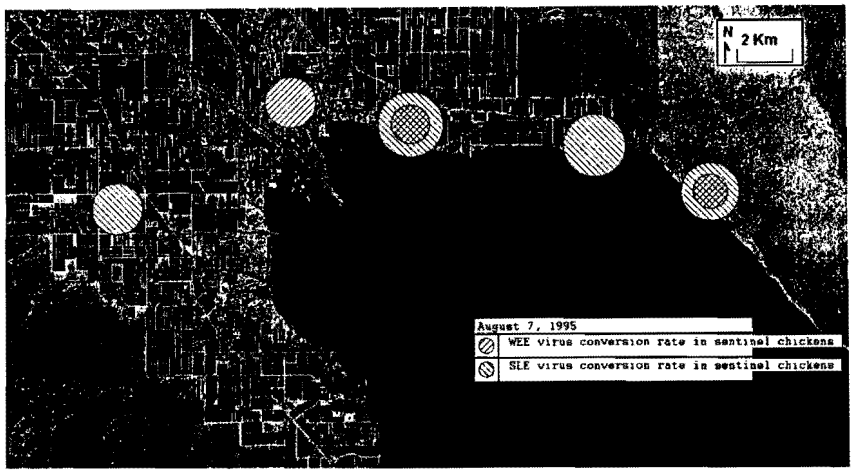

Figure 7. Distribution of SLE and WEE data as indicated by seroconversions among sentinel chickens, August 7, 1995.

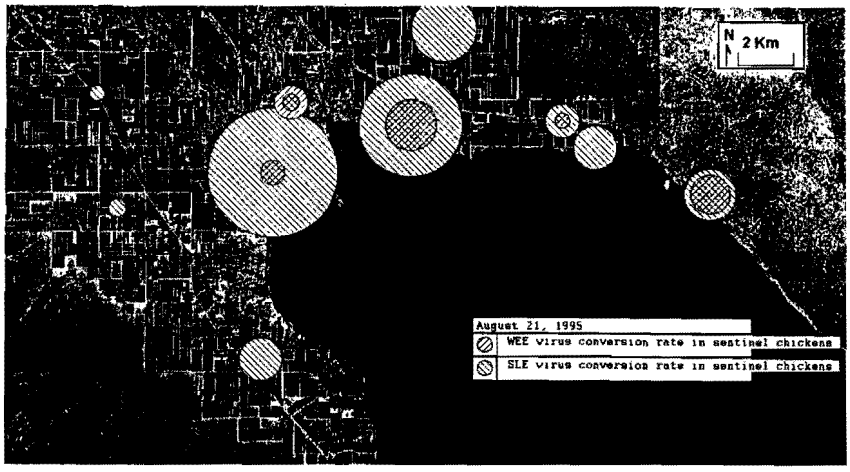

Figure 8. Distribution of SLE and WEE data as indicated by seroconversions among sentinel chickens, August 21, 1995.

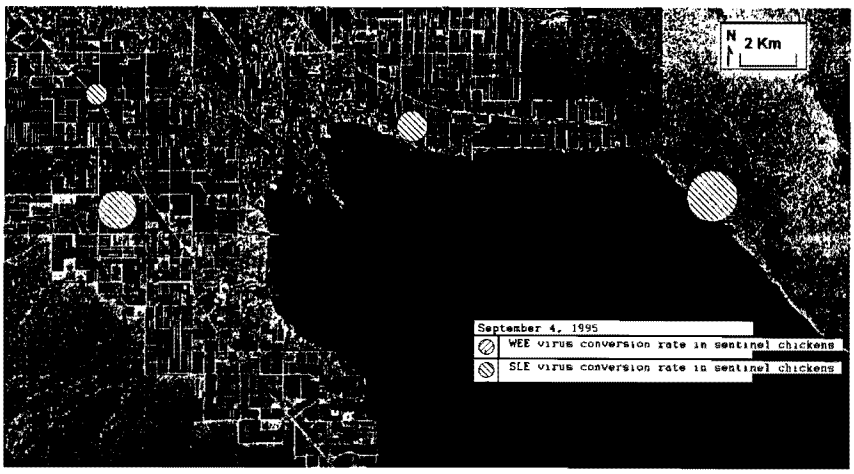

Figure 9. Distribution of SLE and WEE data as indicated by seroconversions among sentinel chickens, September 4, 1995.

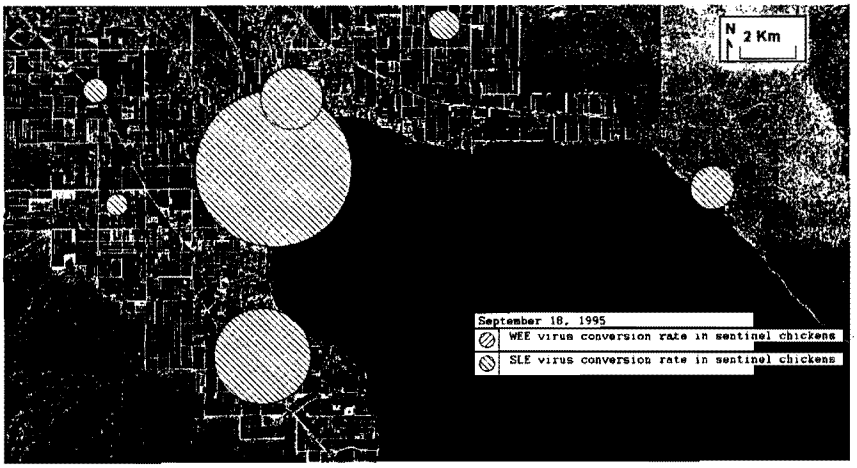

Figure 10. Distribution of SLE and WEE data as indicated by seroconversions among sentinel chickens, September 18, 1995.

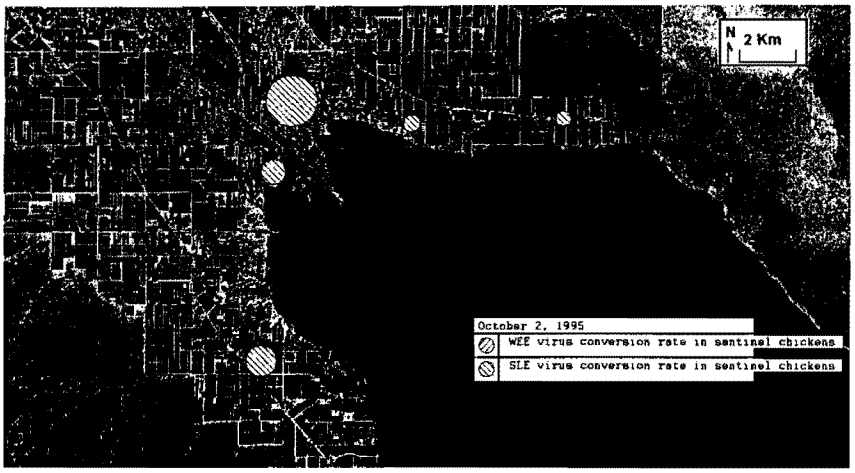

Figure 11. Distribution of SLE and WEE data as indicated by seroconversions among sentinel chickens, October 2, 1995.

DISCUSSION

Although final statistical analysis has not been performed, there appeared to be a positive spatial relationship between the proximity of larval breeding sites and adult host-seeking abundance, as measured by CO_2-baited traps. However, some assumptions are required to support this conclusion. First, trap counts accurately represented regional abundance as displayed as a density halo centered on each trap. Second, all larval breeding sites were sampled and control measures are equally effective at each site. Adult dispersal, up to 6 km from sites at the mouth of the Whitewater Channel, was demonstrated by our previous mark-release-recapture studies (Reisen and Lothrop 1995). This may explain high relative abundance at traps located at sites where host-seeking flight paths may overlap, such as when traps are located in corridors of vegetation or at ecotones.

In accordance with previous data, transmission of both viruses to sentinel chickens was not detected until summer, at which time mosquito abundance was generally low. WEE did not continue into fall and therefore was not present during the fall increase in mosquito abundance associated with breeding duck ponds. SLE showed bimodal transmission intensity, declining during late summer and increasing again concurrent with mosquito abundance in the fall. These and previous data have shown that, in the Coachella Valley, WEE transmission occurs earlier than SLE, but that SLE continues for a longer period and later into the fall.

Planned statistical analysis of the relationship among the ecological factors included in this GIS will determine the extent of their contribution to mosquito abundance and the levels of virus transmission seasonally and regionally.

ACKNOWLEDGEMENTS

We especially thank Stan Husted and the staff of the Coachella Valley Mosquito and Vector Control District for their assistance and support and R.E. Chiles, Center for Vector-borne Disease Research laboratory for his excellent technical support.

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ECOLOGY OF MOSQUITOES AND ARBOVIRUSES AT MORRO BAY, SAN LUIS OBISPO COUNTY, CALIFORNIA[1]

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ABSTRACT

The objective of the present research was to determine if western equine encephalomyelitis (WEE) virus persists at Morro Bay, either by vertical transmission within *Aedes dorsalis* populations or by horizontal transmission among *Aedes* and mammals or *Culex* and birds. During 1994 and 1995, 19 mosquito species including 13,561 females collected by CO_2 -traps and 91,547 adults reared from field-collected immatures were tested for virus infection in 386 and 3,027 pools, respectively, using a plaque assay in Vero cell culture. Numbers tested varied seasonally and depended upon the frequency and abundance of each species in adult and immature collections. *Ae. dorsalis* was most abundant comprising 78% of host-seeking females and 49% of reared adults tested for virus, followed by *Cx. tarsalis* (2 and 17%), *Aedes squamiger* (1 and 4%), *Cs. particeps* (11 and 1%) and *Ae. washinoi* (2 and 9%), respectively. Four of 111 pools of *Ae. squamiger* adults reared from field-collected immatures were positive for Morro Bay virus (minimum field infection rate, MIR, = 1.07 per 1,000 tested). All remaining pools were negative, including 111 pools of *Ae. dorsalis* reared from field-collected immatures that were tested for WEE virus genomic RNA by RT-PCR. Sera collected biweekly from 3 flocks of 10 sentinel chickens and from 1 group of 5 sentinel rabbits were negative for antibodies to WEE, St. Louis encephalitis and California group viruses.

Data indicated that WEE most likely does not persist at Morro Bay, either by vertical or horizontal transmission. None of 44,641 *Ae. dorsalis* reared from field-collected immatures and none of 10,592 trapped females were positive for WEE, indicating that MIRs for WEE were <0.02 and <0.1 per 1,000, respectively. In addition, rabbit sentinels bled during the present study, and wild bush rabbits (n = 26) and Jackrabbits (n = 6) bled by Fulhorst (1994) were negative for WEE antibodies. Similar to Fulhorst (1994), few specimens of the primary vector, *Cx. tarsalis*, were collected host-seeking and these females were not infected with WEE virus. All chicken sera were negative for WEE antibodies, indicating virus was not transmitted at detectable levels among birds.

ACKNOWLEDGEMENTS

We especially thank V.M. Martinez, F. Esposo and Ahgee Guo for technical assistance. This research was supported by special funds for mosquito research allocated annually by the University of California. Logistical support was provided by the Kern Mosquito and Vector Control District and California Department of Parks and Recreation at Morro Bay.

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[1] This research will be submitted for publication to the Journal of the American Mosquito Control Association.

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1996 Proc. Mosq. and Vector Control Assoc. of CA 64: 16-19

ENHANCED SURVEILLANCE FOR MOSQUITOES AND ARBOVIRUSES AT COASTAL WETLANDS IN VENTURA COUNTY, 1994 - 1995

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ABSTRACT

Research was initiated in Ventura County in August 1994 to investigate the hypothesis that fresh and salt water marshes near Point Mugu produce large numbers of mosquitoes that amplify arboviruses and are carried by prevailing onshore wind currents into the Santa Monica Mountains and western Los Angeles County. *Culex tarsalis* abundance peaked in August-September following the flooding of freshwater marshes for duck hunting and also in spring in response to local flooding. *Aedes squamiger* and *Ae. taeniorhynchus* abundance peaked following late winter and mid-summer flood tides, respectively. *Aedes* dispersal appeared to be limited to areas immediately adjacent to emergence sites.

Arboviruses were not isolated from 624 pools of host-seeking mosquitoes. Seven flocks of sentinel chickens and two groups of sentinel rabbits bled biweekly remained seronegative. Morro Bay, a California-group virus, was isolated from one out of 196 pools of *Ae. squamiger* collected as immatures; 218 pools of *Ae. taeniorhynchus* were negative. Collectively, these data indicated that arboviruses were not active at marsh ecotone or upland habitats in Ventura County during 1994 and 1995.

Arbovirus surveillance has intermittently detected western equine encephalomyelitis (WEE) and Saint Louis encephalitis (SLE) virus in the Santa Monica Mountains of western Los Angeles County (Emmons et al. 1994) and in the city of Oxnard in Ventura County. Point Mugu, in Ventura County, ecologically accommodates two 160 hectare freshwater duck marshes and a 400 hectare salt marsh. Large populations of *Culex tarsalis* Coquillett emerge from these marshes. The concurrent appearance of this species in western Los Angeles County and prevailing westerly winds led Saviskas (1992) to suggest the long range downwind easterly dispersal of *Cx. tarsalis* into the Santa Monica Mountains and western Los Angeles County. Recent trapping also demonstrated that the intermittent appearance of *Aedes* species coincided temporarily with the periodic tidal flooding of the salt marsh. Fulhorst et al. (1994) recently recovered WEE virus from *Aedes dorsalis* Meigen collected as immatures at a salt marsh at Morro Bay. Although *Ae. dorsalis* is not found as far south as Point Mugu, other *Aedes* species possibly may maintain WEE or other viruses by vertical transmission.

In 1994 we initiated research to address three main hypotheses:

1. Salt and freshwater marshes near Pt. Mugu serve as enzootic foci for the maintenance and amplification of WEE, SLE and possibly other arboviruses.
2. WEE is maintained horizontally in an *Aedes*-mammal cycle from which it spreads to the primary *Cx. tarsalis*-bird cycle.
3. Vertical transmission of viruses in *Aedes*

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mosquitoes is occurring at the Pt. Mugu salt marsh.

MATERIALS AND METHODS

The following methods address our three hypotheses:

1. Salt and freshwater marshes near Pt. Mugu serve as enzootic foci for the maintenance and amplification of arboviruses. To map the initiation and possible dispersal of arboviruses, seven flocks of ten sentinel chickens were deployed from August 1994 through October 1995 at the following locations: 1) Hueneme Rd. - an upwind negative control, 2) interface of Pt. Mugu Naval Air Weapons System base and the freshwater duck clubs - the hypothesized arbovirus focus, 3) Lewis Rd., 4) Potrero Rd., 5) Cotharin Rd., 6) Mulholland Rd. and 7) Encinal Canyon (Figure 1). Sites 3 to 7 are downwind mosquito dispersal sites. Three additional sentinel chicken flocks at the communities of Thousand Oaks, Fillmore and Simi Valley are maintained by the Ventura County Department of Health Services. These sites determine if arboviruses are widespread within upland agricultural and urban habitats. All sentinel chickens were bled at biweekly intervals and the sera tested for antibodies to WEE and SLE using an enzyme immunoassay (EIA) method (Reisen et al. 1994). In addition, one to two CO_2-baited EVS traps were operated weekly at sites 1 to 7. Additional traps were operated intermittently at the saline marsh and at two perpendicular transects downwind from the marshes. Captured mosquitoes were anesthetized with triethylamine, identified to species and pooled for virus testing. All mosquitoes were tested for virus infection using a plaque assay in Vero cells. Virus isolates were identified by plaque reduction neutralizing assay.

2. WEE virus is maintained by *Aedes* mosquitoes. To determine the role of *Aedes* mosquitoes in virus maintenance and transmission, adult female *Aedes* mosquitoes collected with CO_2-baited EVS traps were tested for infection. Two hutches with five domestic rabbits each were deployed at the salt marsh near site 2 and at site 5, downwind from the marsh and adjacent to that site's chicken flock. Analogous to the chickens, the rabbits were bled biweekly by femoral or ear venipuncture. Sera were screened for virus antibodies using EIA.

3. Vertical transmission of viruses by *Aedes* mosquitoes. Immature *Aedes* collected from the salt marsh were reared to adults, fed a sucrose solution for three to five days, anesthetized, pooled and tested for virus infection as described above.

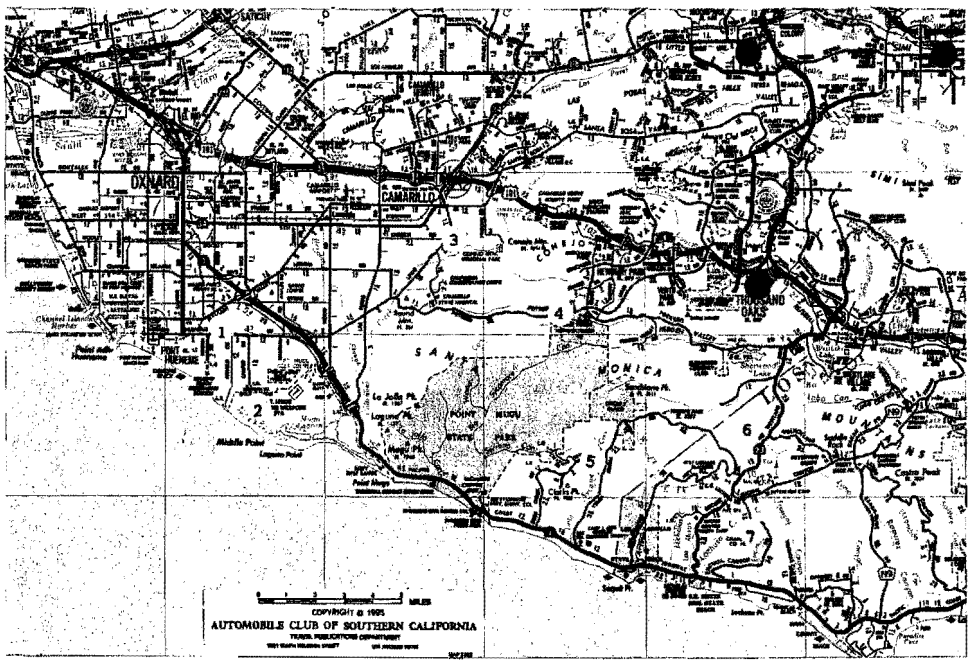

Figure 1. Map of Point Mugu, Santa Monica Mountains and western Los Angeles County showing sentinel chicken flock locations 1 to 7. Also shown are sentinel flock locations maintained by Ventura Co. Environmental Management Branch (V), and traps set along perpendicular transects A and B.

RESULTS AND DISCUSSION

Arbovirus activity. The horizontal transmission of arboviruses was not detected during the August 1994 through October 1995 sampling period. All sentinel chicken flocks and the two groups of rabbits remained negative for arbovirus antibodies. In total, 20,479 *Cx. tarsalis*, 374 *Aedes squamiger* Coquillett and 4,604 *Ae. taeniorhynchus* Wiedemann adult female mosquitoes caught by CO_2-baited traps tested negative for arbovirus infection. There were no human or equine cases of viral encephalitis reported from Ventura County in 1994 -1995.

These data did not support our hypotheses that marshes near Pt. Mugu serve as enzootic foci for the maintenance and amplification of arboviruses in either *Cx. tarsalis*-bird or *Aedes*-mammal cycles. Previously, WEE was isolated from Cx. tarsalis collected at site 7 during September 1991 and one sentinel chicken seroconverted to WEE virus during November 1993. Virus detection occurred concurrently with the large emergence of *Cx. tarsalis* from duck club marshes near Pt. Mugu and extensive searches of the dry canyons near site 7 never revealed local breeding sources of *Cx. tarsalis*. However, sentinel chickens maintained at site 2 by Ventura County Environmental Health Division from 1984 to 1989 never seroconverted to either WEE or SLE viruses, prompting their relocation to upland population centers in 1990. Further research will be necessary to explain the source of intermittent WEE virus activity in western Los Angeles County.

Vertical transmission of WEE and SLE viruses were not detected in *Ae. taeniorhynchus* or *Ae. squamiger* populations. During 1995, 10,710 adult *Ae. taeniorhynchus* collected as immatures were tested in 218 pools with negative results. It was interesting to note that all females (n = 63) in one collection were autogenous. Also, 8,937 *Ae. squamiger* collected as immatures were pooled (196). One pool of males tested positive for Morro Bay, a California encephalitis group virus (Eldridge et al. 1991). Although this was the first isolation of MB virus from *Ae. squamiger* from Ventura County, isolation was expected because Fulhorst (1994) isolated MB virus from almost every population sampled from the Sacramento River estuary to San Diego.

The mosquito abundance patterns of adult female *Cx. tarsalis* collected at the duck marshes (site 2) was similar in August-September and September-October 1995 (Figure 2). Data collected by Ventura County Environmental Management Branch indicated that in 1994, large populations of *Cx. tarsalis* emerged from the Ormond Beach area, northwest and a short distance of both the duck clubs and site 1. Because the duck clubs were not flooded until mid-August in both 1994 and 1995, mosquitoes from the Ormond Beach area probably comprised most of the early August 1994 CO_2 trap collections at the duck marshes and at site 1. Increased abundance observed at downwind sites in 1994 were absent in 1995. Possibly, in 1995, the combination of delayed flooding of the duck marshes, dry conditions at Ormond Beach, and enhanced mosquito control by duck club management contributed to smaller numbers of *Cx. tarsalis* dispersing downwind, even though abundance of *Cx. tarsalis* and both *Aedes* species at the marshes was quite high. Planned mark-release-recapture studies will address more completely the pattern of dispersal of mosquitoes from both fresh and saltwater marshes.

ACKNOWLEDGEMENTS

We thank V.M. Martinez and A. Gou, Center for Vector-Borne Disease Research, Arbovirus Field Station, for their technical assistance. This research was funded in part by funds allocated annually from the Division of Agriculture and Natural Resources, University of California.

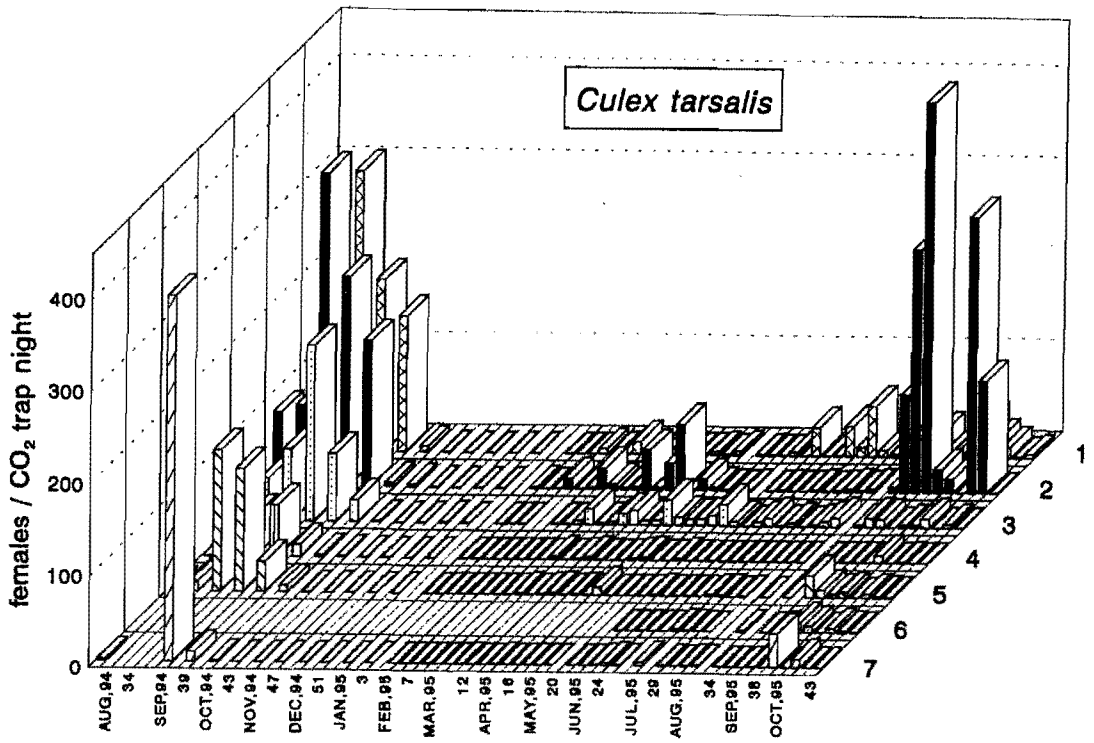

Figure 2. Relative abundance of *Culex tarsalis* females per CO_2 trap night plotted as a function of weekly sampling dates during 1994 and 1995. Sampling sites 1 to 7 described in text and positioned in Figure 1.

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HANTAVIRUSES IN CALIFORNIA - AN UPDATE

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ABSTRACT

To date, a total of 126 human cases of Hantavirus Pulmonary Syndrome (HPS) have been reported from 24 states within the U.S.A. HPS cases are distributed throughout the U.S., but are generally concentrated in the western United States. Current data on the national status of the disease indicates that the median age of victims is 35 years. Fifty-eight percent of victims are males and the fatality rate is 50%.

Currently, four strains have been implicated as the causative agent of HPS in the U.S. These are: Sin Nombre Virus (SNV) in White-footed Mice, (*Peromyscus*), predominantly the Deer Mouse, *Peromyscus maniculatus*, in most of the U.S.; Black Creek Canal Virus (BCCV) in the Cotton Rat, *Sigmodon hispidus*, in Florida; Bayou Virus in a recently suspected reservoir, the Rice Rat, *Oryzomys palustris*, in Louisiana; and an unnamed East Coast strain in *Peromyscus leucopus*, in New York.

Three strains have been presently identified in California: SNV; El Moro Canyon Virus (EMCV) in the Western Harvest Mouse, *Reithrodontomys megalotis*; and the Isla Vista Virus (IVV) in the California Meadow Vole, *Microtus californicus*. EMCV and IVV have not been linked to human illness at the present time. The genus *Peromyscus* has been the most important reservoir of SNV in California. A total of 6,500 mammals (representing 12 families and 44 species) have been tested to date. Ten of 44 species of wild rodents tested were seropositive. Of these, 6.1% had positive antibodies to SNV, EMCV and IVV. Of the 2,700 *Peromyscus maniculatus* tested, 12.2% had SNV positive antibodies from 20 counties.

Much of the ecology of SNV in California is still unknown. An interesting finding in California is that seroprevalence in Deer Mice appears to increase as elevation increases. This may correlate with the higher incidence of human HPS cases observed in higher elevations of the eastern slope of the Sierra Nevada mountain range, where favorable vegetation types encourage higher rodent densities. This factor may result in increased rodent/human contacts within dwellings, especially older structures such as mountain cabins which may not be adequately "rodent proofed". As of December 1995, 13 California residents have been diagnosed with HPS. Eight of these residents have died (61.5% fatality rate). Investigations suggest that nine of the cases may have been acquired in California. The rest may have been acquired either in New Mexico or Washington State. In California, besides the home environment, other settings such as occupational sites and camping and outdoor recreational facilities have been implicated as sites of infection. Some evidence suggests that victims acquired the infection when they either lived or worked in a high risk area. Conversely, in other cases the victims did not appear to be in a high risk area at the time of infection. In many cases, the common factor appears to be cleanup activities at or about the time of infection. Most cleanup and rodent proofing recommendations reflect what vector ecologists have been preaching in the past and are based on common sense and good hygiene practices.

EVALUATION OF MOSQUITO AND ARBOVIRUS ACTIVITY IN ORANGE COUNTY, 1995

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In 1995 the Orange County Vector Control District (OCVCD) continued its mosquito and encephalitis virus surveillance throughout the year. Mosquitoes were collected at 11 permanent sites throughout the County. Eighteen CDC/CO_2 traps were utilized as well as five gravid female traps. The summary of the number of each mosquito species pooled from each trap type is shown in Table 1. A total of 2,243 mosquitoes (81) pools was sent in for virus testing; 76 pools of *Culex quinquefasciatus* and five pools of *Culex tarsalis*. None of the pools tested positive for St. Louis encephalitis (SLE) or Western equine encephalomyelitis (WEE) viruses. No human cases were reported from Orange County. Wild bird sera were tested for SLE and WEE by OCVCD using HAI techniques. Nine modified Australian Crow traps (McClure 1984) were used to trap a total of 6,836 birds and 2,104 blood samples were taken during 1995. The summary of results is given in Table 2. A total of 1,393 House Sparrows was taken, of which only 0.43% tested positive for SLE, while none of 711 House Finches was positive.

Table 1. Number of mosquitoes and mosquito pools submitted for SLE and WEE virus testing by species and trap type from Orange County during 1995.

Species	No. of mosquitoes	Mosquito pools	
		Oviposition traps	CDC traps
Culex quinquefasciatus	2,137	76	0
Culex tarsalis	106	5	0
Totals	2,243	81	0

Table 2. Small bird seroconversions for SLE and WEE antibodies in Orange County during 1995.

Species	SLE	WEE	No. Bloods Sampled	% SLE	% WEE
House Sparrow	6	0	1,393	0.43	-
House Finch	0	0	711	0	-
White Crown Sparrow	0	0	0	0	-
Totals	6	0	2,104	0.49	

1995 represents the lowest percent positive results since 1988 (Table 3). Positive House Sparrows (Figure 1) were found in February, May, June, September, and October with the highest percent positives for SLE occurring on September 11 (1.2 percent, Garden Grove) and October 18 (1.5 percent, Anaheim).

Table 3. Percent positive birds for SLE from 1987 to 1995, Orange County, CA.

	1987	1988	1989	1990	1991	1992	1993	1994	1995
House Sparrow	0.66	0.33	0.72	3.10	4.90	3.43	1.22	0.41	0.43
House Finch	0.36	0.48	0.39	1.36	0.95	1.34	1.85	0.70	0

Sentinel chickens in the San Gabriel Valley seroconverted for SLE on September 25. Modjeska Park in Anaheim produced three positive of 594 sparrows (0.51%) collected throughout the year. Figure 2 shows the SLE positive sparrows from Modjeska Park plotted against host-seeking female mosquitoes. The highest percent positive sparrows (2.5%) at this site occurred in October, during a slight increase in *Cx. quinquefasciatus* activity (1-2 per trap night). Seroconversions in May and June occurred after slight increases in both *Cx. quinquefasciatus* and *Cx. tarsalis* (2-5 per trap night). At the Garden Grove site (Figure 3), there appears to be little correlation between sparrow seroconversions and mosquito activity, which never got above 1.0 per trap night the entire year. The total percent positive sparrows from Garden Grove in 1995 was 0.67%, with the highest in February (7.1%). Traditionally, *Cx. tarsalis* has always been more abundant in rural/suburban areas such as the San Joaquin Freshwater Marsh and Mason Regional Park in Irvine (Figures 4 and 5, respectively). Host-seeking *Cx. tarsalis* numbers in the San Joaquin Freshwater Marsh (Figure 4) were highest in July and August (40-50 per trap night).

Cx. quinquefasciatus was uncommon in the marsh, averaging less than ten per trap night between February and October. In contrast to these low numbers, *Cx. erythrothorax* averaged over 10,000 per trap night during the same period. Figure 6 compares the average number of mosquitoes caught with CDC traps for 1994 and 1995 at the San Joaquin Freshwater Marsh. The average number of mosquitoes per trap night for 1995 was 3 to 6 times greater than 1994 every month of the year. Gravid females of *Cx. quinquefasciatus* were obtained from Reiter ovipositional traps at both suburban and rural sites. Figure 7 illustrates gravid mosquito activity in Irvine (SW county), Huntington Beach (NW county), and Fullerton (N county). All three sites are approximately 13 miles apart and although the numbers of mosquitoes collected were different at each site, activity periods at all the sites were similar. The most productive site for gravid *Cx. quinquefasciatus* in 1995 was Irvine Valley College in the city of Irvine (225 per trap night in July).

In 1996, attempts will be made to re-establish modified stable/crow traps for wild birds which allow access by mosquitoes. The blood-fed mosquitoes that are collected can then be sent in for virus testing.

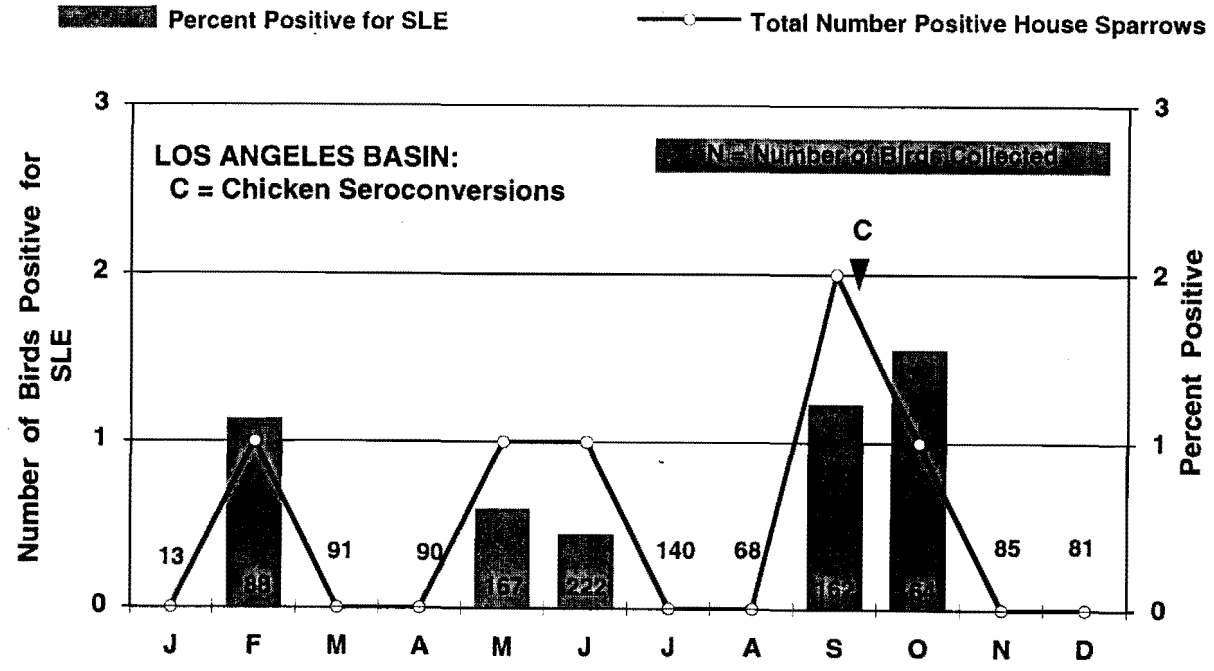

Figure 1. SLE virus activity in the Los Angeles Basin and house sparrow seroconversion in Orange County during 1995.

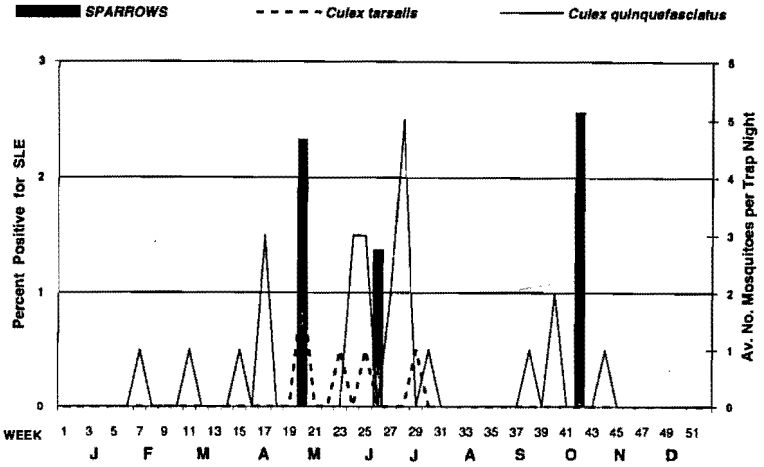

Figure 2. Host-seeking mosquito (CDC traps) activity and SLE positive house sparrows at Modjeska Park in Anaheim during 1995.

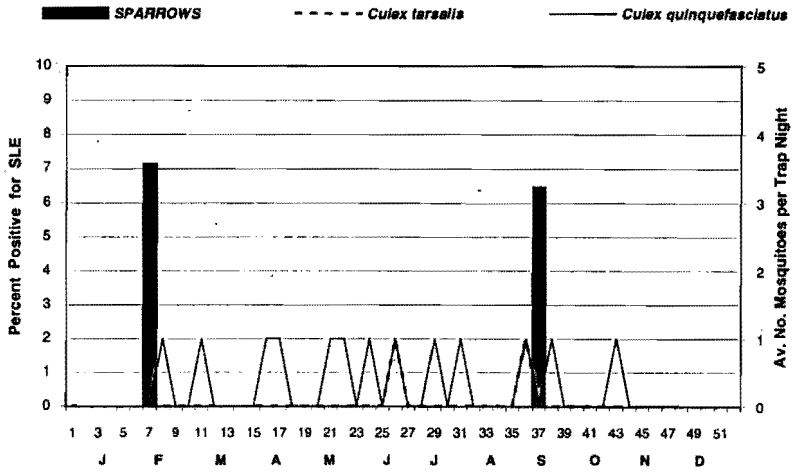

Figure 3. Host-seeking mosquito (CDC traps) activity and SLE positive house sparrows at OCVCD in Garden Grove during 1995.

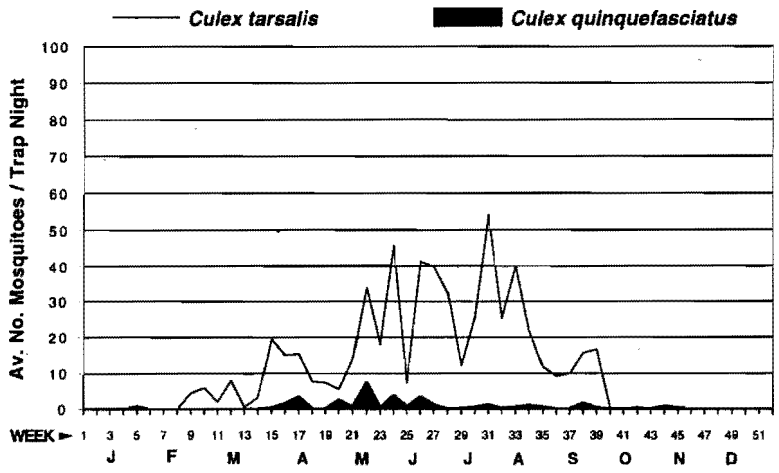

Figure 4. Host-seeking mosquito (CDC traps) activity in the San Joaquin Freshwater Marsh in Irvine during 1995.

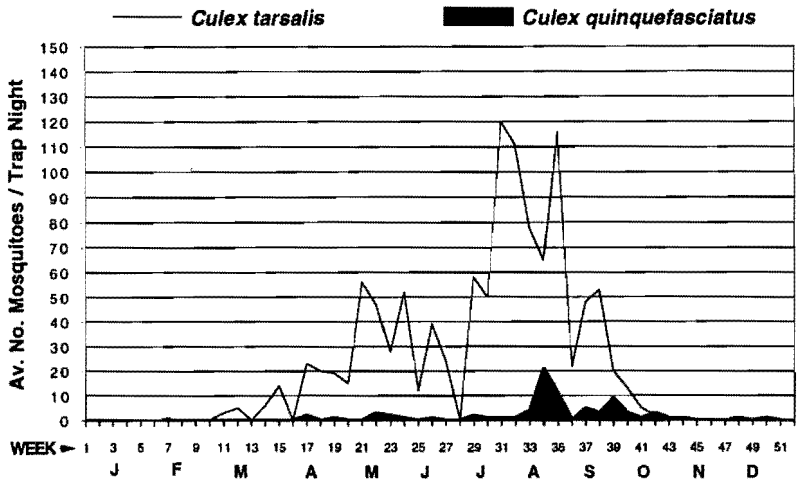

Figure 5. Host-seeking mosquito (CDC traps) activity in the Mason Regional Park in Irvine during 1995.

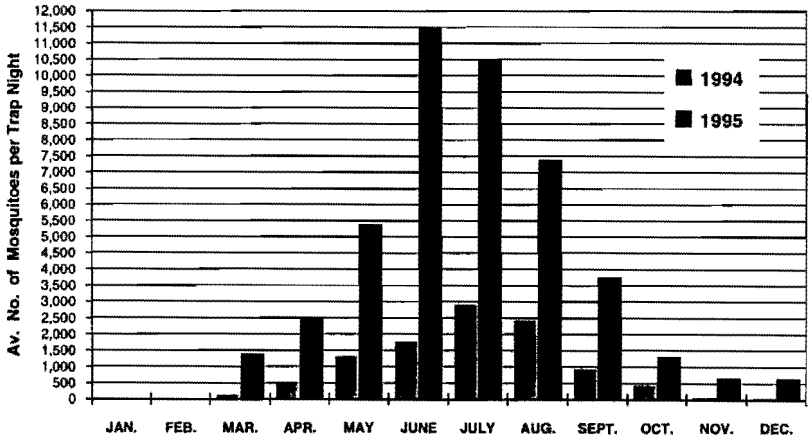

Figure 6. Host-seeking female mosquito activity (CDC traps) in the San Joaquin Freshwater Marsh in Irvine during 1994 and 1995.

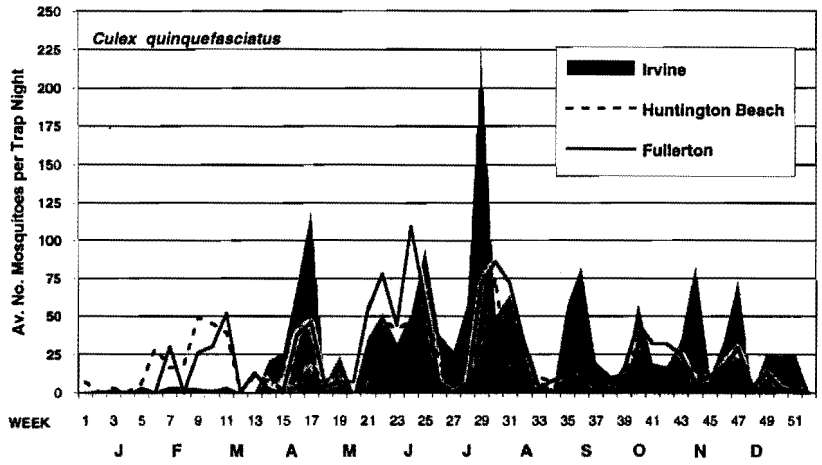

Figure 7. Gravid female mosquito activity at three sites in Orange County, CA, during 1995.

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Gratitude is extended to Dr. D.F. Moore of the Orange County Health Care Agency, for laboratory space and supplies.

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PLAGUE SURVEILLANCE AND DISEASE ACTIVITY IN CALIFORNIA, 1994-1995

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ABSTRACT

Plague is a zoonotic disease that persists in sylvatic rodent and flea populations throughout much of California. Cyclic epizootics of plague occur among susceptible rodent species within disease foci, at times leading to associated human plague cases. Plague is transmitted by flea bite from infected fleas, through direct exposure to contaminated body fluids, or by inhaling the infectious droplets from an animal or human with pulmonary infection. Four human cases, including one fatality occurred in California between January 1, 1994 and December 31, 1995. Two of the four cases were associated with flea bite exposure during an epizootic among California ground squirrels (*Spermophilus beecheyi*). The source of exposure in the other two cases remains undetermined. Surveillance activity by state and local health and vector control agencies confirmed plague in 18 of 36 California counties sampled in 1994, and 15 of 29 counties in 1995. Human case investigations and surveillance activity are reviewed in this two-year summary of plague in California. Broad regional plague foci within the state are described, based on cyclic enzootic and epizootic activity patterns between 1990 and 1995.

Plague continues to persist in California among rodent hosts and reservoirs within a framework of geographical disease foci. These foci occur from the Modoc Plateau and inter-mountain valleys of northern California, through mountainous and coastal habitats, south to the Palomar and Cuyamaca Mountains of San Diego County. Cyclic plague epizootics occur among susceptible rodent species within foci, at times leading to human cases. Cases occur primarily by flea bite transmission during rodent epizootics. Cases have also been associated with aerosol transmission from infected domestic cats.

Smith et al. (1994) described California's cooperative statewide plague surveillance and control program, as coordinated by the Vector-Borne Disease Section, California Department of Health Services (CDHS), and listed the nine components of the program. In addition, these authors reviewed plague surveillance and disease activity, and recorded plague positives among rodents, rodent fleas, wild carnivores, and domestic pets in an average of 18 California counties surveyed between 1990-1993. The current manuscript updates and reviews plague occurrence in California during 1994 and 1995.

Figure 1 demonstrates California human plague cases and fatalities by decade, since 1950. The number of cases increased dramatically in the decades of the 1970's and 1980's, as compared to the previous two decades. This increase correlated with a similar increase of human plague cases in the southwestern United States during these two decades, as reported by Barnes (1982).

The first human plague case in California in the 1990's occurred in the summer of 1992 in a 6 year-old boy who had a flea bite exposure during a family horseback trip into the Ansel Adams Wilderness Area in eastern Madera County. The boy was hospitalized and diagnosed in Fresno. He was treated with antibiotics, and subsequently recovered (Smith et al. 1994).

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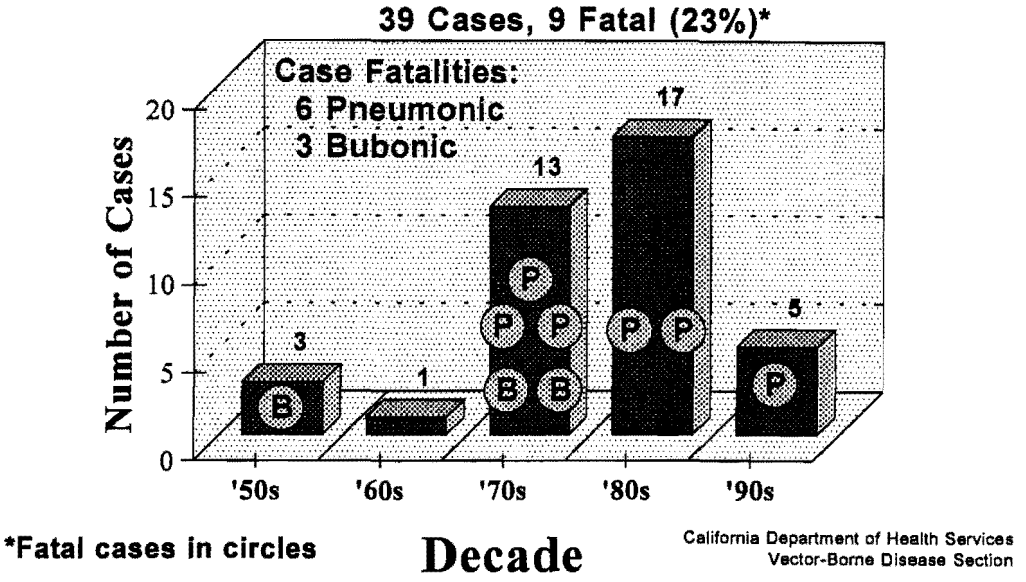

Figure 1. Human plague cases in California to date by decade.

Between January 1, 1994 and December 31, 1995, there were four additional human plague cases in California; two in Kern County, one in Inyo County, and one in El Dorado County (Figure 2).

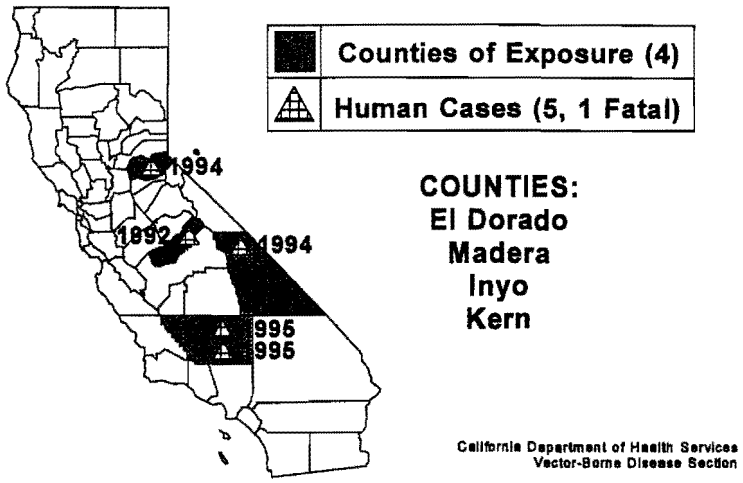

Figure 2. Counties with human plague cases during 1990-1995.

Case #1 occurred in a 56 year-old male resident of Inyo County, who became severely ill January 1, 1994, and was hospitalized in Bishop. The case was confirmed as septicemic plague on blood culture by the Microbial Diseases Laboratory, California Department of Health Services. The man was treated for plague and showed progress in recovery, but died of a heart attack three weeks after being released from the hospital. The man's possible exposure site included his home, three miles west of Bishop, and a mine where he had prospected in the Panamint Mountains. Rodents trapped at the mine were negative for plague, as were rodents and domestic pets serologically sampled by the Vector-Borne Disease Section, CDHS, in the vicinity of the home environment. The source of exposure in this unusual winter time plague case remains undetermined. The case represents California's first

winter plague case since 1928. Winter plague cases have been rather rare in California, as most cases occur in spring and summer months, coinciding with the activity period of rodent hosts.

Case #2 occurred in a 10 year-old resident of San Mateo County who was seen at an emergency room on July 13, 1994, with a painful swelling in the groin. He related bouts of fever, pounding headache, and vomiting for the previous four days. He had returned home recently from a private summer camp at Ice House Reservoir in El Dorado County. He was treated as an outpatient for a possible bacterial infection with oral Keflex, however, his symptoms persisted and the tender swelling progressed to involve several lymph nodes.

Due to the patient's lack of response to antibiotic treatment, persistent lymph node swelling, and a history of camping in the Sierra Nevada mountains, plague was suspected. A blood sample from the boy was sent to the Centers for Disease Control and Prevention, Fort Collins, and was reported as plague positive (titer 1:512).

Epizootiological investigation of the private camp and immediate vicinity at Ice House Reservoir by the Vector-Borne Disease Section, CDHS, and the El Dorado County Environmental Health Department revealed high densities of California ground squirrels (*Spermophilus beecheyi*) and their fleas, and evidence of epizootic die-off in the camp, and in the adjoining U.S. Forest Service campgrounds along the reservoir. Plague bacteria were isolated from one long-eared chipmunk (*Tamias quadrimaculatus*) found dead in the camp, and from one of two flea pools collected from California ground squirrels. Another flea pool from ground squirrels collected in the nearby Strawberry Point campground was plague positive by bacteriological culture. In addition, five of eight (62%) of the ground squirrels sampled in this campground were serologically plague positive.

The private camp, a U.S. Forest Service lease, has over 500 children in attendance each summer. After the suspect plague case was reported over 200 families who had family members in attendance during the week the boy attended the camp were interviewed by epidemiologists of the El Dorado County Environmental Health Department, and the CDHS. No additional plague cases were identified. Vector control operations which were carried out in response to the epizootic and human plague case at Ice House Reservoir have been described by Townzen et al. (1996).

Case #3, the first of 1995, was reported from Tehachapi, Kern County, in a 23 year-old resident, who initially became ill on March 17. He left work on March 20 with symptoms of fever, cough, malaise, and diarrhea. He became extremely ill at home later that evening, and was taken to the hospital. Subsequently, he was transferred to the hospital in Bakersfield in a state of severe respiratory distress. Chest X-rays showed complete "white-out" of the right lung, and infiltrate in the left. He became comatose, was intubated, and placed on mechanical ventilation. The diagnosis of probable plague pneumonia was confirmed by the Microbial Disease Laboratory, CDHS, following direct fluorescent antibody staining, culture, and phage typing. There were no apparent flea bites, and no reported adenopathy.

The man was provided corticosteroids and fluids, and a variety of antibiotics, including streptomycin and doxycycline. Despite close monitoring, respiratory assistance, and life support, he died on April 13, 24 days after admission.

Persons who had face-to-face contact with the man prior to admission, and shortly thereafter, were placed on active surveillance for cough and fever, or any evidence of illness, and were started on a 7-day course of tetracycline or doxycycline equivalent. There were no secondary cases recognized.

A field investigation was begun immediately upon diagnosis by the Kern County Environmental Health Department, and the Vector-Borne Disease Section, CDHS. The county program was instrumental in educating people in the local communities about plague. An extensive plague epizootic was revealed in the vicinity of Tehachapi, and to the northwest and south. A woodrat found dead in a subdivision south of town tested plague positive by bacterial culture. Three house cats, bled by local veterinarians, from residences northwest of Tehachapi were serologically positive for plague antibodies. Two house cats and a bobcat from the small community of Keene, northwest of Tehachapi, were plague positive by bacterial culture. Wild carnivores sampled from Tehachapi and Keene by the U.S. Department of Agriculture, APHIS, Animal Damage Control, Kern County Animal Control, and the Vector-Borne Disease Section showed a 70% rate of positive antibody titers to *Yersinia pestis*, the plague organism (16 of 23 sampled).

Rodents and pets sampled, and bled from the vicinity of the man's home, and at his work site, tested negative for plague. The precise source of the man's exposure in this case could not be determined. A late winter infection, cold weather, lack of ground squirrel activity, and absence of adenopathy and flea bites, point to a possible primary pneumonic exposure in this case from an unknown animal source (California Morbidity, November, 1995).

Case #4 occurred in May, 1995, in a 56 year-old female resident of Thompson Canyon in the Walker Basin, Kern County. Thompson Canyon is 20 miles north of Tehachapi. The woman became ill after first experiencing pain and irritation on her right ankle one day after weed cutting on her property. The irritation continued, during which time she traveled to Downey, Los Angeles County. She was hospitalized in Downey on May 29 with continuing pain in the right ankle, right inguinal lymph node swelling, and fever of 103.8° F. Cultures of lymph node aspirate and blood were sent to the Microbial Diseases Laboratory and confirmed as plague positive. The patient recovered after being treated with antibiotics and was released from the hospital.

Investigation of the patient's property and adjacent properties in the Walker Basin by the Vector-Borne Disease Section and the Kern County Environmental Health Department revealed signs of a plague epizootic among California ground squirrels, which had possibly begun as early as late summer, 1994, and continued in the late spring of 1995 after the squirrels emerged from hibernation. An illness in one of the patient's pet cats in the spring coincided with the approximate time of spring time emergence of ground squirrels in the Walker Basin. This cat, and another of the woman's cats tested plague positive. Two of three wild carnivores sampled in the Walker Basin were serologically positive. All indications in this case point to a flea bite exposure in the home environment during an epizootic among California ground squirrels (California Morbidity, November, 1995).

SYLVATIC PLAGUE DETECTION AND SURVEILLANCE RESULTS

Detection of plague through the cooperative surveillance program in California recorded positives among rodents, rodent fleas, wild carnivores and domestic pets in 18 of 36 counties sampled in 1994, and 15 of 29 counties sampled in 1995 (Table 1). Disease surveillance activity in this two-year period demonstrated plague epizootics among a complex of ground squirrels and chipmunks in montane forest recreational areas of eastern Butte County, at South Lake Tahoe and Ice House Reservoir (El Dorado County), at Prosser Reservoir near Truckee (Nevada County), in the Bishop Creek drainage (Inyo County), and near Mammoth and June Lakes (Mono County). Epizootics occurred among California ground squirrels near Frazier Park (Kern County), near Ojai (Ventura County), at Big Bear Lake (San Bernardino County), in the Angeles National Forest (Los Angeles County), on Mt. San Jacinto (Riverside County), and in the Palomar Mountains (San Diego County). Extensive plague epizootic activity in Kern County from Tehachapi to Lake Isabella led to the two human plague cases in 1995. Backyard plague positive domestic cats were discovered during the two-year period at Truckee (Nevada County), Portola (Plumas County), and Tehachapi (Kern County) through a system of veterinarians alerted to the potential of plague in pets in endemic areas. These cats presented to local veterinarians with illness suggestive of plague. Presence of the disease was confirmed by laboratory testing through the California Department of Health Services surveillance system.

In San Bernardino County in 1995 evidence of plague was found among ground squirrels not only at recreational sites in the San Bernardino Mountains, as in 1994, but also on the fringe of the metropolitan Los Angeles Basin in a city park in Fontana. Flea control and rodent reduction were conducted by the county vector control agency, and plague warning signs were posted to alert park and recreational site visitors. The infringement of plague into major recreational sites within the metropolitan interface presents an extremely important concern in vector-borne disease human case prevention. Human cases and plague epizootics have previously occurred in the Los Angeles Basin and are described by Nelson et al. (1986).

RECREATIONAL PLAGUE FOCI

Poland and Barnes (1979) list criteria for differentiation between enzootic plague and epizootic plague. Animal populations involved in maintenance cycles in enzootic plague may show continuing evidence of plague serologically,

Table 1. Occurrence of enzootic and epizootic plague in California by geographical region, 1990-1995. Enzootic (ENZ) plague defined by serological evidence only, no obvious mortality. Epizootic plague defined by isolation of *Yersinia pestis* from rodents and fleas, or clusters of antibody titers of 1:256 or greater, and apparent mortality. NEG = samples taken, all negative. N.A. = no sample taken.

Geographical Region	1990	1991	1992	1993	1994	1995
Modoc Plateau	ENZ	NEG	ENZ	NEG	NEG	NEG
North Coast	ENZ	NEG	ENZ	ENZ	NEG	NEG
Cascade Mountains/ Inter-Mountain Valleys	EPI	NEG	ENZ	ENZ	ENZ	NEG
North Sierra Nevada Mountains	EPI	EPI	EPI	EPI	EPI	EPI
Central Sierra Nevada Mountains	EPI	EPI	EPI	EPI	EPI	EPI
South Sierra Nevada Mountains	EPI	NEG	EPI	EPI	EPI	EPI
Kern Plateau/Piute Mountains	ENZ	NEG	NEG	ENZ	N.A.	EPI
Tehachapi Mountains	EPI	ENZ	ENZ	EPI	EPI	EPI
Central Coast	EPI	ENZ	ENZ	ENZ	ENZ	EPI
San Gabriel Mountains/ Los Angeles Interface	ENZ	ENZ	NEG	ENZ	ENZ	EPI
San Bernardino Mountains	ENZ	EPI	ENZ	EPI	EPI	EPI
San Jacinto Mountains	ENZ	EPI	ENZ	EPI	EPI	EPI
Palomar and Cuyamaca Mountains	NEG	NEG	EPI	ENZ	ENZ	EPI

however, they do not readily exhibit extensive mortality. In California, at least, in certain regional foci, enzootic reservoir hosts include meadow voles, deermice, and possibly some species of woodrats (Goldenberg et al. 1964, Nelson 1980). Epizootic plague is characterized by animal mortality. *Yersinia pestis* may be isolated from animal carcasses and rodent fleas during epizootics. Epizootic amplifying hosts in California include a complex of ground squirrels, chipmunks, pine squirrels (northern California), and some woodrats (Nelson 1980).

In an effort to more adequately describe regional plague foci within California, the past six years of plague activity (1990-1995) have been analyzed for 13 geographic regions of the state following the criteria of Poland and Barnes (1979). These data are presented in Table 1. Even in this limited analysis, the cyclic nature of plague within these geographic regions of the state becomes evident.

PLAGUE CONTROL

Risk of human exposure to vector fleas and potential plague transmission by flea bite intensifies during epizootic die-offs among susceptible rodent hosts. As animals die, fleas are left without a host, and may readily bite other hosts, including man. The majority of human plague cases in California, since the epidemics of the early 1900's, have involved flea bite exposure and transmission during epizootics involving the California ground squirrel. Cases have also occurred during epizootics among golden-mantled ground squirrels and chipmunks in montane forest habitats of California (California Department of Health Services records).

Positive evidence of epizootic plague with a defined human health risk led to recommendations for flea control at many of the heavily used recreational sites mentioned in the sylvatic plague detection and surveillance results in 1994 and 1995. Flea control was conducted by state and local health and vector control agencies utilizing insecticide dust applications. Application was by direct dusting of insecticide into rodent burrow systems, and by introducing dusts into bait stations, as described by Smith and Lusk (1990).

DISCUSSION

An integrated cooperative system of plague surveillance coordinated by Vector-Borne Disease Section, CDHS, has been established in California since the early 1970's. The integrated system allows for detection of plague in both the enzootic cycle of the disease, through serological evidence of infection among sentinel animal species, and in the epizootic cycle, through testing of suspect animals and

ectoparasites by laboratory culture of *Y. pestis*. An integrated surveillance and sampling system with continuity in monitoring from specific geographic regions provides continuing information on both enzootic and epizootic cycles of plague in California, as well as information on host/ectoparasite complexes within geographic regions.

Plague activity in 1994 and 1995 was reflective of the epizootic explosiveness of the disease in some endemic regions of California. For example, in Kern County, there was little evidence of plague from 1991 to 1993, but extensive epizootic evidence in 1994 and 1995. The extensive epizootics in Kern County resulted in two human plague cases and one fatality, pointing out the critical need to maintain disease awareness, surveillance and control programs within health and vector control agencies. Epizootics within heavily used recreational areas, a city park in a large metropolitan area, and backyard plague in rural residential home sites, further emphasize the continuing need for plague surveillance and management in California.

A continuing awareness of disease activity through a well maintained surveillance system and public education assures rapid, appropriate response by health and vector control agencies to plague epizootics. Where cases do occur, an alerted medical and laboratory community is vital in helping to prevent secondary cases and reduce the potential epidemic spread of the disease.

The cyclic nature of plague within apparent geographic disease foci is evident in this brief analysis of several endemic regions of California. The ecological relationships of plague in established geographic foci are complex, with each focus having its own characteristic maintenance hosts, susceptible rodent hosts, flea vectors and environmental factors which affect the host/ectoparasite relationships and the relationship with *Y. pestis*.

There is a definite need to further delineate plague endemic foci in California where the disease continues to persist, especially where there is a history of human disease associated with epizootics. Knowledge gained from studies of the biotic and abiotic factors that affect vector/host relationships within each focus would allow for better predictability of plague epizootics and could assist in human case prevention.

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VECTORS FOR VECTORS: NEW APPROACHES TO DISEASE CONTROL WITH PANTROPIC RETROVIRAL VECTORS

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Mosquito vector-borne diseases are a major threat to life and health throughout many parts of the world. The long-range objective of our laboratory is to develop methods for the genetic manipulation of vector mosquitoes to provide novel means for control of the diseases they transmit. A vital step in this direction is the development of methods to genetically transform mosquitoes.

Vector-borne parasitic and viral diseases kill many millions of people annually and remain an impediment to improvement in the standard of living in many developing tropical and subtropical countries. In addition, these diseases, specifically some of the flaviviruses, pose a new threat to temperate countries as a result of the invasion of these regions by exotic mosquito vector species. The recent colonization of large portions of the United States by *Aedes albopictus* raises the possibility of efficient transmission of diseases such as Dengue fever (Hawley et al. 1987). In California, various mosquito species transmit alphaviruses including Western equine encephalomyelitis virus and St. Louis encephalitis virus, which can affect both the livestock and human populations.

Intracellular Immunization

The introduction of genes into cells to interfere with the growth of intracellular pathogens has been termed "intracellular immunization". Candidate genes whose expression renders mosquito cell lines resistant to infection with the homologous virus have been identified. Such genes, if expressed in transgenic adult mosquitoes, have the potential to interrupt the cycle of disease transmission (Powers et al. 1994, Higgs et al. 1993). These viral genes include the nucleocapsid genes of bunyaviruses (e.g., La Crosse virus) and the capsid, polymerase, and pre-M genes of flaviviruses (e.g., Dengue, yellow fever). Malaria has also been targeted for this type of genetic control.

Transformation Systems

As studies of intracellular immunization proceed, there is an increasing need to establish techniques for introducing foreign genes into mosquitoes (Crampton et al. 1990, James 1992, Crampton 1994). Such techniques would allow the molecular analysis of important endogenous genes involved in vector-specific behaviors such as bloodfeeding. In addition, transformation techniques would allow the introduction of exogenous genes targeted to interfere with pathogen growth and development in the mosquito vector, thus reducing vector competence. Ultimately, transformation techniques should allow the production of transgenic mosquitoes with decreased vector competence that can be used in release programs to decrease the transmission of vector-borne diseases.

Germline transformation of vector mosquitoes has been hampered by the lack of effective methods to achieve stable integration of exogenous DNA into the mosquito genome. To provide a new tool for genetic manipulation in insects, we developed a novel class of retroviral vectors, called "pantropic vectors", to stably introduce foreign genetic material into the mosquito genome.

Retroviral Vectors

Retroviral vectors based on the murine and avian leukemia viruses have become standard tools for the introduction and expression of foreign genes in mammalian cells, both *in vitro* and *in vivo*. To transform a retrovirus into a retroviral vector, the coding sequences for the structural gene (*gag*), the reverse transcriptase gene (*pol*), and the envelope protein gene (*env*) are removed and replaced

with heterologous (foreign) DNA under the control of the retroviral promoter (long terminal repeat, LTR) or an internal promoter. These replication-incompetent vectors are produced in packaging cell lines that express the retroviral proteins needed for the assembly of an infectious particle. The vector particles can be recovered from the culture supernatant and used to infect target cells. For infection to occur, the retroviral envelope protein must bind to a receptor on the cell surface, uncoat, and release the retroviral genome into the cytoplasm. Here, the RNA viral genome is reverse transcribed, the double stranded DNA molecule is transported to the nucleus, and the provirus integrates into the host cell genome.

Several methods exist to detect the presence of the integrated provirus. For vectors expressing a dominant selectable marker (e.g., neomycin phosphotransferase), infected cells can be selected in medium containing an antibiotic or antimetabolite (e.g., G418) that will kill uninfected cells and allow survival only of those infected cells expressing the transgene product. Alternatively, vectors can carry reporter genes for enzymes that allow detection of infection by histochemical staining, flow cytometry, or other enzymatic assays. Common examples of reporter genes include β-galactosidase, firefly luciferase, chloramphenicol acetyl transferase, green fluorescent protein and neomycin phosphotransferase.

A decade of experience with these replication-incompetent vectors has demonstrated that they can be propagated safely in the laboratory. Because they are incapable of self-propagation, there are no containment issues for infected cells harboring the integrated provirus, other than matters relating to recombinant DNA. These vectors are now widely used for human gene therapy protocols and have been intravenously administered to humans with no untoward effects. Although these retroviral vectors are widely used for gene transfer in mammalian cells, the requirement of a specific protein receptor on the surface of the target cell has limited the host range of these vectors. In addition, the instability of the retroviral envelope protein has precluded concentration of the vector particles by physical methods such as ultracentrifugation. To overcome these disadvantages, we modified the envelope protein of the vectors as described below.

Pantropic Retroviral Vectors

Our laboratory has created modified or pantropic retroviral vectors that contain the envelope glycoprotein of vesicular stomatitis virus (VSV-G) substituted for the retroviral envelope protein (Burns et al. 1993, Yee et al. 1994). The VSV-G protein binds to phospholipid moieties in the cell membrane, thus circumventing the need for a specific protein receptor on the surface of the target cell. These modified retroviral vectors, therefore, have an expanded host cell range (pantropic) and, unlike their amphotropic counterparts, can be concentrated to titers $>10^9$ cfu/ml by ultracentrifugation. Biologic containment issues are the same as for the amphotropic vectors since the pantropic vectors are also replication incompetent. These vectors can attach, uncoat, reverse transcribe, and integrate into the genome of insect cell lines (Matsubara et al. in press). In other species, such as zebrafish, high titer stocks have been microinjected into early embryos to create transgenic organisms (Lin et al. 1994).

Recent experiments from our laboratory demonstrate that the provirus can integrate into the mosquito genome and can mediate expression of high levels of reporter gene products such as firefly luciferase under the control of the *Drosophila* heat shock promoter. Experiments are now in progress to engineer pantropic vectors that will express pesticide resistance (Philips et al. 1990), green fluorescent protein (Chalfie et al. 1994), and the white eye phenotype (Besansky et al. in press) for marker studies to create lines of transgenic mosquitoes. In later studies, viral-specific genes and single chain antibodies to mosquito-transmitted parasites will be engineered into these pantropic vector particles. Concentrated vector stocks will be microinjected into the developing mosquito embryo to create transgenic lines of mosquitoes.

SUMMARY

In summary, these pantropic vectors represent an important innovation that permits the use of retroviral vectors to stably introduce foreign genes into non-mammalian cells. Work in progress will develop these pantropic vectors as tools to mediate stable gene transfer into mosquitoes for the purpose of altering their ability to transmit disease.

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THE INTEGRATED CONTROL OF RICE FIELD BREEDING MOSQUITOES IN CHINA

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ABSTRACT

The dominant mosquito species breeding in rice fields are *Anopheles sinensis* and *Culex tritaeniorhyncus*. The rice culture in most parts of China resulted in high populations of these mosquitoes. The pisciculture and other biocontrol measures combined with chemical control are the main components of integrated management. The applications of other potential biocontrol agents for rice field breeding mosquitoes such as *Azolla* and *Romanomermis* are under investigation.

Effect of rice culture on mosquito population density. Based on investigations conducted during the past few years, the dominant mosquito species breeding in rice fields are *Anopheles sinensis* Wiedmann and *Culex tritaeniorhynchus* Gile. The composition by larval analysis was 21.9-85.9% and 7.2-78.1% for the two species respectively (Table 1). Observations over a 10-year period from 1972 to 1984 in Henan province indicated that the population density of *Cx. tritaeniorhynchus* in a no-rice culture area was 98.3% lower than that in a rice culture area, and 83.1% lower than that in neighboring areas of rice culture. Longitudinal observations revealed that the population density of *Cx. tritaeniorhynchus* in Henan increased 237.25% and 409.80% after the first and second years of rice culture respectively, compared to that of previous years without rice.

Table 1. Species composition of rice field breeding mosquito larvae in China[1].

Locality, Province[2]/ Month, Year	*Anopheles sinensis* Percent (No./Total)	*Culex tritaeniorhynchus* Percent (No./Total)	Others Percent (No./Total)
Guangzhou, GD/Jun., 1983	21.9 (105/480)	78.1 (375/480)	0
Tongbai, HN/Jul., Aug., 1984	69.5 (960/1381)	7.2 (99/1381)	28.3 (322/1381)
Hunan/Jul., Aug., 1984	34.5 (2848/8266)	64.3 (5317/8266)	1.2 (101/8266)
Danyang, JS/Jul., Aug., 1985	49.8 (994/1995)	50.2 (1001/1995)	0
Wanzai, JX/Jul., Aug., 1985	85.9 (413/481)	14.1 (68/481)	0
Shenyang, LN/Jul., Aug., 1984	64.2 (238.371)	31.3 (116/371)	4.5 (17/371)
Zhongwei, NX//Aug., Sep., 1982	23.9 (885/3702)	47.7 (1764/3702)	7.4 (1013/3702)
Yuci, SX/Jul., Aug., 1984	22.5 (50/212)	34.2 (76/212)	27.4 (76/212)
Chengdu, SC/Jul., Aug., 1985	49.7 (485/975)	50.3 (490/975)	0

[1] cited from Lu, 1989.
[2] GD: Guangdong; HN: Henan; JS: Jiangsu;, JX: Jiangxi; LN: Liaoning; NX: Ningxia; SX: Shanxi; SC: Sichuan.

Wet (intermittent) irrigation. Wet irrigation is a means of maintaining a damp soil surface in the rice fields and is accomplished by 10-48 hours of irrigation. The only time when there is standing water is during rice transplantation when water is kept 4 cm deep for 10-15 days to allow seedling survival and establishment. During the rainy period, no irrigation is necessary and excess water is drained

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from the fields. The intermittent irrigation interval is about five days with the total number of irrigations from transplant to harvest being 21-26 times. With this irrigation schedule, water consumption ranges from 600-700M^3 per *mu* (1 *mu* = 1/6 acre). Using the wet irrigation technique, rice harvest yields increased over 10%, water usage decreased one-half to two-thirds, and mosquito larvae breeding was reduced over 85% (Table 2). This irrigation practice has been widely accepted in the provinces and metropolitan areas of Henan, Jiangsu, Hebei, Sichuan, and Shanghai.

Table 2. Relative population index in permanent (P) and intermittent (I) flooded rice fields (Ge et al. 1981).

		Anopheles sinensis			*Culex tritaeniorhynchus*		
	Year	P	I	P:I	P	I	P:I
Larvae/10 dips	1978	0.50	3.13	0.160:1	3.27	33.94	0.096:1
	1979	0.55	4.08	0.135:1	5.20	27.16	0.192:1
Adults/net hour	1978	6.60	14.28	0.462:1	20.91	72.30	0.289:1
	1979	7.67	34.37	0.223:1	32.67	73.38	0.445:1

Rice field pisciculture. The primary fish species cultured in rice paddies were *Ctenopharyngodon idella*, *Cyprinus carpio*, *Carassius auratus* x *C. carpio*, *Tilapia nilotica* and *Gambusia affinis*. The symbiotic relationship of the rice field-pisciculture ecosystem is mutualistic. The grass feeding fish maintain weed control by consuming weeds and its seeds, thereby increasing soil nutrient availability to the rice plants by eliminating competition. They also improve air movement among and illumination to the plants by removing old leaves from the rice stems. The fish also feed on plankton and the immature stages of rice field pests and mosquitoes. The predation on pests and mosquitoes reduces pesticide applications on rice fields. Fish movement and its feeding activities promote root development of rice by improving soil structure, organic substance catabolism, and fertilization by fish excreta. Biological control of rice field breeding mosquitoes with pisciculture has been applied in most of the rice growing areas of central and southern China (Table 3).

Table 3. Reductions (%) of mosquito larval density in rice fields with pisciculture (fish/*mu*[1]) versus without (Su et al. 1977).

Month	Category	500 Common[2] and Red Carp/*mu*	400 Common and Red Carp/*mu*	300 Silver Carp/*mu*
June	Total	85.1	76.3	---
	Anopheles	82.3	1.3	---
	Culex	85.2	80.4	---
July	Total	88.3	75.2	38.1
	Anopheles	35.3	37.7	53.1
	Culex	94.1	82.6	19.8
August	Total	59.9	87.8	82.1
	Anopheles	52.6	88.0	51.8
	Culex	73.7	87.5	95.4
Total	Total	80.9	77.5	73.1
	Anopheles	59.1	51.6	52.2
	Culex	86.3	81.6	84.5

[1] 1 *mu* = 1/6 acre.

[2] Fish survival rate was over 70%.

Romanomermis. Research using the parasitic nematode, *Romanomermis*, is being conducted in the laboratory and rice fields. There is only one family of nematodes that parasitize mosquitoes, Mermithidae, among the five orders and fourteen families that parasitize insects. The known genera of Mermithids are *Hydromermis*, *Epidomermis*, *Octomyomermis* (syn. *Capitomermis*), *Paramermis*, *Romanomermis* (syn. *Reesimermis*), *Perutilimermis*, and *Strelkovimermis* (syn. *Kurshmermis*). Since the discovery of the pathogenic mermithid nematode in *Anopheles gigas baileyi* at Bombi, Tibet by Song

(1983), four species have been found in China. The species are: *Romanomermis jingdeensis* Yang and Chen, *R. sichuanensis* Peng and Song, *R. wuchangensis* Bao. Wang and Wu, and *R. yunanensis* Peng and Song. *Anopheles sinensis* larvae are susceptible to the preparasites of R. *jingdeensis* and *R. sichuanensis*. When first, second, third and fourth instar *An. sinensis* larvae were exposed to the preparasites of *R. jingdeensis* at a ratio of 1:5, the infection rates were 77.2%, 43.0%, 18.4% and 11.9%. The mean infection density was 3.0, 2.4, 1.7 and 1.5 respectively (Yang et al. 1983). Under field conditions with 1000-4000 preparasites per m^2. The parasitism rate of *An. sinensis* larvae was 43.1-95.0% with preparasitic densities of 1.0-4.4 (Yang et al. 1984). Pupal and adult stages of *An. sinensis* were infected by *R. jingdeensis* when fourth instar larvae were exposed to preparasites. The infection ratio in adult mosquitoes was much higher when late fourth instars were exposed compared to early fourths (Chen et al. 1986). The natural parasitism rate of *An. sinensis* larvae in the basin-irrigated rice field was as high as 16.75% (Song et al. 1987). The larvae of *Culex tritaeniorhynchus* were very susceptible to *R. yunanensis* preparasites. The parasitism rate and density were 97.1% and 3.06 respectively with a preparasite:mosquito larvae ratio of 5:1 (Peng et al. 1987a). Release of preparasites at a density of 2000/m^2 in rice fields resulted in a parasitism rate of over 50% in *Cx. tritaeniorhynchus* larvae.

***Azolla* culture in rice fields.** *Azolla*, which grows on the water surface, serves as a mechanical barrier to mosquito larval breeding and adult oviposition. Under laboratory conditions, *Azolla* effectively prohibited adult emergence and oviposition by gravid females. A rice field trial demonstrated that an *A. imbricata* culture could suppress larval and adult densities (Table 4). Species introduced to rice fields are *Azolla filicoides*, *A. caroliniana* and *A. imbricata*. In addition to serving as a biocontrol agent, *Azolla* can also act as bio-fertilizer in the rice fields.

Table 4. Larval and adult *Culex* densities in rice fields with and without *Azolla imbricata* (Xu 1989).

Days after *Azolla* culture	Cover (%)	Larvae/10 dips			Adults/cage[1]		
		Azolla	Control	% Reduction	*Azolla*	Control	% Reduction
0	0	16.4	7.7				
3	20	4.1	29.6	93.5	0	0	
7	40	1.9	9.1	90.2	0.3	3.3	90.0
11	60	4.3	10.0	79.8	0.5	0.3	---
15	80	1.5	37.9	98.1	0	0.8	100
19	90	2.7	34.8	96.4	0	0.5	100
23	95	0.7	1.0	67.1	0.7	2.3	71.2
27	90	1.4	8.7	92.4	0	3.0	100
31	95	0.8	12.3	96.9	0.7	1.2	44
35	95	0.3	1.9	92.6	0.2	3.0	94.3
40	95	0.2	1.8	94.8	0.5	1.7	70.1
44	98	0	0.1	---	0	0	---
48	98	0.8	0	---	0	0	---

[1] Six emergent cages, each with a square meter bottom.

Chemical control. Chemical control was used as a selective and supplementary measure; limited by considerations of economic benefit, equipment availability, labor and material costs, resistance reduction, as well as environmental impact.

Conclusion and perspective. The proportion of rice field area in China to that of the world is over one-third. As soil conditions, water source, climate, etc. vary from one geographic location to another, the integrated control strategy of the rice field breeding mosquito is based on local conditions. The socio-economic conditions and acceptance of control measures by the farmers were also considered. The irrigation (wet) improvement and pisciculture combined with chemical use are the main control

measures in different areas. The application of *Romanomermis* in rice fields will depend on the deep understandings of the rice field ecosystem. Further investigations are needed in this aspect.

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PREDICTING MOSQUITO BREEDING IN A RESTORED OWENS LAKE, CALIFORNIA

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ABSTRACT

Experimental flooding of Owens Lake, Inyo County, California was begun in January 1994 in conjunction with a comprehensive dust mitigation study. As part of that study the possibility was addressed that flooding might result in the production of significant populations of mosquitoes or other potential disease-transmitting arthropods. Lentic aquatic habitats in the Owens Lake area were surveyed and mapped, and mosquito fauna and associated insects and vegetation collected, identified and characterized. Water samples were collected from each site and analyzed for temperature, salinity, pH, conductivity and ionic content. Information from these mature sites was used to compare with the same data collected at an experimental flood site located on the dry lake bed of Owens Lake.

Data were analyzed using common resemblance functions and certain differences in the comparisons were definitive. These data should assist in predicting future breeding of mosquitoes and other biting insects at Owens Lake should large-scale flooding be implemented as part of an overall dust mitigation policy.

Important environmental factors related to mosquito breeding are discussed. Species emphasized were those of greatest significance because of their potential public health importance as vectors of human disease pathogens.

Owens Lake, located in Inyo County, California, was once a saline terminal lake having a depth of approximately 12-13 m. Its principle source of water was the Owens River. In 1913, the Los Angeles Department of Water and Power began diversions from the Owens River, and as a result of those diversions Owens Lake became virtually dry in a little over 10 years. Since becoming dry, fine particulate matter which forms on the surface of the dry lake is easily lofted by the wind, resulting in dust storms that have produced the highest measured levels of particulate air pollution in the United States.

In 1988, one year after new federal particulate air pollution standards were established, the Great Basin Unified Air Pollution Control District, the State Lands Commission and the Los Angeles Department of Water and Power worked together to develop a Long-Range Dust Mitigation Plan for Owens Lake. This Plan included a series of pilot studies designed to result in the development and implementation of an overall strategy for reducing dust emissions from the lake. One of these pilot studies involved the flooding of an experimental test site on the lake bed.

Because of the concern that large populations of mosquitoes might be produced as a result of lake bed flooding, a study was designed to assess this possibility. We originally planned to survey, map and characterize the major components of currently existing mosquito breeding sites in the vicinity of Owens Lake and to monitor conditions in the experimental flood zone in order to be able to predict the changes in the mosquito fauna which might be expected to occur in the event of general flooding of Owens Lake. To be included was a study of vegetation types associated with various mosquito species, as well as a study of water quality parameters of relevance to the establishment of mosquito populations. An important aspect of the study was to be an evaluation of the possibility that populations of mosquito vectors of human diseases

may increase to levels of public health importance. Unfortunately, funding for the study was withdrawn after the first year, precluding a second year of studies involving comparative faunal and floral analyses, and resulting in only a partial data set. Consequently, predictions and conclusions presented here do not have the scientific reliability that a complete study would have provided.

MATERIALS AND METHODS

The initial phase of our project consisted of a survey of existing mosquito populations and aquatic habitats in the Owens Lake area. Aquatic sites were mapped, and immature and adult mosquitoes collected and preserved for identification. To permit similarity analyses, sampling was both quantitative as well as qualitative, using standard sampling methods (Merritt and Cummins 1984).

Water quality parameters were measured using a YSI-33 SCT Meter (Yellow Springs Instrument Co. Inc., Yellow Springs, OH) to determine salinity, conductivity and temperature; a Corning pH 106 Meter (Corning Glass Works, Corning, NY) to determine pH; and a metric ruler to determine depth. Measurements were made concurrently with mosquito collections. Samples of vegetation and other insects associated with each site were collected and preserved for identification. Samples of water from each site were analyzed at the University of California, Davis for carbonate, chloride and sulfate ions using a LaMotte Model DCL-05 Colorimeter Outfit (LaMotte Chemical Products Co., Chestertown, MD).

Studies were confined to shallow lentic habitats because it was considered unlikely that other aquatic habitats would have relevance to future ecological situations associated with a large-scale flood plan for Owens Lake. Samples consisted of 20 random dips from water deep enough to submerge a standard mosquito sampling dipper. Larvae were returned to UC Davis for identification. After flooding was initiated in January 1994, experimental plots and natural habitats were sampled at approximately bimonthly intervals.

Insect and plant samples were identified directly using common identification guides available for California fauna and flora. For predicting future faunistic and floristic components of Owens Lake, we had originally planned to use mathematical methods of ordination and dominance diversity analysis such as those used by Wilhm (1974) and Spellerberg (1991) to assess aquatic pollution. However, because we had only a partial data set, we reverted to the simple resemblance functions of percent dissimilarity and relative Euclidian distance as described by Ludwig and Reynolds (1988). These analyses provide only limited interpretations for site comparisons, but are useful in directing attention to major site differences.

A map of the Owens Lake research area is shown in Figure 1. Most samples were taken in three general areas: 1) the experimental flood site near Keeler, 2) a small former park at Dirty Socks Well near Olancha, and 3) a former salt production facility near Cartago. In order to make comparisons with other types of habitats, single-point collections were made at two irrigated pastures near Big Pine.

SITE DESCRIPTIONS

Experimental flood site. This site, designated the North Test Area, is located near the former northeast shoreline of Owens Lake, ca. 3 km WNW of the town of Keeler (Figures 1, 2). The lake bed within the North Test Area is relatively flat with a gradual downward slope from NE to SW. The dry surface is composed of hard, crusty sand deposits and sand drifts. There was a prevailing south wind at the time of each sampling.

Two primary pipelines had been laid out on the surface of the lake bed (Pipelines A and B, Figure 2), with smaller lateral outlet pipes attached at ca. 12 m intervals along the length of each main pipeline. Water released from these outlet pipes flowed down slope and flooded the site (Main Wetted Area, Figure 2). Water was pumped to the North Test Area from a well located ca. 5 km to the NW.

Sample sites were established at four locations within the flood zone, one site located approximately at the center of each main pipeline and next to one of the smaller lateral outlet pipes, and the other two sites located within the Main Wetted Area ca. 400 m SW (down slope) of each pipeline site (Figure 2).

Dirty Socks Well. This sample site is located close to the former SE shoreline of Owens Lake, near its southern end, ca. 6.5 km NE of the town of Olancha (Figure 1). It is a natural hot spring (27.5°-34° C) and water flows year-around. At one time the spring had been developed as a park, and a concrete basin fitted with steps and handrails was constructed for the spring water to flow into. Although abandoned years ago, the spring still flows into the basin and a few people continue to use it as a

recreation site.

Located a short distance to the north of the spring is a large pond. This pond receives some rain water but appears to exist primarily from runoff originating from the spring. Additional runoff from the spring flows to the NW and a marsh area has been formed there. Samples were randomly taken along the south edge of the pond and the north edge of the marsh area.

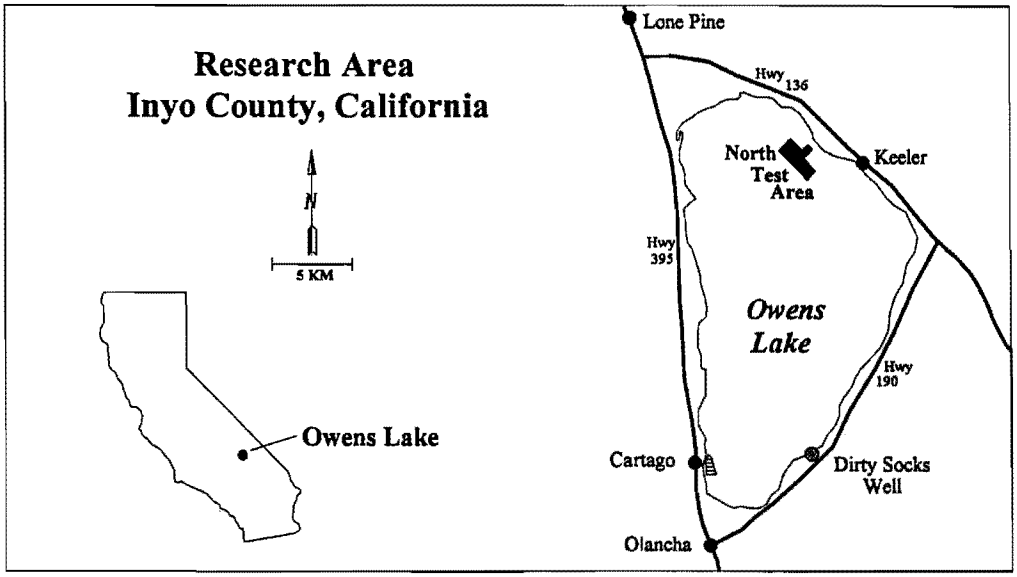

Figure 1. Map of the Owens Lake research area, Inyo County, California.

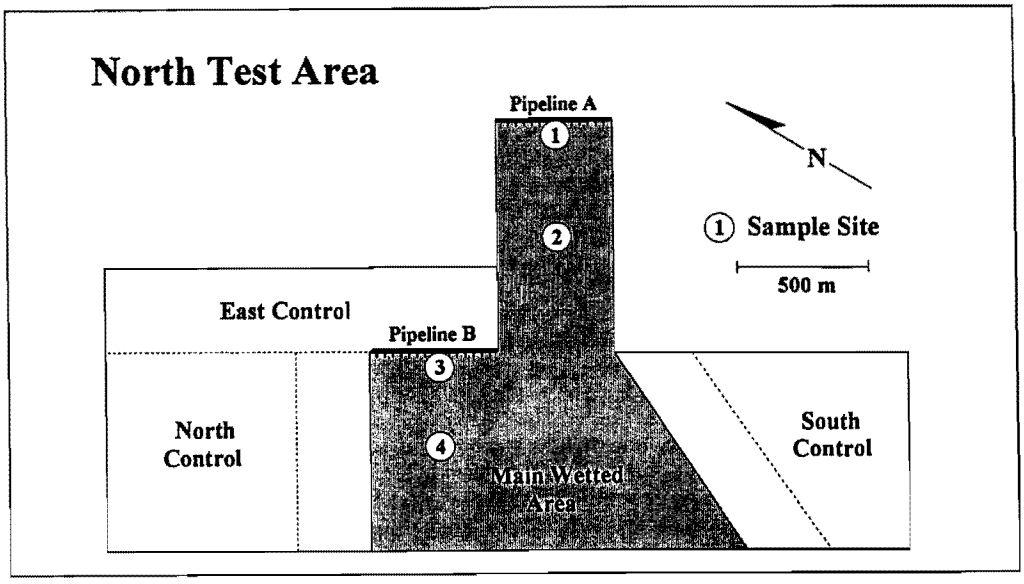

Figure 2. Map of the North Test Area experimental flood site.

Cartago. The third primary sample site was located just east of the town of Cartago, near the southern tip of Owens Lake on its former southwest shoreline (Figure 1). Water from an artesian well near Cartago flows down to a series of abandoned soda evaporation ponds. A pasture runs along the

west side of these ponds and a marsh area has formed adjacent to the western side of the southernmost ponds. This marsh area contains standing water much of the year. A few small pools of standing water are found in the pasture north of the marsh area. Samples were taken randomly from the marsh area, evaporation ponds and salt grass areas east and west of the ponds.

Irrigated pastures. On June 29, 1994 two irrigated pasture sites near the town of Big Pine were sampled. Big Pine is located toward the northern end of Owens Valley, ca. 62 km from Owens Lake. Although these sites were some distance from the North Test Area, they were typical of pasture lands found throughout Owens Valley.

The first pasture was located at the north edge of Big Pine, ca. 0.5 km west of Highway 395 on Reynolds Road. The pasture consisted mostly of open grassland, with scattered trees and a few small but dense stands of willows. Immature and adult *Aedes melanimon* mosquitoes were abundant here.

The second pasture was located ca. 3 km north of Big Pine and ca. 0.5 km east of Highway 395 near Klondike Lake. The area was open with a variety of shrubs, grasses and scattered stands of bulrushes. All stages of *Aedes melanimon* were also present in large numbers at this site.

RESULTS

Flooding was initiated at the North Test Area in January 1994. On February 16, 1994 water was flowing smoothly down slope from Pipelines A and B (Figure 2) at a depth of 5-8 cm in many places. Due to the natural undulations of the lake bed surface, water flowed down slope along the depressed areas, leaving many "islands" of varying size that did not become completely immersed. No pools of standing water were observed in the vicinity of the sites sampled. Water tended to spread out more uniformly in the area down slope from Pipeline B and usually was deeper (up to 10-12 cm) than in the area down slope from Pipeline A. Later in the year, water releases were reduced, resulting in water depths ranging only from 1.5-5 cm.

Vegetation. The vegetation which developed at the North Test Area varied considerably depending upon the particular combination of characteristics of topography and flooding. When flooding was initiated, the North Test Area was essentially barren of vegetation, except for a scattering of salt grass (*Distichlis spicata*). Within a few months, salt grass was observed in greater quantities and various other plants began to appear. Important among the new colonizers were cattails (*Typha latifolia*) and sedges (*Scirpus robustus*). These plants first became established around each of the individual lateral outlet pipes coming off the two main pipelines (Figure 2). The scouring action of water flowing out of the lateral outlet pipes caused sand to be washed away, resulting in the formation of small pools (ca. 1 m wide by 2 m long and 10-15 cm deep). By June 29, 1994 some of the cattails around these pools had grown to a height of over one meter. As flooding continued, the cattails and sedges increased in size and density around the lateral outlet pipes. By the termination of our study in June 1995, the cattails and sedges had spread to form a dense and almost continuous line of vegetation along the length of each of the two main pipelines.

At the Dirty Socks Well site, salt grass was widespread around the edges of the pond and marsh areas, although the marsh area itself was dominated by bulrushes (*Scirpus americanus*). Other small shrubs were scattered widely around the site. There was abundant algae growth in the basin, pond and marsh area.

At Cartago, salt grass was present on the east and west sides of the soda ponds, and cattails, bulrushes and other plants were prevalent in the marsh area as well as in some of the ponds themselves. Within the ponds, vegetation was concentrated primarily along the levees which form the ponds. Algae was present in some of the pools within the salt grass areas, and in the evaporation ponds themselves.

Water Quality Characteristics. A summary of water analysis data for all sample sites is shown in Table 1. At the North Test Area salinity ranged from 0-2.5 parts per thousand ($^{o}/_{oo}$) and conductivity ranged from 600-3,100 μmhos/cm. Generally, readings for samples at these sites were less than 1 $^{o}/_{oo}$ for salinity and less than 1,000 μmhos/cm for conductivity. However, samples collected 400 m from the water outlets (Sample Sites 2 and 4, Figure 2) always had higher salinity readings than did samples collected at the outlet pipes (Sample Sites 1 and 3, Figure 2). The latter measurements should be close to the salinity of the source well.

In contrast to samples from the North Test Area, salinity and conductivity measurements at Dirty Socks Well and Cartago were considerably higher,

with salinity values of up to 6.7°/$_{oo}$ and conductivity values as high as 24,000 µmhos/cm. None of these aquatic sites are stable, and water flows and depths varied throughout the period of this study. Nevertheless, it is obvious that the North Test Area on the whole was less saline than the more mature sites at Dirty Socks Well and Cartago. This comparison is valid only for the period sampled. In time, the North Test Area could increase in salinity.

Table 1. Water analysis data for samples collected at various Owens Valley sites, February 1994 - June 1995. Data shown represent mean values for all samples collected at each site.

Sample Site	No. of Samples	Temperature (°C)	Salinity (°/$_{oo}$)	pH	Conductivity (µmhos/cm)
North Test Area 1	7	21.6	0.43	7.49	990
North Test Area 2	7	23.9	1.01	9.12	1,757
North Test Area 3	7	22.2	0.47	7.51	914
North Test Area 4	7	26.6	0.74	9.00	1,374
Dirty Socks Pond	3	18.6	5.56	8.59	10,000
Dirty Socks Well	3	31.2	4.83	6.79	11,650
Dirty Socks Marsh	6	27.1	5.28	7.57	14,717
Cartago Pond	3	20.7	0.66	8.07	937
Cartago West Marsh	4	27.0	0.65	7.50	1,350
Cartago East Pools	1	31.0	3.50	9.59	13,350
Cartago Pasture	5	30.1	3.36	8.88	8,770
Big Pine (Reynolds Road)	1	22.0	0	7.39	140
Big Pine (Klondike Lake)	1	29.0	0.5	8.15	1,100

Water quality data is shown graphically using limnology triangles (Cole 1983), where points on the triangle represent the relative proportion of common ions (e.g., carbonate, chloride and sulfate). When comparing data from all sites using this method (Figure 3), it can be seen that there was considerable variation in the relative composition of ions among the sites. As expected, the less saline North Test Area had water which tended to be higher in carbonate ions when sampled close to the water outlet pipes, and higher in chloride ions when sampled away from the outlets. With the single exception of water collected at Sample Site 2 on June 29, 1994 none of the water samples from the North Test Area were relatively high in sulfate ions.

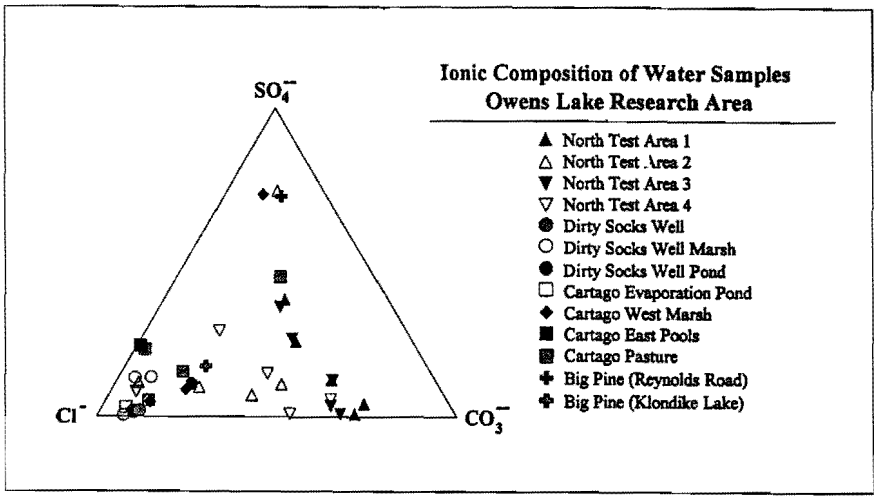

Figure 3. Limnology triangle showing ionic composition of water samples from all Owens Valley sampling sites.

Ionic composition of water samples from the North Test Area varied considerably over time (Figure 4). This variation appeared to coincide with the regulation of the flow of water from the outlet pipes by project personnel. Measurements made in May 1994, a few months after the initiation of flooding, showed that water was relatively high in carbonate ions (suggesting that the water was relatively fresh). In June and July, water samples showed higher levels of chloride ions, then sulfate ions, respectively, as the water flow to the Main Wetted Area (Figure 2), especially from Pipeline A, was reduced during that period of time. By September, the flow of water to the Main Wetted Area had been increased and water samples again showed relatively higher levels of carbonate ions. However, these estimates of ionic composition are not completely consistent with salinity levels. The lowest salinity readings at all North Test Area sites were found in July, while September readings were about average.

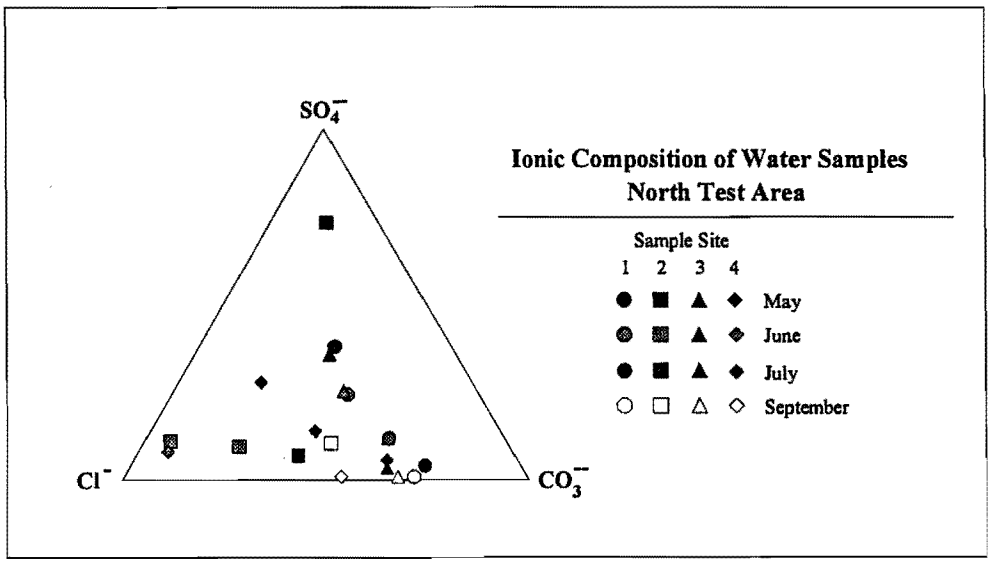

Figure 4. Limnology triangle showing ionic composition of water samples from the North Test Area over time.

Mosquitoes. The Owens Valley presents a significant mosquito control challenge because of the abundance of mosquito breeding habitat. There are vast acreages of pasture lands throughout the valley which, when irrigated, become attractive breeding sites for mosquitoes such as *Aedes melanimon*. Another periodic source of habitat comes from the Owens River itself. Water flow to the river is regulated and at certain times of the year an increase in the flow rate results in localized flooding. When the flow is reduced and the river recedes, the flooded land left behind creates large quantities of habitat attractive to mosquitoes such as *Culex tarsalis*. These flooded areas are very difficult to treat because the dense vegetation growing near the river severely restricts access. Other mosquito breeding habitat can be found in the area around Owens Lake itself where there are numerous natural springs and their associated marshlands, as well as the aforementioned abandoned soda evaporation ponds.

The experimental flood site was compared to sites it was felt might represent what the North Test Area would resemble in the future, and also with other habitats in the Owens Valley known to cause mosquito problems. However, since no flooding of the Owens River occurred coincident to any of our collecting trips, this habitat could not be sampled.

Mosquitoes collected during the study are shown in Table 2. Generally, higher numbers of mosquitoes collected is indicative of higher numbers of larvae present in the habitats. A few mosquitoes were collected at Dirty Socks Well in February and March of 1994, and at Cartago beginning in May.

No mosquito larvae were detected at the North Test Area until July 27, 1994. On that date, second and third stage larvae were collected from a small

pool which had formed around the lateral outlet pipe at Sample Site 1 (Figure 2). Prior to this visit, water flowing from both Pipelines A and B had been fairly uniform and constant. Sometime after June 29, but prior to July 27, the flow from Pipeline A had been greatly reduced. This resulted in stagnant pools, as opposed to pools which were constantly being washed out by water released from the outlet pipes. No larvae were found at Sample Site 3 (Pipeline B), presumably because the water flow there remained constant. No mosquitoes were ever found at Sample Sites 2 or 4. Water flow from Pipeline A remained very low until September 1994, although pools of water remained at each lateral outlet pipe and mosquito larvae continued to occur at Sample Site 1.

The species of mosquitoes collected at the test sites were the same as those collected at the comparison sites at Dirty Socks Well and Cartago: *Cx. tarsalis* and *Cs. inornata.* However, *Ae. melanimon* were collected only at the two irrigated pasture sites.

Table 2. Summary of the number of mosquitoes collected per 20 dips at each site where mosquitoes were found in the Owens Valley Research Area, February 1994 - June 1995.

Date	Site	Species	Number
2 - 16 - 94	Dirty Socks Well Pond	*Cs. inornata*	3
3 - 23 -94	Dirty Socks Well Pond	*Cs. inornata*	1
	Dirty Socks Well Marsh	*Cs. inornata*	1
		Cx. tarsalis	6
5 - 11 - 94	Dirty Socks Well Marsh	*Cx. tarsalis*	3
	Cartago Pasture	*Cx. tarsalis*	18
	Cartago Evaporation Pond	*Cx. tarsalis*	1
6 - 29 - 94	Dirty Socks Well Marsh	*Cx. tarsalis*	78
	Cartago Pasture	*Cx. tarsalis*	1
	Big Pine (Reynolds Road)	*Ae. melanimon*	46
	Big Pine (Klondike Lake)	*Ae. melanimon*	75
7 - 27 - 94	North Test Site 1	*Cx. tarsalis*	60
	Dirty Socks Well Marsh	*Cx. tarsalis*	5
	Cartago West Marsh	*Cx. tarsalis*	1
	Cartago Pasture	*Cx. tarsalis*	29
9 - 15 - 94	North Test Site 1	*Cx. tarsalis*	130
	Dirty Socks Well Pond	*Cs. inornata*	2
		Cx. tarsalis	24
	Cartago Pasture	*Cx. tarsalis*	27
	Cartago East Pools	*Cx. tarsalis*	8
6 - 6 - 95	North Test Site 1	*Cx. tarsalis*	76
	North Test Site 3	*Cs. inornata*	2
		Cx. tarsalis	271
	Dirty Socks Well Marsh	*Cx. tarsalis*	192
	Cartago Pasture	*Cx. tarsalis*	142
	Cartago West Marsh	*Cx. tarsalis*	24

Other Insects Collected. Large numbers of adult shore flies (Diptera: Ephydridae) were observed in the wetted areas around Pipeline A two months after the beginning of flooding. Adult biting gnats (Diptera: Ceratopogonidae) were abundant during the spring months of 1994 in the vicinity of Pipeline A, as were adult tiger beetles (Coleoptera: Cicindellidae). By May 1994, water boatmen (Hemiptera: Corixidae) had become well established at Sample Sites 1 and 3. In June 1994, numerous adult dragonflies and damselflies were observed in the areas around the cattails and sedges at both main pipelines.

Representative insect and plant specimens were collected when they were first encountered at the North Test Area. Representative insect and plant specimens from Dirty Socks Well and Cartago were also collected for purposes of comparison with those collected at the North Test Area. A summary of these specimens is presented in Table 3.

Table 3. Insects and plants collected, Owens Lake Research Area, Inyo County, California, 1994-95.

Insects				
Order	**Family**	**Genus-species**	**Developmental Stage[a]**	**Collection Site[b]**
Coleoptera	Carabidae		A	1
	Cicindellidae		A	1
	Dytiscidae		A	1
	Hydrophilidae		A	1, 3
Diptera	Ceratopogonidae	*Culicoides sp.*	L, P, A	1, 2, 3
	Chironomidae		L, A	1, 2, 3
	Culicidae	*Culex tarsalis*	L	1, 2, 3
		Culiseta inornata	L	1, 2
	Dolichopodidae		A	1
	Ephydridae		L, P, A	1
	Stratiomyidae		L	2
	Syrphidae		L	2
	Tabanidae		A	1
Ephemeroptera	Baetidae		N	1, 3
Hemiptera	Corixidae		N, A	1, 3
Odonata	Aeschnidae		N, A	1, 2, 3
	Coenagrionidae		N, A	1, 3
	Libellulidae		N, A	1, 2, 3

Plants		
Family	**Genus-species**	**Collection Site[b]**
Cyperaceae	*Scirpus americanus*	2, 3
	Scirpus robustus	1
Gramineae	*Distichlis spicata*	1, 2, 3
Juncaceae	*Juncus textilus*	3
Typhaceae	*Typha latifolia*	1, 3

[a] Key to Developmental Stage: L = Larva; N = Naiad; P = Pupa; A = Adult.
[b] Key to Collection Site: 1 = North Test Area; 2 = Dirty Socks Well; 3 = Cartago.

Data Analyses. Using the number of mosquitoes collected (Table 2), comparisons of sites and areas were made using two resemblance functions (Ludwig and Reynolds 1988): percent dissimilarity (PD) and relative Euclidian distance (RED). With the PD method, a value of 1.00 is obtained when two sites are completely dissimilar based on the organisms used for the comparison (in this case, mosquitoes). The irrigated pastures at Big Pine, the only sites yielding *Aedes melanimon*, provided a value of 1.00 in comparison with all other sites. For the most part, we found values within the same collecting areas to be low, showing high constancy. There were some exceptions, however, where values tended to indicate more dissimilarity than actually existed. This is because PD is sensitive to total numbers of organisms collected, and is not a good method to use when there is considerable variation in population sizes among sampling units, which was the case in this study. The RED method provides a more useful tool for samples of varying size. Using RED comparisons, we found that all

of the sites except the irrigated pastures at Big Pine appeared similar with regard to the proportions of the mosquito species present.

Similar analyses were made using the salinity data. With the PD method we found that the Dirty Socks Well pond and marsh sites provided values of 1.00 when compared to all other sites, which would be expected when looking at the much higher salinities found at those two sites. Using the RED comparisons we obtained similar results. We also found that there was much more variation between sites within sampling areas than there were for the PD and RED comparisons using mosquito species.

The salinity data can be better understood by examining the figures on which the data are plotted using limnology triangles (Figures 3,4). As can be seen in Figure 3, there is considerable variation in the relative composition of ions among the sites, with even the Big Pine irrigated pasture sites having relatively high amounts of sulfate ions (Reynolds Road) and chloride ions (Klondike Lake). The changes in ionic composition of water at the North Test Area from May to September of 1994 are shown clearly in Figure 4.

Data for ionic composition of water samples from sites where mosquito species were present revealed little except that the majority of mosquitoes collected came from water that was relatively saline. However, it was surprising to find the Big Pine water samples, where *Ae. melanimon* was collected, showing high amounts of sulfate (Reynolds Road) and chloride (Klondike Lake) ions, but these data are based on only one collection from each site.

A better way to understand the salinity tolerances of the species involved during this study is to examine the range of salinities in which they were collected: *Ae. melanimon* was never collected in water where the salinity exceeded 0.5°/$_{oo}$, whereas *Cs. inornata* was found in water having salinities of 0.4-5.9°/$_{oo}$ and *Cx. tarsalis* was found in water having salinities of 0.0-6.7°/$_{oo}$.

DISCUSSION

In spite of a relatively small and incomplete data set, certain trends emerge from the comparisons which were made which should be of value in designing and managing a large-scale flood system for Owens Lake in a way which will minimize mosquito breeding.

Habitat characteristics. There are several characteristics of aquatic habitats which seem to favor breeding of mosquito larvae. The following discussion considers these characteristics in the context of the North Test Area and the two primary comparison sites.

1) Water depth. Most mosquito larvae are found in water that is at least 5 cm deep and not more than 0.5 m. At depths of less than ca. 5 cm, mosquito larvae cannot perform their normal functions of feeding, moving and breathing, and when aquatic habitats which contain pool-breeding mosquito larvae (mosquitoes in the genera *Aedes*, *Culex*, and *Culiseta*) evaporate to such depths, mortality usually is high. On the other hand, mosquito larvae are relatively rare in water deeper than a meter or so because there is little emergent vegetation in such habitats, and thus little protection from aquatic predators (fish and predacious insects such as water beetles).

Water at both Dirty Socks Well and Cartago is present throughout most of the year in depths varying from a few centimeters to approximately one meter. Most mosquito larvae were found at these sites where water depths ranged from 10-20 cm. At the North Test Area, water depths in most areas were too shallow to permit mosquito breeding. However, exceptions did develop in the vicinity of the outlet pipes where sand was scoured out. These areas were characterized by lush growths of cattails and sedges. The dense vegetation tended to impede water flow, resulting in small sand dams, which in turn further reduced flow and increased water depths. It was in such areas that mosquito larvae were found. As the North Test Area grows older, such situations should increase, because the combination of vegetation growth and reduced water movement tends to reinforce the circumstances which result in ponding.

2) Water movement. Most mosquito habitats are lentic habitats, and only rarely are mosquito larvae found where water movement is more than a fraction of a centimeter per second. The reasons for this are closely related to the factors discussed under water depth. All of the behavioral functions of mosquito larvae (feeding, breathing, avoidance of predators) are adapted to lentic habitats. When water movement was significant at the outlet pipes early in this study, mosquito breeding was not observed. It was only after water movement had been impeded by vegetation growth several months into the study that mosquito larvae appeared at the North Test Area. Thus, mosquito larvae were

detected at Sample Sites 1 and 3 (at the outlets) and never at Sample Sites 2 and 4 (400 m from the outlets).

3) Vegetation. Mosquito larvae are rarely found in open water. There is little protection from aquatic predators in such situations, and usually only early-season *Aedes* mosquitoes inhabit unvegetated situations, when predators are usually absent. There was adequate vegetation present in the areas where we collected mosquito larvae at the Dirty Socks Well and Cartago sites. Vegetation at the North Test Area is discussed above.

4) Salinity. Mosquitoes vary considerably from species to species in their ability to develop in saline habitats. Generally, mosquitoes can be separated into three groups on this basis: 1) strictly fresh water species which cannot tolerate water which is more saline than haemolymph (i.e., ca. 0.5°/$_{oo}$); 2) brackish water species which can live in water having salinities up to about the concentration of sea water (i.e., ca. 35°/$_{oo}$); and 3) saline-tolerant species which can live in water equal to or greater than the salinity of sea water. *Aedes melanimon* is an example of a fresh water species, and we found this species only in the Big Pine irrigated pasture sites. Some investigators have considered this species to fall into the brackish water category, and in fact it may be able to tolerate more saline habitats than most fresh water species. This species has been collected by the authors in Contra Costa County, CA in water having salinity of 1.1°/$_{oo}$. However, it is unlikely to breed in any of the sites around Owens Lake unless unusually fresh water habitats were produced in connection with flooding, similar to what is found in irrigated pastures.

Culex tarsalis and *Cs. inornata* are both brackish water species, and both were found in highly saline situations at all of the sites we sampled, including the North Test Area. From the standpoint of the objectives of this study, the most important observation was that flood water from the North Test Area never became so saline that *Cx. tarsalis* and *Cs. inornata* could not breed there. For that matter, the salinities we found at the North Test Site would not preclude *Ae. melanimon*, but in time this species might be excluded. Comparisons with the mature sites at Dirty Socks Well and Cartago suggest that maturation of a re-flooded Owens Lake would probably not eventually exclude *Cx. tarsalis* and *Cs. inornata* on the basis of excess salinity.

Public Health Significance of Findings. If large-scale flooding is selected as part of an overall dust mitigation plan for Owens Lake, a significant amount of suitable mosquito breeding habitat is likely to develop. This would raise serious public health concerns because, of the mosquito species sampled, *Cx. tarsalis* is the primary vector of both western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses in the western United States (Reeves and Hammon 1962). The creation of large amounts of habitat favoring the breeding of *Cx. tarsalis* would be a concern not just for Inyo County, but for the western U.S. as well.

California encephalitis (CE) virus has been isolated from *Ae. melanimon* in Owens Valley (Work et al. 1969). However, as discussed earlier, it is not likely that suitable breeding habitat for *Ae. melanimon* would develop in a re-flooded Owens Lake.

Two saline-tolerant species which occur in the western U.S. are *Aedes dorsalis* and *Ae. campestris*. They often are found in habitats so saline that no other mosquito species can survive. Such habitats usually are characterized by encrusted salt. Although there are several sites around Owens Lake which fit this description, neither of these two species was collected during this study, nor was the development of any such habitat at the North Test Area observed during this study. However, there is the possibility that populations of these species may exist in the Owens Lake area and their presence would also contribute to public health concerns because both are potential vectors of both WEE and SLE. In addition, *Ae. dorsalis* is a vector of California encephalitis in various regions of the Great Basin (Crane et al. 1977).

In conclusion, observations made during this study should help with the design and management of a large-scale flood plan that would minimize mosquito breeding in a restored Owens Lake.

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TEMPORAL AND SPATIAL DISTRIBUTION OF *AEDES SIERRENSIS* OVIPOSITION

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The larval habitat and adult behavior of the western treehole mosquito, *Aedes sierrensis* (Ludlow), have made it difficult to study certain aspects of its life history or to formulate an economical surveillance program. This mosquito is widely distributed in lower elevation woodlands of western North America from British Columbia to Baja California (Darsie and Ward 1981). Immatures are restricted to water-filled treeholes and, less commonly, other containers holding leaf sediment (Bohart and Washino 1978). These habitats are often cryptic or inaccessible, requiring labor-intensive searches that may not locate large portions of a population (Papineau 1984). Even when larval habitats are located, larval abundance may have a low correlation with subsequent adult emergence from sampled treeholes because of environmental (Colwell et al. 1995) or competitive, pathogenic and parasitic interactions (Hawley 1985, Washburn et al. 1991). Adult *Ae. sierrensis* are rarely collected by light traps (Mortenson et al. 1978), rendering this economical tool ineffective for their surveillance. However, both sexes (males seek the hosts of females to locate mates) can be monitored with human sentinel collections (Washburn et al. 1992) or with CO_2-baited Fay traps (Garcia et al. 1989). These are important methods for life history studies or site-specific monitoring but are impractical for routine surveillance. For example, since *Ae. sierrensis* adults do not fly far (Bennett 1980) many sentinels or traps would be needed to survey large areas.

Similar difficulties have been encountered in surveillance studies of the eastern treehole mosquito, *Aedes triseriatus* (Say) (Craig 1983). Ovitraps (Loor and DeFoliart 1969) have become a preferred method for surveillance of *Ae. triseriatus* because they are an economical alternative to larval surveys (Furlow and Young 1970, Leiser 1981) and they are useful for studies of the temporal and spatial distribution of oviposition (e.g., Kitron et al. 1989, Beehler and DeFoliart 1990). There has been less effort to utilize ovitraps for surveillance of *Ae. sierrensis*; however Mortenson et al. (1978) constructed ovitraps modeled after those used as a standard for *Aedes aegypti* (L) (Fay and Eliason 1966) and demonstrated their ability for detecting the presence of *Ae. sierrensis* in a routine surveillance program.

Further efforts to utilize ovitraps for surveillance and life history studies of *Ae. sierrensis* are warranted. The adult female is a biting pest of humans in wooded recreational and residential areas and is an important vector of *Dirofilaria immitis* (Leidy), the dog heartworm (Weinmann and Garcia 1974, Walters and Lavoipierre 1982). The present study was conducted in order to identify factors which increase the sensitivity of ovitraps for detecting the presence of *Ae. sierrensis* and to utilize ovitraps for examining the temporal and spatial distribution of eggs.

MATERIALS AND METHODS

Study Sites. Following the classification of Munz (1965), the three sites used for the study were a foothill woodland of blue oak (*Quercus douglasii* Hooker and Arnott) near Lakeport, Lake County, CA, a northern oak woodland of California black oak (*Quercus kelloggii* Newberry) on Cobb Mountain, Lake County, CA, and a northern oak woodland dominated by interior live oak (*Quercus wislizenii* Candolle) and Pacific madrone (*Arbutus menziessii* Pursh) near Potter Valley, Mendocino County, CA. Previous sampling indicated all three sites held large populations of *Ae. sierrensis*.

Standard Ovitrap Design. Translucent polypropylene cups (473 ml) were used to hold treehole water (380 ml) as an oviposition attractant.

Strips of Teri-wiper® towels (26.7 by 10.8 cm), the oviposition substrate, lined the insides of the cups and overlapped the tops. These rough-textured towels were manufactured with a grid of nylon fibers (ca. 1.0 x 0.9 cm) which improved longevity and facilitated counting of eggs. Cups were placed inside of plywood boxes painted gloss black inside and out. These boxes (hereafter called oviboxes) (Figure 1) were constructed from 1.3 cm thick plywood with external dimensions of 29.2 cm height, 15.2 cm width and 17.8 cm depth. An 11.4 x 11.4 cm centered vertical entrance hole was cut into the front of each ovibox at a point 7.7 cm below the top. Hardware cloth (2.5 cm mesh) was stapled onto the back of each entrance to prevent disturbance by wildlife. A handle was attached to the lid of each ovibox and the lids were hinged and hasped to allow access. After construction and painting, oviboxes were left outside with the lids open for at least one month prior to use.

At the sites, oviboxes were staked to the ground on the north sides of the bases of trees with the entrances facing north. With this placement the ovitrap entrances were 0.2 m above ground level. Treehole water and liners were collected and replaced once per week. A large blue oak treehole (volume >85 l) was the source of all water utilized in standard ovitraps. After each collection, treehole water was replaced with enough deionized water to refill the treehole. Invertebrates were excluded from the ovitraps by sieving (0.5 mm mesh) the treehole water before use. *Aedes sierrensis* eggs oviposited onto liners were counted in the laboratory with a dissecting microscope (20X).

Comparisons of Modified Ovitraps to the Standard Ovitrap. Four modifications were compared to the standard ovitrap. Study sites, inclusive dates and numbers of repetitions completed are listed in Table 1. Modifications tested were: 1). No ovibox. Cups in the "no ovibox" treatment group were painted gloss black and placed inside a 15 x 15x 15 cm hardware cloth cage (2.5 cm mesh). All ovitraps in this comparison received 400 ml of treehole water. The volume was increased for this comparison because of the high rates of evaporation from traps operated without an ovibox. 2). Deionized water. Deionized water was compared to treehole water as on oviposition attractant. 3). Horizontal entrance. An entrance, otherwise identical to that of the standard ovitrap, was centered in the lids of the horizontal entrance oviboxes. Two disturbed repetitions were eliminated from the analysis. 4). Oak leaf infusions. Blue oak leaves (4.5 kg) were collected from the ground, mixed with deionized water (37.9 l) and treehole water (1.9 l) in polypropylene containers and stored at ambient outdoor temperatures. Water used or evaporated was replaced with deionized water. Sieved oak leaf infusion (4-89 days old during the experiment) and aged oak leaf infusion (13 to 16 months old) were compared to standard ovitraps holding treehole water.

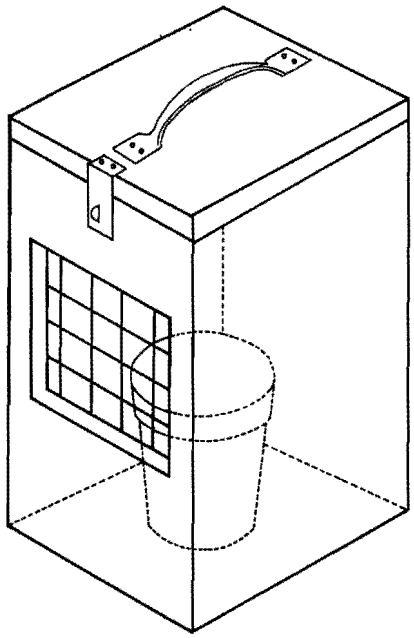

Figure 1. The standard ovitrap for monitoring *Ae. sierrensis* oviposition

Similar methods were used to complete each comparison. Repetitions were located at stations >25 m apart. For tests 1, 2, and 4, ovitraps at each station were located at trees ca. 2 m apart. Test 3 was done by placing standard and horizontal entrance ovitraps against each other at the base of the same tree. Ovitrap locations were initially determined randomly at each station and their positions were rotated during subsequent trap periods. Pearson's chi-square statistic was calculated for each of the comparisons to determine if the frequencies of positive traps were different in standard and modified ovitraps. Numbers of eggs collected by standard and modified ovitraps were compared by Mann-Whitney tests for ranked data (Tests 1-3) or Kruskal-Wallis analysis of variance by ranks (Test 4).

Table 1. Sites, dates and numbers of repetitions of tests comparing the effectiveness of standard and modified ovitraps for collection of *Ae. sierrensis* eggs.

Modification	Site	Inclusive test dates	Number of trap days	Number of trap periods	Repetitions per trap period
1) No ovibox	Cobb Mountain	5/15 - 6/23/92	39	6	5
2) Horizontal entrance	Potter Valley	6/23 - 9/26/94	95	13	4
3) Deionized water	Cobb Mountain	6/17 - 9/10/91	53	8	4
4) Oak leaf infusion	Lakeport	6/19 - 9/12/94	85	9	3

Seasonal Distribution of *Aedes sierrensis* eggs. Seven standard ovitraps were operated for 26 trap periods between April 2 and October 29, 1992, at the Potter Valley woodland using the methods described above. Stations were located between 25 to 50 m apart. The ovitraps were serviced weekly with the exception of a two week trap period ending August 03. Mean high daily air temperature and total rainfall during each trap period were determined with data collected by the National Weather Service in Ukiah, Mendocino County, CA., 14.4 km southeast of the study site. Spearman's correlation analysis by ranks was used to compare mean high temperatures and numbers of *Ae. sierrensis* eggs trapped during nine trap periods (April 30 to July 6) that included the seasonal peak activity period of adult females.

Ten treeholes in the Potter Valley woodland (volumes ranged from 0.4 to 10.2 l) were monitored March 19 and on a weekly basis from April 23 to October 20, 1992 for the presence of water and *Ae. sierrensis* immatures. Treeholes holding water were sampled by removing three aliquots (ca. 45 ml each) with a polyethylene pipette (5 mm diameter mouth). The presence of early larvae (first and second instars), late larvae (third and fourth instars) and pupae were noted for each treehole.

Samples of the culicid eggs collected on 11 dates (April 30, May 7, 14, and 28, June 4 and 18, July 6, August 3, 17 and 31 and September 21) were reared to fourth instar or adult stages (minimum 50 per date) for identification according to Bohart and Washino (1978). Other insect eggs were reared to larvae or adults and identified according to McAlpine et al. (1981) on at least one date.

Vertical Distribution of *Aedes sierrensis* Eggs and Host-Seeking Adults. An interior live oak tree near the center of the Potter Valley woodland was used to monitor the vertical distribution of oviposited *Ae. sierrensis* eggs over the course of the 1994 season. This tree had a single primary stem and lacked secondary branches on its north side. Six standard ovitraps were mounted on the north side of the trunk with the entrances 0.2, 1.0, 3.0, 5.0, 7.5 and 10.0 m above the ground. All traps were operated according to the methods described for standard ovitraps. Ovitrap liners and treehole water were replaced once per week for each of 23 trap periods between May 9 and November 2 with the exception of a two-week trap period ending October 25. The numbers of eggs collected at each height were compared using a Kruskal-Wallis analysis of variance by ranks both for data collected over the entire season (n = 23) and for the peak trap periods from May 31 to July 19 (n = 7). A Spearman rank correlation analysis was used to determine if egg catches at each height were linearly correlated over the course of the season.

A second interior live oak tree (20 m north of the first) was used to monitor the vertical distribution of host-seeking *Ae. sierrensis* adults. The tree had a single primary stem and extensive secondary branching forming a canopy with a diameter of ca. 17 m. Fay traps (Fay and Prince 1970) baited with 3.2 kg of dry ice were hung in the tree with the trap openings at 0.2, 1.0, 3.0, 5.0, 7.5 and 10.0 m above the ground on May 10 and 23 and June 1, 6 and 14, 1994. By utilizing the large canopy of the tree all traps were >8 m apart. Traps were set at 1400 h and collected at 1100 h the following day. Adults were anesthetized with carbon dioxide and released after being counted and identified in the field with a 10X lens. Numbers of females and males collected at each height were compared by a Kruskal-Wallis analysis of variance by ranks followed by a multiple range test.

RESULTS AND DISCUSSION

Comparisons of Modified Ovitraps to the Standard Ovitrap. 1). No ovibox. Standard ovitraps were significantly more effective for detecting the presence of *Ae. sierrensis* than ovitraps operated without an ovibox. In 30 paired

comparisons, 89.5% of the *Ae. sierrensis* eggs were oviposited into standard ovitraps (Table 2). Field observations indicated evaporation rates were high in the "no ovibox" treatment group. Most of the cups in this group were nearly dry when collected and six were noted to have less than 10 ml of treehole water remaining. The oviboxes sheltered the attractant water in the standard ovitraps and all were observed to hold sufficient volumes for effective operation at the ends of the trap periods.

Table 2. Results of comparisons of modified ovitraps to standard ovitraps. Standard ovitraps utilized the ovibox with a vertical entrance and held treehole water as an attractant.

		Aedes sierrensis eggs	
Ovitrap comparison	Total repetitions	Number of positive traps	Mean ± std. dev. per day
1) No ovibox	30	8[a]	3.3 ± 7.0[b]
Standard		29[a]	25.9 ± 22.8[b]
2) Horizontal entrance	50	45	15.8 ± 11.9
Standard		43	16.5 ± 12.5
3) Deionized water	32	6[a]	2.1 ± 5.6[b]
Standard		29[a]	30.8 ± 22.1[b]
4) Oak leaf infusion	27	25	25.2 ± 28.2
Aged oak leaf infusion		24	18.9 ± 15.8
Standard		25	20.2 ± 21.8

[a]Frequencies of positive ovitraps are significantly different by a Pearson's Chi-square test ($p<0.01$).
[b]Mean numbers of eggs are significantly different by a Mann-Whitney test ($p<0.01$).

Lewis and Tucker (1978) found that water-filled wooden boxes with small entrance holes were attractive sites for *Ae. sierrensis* oviposition. Ovitraps without boxes have been utilized in studies of *Ae. triseriatus*, *Ae. aegypti* and *Ae. albopictus* (Skuse) (Furlow and Young 1970, O'Meara et al. 1995). Table 2 indicates the importance of the ovibox in ovitrap studies of *Ae. sierrensis*. The darkened cavity inside the ovibox may enhance visual oviposition cues or hold chemical odors evaporated from treehole water that increase the attractiveness of the site for oviposition. The larger size of the standard ovitrap may attract more *Ae. sierrensis* females to the location. Reduced wind speed inside the box may facilitate oviposition. Also, the effectiveness of the "no ovibox" traps may have been reduced near the ends of trap periods when most of the water had evaporated from the cups.

2). Horizontal entrance. Previous laboratory (Wilton 1968) and field (Lang 1990) tests have shown that *Ae. triseriatus* females prefer horizontal over vertical entrance containers as oviposition sites. In the present study, *Ae. sierrensis* females showed no preference for vertical or horizontal ovitrap entrances in 50 paired comparisons. There were no identifiable seasonal trends in oviposition site selection related to the orientation of the ovitrap entrance (Figure 2).

3). Deionized water. Deionized water was not an effective attractant to *Ae. sierrensis* females seeking oviposition sites (Table 2). The total numbers of *Ae. sierrensis* eggs oviposited into standard ovitraps was more than an order of magnitude greater than in traps holding deionized water. The frequency of positive traps was significantly higher in standard ovitraps (90.6%) than in deionized water ovitraps (18.8%).

4). Oak leaf infusions. Since prepared infusions could be standardized and used in other areas within the range of *Ae. sierrensis*, oak leaf infusions and treehole water were compared as oviposition attractants. Standardization of the chemical and microbial components of attractant water used in ovitraps is an important consideration for some species of mosquitoes (Reiter 1986, Isoe et al. 1995). Some infusions may attract or repel oviposition depending upon their age. To assess these possibilities both oak leaf infusion and aged oak leaf infusion were compared to treehole water as oviposition attractants during an 85 day period. There were no significant differences in the mean numbers of *Ae. sierrensis* eggs oviposited into traps holding oak leaf infusion, aged oak leaf infusion

or treehole water. Seasonal trends were similar for each of the three waters tested (Figure 3). Declines in oviposition during the late summer are related to seasonal population declines among adult females and not to the attractancy of the oak leaf infusions or treehole water. These results indicate an oak leaf infusion is an acceptable alternative to treehole water in *Ae. sierrensis* ovitraps. The age of the attractant may be less important for *Ae. sierrensis* than for some other mosquitoes.

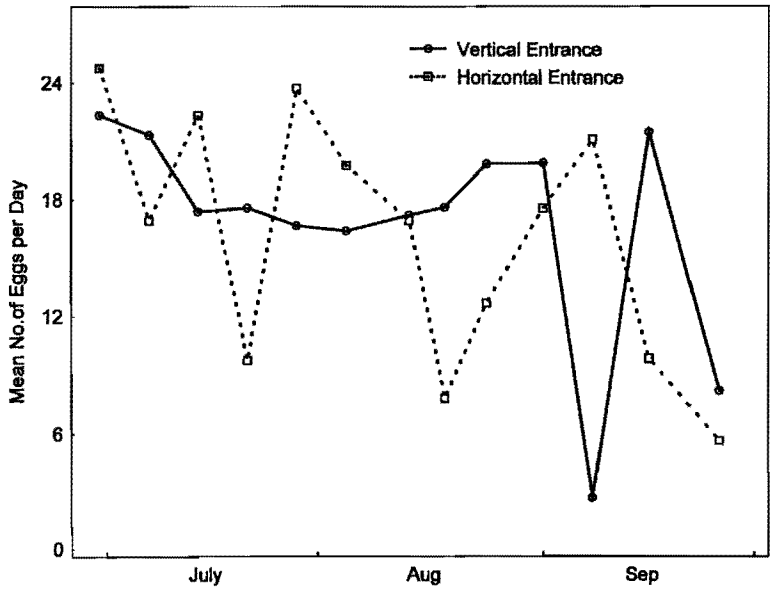

Figure 2. *Aedes sierrensis* eggs trapped with standard (vertical entrance) and horizontal entrance ovitraps at the Potter Valley woodland in 1994.

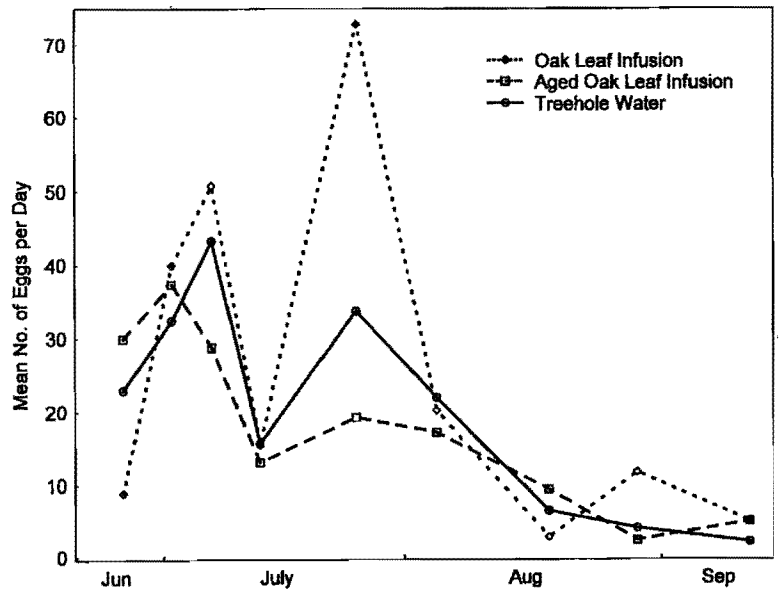

Figure 3. *Aedes sierrensis* eggs trapped in ovitraps holding oak leaf infusion, aged oak leaf infusion and treehole water at the Lakeport woodland in 1994.

Seasonal Distribution of *Aedes sierrensis* Eggs. At the Potter Valley site, *Ae. sierrensis* females oviposited 45,147 eggs into seven standard ovitraps during 26 trap periods between April 2 and October 29, 1992 (Figure 4). Identification of all mature larvae and adults reared from *Aedes* eggs confirmed this species determination. A total of 2,173 *Orthopodomyia signifera* (Coq.) eggs were also collected, all between July 20 and September 21. Eggs of this species have external membranous sheaths (Chapman 1964) making them easily separable from *Ae. sierrensis* eggs. *Orthopodomyia signifera* and *Ae. sierrensis* are the only mosquitoes that regularly inhabit northern California treeholes (Zavortink 1985) and no other culicid eggs were found in the ovitraps. Larvae and adults of other insects reared from eggs oviposited into the traps were identified from the following families: Ceratopogonidae, Psychodidae, Chironomidae and Empididae.

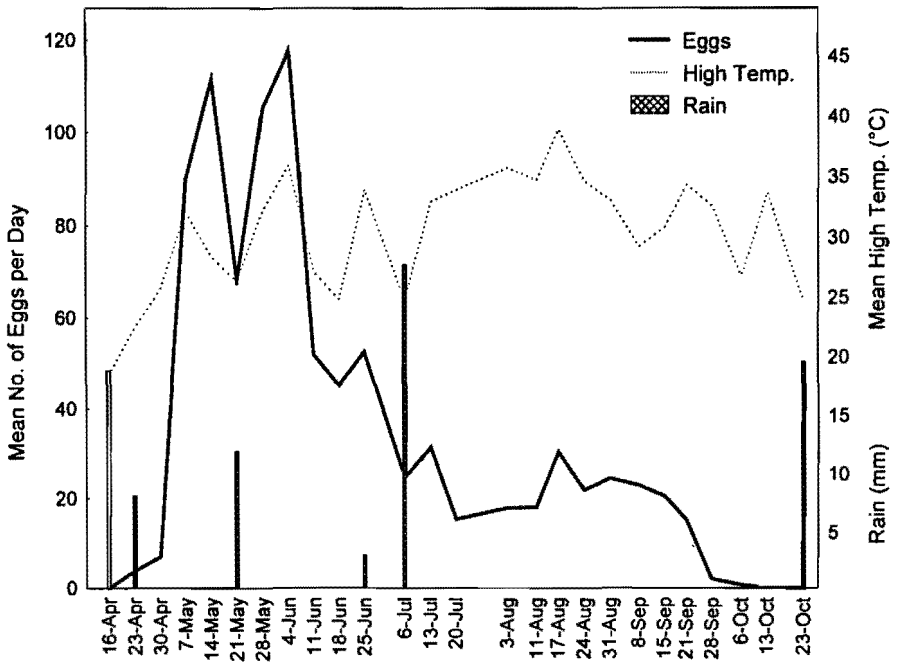

Figure 4. *Aedes sierrensis* eggs trapped in seven standard ovitraps at the Potter Valley woodland in 1992. Mean high temperature and total rainfall during each trap period are also shown.

In the 1992 season, *Ae. sierrensis* eggs were first detected in a single ovitrap on April 23. Most of the eggs (71.3%) were oviposited during the months of May and June. Oviposition peaked on June 4 when the catch averaged 117.7 eggs per trap per day. From April 30 to July 6, mean daily high temperatures and numbers of eggs oviposited into traps were positively correlated ($R=0.73$, $p<0.03$). During the same time period ovipositional declines also corresponded to four of five trap periods that included rainfall. *Aedes sierrensis* egg collections declined in July during a period of hot and dry weather, suggesting that mortality had reduced the size of the female population ovipositing in the woodland. *Aedes sierrensis* eggs were last detected in the ovitraps on October 13. The seasonal distribution of oviposition shown in Figure 4 is similar to the distribution of host-seeking female *Ae. sierrensis* found by Garcia et al. (1989) at the same study site.

At least two factors may be responsible for the observed increase in numbers of *Ae. sierrensis* eggs collected from early August to mid-September. Most importantly, an increase in ovitrap catch occurred in late summer as the availability of natural water-filled treeholes diminished. Only two of 10 monitored treeholes held water on August 3 and all were dry by August 25 (Table 3). By September, more than 90% of the treeholes are usually dry in this area of northern California, even in years with above-average precipitation (Washburn and Hartmann 1992). Secondly, rainfall on June 29 (after most of the seasonal oviposition had occurred) raised the water level in seven of 10 treeholes monitored in the

Table 3. Results of pipette sampling of ten treeholes at Potter Valley in 1992.

Date	Percent holding water	Percent positive for *Aedes sierrensis* immatures		
		Early larvae	Late Larvae	Pupae
3/19	100	0	100	50
4/23	100	0	90	90
4/30	90	10	90	80
5/7	70	0	60	50
5/14	70	0	40	50
5/22	60	0	40	30
5/28	50	0	30	30
6/4	40	0	0	20
6/12	30	0	0	0
6/19	30	0	0	0
6/26	20	0	0	0
6/30	70	70	0	0
7/6	60	70	70	0
7/13	50	10	40	40
7/20	40	0	40	40
7/27	40	10	40	40
8/3	20	0	10	10
8/11	20	0	0	0
8/17	10	0	0	0
8/24 - 10/20	0	0	0	0

woodland. Although a summer egg diapause has been reported for local *Ae. sierrensis* populations (Jordan 1980), in this study, first instars hatched in all seven censused treeholes by June 30. These larvae pupated in the treeholes from mid-July to early August and ultimately produced a small second generation of adults which probably contributed to egg production during late summer.

Seasonal totals of *Ae. sierrensis* eggs in individual ovitraps ranged from a minimum of 2,951 to a maximum of 12,969 eggs indicating that trap location within the woodland had an important influence on their attractiveness as oviposition sites. The results show that standard ovitraps are useful for measuring seasonal variation in oviposition at particular locations, but estimation of biting intensity based on results from small numbers of ovitraps would not be reliable. Standard ovitraps were also very effective for detecting the presence of *Ae. sierrensis* over the course of the season. Using seven ovitraps in the woodland, *Ae. sierrensis* were detected for 24 consecutive trap periods from April 23 to October 13. From May 7 to September 21, 96.2% of the ovitraps were positive for *Ae. sierrensis* eggs.

Vertical Distribution of *Aedes sierrensis* Eggs and Host-Seeking Adults. The vertical distribution of oviposited *Ae. sierrensis* eggs was monitored for the entire 1994 season at the Potter Valley site during which a total of 21,538 eggs were collected (Table 4). Seasonal totals for individual ovitraps ranged from a minimum of 2,096 eggs at 5.0 m to a maximum of 4,123 eggs at 3.0 m above ground; however, there were no significant differences in the number of eggs collected at any height ($p>0.65$). The data indicated *Ae. sierrensis* females were just as likely to oviposit in a trap 10.0 m above ground as at any other height tested. Some treehole mosquitoes in eastern North America also oviposit high in the tree canopy. Sinsko and Grimstad (1977) found that the greatest number of *Aedes hendersoni* Cockerell eggs were collected in ovitraps 6.1 m above ground, the highest level tested. In the same study, eggs of *Aedes triseriatus* were also collected 6.1 m above ground, but they were most prevalent in ovitraps at ground level.

Seasonal variation in the numbers of *Ae. sierrensis* eggs trapped was not affected by the height of the ovitrap above ground (Figure 5). A Spearman's linear correlation matrix showed the catch at each height was significantly correlated

Table 4. Numbers of *Ae. sierrensis* eggs caught per trap period at each of six heights above ground at the Potter Valley woodland in 1994.

	Height above ground (m)						
Date	0.2	1.0	3.0	5.0	7.5	10.0	Totals
May 16	0	0	0	0	0	0	0
May 24	0	115	0	0	102	0	217
May 31	855	424	861	369	1241	754	4504
June 6	382	397	164	117	530	116	1706
June 14	675	397	893	584	739	947	4235
June 21	564	561	620	352	96	453	2646
June 27	218	205	297	12	171	435	1338
July 5	351	298	225	297	80	608	1859
July 12	279	426	250	135	209	2	1301
July 19	0	161	259	0	202	197	819
July 25	99	87	0	11	0	108	305
Aug. 1	103	182	91	0	49	38	463
Aug. 9	198	0	58	32	48	0	336
Aug. 18	46	96	56	0	80	65	343
Aug. 24	85	79	32	58	18	63	335
Sept. 1	67	0	158	0	0	0	225
Sept. 8	46	0	75	0	59	0	180
Sept. 16	36	27	44	0	0	0	107
Sept. 26	119	0	151	9	45	32	356
Oct. 4	0	0	0	120	34	0	154
Oct. 11	0	0	0	0	0	0	0
Oct. 25	0	0	0	0	0	109	109
Nov. 2	0	0	0	0	0	0	0
TOTALS	**4123**	**3455**	**4234**	**2096**	**3703**	**3927**	**21538**

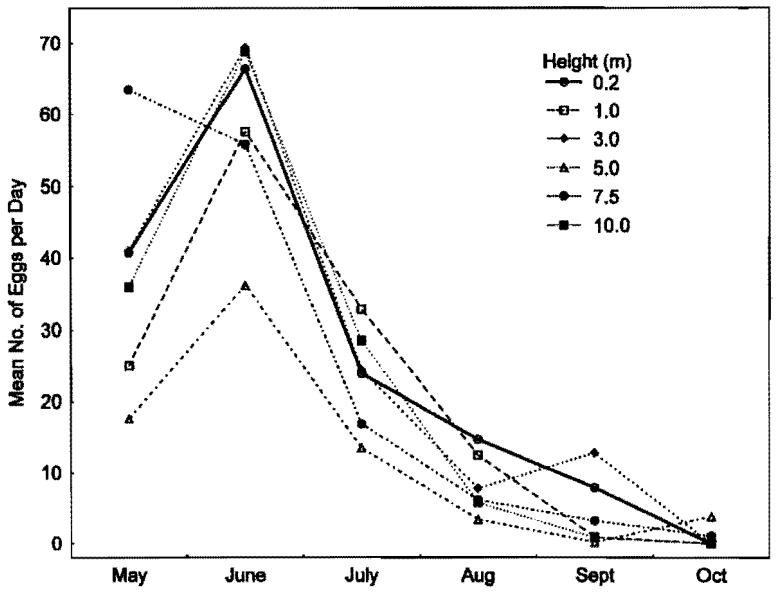

Figure 5. *Aedes sierrensis* eggs trapped at six heights above ground in each month from May to October, 1994, at the Potter Valley woodland.

($R>0.57$, $p<0.01$) with all other heights over the course of the study. These results do not provide any evidence that *Ae. sierrensis* females shifted the vertical space searched for oviposition sites during the season.

In contrast to the even vertical distribution of oviposited eggs, host-seeking female *Ae. sierrensis* were caught in significantly larger numbers in Fay traps operated near ground level (Table 5). Over the course of the test, 94% of the adult females were caught in traps operated at 0.2 and 1.0 m above ground (Figure 6). Since females blood-feed on mammals (Tempelis and Washino 1967, Garcia et al. 1988) and are not known to feed on birds, this distribution is similar to that of the known hosts of females. Less than 2% of the females were caught in traps between 5.0 and 10.0 m above ground, the zones likely to hold the largest numbers of birds.

The vertical distribution of male *Ae. sierrensis* was very similar to that of the females (Table 6). Males seek the hosts of females to locate mates (Peyton 1956) so a similar distribution in carbon dioxide baited Fay trap catches is not surprising. More than 94% of males were caught in traps operated at 0.2 and 1.0 m above ground, and less than 3% were caught between 5.0 and 10.0 m above ground.

Table 5. Numbers of female *Aedes sierrensis* caught per day in carbon dioxide baited Fay traps operated at six heights above ground at the Potter Valley site in 1994.

	Height above ground (m)					
Date	0.2	1.0	3.0	5.0	7.5	10.0
May 10	51	92	5	0	0	0
May 23	164	71	12	2	2	0
June 1	72	47	9	4	1	0
June 6	34	39	5	1	0	1
June 14	79	21	1	1	1	0
MEAN	80.8a	54.0a	6.4b	1.6c	0.8c	0.2c

Means followed by the same letter are not significantly ($p>0.05$) different by a Kruskal-Wallis analysis of variance by ranks followed by a multiple range test.

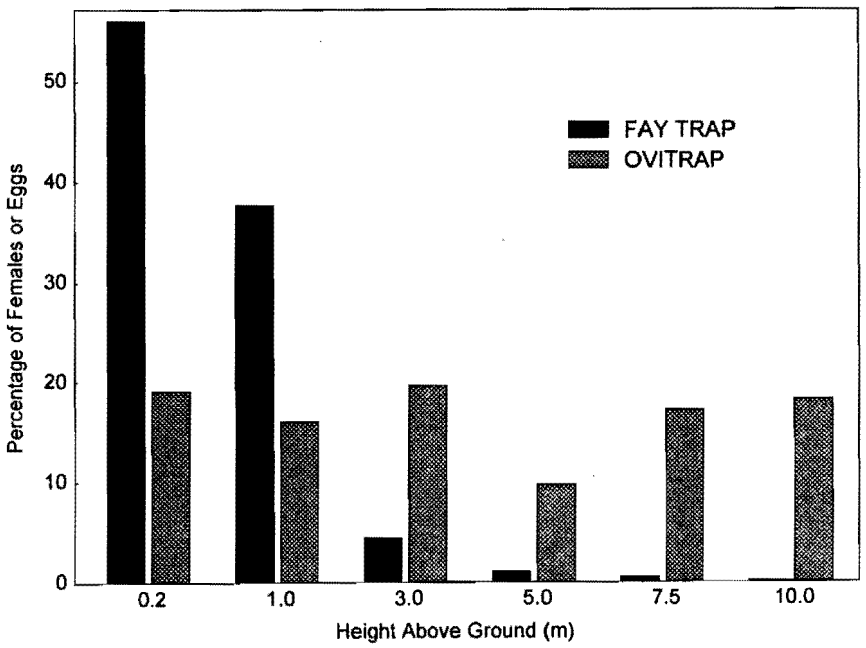

Figure 6. Percentage of host-seeking females caught in Fay traps and ovitrapped eggs captured at each of six heights above ground at the Potter Valley woodland in May and June, 1994.

Table 6. Numbers of male *Ae. sierrensis* caught per day in carbon dioxide baited Fay traps operated at six heights above ground at the Potter Valley site in 1994.

	Height above ground (m)					
Date	0.2	1.0	3.0	5.0	7.5	10.0
May 10	434	570	23	10	13	5
May 23	489	194	27	4	3	3
June 1	103	66	15	3	2	1
June 6	20	17	3	1	0	1
June 14	59	24	0	0	0	1
MEAN	221.0a	174.2a	13.6b	3.6b	3.6b	2.2b

Means followed by the same letter are not significantly different ($p>0.05$) by a Kruskal-Wallis analysis of variance by ranks followed by a multiple range test.

SUMMARY

Several factors affecting the sensitivity of ovitraps for detecting the presence of *Ae. sierrensis* were identified in this study. Use of the ovibox significantly increased the frequency of positive traps and the total numbers of eggs oviposited into standard ovitraps. There were no measurable differences in sensitivity related to a vertical or horizontal ovitrap entrance. Treehole water and oak leaf infusions were found to be effective attractants in *Ae. sierrensis* ovitraps. The age of the oak leaf infusion was not found to be a critical factor affecting sensitivity of the ovitraps. Deionized water was not an effective oviposition attractant for *Ae. sierrensis*.

Standard ovitraps were very effective for detecting the presence of *Ae. sierrensis* from April to October, the entire activity period of adult females. The seasonal distribution of oviposition in standard ovitraps showed peak activity in May and June. This is similar to a distribution of host-seeking females previously described for the same location and indicates standard ovitraps are useful for measuring seasonal ovipositional trends. Oviposition was positively correlated with mean high temperatures from late April to early July, but this correlation was not apparent as adult populations declined in late summer. Monitoring of treeholes at the study site indicated a small oviposition peak in August and September occurred as natural treeholes dried out, reducing the numbers of available oviposition sites. A small second generation of *Ae. sierrensis* adults emerged after unusual summer rain and may have also contributed to oviposition in late summer.

Analysis of the vertical distribution of *Ae. sierrensis* oviposition showed that females searched from ground level to near the top of the tree canopy for oviposition sites. The lack of a measurable vertical limitation to oviposition site searching behavior might indicate the *Ae. sierrensis* population was limited by lack of suitable larval habitats. Host-seeking behavior of adults of both sexes was concentrated near the ground where the mammalian hosts of the species are most abundant.

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MOSQUITO ABUNDANCE AND ARBOVIRAL ACTIVITY IN SAN BERNARDINO COUNTY DURING 1986-95: A DECADE IN REVIEW

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ABSTRACT

Of the 155,420 mosquitoes collected in New Jersey light traps and CO_2 -baited CDC traps in San Bernardino County during 1986 through 1995, 83.7% were from the desert region (Colorado River-Needles) and 16.3% from the valley region. The dominant species in the desert region were *Culex tarsalis* (47.3%), *Aedes vexans* (33.6%) and *Culiseta inornata* (16.4%). In the valley region, the dominant fauna consisted of *Cx. tarsalis* (31.0%), *Cx. stigmatosoma* (25.5%), *Cx. quinquefasciatus* (21.4%), and *Culiseta incidens* (10.6%). Based on 1,014 chicken sera tested during the period, the desert region showed 16 and 11 samples positive for Saint Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) viruses, respectively. One thousand twenty-one sera tested from the valley region gave only six SLE positives. Similarly, 860 mosquito pools tested from the desert region had five and seven positive pools for SLE and WEE, respectively; none of the 503 pools from the valley region tested positive. Based on both chicken serology and mosquito pool data, the 1993 season had the highest number of positives for both SLE and WEE.

In contrast to relative mosquito abundance, the data on confirmed human cases of encephalitis (SLE) during the last ten years in San Bernardino County showed more SLE cases (four) in the valley area than in the desert region (two). This indicates that apart from high mosquito numbers, other factors such as mosquito age, infection rate, breeding and resting sites, host availability, and demographic factors, all play an important role in disease transmission.

As part of the state-wide encephalitis virus surveillance (EVS) program in California, the San Bernardino County Vector Control Program (SBCVCP) has carried out EVS and other mosquito control activities in both the desert and valley areas of San Bernardino County for several years. Geographically, the county consists of desert, mountain and valley regions. Demographically, the valley region houses over 80% of the nearly 1.6 million population. Historically, occasional cases of both Saint Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) have been reported in the desert and valley regions.

The occurrence of 26 human cases of SLE in southern California during 1984, gave a fresh impetus to both operational and research aspects of vector ecology and control in California. The research was shouldered by personnel at the University of California at Berkeley, Davis, Los Angeles and Riverside (Barr et al. 1986, Palchick and Washino 1986, Mulla et al. 1988, Reeves 1988, Reisen 1988, Schreiber et al. 1988, Mian et al. 1990). Field surveillance and control operations remained with the California Department of Health Services, Vector-Borne Disease Section (CDHS-VBDS), and local mosquito and vector control agencies, both individually within their boundaries and collectively through the Mosquito and Vector Control Association of California (MVCAC).

New information made available through university research, helped local agencies to improve the efficiency, reliability and cost effectiveness of their surveillance methods. As a local agency, SBCVCP has been involved in carrying out routine

encephalitis virus surveillance within its operational boundaries in San Bernardino County. The present report, therefore, provides a 10-year update on the data generated in routine EVS activities during 1986 through 1995.

MATERIALS AND METHODS

General EVS procedures followed in these studies were as follows:

Adult Mosquito Population Dynamics

The abundance of various mosquito species was monitored on a weekly basis through a number of New Jersey light traps. In the valley and mountain regions, the traps were stationed at Yucaipa, Redlands, Highland, San Bernardino, Loma Linda, Colton, Fontana, Ontario, Upland and Jenks Lake. Within the valley region there were at least two traps each in urban, suburban and rural environments. In the desert region (Needles area), one trap each was operated in urban, suburban and rural areas along the Colorado River.

Adult mosquitoes collected in the traps were counted, sexed and identified to species and the Adult Mosquito Occurrence Reports submitted to the California Department of Health Services, Vector-Borne Disease Section.

Arboviral Activity in Female Mosquitoes

Arboviral activity in local mosquito populations was monitored in all three regions using CO_2 -baited CDC (Centers for Disease Control and Prevention) traps to collect host-seeking adult female mosquitoes. Eight or more such traps were operated twice a month in the valley area and once a month in the Colorado River (desert) area. Traps operated twice during the 1993 and 1994 seasons in the mountain area did not yield a significant number of adult mosquitoes. Female mosquitoes collected overnight were anesthetized using triethylamine (TEA), counted, identified to species and pooled by species with 10-50 specimens per labeled vial. All pools were stored on dry ice in the field or in a deep freezer at -60° F in the laboratory before being shipped in dry ice-packed containers by overnight express mail to the Viral and Rickettsial Disease Laboratory (VRDL) in Berkeley for virus testing.

Arboviral Activity in Sentinel Chickens

Both wild and domestic birds are known to play a significant role in the epidemiology of mosquito-borne encephalitides by acting as reservoir hosts. Therefore, a sentinel flock of 10 to 25 white leghorn chickens was maintained in both the valley area in San Bernardino/Colton and the desert area in Needles. The valley flock of 25 birds was stationed at the wastewater treatment plant in San Bernardino. Due to a human case of SLE in 1987, twelve birds from the 1988 valley flock were maintained at the southwestern corner of Mill Street and Meridian Ave., where the human case occurred. In 1989, the flock was relocated about a block away from the current location at the northeastern corner of Meridian Ave., and Olive Street in Colton. This site is within one mile of the two SLE human cases reported in 1987 and 1988 in the city of San Bernardino. The desert flock was maintained at the sewage treatment plant in Needles. During the 1995 season, this flock was temporarily moved to Park Moabi Regional Park following two SLE human cases from the area during 1993 and 1994. Since the 1990 season, the flock size and bleeding frequency were changed from 25 to 10 birds and from once a month to biweekly bleeding, respectively. Similarly, the old method of drawing approximately two ml of blood from the jugular vein was replaced by the comb-prick method developed by Dr. Reisen of the Arbovirus Field Station in Bakersfield. All chicken blood samples were mailed to VRDL for detection of arboviral activity.

Notification of seroconversions and/or positive mosquito pools by VRDL was made to SBCVCP over the phone. Upon receipt of such notification, the affected areas were posted with "Mosquito Warning" signs. Area residents were informed through local media - newspapers, radio and television, about precautions necessary to minimize the risk of encephalitis infection. They were also advised to report all standing water sources for inspection by SBCVCP. In the wake of positive serology or mosquito pools, increased larvicidal and adulticidal applications were carried out in the affected areas when necessary.

RESULTS AND DISCUSSION

Of the 155,420 mosquitoes collected at all sites during the 1986-95 decade, 83.7% were trapped in the desert area and 16.3% from the valley region. Based on total combined data, the percent species composition county-wide ranks *Culex tarsalis* Coquillett the most abundant species (44.6%), followed by *Aedes vexans* Meigen (28.1%), *Culiseta inornata* Williston (14.6%), *Culex stigmatosoma* Dyar (4.3%), *Culex quinquefasciatus* Say (3.8%), *Culiseta incidens* (Thompson)(1.7%), *Anopheles*

franciscanus McCracken (1.5%), and *Culex erythrothorax* Dyar (1.1%). Other species accounting for ≤ 0.1% each were *Aedes washinoi* Lanzaro and Eldridge, *Anopheles freeborni* Atkin, *Culiseta particeps* Adams, *Psorophora columbiae* Dyar and Knab, and *Psorophora signipennis* Coquillett (Table 1).

Species composition by region showed some disparity. Faunal abundance by species in the desert region showed *Cx. tarsalis*, the most abundant species followed by *Ae. vexans*, *Cs. inornata*, *An. franciscanus*, *Culex erythrothorax*, *Culex quinquefasciatus*, *Cx. stigmatosoma*, *An. freeborni*, *Cs. particeps*, *Ps. signipennis*, *Ps. columbiae* and *Cs. incidens*. Species composition in the valley region was somewhat different, with *Cx. tarsalis* as the most abundant followed by *Cx. stigmatosoma*, *Cx. quinquefasciatus*, *Cs. incidens*, *Cs. inornata*, *An. franciscanus*, *Cx. erythrothorax*, *Ae. washinoi*, *An. freeborni*, *Cs. particeps* and *Ae. vexans*.

Data on the chicken serology show that the 1993 season had the highest number of seroconversions for both SLE and WEE, especially in the desert flock, which had 100% and 60% sero-positive chickens, respectively (Table 2). There were no seroconversions noted in 1986 and 1987; there was however an SLE human case confirmed late in the 1987 season. The area where the human case occurred had not been surveyed for encephalitis viruses. The following year, two out of 12 chickens seroconverted to the SLE virus. The desert region showed more virus activity than the valley area as evident from seroconversions in 1988, 1989, 1991, 1992, 1993 and 1994. This region also had more mosquito pools positive in 1986, 1990, 1992 and 1993 than the valley area which had no positive pools during the entire decade of EVS in San Bernardino County.

Although no viruses were detected in mosquito pools in the valley area, there were several confirmed human SLE cases (1987, 1988, 1991, 1993). As compared regionally, San Bernardino County had almost one-fourth (4/17) of the total number of SLE human cases in southern California and one-twelfth (4/48) of all the encephalitis cases confirmed in California during the last 10 years (Table 3). Moreover, based on the travel history of two SLE cases, one from Orange County in 1993 and another from Riverside County in 1994, these individuals are believed to have contracted the disease in the Colorado River area of San Bernardino County. This area is relatively thinly populated but it's recreation sites are frequented by a large number of tourists during the mosquito season. It is important to point out that in the presence of higher viral activity detected in both chickens and mosquitoes in the desert area, human cases (2) were lower by 1 to 2 as compared with those (4) of the valley region. It also indicates that apart from mosquito numbers, there are other factors such as mosquito physiological age, infection rate, breeding sites, resting sites, host availability and possibly other demographic factors, that play significant roles in the epidemiology of arthropod-borne diseases.

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Table 1. Mosquito distribution by area and species in San Bernardino County during 1986 - 1995.[a/]

		Number of Mosquitoes Trapped													
Mosquito Species	Area	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	Area Total	%	Species Total	%
Aedes vexans	D	3279	265	8191	12560	2749	540	842	12671	2510	66	43673	33.6		
	V	0	0	0	4	4	0	0	3	0	0	11	<0.1	43684	28.1
Ae. washinoi	D	0	0	0	0	0	0	0	0	0	0	0	0		
	V	0	0	0	0	0	3	33	3	0	9	48	0.2	48	<0.1
Anopheles	D	81	10	384	25	217	0	829	14	58	7	1625	1.2		
franciscanus	V	1	1	50	81	189	0	160	20	229	12	743	2.9	2368	1.5
An. freeborni	D	1	0	34	0	0	32	0	0	0	0	67	<0.1		
	V	8	4	5	0	0	5	0	0	0	0	22	<0.1	89	0.1
Culex erythrothorax	D	212	81	190	13	149	48	6	19	44	346	1108	0.8		
	V	89	58	54	30	343	22	1	13	18	11	639	2.5	1748	1.1
Cx. quinquefasciatus	D	5	1	82	13	27	24	268	38	15	33	506	0.4		
	V	209	109	604	700	921	370	771	614	213	908	5419	21.4	5925	3.8
Cx. stigmatosoma	D	43	25	84	0	14	72	6	4	0	7	255	0.2		
	V	176	82	495	1238	928	964	728	591	442	802	6446	25.5	6701	4.3
Cx. tarsalis	D	5841	5143	7610	7733	7503	5549	3506	4045	4152	10466	61548	47.3		
	V	26	98	363	1484	1309	932	1525	947	355	797	7836	31.0	69384	44.6
Culiseta incidens	D	2	6	0	0	0	0	0	0	0	1	9	<0.1		
	V	0	26	61	174	45	85	341	977	446	528	2683	10.6	2692	1.7
Cs. inornata	D	521	1387	4742	4928	2871	1677	918	3034	496	713	21287	16.4		
	V	2	22	37	517	34	51	62	161	101	408	1395	5.5	22682	14.6
Cs. particeps	D	4	0	39	0	0	0	0	0	0	0	43	<0.1		
	V	0	0	0	13	0	1	0	0	0	0	14	<0.1	57	<0.1
Psorophora columbiae	D	0	0	0	0	13	0	0	0	0	0	13	<0.1		
	V	0	0	0	0	0	0	0	0	0	0	0	0	13	<0.1
Ps. signipennis	D	0	0	0	0	0	8	0	0	22	0	30	<0.1		
	V	0	0	0	0	0	0	0	0	0	0	0	0	30	<0.1
TOTAL	D	9989	6918	21356	25272	13530	7942	6375	19825	7275	11639	130121	100	130121	83.7
	V	511	400	1669	4241	3786	2441	3621	3329	1826	3475	25229	100	25299	16.3
Column Total		10500	7318	23025	29513	17316	10383	9996	23154	9101	15114	155420		155420	100
Annual percentage		6.8	4.7	14.8	19.0	11.1	6.7	6.4	14.9	5.9	9.7	100			

[a/] Mian (1988, 1989, 1995); Mian and Prochaska (1991 - 1993); Mian et al. (1994, 1995).
[b/] D = Desert, V = Valley.

Table 2. Summary of encephalitis virus activity in San Bernardino County during 1986 - 1995.[a/]

	Chicken Seroconversion SLE/WEE/# Sera tested		Positive Mosquito Pools SLE/WEE/# Pools tested	
Year	Desert	Valley	Desert	Valley
1986	0/0/6	0/0/130	0/3/115	0/0/91
1987	0/0/71	0/0/82	0/0/120	0/0/27
1988	1/0/136	2/0/84	0/0/160	0/0/25
1989	0/1/130	0/0/199	0/0/39	0/0/66
1990	0/0/56	0/0/87	1/0/105	0/0/63
1991	1/0/145	0/0/99	0/0/92	0/0/38
1992	0/4/110	0/0/180	0/1/40	0/0/77
1993	10/6/110	4/0/110	4/3/65	0/0/46
1994	4/0/120	0/0/120	0/0/52	0/0/24
1995	0/0/130	0/0/130	0/0/72	0/0/46
Total:	16/11/1014	6/0/1221	5/7/860	0/0/503

[a/] Mian (1988, 1995), Mian and Prochaska (1990 - 1993) and Mian et al. (1994, 1995).

Table 3. Summary of human cases of encephalitis in San Bernardino County compared with regional and statewide data during 1986 - 1995[a/].

	Number of confirmed human cases of SLE/WEE		
Year	San Bernardino County	Southern California	State-Wide
1986	0/0	3/0	3/2[b/]
1987	1/0	1/0	1/0
1988	1/0	2/0	2/0
1989	0/0	1/0	29/0
1990	0/0	1/0[c/]	2/0
1991	1/0[d/]	3/0[d/]	3/0
1992	0/0	2/0[e/]	2/0
1993	1/0	3/0[f/]	3/0[g/]
1994	0/0	1/0[h/]	1/0
1995	0/0	0/0	0/0
Total	4	17	48

[a/] Emmons et al. (1987 - 1994) and Reilly et al. 1995.
[b/] Additionally, one case of WEE in a two-year child in northern California.
[c/] Patient had travel history to Colorado River area of CA/AZ.
[d/] Based on travel history, disease was contracted in New Mexico and Arizona.
[e/] Additionally, one case of WEE in a horse in Imperial, CA.
[f/] Based on travel history, one SLE case from Orange County was presumably contracted in the Colorado River (Needles) area, San Bernardino County.
[g/] There were two WEE cases: in a horse and an emu.
[h/] Based on travel history, the disease was most probably contracted in the Colorado River (Needles) area, San Bernardino County.

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EFFECTIVE CONTROL OF *CULEX TARSALIS* BY *LAGENIDIUM GIGANTEUM* IN SAN JOAQUIN RICE FIELDS

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ABSTRACT

Lagenidium giganteum was applied by air in late June to approximately 100 acres of emergent rice. *Cx. tarsalis* larval populations decreased dramatically between five and twelve days after application when infected larvae were abundant, and remained low for the rest of the season. A New Jersey light trap monitoring nearby untreated fields continued to yield very high adult *Cx. tarsalis* counts, providing evidence of the efficacy of *L. giganteum* in this habitat.

L. giganteum was isolated and described by Couch (1935) as weakly parasitic of mosquito larvae. It was "rediscovered" thirty four years later when Umphlett and Huang (1972) tested a North Carolina isolate and demonstrated its larvicidal properties. Since then a number of *L. giganteum* strains have been isolated. These strains have generally been intolerant of polluted or saline water, and have also been more efficacious against some mosquito species than others (Jaronski and Axtell 1982, Merriam and Axtell 1982, Goettel et al. 1983, Lord and Roberts 1985, Orduz et al. 1992).

L. giganteum is still a very promising biological control agent. It is EPA registered and a commercial product of fermented mycelium is tantalizingly close. When the mycelial fermentation product becomes diluted through application in an aquatic environment the resulting low nutrient concentration promotes asexual reproduction and initiates production of zoosporangia. The free swimming, short-lived biflagellate zoospores that are released from the zoosporangia are either engulfed during larval feeding, or attach to the mosquito larva cuticle, encyst and subsequently initiate mycelial invasion of the larval body. Within one to three days the nutrients in the infected larva are exhausted, and this asexual reproduction cycle is repeated. Thus a single application of *L. giganteum* culture to a field population of mosquito larvae should result in continued cycles of infection of new cohorts (Kerwin et al. 1994).

Of the isolates studied hitherto, the California strain 52675 (Lord and Roberts 1985) and the isolate from Colombia (Orduz et al. 1992) have proven to be the most efficacious. Studies of the Colombia isolate of *L. giganteum* under laboratory conditions using *Cx. tarsalis* Coquillett as a test larva show it is more tolerant to salinity and effluent than the California strain (Reid 1994), but that good water quality is still required for optimum results. To test efficacy in the field, the Colombia strain was applied to commercial rice fields this past summer.

MATERIALS AND METHODS

The Colombia strain of *L. giganteum* was maintained in sterile culture in fish peptone and yeast extract based media. Stock cultures of L. giganteum were grown on agar plates on PEP2 medium (15g agar, 3.6g fish peptone, 3g yeast extract, 2g glucose, 1.8g cottonseed extract, 0.6g $CaCl_2.H_2O$ and 0.2g $MgCl_2.6H_2O$ per liter) or in liquid culture, either in rotary shaken flasks or in a fermenter, using PEP1 liquid medium (3.6g fish peptone, 3g yeast extract, 2g glucose, 1.8g cottonseed extract, 0.2g cholesterol, 0.6g $CaCl_2.H_2O$, 0.5g NaOH, 0.2g $MgCl_2.6H_2O$, 2.5ml commercial soybean oil and 15ml blended whole egg

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per liter). The production of the 100L volume necessary for mosquito control in rice fields was undertaken by a commercial laboratory. Larval *Cx. tarsalis* were detected in rice fields in San Joaquin County in early June, and experimental and control sites selected following dipping to determine larval population distribution data. Baseline water quality was also monitored. Large scale *L. giganteum* production was scheduled for the end of June to coincide with a predicted peak of early instar *Cx. tarsalis* larvae.

Unforeseen cultural practices associated with seedling rice rendered unusable the preselected fields in which *Cx. tarsalis* larval populations had been present. Since the *L. giganteum* fermentation product was almost ready for application, this necessitated a hurried selection of alternative sites. Pre-dipping located a suitable area for larvicide application with one rice field adjacent to an unused airstrip north of Escalon containing a particularly dense aggregate of larvae. A New Jersey Light Trap was in place on the airstrip. A comparable larval aggregate could not be found in the selected control area about 3 miles away, just south of the Escalon stockyards, even though that area historically produced large numbers of larvae.

The larvicide was diluted 1:4 (1L per 4L of water) in the tanks of the helicopter, the pumps set at minimum agitation, and the larvicide applied at the rate of 4L per acre (ca. 1 ml/m^2). The control site was left unsprayed. Laboratory reared second instar *Cx. tarsalis* larvae were placed in treated and control fields in sentinel buckets immediately prior to aerial application. To half of these cages in the experimental site was added *L. giganteum* to evaluate in-field efficacy of the product. Following application, field and sentinel bucket larvae were monitored on day five, and field larvae were sampled at the control site with a minimum of 30 dips, and the experimental site sampled with 110 dips per visit, for two months post-treatment. Water quality also was measured at these times. Laboratory reared *Cx. tarsalis* larvae were used to test infectivity of field caught, *L. giganteum* infected wild larvae.

RESULTS AND DISCUSSION

The control and experimental rice fields contained water suitable for *L. giganteum* activity. Mean conductivity was 90 and 93 µmhos/cm and pH 5.5 and 5.6 respectively. Water temperature at 1000 h ranged between 19 °C and 24 °C. The day prior to application (June 29) the control field yielded an average of 0.3 larva/dip. The highest average count per 10 dips in the experimental field aggregate was 3.6 larvae/dip (Figure 1), and the median was 2.1 larvae/dip.

There was no larval infection in any of the sentinel buckets five days post-application (July 5), and the highest concentration of larvae in the experimental field had climbed to 7.4 larvae/dip and the median value had risen to 3.9, indicating that the commercial fermentation product was slow to produce zoospores. By day 12 (July 12), larvae were still difficult to find in the control field (0.1/dip) but larval numbers in the experimental field aggregate had fallen substantially and the median value had decreased from 3.9/dip to 0.1/dip with three to four dead, infected larvae per dip. One more infected larva was found on day 19 (July 19). All the dead larvae from the field induced *L. giganteum* infection in laboratory reared larvae.

As the season progressed, the species composition of the larval population began to change. On July 19, 14% of the larvae were *Anopheles freeborni* Aitken, as were the few larvae collected thereafter.

Sampling on July 17 of other rice fields to which *L. giganteum* was applied in this 100 acre trial yielded six dead *Cx. tarsalis*, one live *Cx. tarsalis* and two live *An. freeborni* in 200 dips, providing evidence of the widespread effectiveness of this single application of *Lagenidium giganteum*.

Establishing a qualitative relationship in rice fields between the number of adult *Cx. tarsalis* caught in a light trap and larval *Cx. tarsalis* numbers in adjacent fields is very difficult, the major problems being the ability of adults to fly and the uneven dispersion of larvae in the habitat. Nevertheless, one could argue that the initial high larval counts in the experimental field are reflected in the subsequent high adult counts from untreated fields (Figure 2), and as *L. giganteum* recycled through the *Cx. tarsalis* larval population in the treated field during the summer, maintaining a low larval population, untreated fields continued to produce significant numbers of adults.

Unpredictable water management practices and difficulty in precisely predicting the location of larval populations in the field provide real constraints in selecting, ahead of time, experimental sites for studies such as this. Nevertheless, the

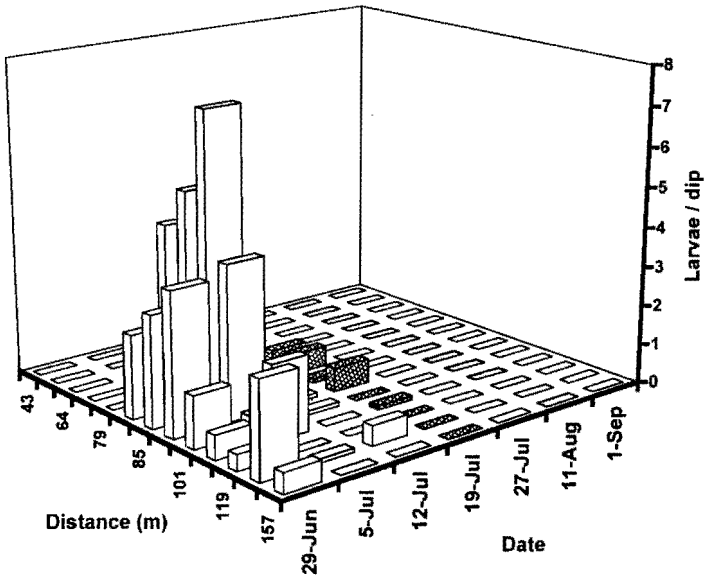

Figure 1. Changes in the number of larvae/dip in a larval aggregate for *Cx. tarsalis* found along one edge of a rice field, following an application of the Columbia strain of *L. giganteum* on June 30, 1995.

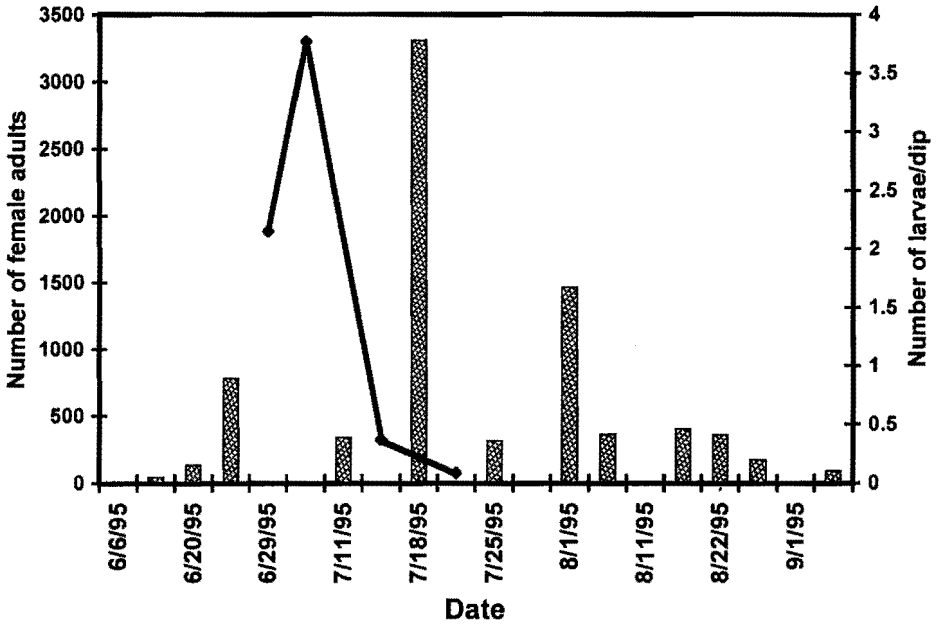

Figure 2. Comparison between the number of adult *Cx. tarsalis* caught in a New Jersey light trap (bar graph) from both treated and untreated rice fields, and the number of *Cx. tarsalis* larvae (line graph) in a treated rice field following application on June 30 of the Columbia strain of *L. giganteum*.

Colombia strain of *L. giganteum* proved to be very effective for long term control of *Cx. tarsalis* in commercial rice fields following a single application in early summer. In addition, the excellent recovery of infected larvae in subsequently infected laboratory reared *Cx. tarsalis* larvae offers the opportunity to further investigate *L. giganteum*'s reported ability to overwinter and subsequently infect (Fetter-Lasko and Washino 1983). We propose to revisit the experimental site this summer and undertake a number of sampling strategies to see if overwintering has occurred.

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SIN NOMBRE VIRUS AND EL MORO CANYON VIRUS (BUNYAVIRIDAE: *HANTAVIRUS*) IN RODENTS (MURIDAE: SIGMODONTINAE) FROM ORANGE COUNTY, CALIFORNIA

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ABSTRACT

During a screening program for Hantavirus activity in Orange County and adjacent San Diego County, blood serum samples from 1,120 rodents representing eight genera and 14 species were tested. Samples dating from December 1993 through 1995 were included in the survey. Rodents seropositive for Sin Nombre virus (SNV) included 39 of 480 (8.1%) deer mice (*Peromyscus maniculatus*) and three of 102 (2.9%) California mice (*Peromyscus californicus*). In addition, 24 of 202 (11.9%) harvest mice (*Reithrodontomys megalotis*) were seropositive for El Moro Canyon virus (ELMCV). All other species tested were negative. There appears to be a correlation between age and sex of the reservoir host and seroprevalence of virus, especially among males of both deer mice and harvest mice. No seasonal fluctuation in seroprevalence was observed. Many of the hantavirus-positive rodents were identified from localities along coastal bluffs and the foothills of the Santa Ana Mountains where several residential, commercial, and industrial sites (old and new) exist and the potential health risk should not be overlooked.

European and Asian Hantavirus infections in humans and reservoir hosts, their human etiologic profiles, and their associated epidemiological histories have been topics of health interest during recent years (LeDuc 1987, Lee et al. 1990, McKee et al. 1991). Follow-up studies in North America (LeDuc et al. 1984, Tsai et al. 1985) documented Hantaan (HTN)-related viruses from rats and mice, including the sigmodontines, *Peromyscus maniculatus* and *Neotoma mexicana*, taken from coastal, inland, urban and rural areas. There are approximately 20 species of *Hantavirus* described and collectively, they comprise a genus of the family Bunyaviridae. The emergence in the spring of 1993 involving a new disease-causing *Hantavirus* (Hughes et al. 1993, Hjelle et al. 1994a) in the southwestern United States caught all health officials unawares and prompted federal, state, and local public health authorities to initiate immediate intensive and extensive field investigations to determine the source(s) of this new disease. As a result, serological and recently developed viral nucleotide sequencing protocols provided the identification of a new virus (Four Corners virus, FCV, Hjelle et al. 1994b; since renamed Sin Nombre virus, SNV) and a definite correlation between SNV-caused Hantavirus Pulmonary Syndrome (HPS) in humans and deer mice (*Peromyscus maniculatus*) carrying SNV (CDC 1993a, CDC 1993b, CDC 1994, Hjelle 1994b). Two other hantaviruses are known to occur in California. El Moro Canyon virus (ELMCV), which is closely related to SNV, has been associated with the harvest mouse, *Reithrodontomys megalotis* (Hjelle et al. 1994c) and Isla Vista virus (ISLAV), which is a genetically distinct virus (related to Prospect Hill virus) in the California vole, *Microtus californicus* (Hjelle, pers. comm.). Neither ELMCV nor ISLAV are known to cause human disease. Shortly after the Centers for Disease Control and Prevention (CDC) in Atlanta determined the association between HTN

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virus and HPS in June 1993, a Hantavirus Serosurveillance Program (HSP) was officially initiated by the Orange County Vector District Laboratory on June 25, 1993. Dr. James E. Childs (CDC) was contacted and agreed to provide laboratory testing of any deer mice and related rodent sera for HTN viral antibodies. The decision to begin a serosurveillance program was predicated upon the idea that etiologic agents (e.g., bacteria, viruses, etc.) are often associated with their reservoir host species throughout their distributional range and the fact we had a large number of sera samples from rodents acquired during the District's Lyme Disease Serosurveillance Program (Webb et al. 1994). In the early phase of the Orange County Program, small mammals and sera samples were collected during the period of June through July 1993. In August, our laboratory was contacted by the California Department of Health Services (CDHS) and advised to postpone our HSP because two human Hantavirus cases (both fatal) had been identified in California. Shortly thereafter, with oversight by safety staff from the Orange County Environmental Health Department, a workable and safe rodent trapping, handling, and bleeding protocol was developed. In the meantime, the sera accumulated since June and banked, frozen (-70 °C) rodent sera (total = 277 samples) accumulated during the District's Lyme Disease and Bubonic Plague Surveillance Programs, including 55 samples from *P. maniculatus*, were sent to Dr. Thomas Ksiazek (CDC, Atlanta) on August 24, 1993. As a result of testing at the CDC Laboratory, five of 34 *P. maniculatus* sera collected from a single site in 1992 were seropositive for Hantavirus, giving an impetus to the development of the Hantavirus Serosurveillance Program in Orange County. Subsequent sera samples taken in 1993 were sent to CDC, Atlanta; in 1994 sera were sent to the California Department of Health Services (Dr. Michael Ascher) and to the University of New Mexico School of Medicine, Albuquerque (Dr. Brian Hjelle); and 1995 samples were sent only to the University of New Mexico.

MATERIALS AND METHODS

COLLECTION SITES

Rodents were collected from several sites throughout Orange County. Most of the collection locations where *Peromyscus maniculatus* and *Reithrodontomys megalotis* were trapped were Sage Scrub habitats often associated with grasslands and disturbed, weedy (ruderal) vegetation or Sumac savannah grassland. Occasionally these two species were collected in chaparral, woodland, or riparian habitats. On a few occasions both species of mice were collected from suburban residential sites that were significant distances from natural habitats.

RODENT TRAPPING AND PROCESSING

Mice were trapped in Sherman live box traps (7.6 x 8.9 x 22.9 cm) baited with dry oats that had been placed during the early evening at sites considered to be suitable rodent habitat. The traps were picked up early the next morning using a Pilstrom® snake tongs by one of the field staff who wore rubber gloves. Traps were placed inside a red plastic biohazard material bag and transported to a staging and processing area.

Handling and processing the rodents were done outdoors in the sunlight which affords additional protection from active virus infection. Inactivation by UV light exposure has been demonstrated in related Hantaviruses (Karabatsos 1985). The rodents were usually processed by a two-person team who were clad in polylaminated Tyvek® full body coveralls. Eyes and lungs of each field team member were protected during the rodent processing procedures by a FTHC face shield outfitted with a powered air purifying respirator (PAPR; Neoterik Type CF60), and a high efficiency purifying air (HEPA) filter (NP1050). Hands were protected with double pairs of Best N-Dex® soft-nitrile disposable gloves.

Rodents retrieved from the field were placed individually, while still in the box trap, into an ice chest containing dry ice. Within a few minutes, the rodent was narcotized and immobilized. It was then emptied from the trap onto a dissection tray. Identification to species was done at this time and this information along with locality and other pertinent data were entered into a field catalogue with each entry designated with a unique field identification number. Concurrently, the rodent specimen was bled infrasternally by cardiac puncture with a 3/8 inch, 25 or 26 gauge needle and a 1 cc tuberculin syringe. As much blood as possible was removed from each specimen. The blood was ejected from the syringe into a 25 ml plastic tube which was then capped. The vial was marked with an appropriate field number and placed in a test tube rack until all the rodents had been bled.

Spent needles and syringes were disposed of in a Sharps container after being rinsed in a 5-10% bleach solution. The blood-sampled rodent was then placed in a plastic sandwich bag which was itself placed in a zip-lock freezer bag. Both bags had been marked with the appropriate host/field identification number. The bagged rodent was immediately put into a dry ice container for temporary storage. All of the rodent specimen bags processed that day were placed in a single marked plastic bag and then stored in a Revco freezer (-70 °C).

BLOOD PROCESSING

The rodent whole blood samples were stored in the refrigerator (4 °C) until they were centrifuged and the sera transferred to 0.5 ml disposable microcentrifuge tubes. These tubes were packed in dry ice and shipped to the University of New Mexico School of Medicine (UNMSM) where serologic screening analyses of the sera was conducted.

RODENT CARCASS PROCESSING

Selected carcasses of seropositive mice were sent on dry ice to Dr. Brian Hjelle at UNMSM where RT-PCR and sequencing analyses were performed as described elsewhere (Hjelle et al. 1994b, 1994c). A specimen tag containing the field identification number, collection locality, date collected, and species identification was tied to the right hind leg of each carcass.

RESULTS

A total of 1,120 rodents representing eight genera and 14 species was tested (Table 1) and included specimens dated from December 1993 through December 1995. Sixty-six (5.9%) of the specimens were seropositive for *Hantavirus* (SNV or ELMCV). Of the 66 positive rodents, 59% were *Peromyscus maniculatus*, 36% were *Reithrodontomys megalotis*, and 5% were *Peromyscus californicus*. All other rodent species tested negative. Seropositive rodents (SNV and ELMCV) were collected every month of the year. There appears to be a correlation between age and sex of the reservoir host and seroprevalence of virus, especially among males. Of the 39 seropositive *P. maniculatus*, 77% were males and all 39 were adults. Of the 24 seropositive *R. megalotis*, 96% were males and all were adults. In contrast, the overall sex ratio for the total number collected was approximately 60/40 (283 males/197 females and 128/74 for deer mice and harvest mice, respectively).

Table 1. Rodents tested for Hantavirus antibodies from Orange County, CA, December 1993 through December 1995.

Rodent Species	No. of rodents	Hantavirus positive	Percent Positive
Chaetodipus californicus	9	0	0
Microtus californicus	11	0	0
Mus musculus	93	0	0
Neotoma fuscipes	28	0	0
Neotoma lepida	45	0	0
Peromyscus boylii	11	0	0
Peromyscus californicus	102	3	2.9
Peromyscus eremicus	99	0	0
Peromyscus maniculatus	480	39	8.1
Rattus norvegicus	1	0	0
Rattus rattus	19	0	0
Reithrodontomys megalotis	202	24	11.9
Spermophilus beecheyi	19	0	0
Thomomys bottae	1	0	0
TOTALS	**1120**	**66**	**5.9**

Figure 1 shows the proximity of all positive sites to each other within the county. Note that all three seropositive *P. californicus* were collected in areas where positive *P. maniculatus* occur and, in the case of Newport Coast, both *P. californicus* were taken along the same 300 m trapline as positive *P. maniculatus.* *Reithrodontomys megalotis* seropositive for ELMCV were also collected concurrently with SNV positive *P. maniculatus* from San Clemente, San Juan Capistrano, and Rancho Mission Viejo (Figure 1). Many of the SNV seropositive rodents were identified from localities along coastal bluffs and the foothills of the Santa Ana Mountains where several residential, commercial, and industrial sites (old and new) exist or where construction is currently underway. These include: a residential backyard, a retirement community, office trailers at an environmental engineering firm, a sand and gravel company, a farm school, a preschool, fields and canyons adjacent to a government research facility, a microwave relay building, flood control channels in urban areas, and open fields near shopping malls. Other less populated sites include: one state park, five county regional parks, private ranch land, a county landfill, and proposed housing and condominium developments. These records represent both SNV and ELMCV positive rodents and even though no human cases of either virus have been reported from Orange County, the potential health risk should not be overlooked.

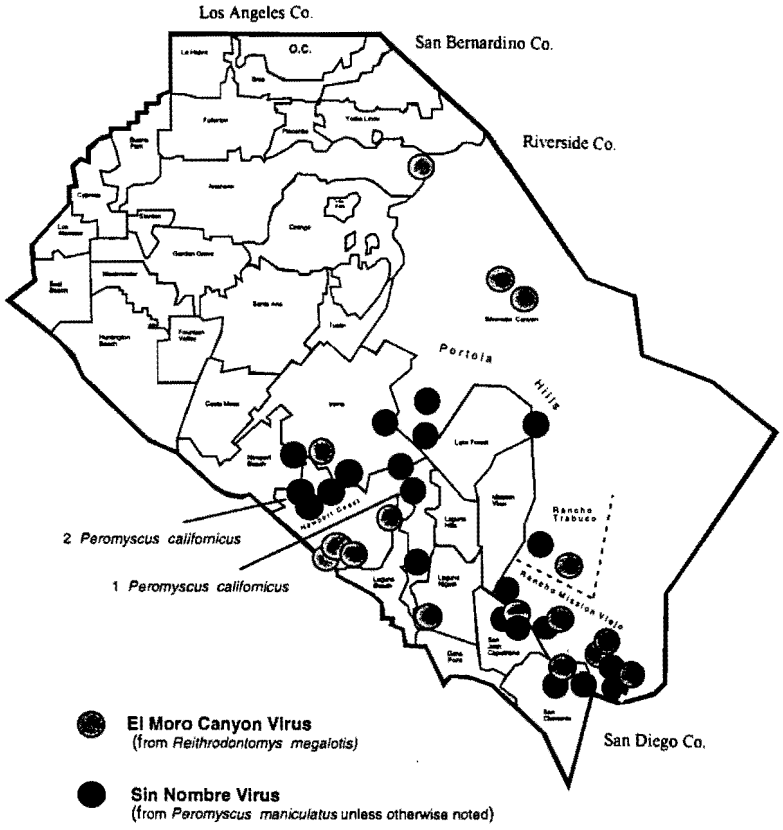

Figure 1. Distribution of Hantaviruses in Orange County, CA, as of December 31, 1995.

Deer Mice (*Peromyscus maniculatus*)

The total number of sera collected through December, 1995 was 480. Thirty-nine of the 480 (8.1%) deer mice were seropositive for SNV. A summary of all cities and unincorporated areas of Orange County with positive *P. maniculatus* and seroprevalence is given in Table 2. Seroprevalence ranged from three to 33 percent in these areas and at specific sampling sites within them the range was slightly higher (7 to 40%). At 17 of 20 positive sampling sites, small sample sizes of 3-12 rodents

were sufficient to obtain one positive mouse. SNV seropositive mice were taken every month of the year except in January and October. However, only two deer mice of a total of ten rodents were collected from a single site in January, 1995. Because of severe weather, no rodents were trapped in January, 1994.

Table 2. Deer mice (*Peromyscus maniculatus*) seropositive for Sin Nombre Virus in Orange County, CA, December 1993 to December 1995.

Location	No. of trap sites	No. of Positive sites	Total No. Deer mice	Total No. Positive	Percent Positive
Irvine	9	4	97	8	8.2
Laguna Beach	4	1	31	1	3.2
Mission Viejo	2	2	8	2	25.0
Newport Coast	6	4	80	12	15.0
Portola Hills	7	3	34	4	11.8
Rancho Mission Viejo	5	1	31	3	9.7
Rancho Trabuco	2	1	3	1	33.3
San Clemente	5	1	39	1	2.6
San Juan Capistrano	4	2	23	7	30.4

Orange County rodent trapping records indicated that *Peromyscus maniculatus* was collected more frequently in disturbed grassland and sage scrub habitats, or grassland / sage scrub / chaparral ecotones and was much less common in chaparral, woodland and riparian habitats (Table 3). Selection of trapping localities in 1994 and 1995 was based on this information and therefore, most were represented by elements of grassland and sage scrub plant communities (grassland-ruderal, floodplain sage scrub, buckwheat sage scrub, etc.; Mathews 1992). This bias may give the impression that SNV is only found in rodents inhabiting particular plant communities when, in fact, other plant communities were deliberately avoided in order to assure a greater sample size of the reservoir host *(P. maniculatus)*. It is possible that the apparent scarcity of *P. maniculatus* in chaparral and woodland habitats in Orange County may influence the incidence and distribution of SNV among populations of *P. maniculatus* (as well as other species of *Peromyscus*) in these habitats, but no detailed population density study of rodents from different plant communities in conjunction with seroprevalence study has been done to provide more convincing evidence.

Western Harvest Mice *(Reithrodontomys megalotis)*

In March of 1994, a number of sera from *R. megalotis* was sent with others for Hantavirus screening to the University of New Mexico, School of Medicine (UNMSM). Among these sera a single SNV seropositive mouse was found that had been trapped in Laguna Beach (El Moro Canyon), but lacked detectable SNV rNA. Reverse transcription PCR techniques were used to amplify cDNA from four specimens of *R. megalotis* from El Moro Canyon and led to the description of a phylogenetically distinct *Hantavirus* in harvest mice in October, 1994, eventually being named El Moro Canyon virus (Hjelle et al. 1994c). Subsequently in April, additional sera samples from *R. megalotis*, including stored frozen samples, were tested by UNMSM. Two retrospective ELMCV positives were found in these samples; one from near San Clemente (TRW test site) taken in August, 1992 and another from Laguna Beach (El Moro Canyon) taken in July, 1993. Twenty-four of 202 (11.9%) harvest mice collected from December 1993 to 1995 were seropositive for ELMCV. Seroprevalence ranged from two to 50 percent in these areas and at specific sampling sites within them the range was slightly higher (3 to 60%). At 14 of 16 positive sampling sites, small sample sizes of 1-10 rodents were sufficient to obtain one positive mouse. ELMCV seropositive mice were not taken in February, April, May, June, or October, but sample sizes were small for all of those months. A summary of all localities and seroprevalence in *R. megalotis* is given in Table 4.

Table 3. Selected rodent trapping records and habitat types in Orange County, CA, from 1984 through 1995.

Rodent species	Grassland-Sage Scrub-Ruderal Habitats	Percent of Total	Chaparral-Woodland-Riparian Habitats	Percent of Total
Chaetodipus californicus	27	3.2	12	3.8
Dipodomys agilis	3	0.4	-	-
Microtus californicus	10	1.2	1	0.3
Neotoma fuscipes	13	1.6	46	14.7
Neotoma lepida	51	6.1	55	17.6
Peromyscus boylii	3	0.4	16	5.1
Peromyscus californicus	43	5.2	63	20.1
Peromyscus eremicus	74	8.9	26	8.3
Peromyscus maniculatus	**398**	**47.8**	**38**	**12.1**
Reithrodontomys megalotis	**175**	**21.0**	**16**	**5.1**
Spermophilus beecheyi	35	4.2	40	12.8
Total No. Records	**832**		**313**	

Table 4. Harvest mice (*Reithrodontomys megalotis*) seropositive for El Moro Canyon Virus Orange County, CA, December 1993 to December 1995.

Location	No. of trap sites	No. of Positive sites	Total No. Harvest Mice	Total No. Positive	Percent Positive
Anaheim	4	1	15	1	6.7
Irvine	5	1	17	1	5.9
Laguna Beach	3	2	8	4	50.0
Laguna Niguel	3	1	6	1	16.7
Rancho Mission Viejo	5	2	33	11	33.3
Rancho Trabuco	1	1	4	1	25.0
San Clemente	5	1	51	1	2.0
San Juan Capistrano	5	1	23	1	4.3
Santa Ana Mountains (near Silverado Canyon)	4	2	8	3	37.5

California Mice (*Peromyscus californicus*)

The total number of sera collected was 102. Three of the 102 (2.9%) *P. californicus* were seropositive for SNV. Two of 31 (6.5%) were seropositive from one Newport Coast trap site along the same trapline and a single mouse from Irvine (caught on the other side of a brick wall surrounding a retirement community) was positive. Although it was the only rodent trapped from that site, positive *P. maniculatus* were taken 1.5 miles north and 3.0 miles west of there. The two Newport Coast specimens were taken from an area where 15 percent of the *P. maniculatus* are seropositive for SNV (Table 2).

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1996 Proc. Mosq. and Vector Control Assoc. of CA 64:80-84

PLAGUE ACTIVITY IN SAN BERNARDINO COUNTY DURING 1995

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ABSTRACT

In 1995, the San Bernardino County Vector Control Program carried out 24 plague surveys at various sites in San Bernardino County. Of the 271 live trap-collected rodents, 98.9% were ground squirrels, including 258 *Spermophilus beecheyi* and 10 *Spermophilus lateralis*. The remaining rodents included three *Tamias merriami*. None of the 932 fleas collected from these animals tested positive for plague.

Of the total sera drawn from 271 rodents, 32 tested positive for plague antibodies from seven different locations: Hanna Flat campground and Camp Whittle in Fawnskin, Cedar Lake Camp in Big Bear, Forest Falls picnic site, Silverwood Lake campground and Miller Canyon Group Camp in the Silverwood area and Jurupa Hills Regional Park in Fontana. Upon receipt of laboratory results on plague positive samples, all public use sites showing plague activity were closed to allow for appropriate ectoparasite and rodent control. Public educational information on plague was disseminated through the local press.

Plague is an enzootic rodent disease communicable to humans. The disease, caused by a bacterium, *Yersinia pestis*, is transmitted to man and other animals through the bite of fleas. Humans are exposed to the disease if they enter plague infected areas or if the disease is transmitted from feral rodents to commensal rats or cats that cohabit human environments. The occurrence of rural plague cases has been attributed to the extension of human habitations into wilderness areas.

Historically, plague is reported to have originated in Central Asia and spread to almost all continents of the world. This spread is evidenced from the first pandemic of AD 542, which involved Arabia, Europe and North Africa. The second pandemic, the "Black Death" of the Middle Ages (1300's) covered parts of both Asia and Europe. The third and last pandemic originated in Asia (Southwest China) and spread to South Africa and South America by 1899, then to North America (San Francisco) and Australia (Brisbane and Sidney) by 1900 (Twiggy 1978, Kettle 1984).

Following the introduction of plague in 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 its ectoparasitic fleas. Plague infection in wild rodents is widely distributed in California including the coastal counties south of San Francisco Bay; inter-mountain valleys of northern California, the Sierra Nevada from Lassen Peak to the Kern Plateau, and the Tehachapi, San Gabriel, San Bernardino and San Jacinto Mountains of southern California (Salmon and Gorenzel 1981, Anonymous 1983).

Known foci of plague epizootics are distributed throughout the mountains and foothill areas of San Bernardino County. The mountain ranges along with natural recreational lakes provide a wide variety of camping, hiking and water sport facilities to both local and out-of-county visitors. To safeguard public health and safety in these areas, the San Bernardino County Vector Control Program (SBCVCP) in

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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 in plague enzootic areas during the season (April through November). The data generated in routine plague surveillance during 1995 are presented in this paper.

MATERIALS AND METHODS

In routine plague surveys, the general method described by Lang and Wills (1991) was used as follows: In a typical survey, 30-35 Tomahawk #103 live traps (Tomahawk Live Trap Co., Tomahawk, WI) baited with peanut butter and rolled oats were set at appropriate shaded locations close to rodent burrows in the survey area. In a campground situation, traps were also set near picnic tables that attract wild rodents, especially ground squirrels and chipmunks. For location purposes, each trap site was flagged with orange nylon ribbon hung from an adjacent bush or tree. The traps were set by 10:00 a.m. and picked up in the early afternoon the same day.

All traps with live animals were brought to a shady site for processing. Traps with animals were transferred into 45 x 90 cm clear polyethylene bags (3 mil). For each trap, a ball of cotton drenched in ethyl ether was introduced into the bag which was then kept tightly closed with a rubber band until the animal was anesthetized (usually in 5-15 min.). The unconscious animal was taken out of the bag and cage and transferred to a white enamel pan (30 x 20 x 5 cm deep), where the fleas were combed out using a stiff bristled brush. The fleas from each animal were collected in labeled 2 ml polypropylene screw cap tubes containing a 2% saline solution. Next, through cardiac puncture, a 3 ml blood sample was drawn from each animal using a 23 gauge syringe. Before the animal regained consciousness and was released back into its habitat, all pertinent data such as species, sex, and reproductive stage were recorded. All necessary survey site information was recorded before leaving the area.

All collected blood samples were brought to the laboratory, centrifuged for 20 minutes at 2000 rpm, and the serum from each sample was transferred to labeled 2 ml polypropylene screw cap tubes. All sera and flea samples, along with completed paperwork, were sent on blue ice by overnight mail to the California Department of Health Services, Vector-Borne Disease Section (CDHS-VBDS) in Sacramento for laboratory analysis.

The CDHS-VBDS laboratory immediately informed us via telephone of any plague-positive samples. In the event of plague-positive sample confirmation, the standard plague epizootic protocol would be followed. The protocol includes posting the area with "Plague Warning" signs, public education, press releases (if warranted), immediate evacuation (if a campground), pre-treatment flea index evaluation depending on the date of the original survey, burrow dusting with an insecticide (Diazinon® 2D) for fleas followed by rodent control using a rodenticide (Diphacinone bait blocks or aluminum phosphide tablets), if necessary, and finally post-treatment evaluation flea index prior to re-opening the area for public use, especially a park or campground. Rodenticide treatment was done by placing two aluminum phosphide (Fumitoxin®) tablets into each active burrow which was then closed with a ball of crumpled newspaper and sealed with dirt. For follow-up if any, each burrow site was flagged with a bright colored nylon ribbon.

RESULTS AND DISCUSSION

During the 1995 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 271 rodents collected in live traps, 168 (98.9%) were ground squirrels, namely, 258 (102M, 156F) *Spermophilus beecheyi*, and 10 (4M, 6F) *Spermophilus lateralis*. The remaining rodents included three female chipmunks, *Tamias merriami*.

All 932 fleas combed out from the animals tested negative for the plague bacterium. Of the total fleas collected, 90.0% were *Oropsylla (Diamanus) montanus* (412M and 427F) and 9.6% *Hoplopsyllus anomalus* (33M and 56F). The remaining 0.4% were unidentified.

Based on rodent serology results, there were 32 seropositive samples, showing plague epizootics at seven different sites (Table 1). The number of plague positive rodents and localities were almost 2x and 3x higher, respectively, in 1995 than 1994. During the 1995 surveys, the seven sites with seropositive rodents were Hanna Flat campground, Camp Whittle, Cedar Lake Camp, Forest Falls picnic area, Silverwood Lake campground, Miller Canyon Group Camp and Jurupa Hills Regional Park. Most of the positive rodents were ground

Table 1. Summary of plague surveys carried out in San Bernardino County during 1995.

Survey Date	Location	Altitude (ft)	Sera Samples Tested (♂,♀)	Positive	Titer	Ectoparasites Tested (♂,♀)	Flea Index
5-9	Hanna Flat I C.G.[a]	7000	12 (5,7)	4	(1:32-1024)	20 (8,12)	1.6
5-22	Hanna Flat II C.G.	7000	10 (1,9)	5	(1:32-512)	8 (0,8)	0.8
6-1	Camp Whittle	7000	9 (4,5)	6	(1:32-256)	32 (13,19)	3.5
6-15	Heart Bar C.G.	6900	10 (2,8)	-[c]		23 (7,16)	2.3
6-27	Dogwood C.G.	5800	11 (4,7)	0		32 (12,20)	2.9
7-7	Camp Whittle	7000	21 (6,15)	-		7 (4,3)	0.3
7-11	6200-6300 Blk Pine Kendall Dr.	1100	4 (2,2)	0		30 (13,17)	7.5
7-25	Applewhite C.G.	3300	16 (5,11)	0		15 (7,8)	0.9
7-31	Yucaipa Regional Park	2700	4 (1,3)	0		0	0
8-7	San Gorgonio	7000	14 (5,9)	0		67 (41,26)	4.7
8-15	Forest Falls picnic site	6000	5 (1,4)	2	(1:32-64)	17 (10,7)	3.4
8-21	Cedar Lake Camp	6700	19 (5,14)	6	(1:32-256)	87 (50,37)	4.5
9-5	Northshore C.G.	5300	16 (6,10)	0		53 (20,33)	3.3
9-11	Cedar Lake Camp	6700	4 (3,1)	-		10 (4,6)	2.5
9-12	Forest Falls picnic site	6000	8 (4,4)	-		1 (1,0)	0.1
9-25	Lytle Creek	3500	4 (0,4)	0		43 (14,29)	10.7
10-16	Rio-Barranca G.C.[b]	4200	23 (10,13)	0		108 (49,59)	4.7
10-17	Barton Flats C.G.	7000	10 (6,4)	0		72 (29,43)	7.2
10-19	Jurupa Hills Regional Park	1200	17 (10,7)	2	(1:64-128)	105 (46,59)	6.1
10-23	Silverwood Lake C.G.	3800	27 (15,12)	6	(1:32-512)	143 (105,38)	5.2
10-24	Miller Canyon G.C.	4200	6 (1,5)	1	(1:128)	48 (23,25)	8.0
11-9	Oak Glen County Park	3500	1 (0,1)	-		0	0
11-21	Jurupa Hills Regional Park	1200	20 (10,10)	-		6 (3,3)	0.3
11-30	Silverwood Lake C.G.	3800				5 (5,0)	0.1[d]
	Totals		271 (106,165)	32		932 (464,468)	

[a] Campground; [b] Group Camp; [c] Not tested; [d] Based on 28 burrows flagged post-treatment.

squirrels with high flea indices. Detailed information on these sites is shown in Table 2. Hanna Flat campground, Camp Whittle and Cedar Lake Camp had high rodent populations, resulting in ectoparasite and rodent control (with diphacinone bait blocks) operations according to the method used in earlier surveys (Mian et al. 1994, 1995). The Forest Falls picnic site and the Jurupa Hills Regional Park in Fontana was burrow dusted with Diazinon 2D. The Silverwood Lake campground and the Miller Canyon Group Camp were treated for both flea and rodent control by the California Department of Parks and Recreation (CDPR) certified staff with guidance from both CDHS-VBDS and SBCVCP. Prior to re-opening loops 4, 5 and 6 for public use, 419 applications of aluminum phosphide were made. A joint post-treatment evaluation by CDHS-VBDS, SBCVCP and CDPR included a random sampling of 28 treated rodent burrows that were re-opened and flagged with a flannel cloth for live fleas. Other sites such as loops 1, 2 and 3 at Silverwood Lake campground and the Miller Canyon Group Camp, already closed for the season, were also treated with aluminum phosphide tablets during December 1995. Thus a total of 533 burrows were treated with 14.90 lb. of aluminum phosphide over the entire Silverwood Lake campground and the Miller Canyon Group Camp. Loops 1, 2 and 3 of Silverwood Lake campground and the Miller Canyon Group Camp will be evaluated prior to re-opening next year.

Table 2. Summary of plague epizootics in San Bernardino County during 1995.

	Campsite						
Activity	Hanna Flat Campground Fawnskin	Camp Whittle Fawnskin	Cedar Lake Christian Camp Big Bear	Forest Falls picnic area Forest Falls	Jurupa Hills Regional Park Fontana	Silverwood Lake Campground Silverwood Lake	Miller Canyon Group Camp Silverwood Lake
Initial Survey Date	5/9	6/1	8/21	8/15	10/19	10/23	10/24
Rodents trapped (per 35 live traps)	21	9	19	5	17	27	6
Flea Index	1.4	3.3	4.5	3.4	6.1	5.2	8
Serology results							
Date of positives	8/21	6/9	6/19	2/5	2/17	6/27	1/6
Titer (≥ 1:16)	(1:32-1024)	(1:32-256)	(1:32-256)	(1:32-64)	(1:64-128)	(1:32-512)	(1:128)
Site Closure Date	6/26[c]	7/1	9/5	9/5	11/14	11/14	Already closed for season
Flea Control Date[a]	5/18,6/26	7/1	9/6	9/7	9/15	11/20[e]	
Rodent Control Date[b]	5/18, 6/26	7/1	9/6	NA[d]	NA	11/20	12/12
Post-treatment Survey	5/22	7/7	9/11	9/12	11/21	11/30[f]	NA
Rodents trapped (per 35 live traps)	10	21	4	8	6		NA
Flea Index	0.8	0.3	2.5	0.2	0.3	0.1	NA
Site reopening date	5/22,6/29	7/7	9/11	9/12	11/22	12/8	1996 season

[a] Flea control included the use of diazinon 2D (dust) either in bait stations or directly in rodent burrows.
[b] Rodent control included the use of a poison bait containing an anticoagulant rodenticide, diphacinone.
[c] Due to positive serology of post-treatment survey, the camp was temporarily closed between June 26 and 29 to allow for flea and rodent control.
[d] Not available
[e] Following the closure, active burrows were treated (fumigated) with aluminum phosphide tablets (Fumitoxin®) at the rate of 2 tablets/burrow.
[f] Twenty eight treated burrows were reopened at random and flagged for fleas. Flea index calculated as fleas/burrow.

During the foregoing plague epizootics, public educational information was provided through local newspapers, radio and television. Plague warning signs were posted immediately and left in place for the remainder of the season in affected areas. There were no human plague cases in San Bernardino County; there was however, one female dog infected with plague at Cedar Lake Camp, prompting us to conduct plague surveys for the first time at this location. The dog recovered from the disease through antibiotic treatments.

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"LISTENING" TO MOSQUITO ANTENNAE: ELECTROANNTENNOGRAMS AID IN IDENTIFICATION OF OVIPOSITION ATTRACTANTS

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Effective monitoring of populations of *Culex* mosquitoes vectoring human diseases, and particularly, the presence of infective individuals within those populations, is a continuing problem for mosquito control and public health agencies. Attractants which selectively attract blood-fed, gravid female mosquitoes, the segment of the population most likely to be infected, would find immediate use in monitoring programs. The volatile chemicals which gravid mosquitoes use to locate suitable oviposition sites have the potential to fill this selective attractant role.

It has been known for some time that gravid mosquitoes are attracted to and stimulated to oviposit by chemical cues associated with desirable oviposition sites (reviewed in Bentley and Day 1989). The chemicals are generally produced by the microbial degradation of organic debris in the oviposition waters, such as may occur in dairy lagoons or seasonally flooded ponds. While the literature on various sources of oviposition attractants is extensive, relatively few of the attractive compounds have been identified. Usually, crude sources of oviposition attractants such as pond water contain complex mixtures of hundreds of compounds, making bioassay-driven fractionation to isolate active compounds long and difficult, especially when trying to identify trace components. Furthermore, behaviors such as attraction to an oviposition site are probably mediated by blends of chemicals rather than a single chemical. Consequently, identifying each active component and reconstructing the active blend by iterative trial and error may be required.

One possible way to shorten the identification process is to use a mosquito antenna as a detector to screen potential attractants or repellents. Most of a mosquito's receptors for volatile compounds are located on the antennae, and the receptors are tuned to detect compounds of importance to the mosquito. Thus, if a mosquito antenna is treated with a puff of a volatile stimulus, and the resulting electrical signal produced by the antenna is amplified and measured, one can quantify how sensitive the antenna is to the stimulus. On the reasonable assumption that the antenna will be most sensitive to compounds of importance to the mosquito, stimuli from a crude attractant source which give strong antennal responses are good candidates for attractants. In similar fashion, strong repellents should also give strong antennal signals. This use of an insect antenna for testing volatile compounds is known as electroantennogram detection (EAD). It has been used for a number of years to study responses of mosquito antennae to known stimulants (Davis 1976, 1984, Mordue et al. 1992, Blackwell et al. 1993).

EAD alone is of limited use for identification of compounds when faced with a crude extract containing many compounds. However, the power of the EAD method applied to the identification of new compounds is increased enormously by coupling EAD with gas chromatography (GC), which separates an attractant extract into its individual components. As the individual components are eluted from the gas chromatograph, the effluent is split, with half going to the GC detector, and the other half being passed over the wired-up mosquito antenna (Figure 1). By comparing the pattern of antennal responses to the pattern of compounds detected by the GC, it is immediately obvious which compounds in the extract the antenna is most sensitive to, i.e., which chemicals are strong candidates for attractants. Thus, in one pass, the compounds with the best potential as attractants are

located from amongst the many chemicals in the crude extract. It is then relatively straightforward to isolate and identify these compounds by standard analytical chemistry methods such as mass spectrometry.

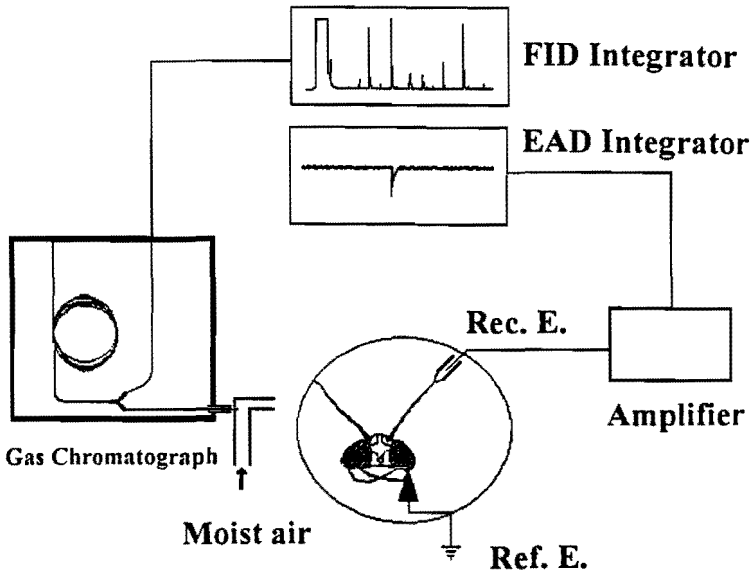

Figure 1. Schematic of a coupled gas chromatograph-electroantennogram detector.

Our objectives in the work described here were:

1. To use GC-EAD to locate and identify potential oviposition attractants for *Culex* mosquitoes from extracts of oviposition waters.
2. To determine the changes in the odor profile of oviposition water over a period of several weeks.
3. To compare the odor profiles of oviposition waters prepared from different materials, such as alfalfa and grass.

METHODS

Insects. Colonies of *Cx. tarsalis* and *Cx. quinquefasciatus* were established at UCR, and augmented periodically with individuals reared from field-collected egg masses.

Infusions and Extracts. Bermuda grass, alfalfa, and other infusions were prepared as previously described (Isoe and Millar 1995, Reiter 1983, Reisen and Meyer 1990, Kramer and Muller 1979). Biologically active extracts were prepared by sweeping the airspace above a stirred infusion with purified nitrogen, trapping the volatiles from the airspace on traps prepared from the adsorbent Porapak Q. Aerations were carried out at room temperature for 24 hours, at a nitrogen flow rate of 1 liter/minute. The trapped volatiles were then eluted with a small amount of solvent (e.g., ether). Extracts prepared in this fashion elicited activity from mosquito antennae in GC-EAD tests, were attractive to gravid mosquitoes, and/or stimulated oviposition by gravid females.

Bioassays. Porapak Q extracts were assayed by coupled gas chromatography-electroantennogram detection. Briefly, saline-filled glass microelectrodes were attached to the cut tip and at the base of an antenna of a gravid female mosquito. An extract was injected onto the GC, and the GC effluent was split, with part going to the GC detector, and the rest being directed over the antennal preparation. The signals from the GC detector and the insect were simultaneously recorded, so that antennal spikes could be easily matched with the corresponding GC peaks. Each analysis was replicated at least six times with different antennal preparations. Compounds corresponding to GC-EAD active peaks were identified by GC and GC-mass spectrometric analysis.

Sticky screen bioassays developed in our laboratory (Isoe et al. 1995) were used to evaluate the attractancy of the compounds located and identified by GC-EAD and GC-MS analyses. Briefly, glass oviposition cups were half-filled with a treatment solution or a distilled water control, with the mouths of the cups covered with hardware cloth screens coated with Tanglefoot. Pairs of cups were

placed in screen cages, and blood-fed, gravid female mosquitoes were added (minimum eight replicates of 20 mosquitoes per replicate). The next morning, the numbers of mosquitoes trapped on treatment and control screens were compared.

RESULTS AND DISCUSSION

Coupled GC-EAD analyses. A typical coupled GC-EAD trace to an extract of Bermuda grass infusion is shown in Figure 2. The antenna responded to a number of compounds in the extract. The signal from the antenna was "noisy", but by running a number of replicates, the reproducible antennal responses were quickly separated from random electrical noise spikes from the detector system. Compounds which consistently elicited an antennal response were subsequently identified by mass spectrometry as dimethyltrisulfide, phenol, para-cresol (4-methylphenol), nonanal, 4-ethylphenol, napthalene, 2-undecanone + indole (one overlapped peak), 3-methylindole (skatole), and 2-tridecanone.

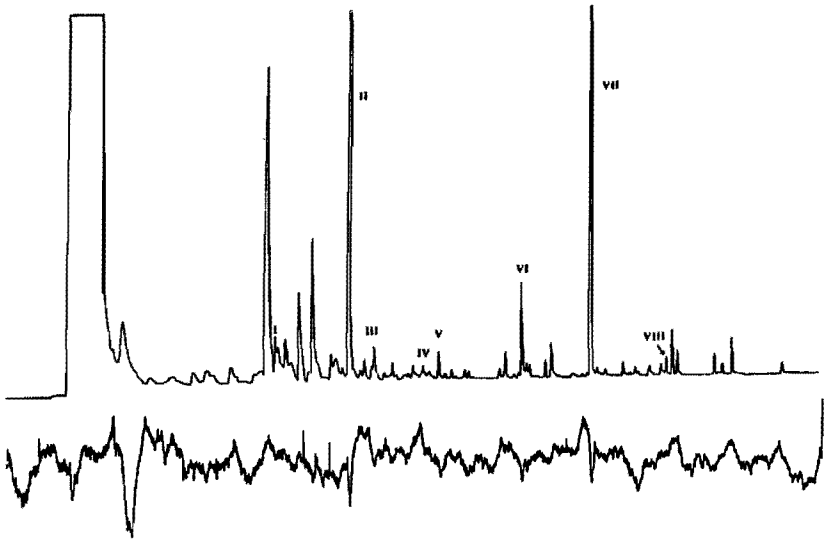

Figure 2. Typical GC-EAD traces obtained with an antenna from a gravid female *Cx. quinquefasciatus* mosquito challenged with a Bermuda grass extract. The top trace shows the profile of compounds detected by the gas chromatograph detector. The bottom trace shows the antennal responses.

A second study compared the antennal response of gravid females of two species, *Cx. tarsalis* and *Cx. quinquefasciatus* (Figure 3). The response profiles were not identical. In particular, *Cx. quinquefasciatus* appeared to be more sensitive to indole and 3-methylindole. These results may reflect the known oviposition preferences of these two species; *Cx. quinquefasciatus* is known to oviposit in highly polluted waters such as dairy lagoons, which contain large amounts of indole and 3-methylindole characteristic of animal wastes, while *Cx. tarsalis* prefers less polluted waters.

The compounds were then tested in laboratory bioassays as oviposition attractants, using sticky screen bioassays. *Cx. quinquefasciatus* females were neither attracted nor repelled by napthalene, phenol, 4-ethylphenol, and 2-undecanone at concentrations ranging from 0.01 to 10 micrograms/liter. At low concentrations (0.01 µg/l) 3-methylindole was attractive, while at higher concentrations it became repellent. Both para-cresol and nonanal were attractive at concentrations of 0.01 and 0.1 µg/liter respectively.

Change in the Odor Profile of Bermuda Grass Infusion with Time.

It is commonly known that *Culex* mosquito oviposition follows a distinct pattern in relation to the period of time that a particular oviposition site has been flooded. It has been documented that oviposition rates increase with time after flooding as the dead plant materials and other organic matter in the pond decay (Beehler and Mulla 1993). Oviposition rates peak several weeks after flooding, and then decline. While it has been shown that the buildup of predators may deter oviposition (Chesson 1984, Tietze and Mulla 1991), the profile of volatile

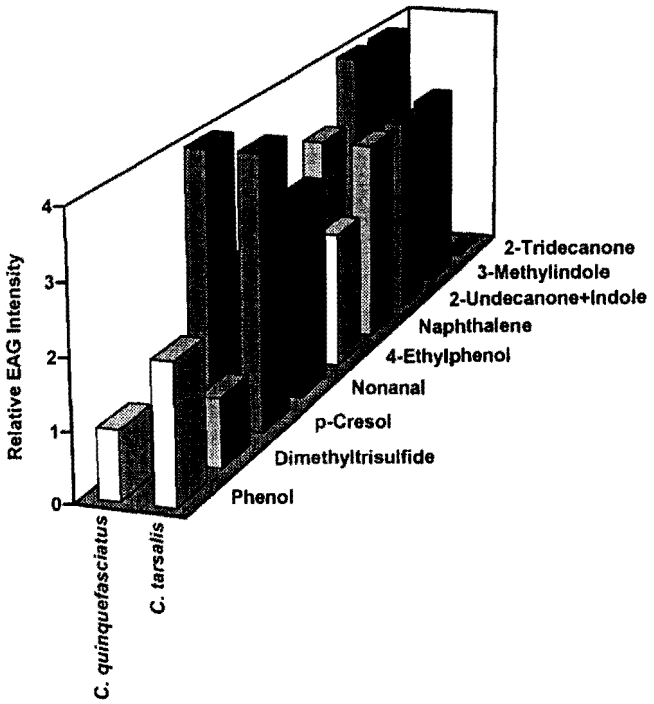

Figure 3. Relative responses of antennae from *Cx. tarsalis* and *Cx. quinquefasciatus* to EAD-active compounds in Bermuda grass extracts.

compounds, some of which undoubtedly function as oviposition attractants, had not been examined previously.

Our investigations showed that the volatiles profile from a fermenting Bermuda grass infusion sequentially sampled over a period of several weeks was dynamic. Some compounds, such as indole, were initially high and decreased steadily with time. Other compounds, such as dimethyltrisulfide, followed the reverse trend, being initially low, and building steadily. A number of compounds, including phenol, para-cresol, and 3-methylindole increased and peaked at about two weeks; then dropped rapidly. By the end of four weeks of fermentation, concentrations of most volatiles had fallen to low levels. These results suggest that the dynamics of the volatiles being emitted by oviposition waters is linked with oviposition. Volatiles concentrations are initially low, when oviposition is minimal, but increase over time as a result of microbial degradation of the organic matter in a pond. Thus, significant amounts of volatiles may signal the presence of abundant nutrients for mosquito larval development, such as microbial fauna and partially degraded particulate organic matter from ongoing decay processes. Several weeks later, when most of the organic matter available for decomposition has been exhausted, production of volatiles slows as microbial populations senesce, and the site decreases in attractiveness.

Comparison of Volatiles Profiles of Different Infusions.

It has been demonstrated that mosquito exhibit distinct preferences for infusions made from different fermented substrates (Kramer and Mulla 1979, Reisen and Meyer 1990). For example, we have found alfalfa infusion to be repellent to gravid *Cx. tarsalis*, and attractive to *Cx. quinquefasciatus* (Millar and Du, unpublished data), while both species are attracted to Bermuda grass infusions (Isoe et al. 1995). Consequently, an experiment was conducted to compare the volatiles profiles of alfalfa and Bermuda grass infusions (Figure 4). Four of the five most abundant compounds in the Bermuda grass extract (dimethyltrisulfide, para-cresol, indole, 3-methylindole) were also present in significant amounts in the alfalfa extract. However, the alfalfa extract contained significant quantities of a number of compounds which were not present in the Bermuda grass, such as octene, linalool, and methyl salicylate. Because Bermuda grass extract is attractive to both *Cx. tarsalis* and *Cx.*

quinquefasciatus, while alfalfa extract is repellent to *Cx. tarsalis*, one or more of the additional compounds in the alfalfa extract appear to selectively repel *Cx. tarsalis*.

In summary, we have given examples of how GC-EAD and GC-MS analysis of oviposition water volatiles can be used to examine questions relating to mosquito oviposition attractants and repellents. Our studies have indicated that the attraction of gravid female mosquitoes to oviposition sites is mediated by volatile attractants, and a number of potential attractants have been identified. Furthermore, we have shown that the volatiles profiles are dynamic, changing both qualitatively and quantitatively over time. Finally, we have begun to investigate the mechanisms which cause some infusions to be attractive and others to be repellent to various mosquito species. During the coming year, we aim to reinforce our analytical results with both laboratory and field bioassays.

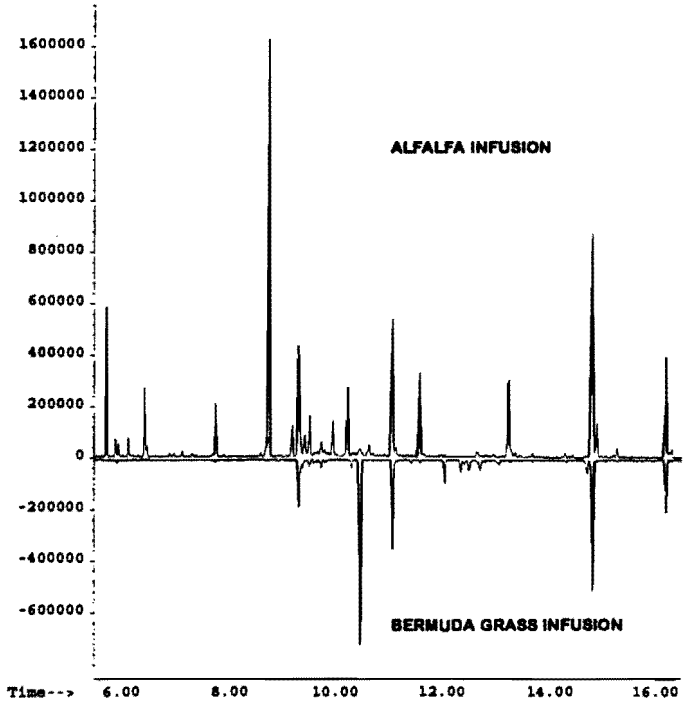

Figure 4. Volatiles profiles of extracts of Bermuda grass and alfalfa infusions aged for one week, as determined by coupled gas chromatography-mass spectrometry.

ACKNOWLEDGEMENTS

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1996 Proc. Mosq. and Vector Control Assoc. of CA 64:91-95

DISTRIBUTION AND SEASONAL ABUNDANCE OF TICKS IN SACRAMENTO COUNTY

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ABSTRACT

The distribution and abundance of ticks in Sacramento County was monitored at selected sites from November to May, 1990 to 1995. The distribution of two common human biting species, *Dermacentor occidentalis* and *Ixodes pacificus* is strongly associated with yellow pine, oak woodlands and riparian habitat with high abundance of these two species being geographically clumped. *Dermacentor variabilis* is widely distributed throughout the county, frequently associated with grasslands, riparian habitat, pastures and rural residential locales. *Dermacentor occidentalis* and *Ixodes pacificus* are winter and spring ticks while *Dermacentor variabilis* is a spring and summer tick. Other species of ticks were rarely collected from vegetation in various locations along the American River riparian corridor.

Beginning in 1990, from November to May, the Sacramento Co./Yolo Co. Mosquito and Vector Control District (SYMVCD) conducted regular surveillance for three human biting tick species, *Dermacentor occidentalis* Marx, *Dermacentor variabilis* (Say), and *Ixodes pacificus* Colley and Kohls. *Ixodes pacificus* has been of primary interest because of its strong association with the transmission of Lyme disease in the west, Burgdorfer et al. (1985). The other ticks can be important in the transmission of viral, bacterial and rickettsial diseases and can be a serious public nuisance in parks and recreation areas. Our objective has been to establish the geographic distribution and relative abundance of tick populations within the county, and to produce a site-oriented risk assessment for *Ixodes pacificus* exposure.

A generalized state-wide distribution for *Ixodes pacificus* has been established by the California Department of Health Services, Vector-Borne Disease Section, which fits nicely with distributions determined for *I. pacificus* by Bishop and Trembley (1945), Arthur and Snow (1968), and Furman and Loomis (1984). More specific distributions and seasonality of *I. pacificus* have been determined in localized areas by Westrom (1985), Lane and Lavoie (1988), Monsen et al. (1990), Lane (1990), and Kramer and Beesley (1993). Most of the distribution and seasonal studies of *I. pacificus* have been conducted in the moist coastal communities where this desiccation vulnerable tick is more abundant (Furman and Loomis, 1984). East of the moist coastal zone, a smaller population of *I. pacificus* exists on the west slope of the Sierra Nevada Mountains. The two populations are separated from each other by the dry central valley of California. In the Sierra Nevada, drier conditions restrict the distribution of *I. pacificus* to riparian habitats and moist elevations. Less favorable conditions can create different behavioral adaptations for *I. pacificus*, which in turn may alter the risk of Lyme disease exposure in the Sierra Nevada.

In Sacramento County, there exists three major vegetation habitats (Hickman 1993). The yellow pine dominated foothills in the extreme northeastern portion of the county is characterized by a tall, moderately dense gray or ponderosa pine upper canopy, a black and blue oak mid-canopy and an evergreen and deciduous manzanita, toyon and poison oak understory. Moving west, it grades into an oak woodland scrub which is characterized by medium tall, dense to open, broad leaf deciduous forests, dominated by blue oak with a scrub oak mid-canopy and a coyote brush, poison oak and chamise understory. Finally, the oak woodland grades into open grassland which is characterized by tall, widely

spaced valley oaks with a variety of grasses covering the ground. These areas may have an understory of poison oak, especially in riparian areas.

METHODS AND MATERIALS

The surveillance for *I. pacificus* coincided with the adult activity period for the species. Different sites within Sacramento County were sampled for ticks on a monthly basis from November to May. Collections were made using standard square meter flannel flags which were swept over road and trail side vegetation. At each collection site, a specific route was repeatedly used to allow flag-hours to remain reasonably consistent for a given site.

During the initial two years surveillance, a large number of sites throughout the county were sampled to determine the geographic distribution of the three tick species. Veterinary collections of ticks removed from dogs were also used as a surveillance tool. Information from the veterinarians included the dogs' residences and travel prior to the tick infestation. Combining the two surveillance procedures, a map of the geographic distribution of the three tick species was constructed. The Sacramento River and other low valley waterways and recreation sites were only sampled during this two-year time period to determine tick distribution. Sampling sites were also selected along the two major waterways which originate from the Sierra Nevada, the American and Cosumnes Rivers, where *I. pacificus* presence had already been established.

After the distribution of *I. pacificus* within Sacramento County was established, thirteen sites were selected to observe abundance and seasonality of the three tick species within the *I. pacificus* range. Twelve sites were chosen along the American River where I. pacificus has a greater distribution, and one on the Consumnes River. These sites consisted of state and city parks, recreational areas, and residential locations. Following an westerly transect along the American River, the 12 sampling sites were Folsom Dam, Negro Bar, Folsom City Park, Willow Creek State Park, Michigan Bar, Mississippi Bar, Orangevale Park, Nimbus Dam, Rossmoor Bar, Sacramento Bar, Ancil Hoffman Park, and Goethe Park. These 12 sites are located between Folsom Lake and Rancho Cordova. The Consumnes River site was at the Rancho Murieta Country Club.

The selected sites represented locations in each of the three floral habitats as described in the Jepson Manual for this geographic region. These sites were also influenced by riparian flora which dominated at higher elevations. Changing topography also contributed to the dominance of the various local flora.

Each site was usually sampled monthly and the collected ticks were brought back to the laboratory for identification, sampling counts and disease testing. Most *I. pacificus* specimens were sent to various laboratories for testing, while the *Dermacentor* species were preserved in alcohol. Recent investigation suggests that repeated sampling of a given site will eventually deplete the tick population thereby influencing estimates of abundance and seasonal activity (Kramer et al. 1993). Our numbers may therefore present an underestimate of what may have existed without sampling, however, enough ticks were likely overlooked during sampling or gained through recruitment that the general distribution and overall population trends remained similar from year to year. One site, Willow Creek State Park, has been more intensely sampled since 1990 and has shown a slow but recognizable reduction in numbers of *I. pacificus* over the years which may be a result of over sampling.

RESULTS

During the initial survey in Sacramento County it was found that the greatest tick concentrations occurred along the major waterways. The Sacramento River, being the western margin of the county, supports only populations of *Dermacentor variabilis*. Two major rivers transect Sacramento County. In the north, the American River flows from Folsom Lake in the northeast to its confluence with the Sacramento River just north of the city of Sacramento at Discovery Park. In the central part of the county, the Consumnes River originates from Michigan Bar to its confluence with the Mokelumne River near Thornton. *Dermacentor variabilis* was the only tick collected in the low land areas around the city of Sacramento along the American River and from the Mokelumne River to the Highway 99 overcrossing along the riparian corridor of the Consumnes River. It was also the only tick collected in all other riparian corridors and wetland habitats throughout the county. Moving east and upstream along the American River, *Dermacentor occidentalis* was collected from the Howe Avenue overcrossing throughout the riparian corridor to the eastern border of the county at Folsom Lake. It was also collected

along the Cosumnes River east from the Hwy. 99 overcrossing to Michigan Bar. It was not collected outside of the riparian corridors of these two river systems. *Ixodes pacificus* was collected only along the American River from Rancho Cordova to the eastern border at Folsom Lake and only from three sites on the Cosumnes River between Rancho Murieta to the county border at Michigan Bar.

Ixodes pacificus occurred in twelve of the thirteen sites routinely surveyed but varied in abundance, decreasing from east to west. *Dermacentor occidentalis* occurred in all thirteen sites increasing in abundance inversely to *I. pacificus*. *Dermacentor variabilis* was common throughout all the sample sites. Both *I. pacificus* and *D. occidentalis* shared the same seasonality, beginning their activity in late October or early November and ending in late May. *D. variabilis* was found to have a later activity period starting in early April and ending in late August.

The areas of abundance for the three tick species closely corresponded with three definable habitat types. The easternmost yellow pine habitat sites along the American River were dominated by *I. pacificus*. At sites such as Folsom Dam, Willow Creek to Mississippi Bar and Nimbus Dam, *I. pacificus* collections easily dominated those of *D. occidentalis*. Sites farther west, such as Rossmoor Bar, Sacramento Bar and Ancil Hoffman Park, are at the margin of yellow pine dominated habitat and oak dominated woodlands. In these sites *I. pacificus* numbers were consistently less than those of *D. occidentalis*, which was the dominant tick. The site farthest west, Goethe Park, is an oak woodland habitat where *D. occidentalis* occurs abundantly from November to April, and *I. pacificus* has not been collected. At sites investigated farther down the American River, *D. occidentalis* gradually diminishes in numbers until it completely disappears in collections from west of the Howe Avenue overcrossing to the confluence with the Sacramento River at Discovery Park.

The Cosumnes River site at Rancho Murieta represents a transition from yellow pine dominated habitat to oak woodland dominated habitat. At this site, *D. occidentalis* dominates as it does in this same habitat on the American River, while *I. pacificus* is rare, similar to the situation at the Ancil Hoffman site on the American River.

Seasonal abundance of the three tick species was relatively consistent during each year of the surveillance. *I. pacificus* adults were usually first observed questing in late October, generally after the first strong rains, but not contingent on rain, for in 1995 *I. pacificus* adults were first noted on October 25, three weeks prior to the first rain for that year. The final month at any site in which adult *I. pacificus* were collected was May, after which they would disappear from collections until October. In each year, *I. pacificus* had a fall and spring peak of abundance. The first abundance peak was the greatest and occurred in December. The two following months showed a decline and then a rise in March when a second lesser peak would occur. Through April and May, *I. pacificus* would decline in numbers at all sites (Figure 1).

The seasonal abundance of adult *D. occidentalis* was similar to that of *I. pacificus*, occurring from late October to late May with a bimodal abundance curve. *D. occidentalis* disappeared a few weeks earlier in the western sites, such as Ancil Hoffman Park, than in the eastern sites, such as Folsom Dam.

D. variabilis could be collected at all the sites in Sacramento County, but was most abundant in the valley grassland and agricultural areas. It was found to be most abundant in protected lowland areas such as the Stone Lakes Wildlife Reserve, the Putah sink and in riparian habitat along the Sacramento River.

DISCUSSION

Distribution of ticks in Sacramento County appears to be based on two closely linked environmental factors, elevation and ambient moisture. *I. pacificus* is most abundant in the yellow pine foothill zone. *D. occidentalis* predominates in the oak woodland foothill zone and *D. variabilis* is most abundant in the valley grassland and agricultural zones. At the highest elevations in Sacramento County, the yellow pine foothills receive slightly more annual rainfall and experience greater relative humidity than do lower elevational sites where oak woodland and grassland habitats predominate. *I. pacificus*, being the most dessication vulnerable tick of the three, would be expected to do best at the higher elevation locations which mimic the wet and cool temperate and coniferous biomes where other members of the complex predominate. According to two floral maps (Kuchler 1973, Hickman 1993), *I. pacificus* has its greatest abundance in Sacramento County in the small area of greatest elevation and covered by a margin of the

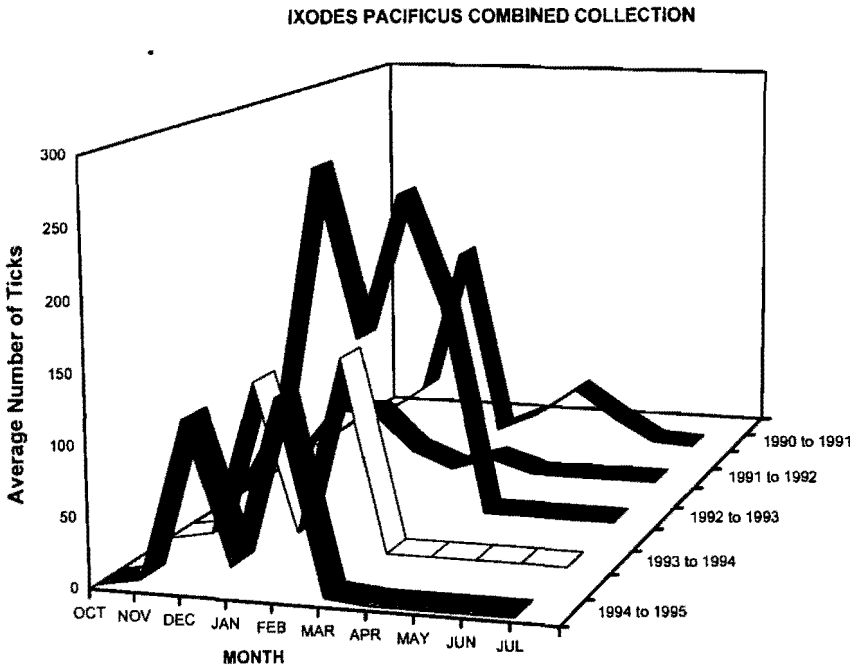

Figure 1. Seasonal distribution of *Ixodes pacificus* collected in Sacramento County, 1990-1995.

yellow pine zone in the extreme northeastern portion, along the American River. This area is also influenced by the riparian conditions associated with the river.

The oak woodland foothill zone where *D. occidentalis* dominates is slightly drier than the higher elevation sites, especially during the summer months. This may be enough to prevent *I. pacificus* from becoming firmly established. Individual *I. pacificus* can be collected in this zone, but only in very small numbers and not on a reliable basis. *D. occidentalis*, a more desiccation resistant tick, can do well in these foothill zones, but its numbers gradually decrease and eventually the tick disappears in the driest valley zones.

The valley grassland and agricultural zones of Sacramento County cover the greatest area of the county. *D. variabilis* occurs throughout the county but is most abundant in these lowland areas, especially in habitat along the Sacramento River. These low areas are too dry during much of the year to support *D. occidentalis* or *I. pacificus* life cycles as neither species has been collected in this habitat. After the seasonal decline of *I. pacificus* in the yellow pine zone and *D. occidentalis* in the oak woodland zone, *D. variabilis* can be collected in these habitats as well but at lesser numbers than the lowland sites.

On rare occasions, other tick species were collected during the routine surveillance. Three individuals of adult *Dermacentor albipictus* (Packard) were flagged along the American River, two near the Sunrise overcrossing and one at Folsom Dam. The collections of these ticks were always during the month of December. On one occasion, a single individual of *Ixodes spinipalpis* Nuttall was collected during a January flagging survey along Humbug Creek in Folsom.

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EFFICACY OF LARVIVOROUS FISH AGAINST *CULEX* SPP. IN EXPERIMENTAL WETLANDS

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ABSTRACT

The efficacy of sticklebacks (*Gasterosteus aculeatus*) as a biological control agent for mosquitoes inhabiting constructed wetlands was evaluated in small experimental marshes in San Jacinto, CA. Sticklebacks were stocked into one half of the wetlands at a final rate of 0.75 kg/ha^{-1} in late June and early July. *G. aculeatus* was ineffective as a biological control agent for mosquitoes. Environmental stresses associated with an especially large die off of *Scirpus californicus* and secondary treated effluent may have contributed to the demise of the sticklebacks. Mosquitofish, *Gambusia affinis*, were unexpectedly found in one of the marshes. The restricted distribution and the failure of the population to proliferate suggest that conditions may also have been stressful for mosquitofish. Constructed wetlands have an enormous potential for mosquito production and our options for biological control agents may be somewhat limited during particular periods of marsh succession.

Man-made wetlands are likely to have an increasing role in water reclamation strategies in arid regions of the United States such as southern California. In order to meet the ever increasing demands for water treatment by the burgeoning human populations in arid regions of the U.S., constructed wetlands will be incorporated as part of water resources management programs. The Multipurpose Wetlands Research and Demonstration Project in San Jacinto, CA is a five year multiagency (Eastern Municipal Water District, U.S. Department of the Interior, and U.S. Bureau of Reclamation) effort with the goal of evaluating and expanding the use of reclaimed water and contaminated groundwater through the incorporation of multipurpose constructed wetlands into Eastern Municipal Water District's total water resources management program (USBR, NBS and EMWD 1994). The focus of the project is the development of design, construction and operational criteria that will provide a cost-effective and innovative alternative for managing water resources and other public benefits such as wildlife conservation and public education.

Effective mosquito abatement strategies must be developed in conjunction with the aforementioned criteria, particularly if new wetlands are to be located near residential areas. Ideally, multipurpose constructed wetlands will remove nutrients, particularly nitrogen, from secondary treated water and provide habitat for endemic wildlife species. However, vegetation that strips wastewater of nutrients and provides cover for waterfowl and fishes also creates harborage for mosquitoes. Because multipurpose wetlands are intended to preserve local biodiversity, the placement of non-endemic species into such wetlands is discouraged. Alternatives to the non-endemic larvivorous fishes commonly used to control larval mosquito populations, such as the mosquitofish (*Gambusia affinis*), are therefore needed.

The three-spined stickleback, *Gasterosteus aculeatus*, is an ideal potential biological control agent for mosquitoes because this species is indigenous to California (Swift et al. 1993) and feeds readily on aquatic invertebrates (Coykendall 1980, Bay 1985). Hubbs (1919) suggested that the stickleback was well suited for mosquito control

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because of its mouth morphology, behavior, and hardiness. Vegetated habitats, like those found in multipurpose wetlands, are among those preferred by sticklebacks (Moyle 1976). Even though *G. aculeatus* has many positive attributes for use in biological control programs, definitive studies on the effectiveness of sticklebacks as mosquito control agents are lacking (Coykendall 1980, Bay 1985, Ahmed et al. 1988). Our objective was to evaluate the effectiveness of the stickleback as a mosquito control agent for multipurpose constructed wetlands.

MATERIALS AND METHODS

Our studies were carried out in eight experimental marshes (research cells: 14 m x 69 m) that were divided into two groups based on the spatial distribution of bulrush (predominantly *Scirpus californicus*). One-phase marshes contain vegetation throughout the entire marsh except for a narrow zone (approximately 3 m) at the inflow and outflow. Water depth is approximately 0.5 m. Three-phase marshes consist of inflow and outflow marshes that are separated by an area of open water which is 1.2 m deeper than the inlet and outlet marshes. Water depth in the inlet and outlet marshes is approximately 0.5 m. During the first year of operation, secondary treated wastewater was pumped into the ponds at an average rate between 20 and 35 l/min^{-1}. Water retention time was approximately two weeks. In order to examine the impact of flow rates and water retention time on nutrient concentrations in these model wetlands, flow rates were varied during the third year of operation and, consequently, water flow during our study was less constant (0-228 l/min^{-1}) than previous years.

Larval mosquito populations. Mosquito larvae were sampled weekly with a 450 ml dipper at 16 stations in the vegetated regions along the periphery of each marsh from July 7 until September 1. A final set of samples was taken on September 15. On each date, three 450 ml dips were taken along a 2 m transect at each station, combined and preserved. An initial survey of mosquito larval populations was conducted on June 26 by taking five 450 ml dip samples at 32 stations. Because water levels in the marshes fluctuated and thick stands of hydrophilic terrestrial vegetation (i.e., willows) developed along the perimeters of some marshes, it was not always possible to take three dips at all stations as time progressed. Statistical comparisons were based on the average number of larvae per dip for each research cell using a repeated measures ANOVA.

Fish. Sticklebacks were collected by dip net from the Mojave River near Victorville, CA. Fish were returned to the laboratory in aerated coolers containing Mojave River water. The standard length (SL in mm) and wet weight (g) were measured for a representative sample of the fish. The entire catch was weighed and separated into four buckets (20 l) containing dechlorinated, aged tap water and were maintained under continuous aeration. The fish were transported the following morning to the field site and, after a 30 minute acclimation period in 50% research cell water and 50% aged tap water, were stocked into four of the research cells (cell nos. 1, 4, 7 and 8) on June 29 and July 13 at a final rate of 0.75 kg/ha^{-1}.

Fish were sampled using minnow traps on July 7 and on alternate weeks beginning on July 14. Gee minnow traps (Cuba Specialty Mfg. Co., Fillmore, NY) were lined with fiberglass window screen and placed in the corners of the marshes for 24 h. Traps were baited with dog food except on July 27-28 and Aug. 10-11 when analyses of inorganic nutrients were carried out by another research group.

RESULTS AND DISCUSSION

Larval mosquito populations: Larval mosquito populations declined from approximately 20 larvae/dip^{-1} in mid-July to approximately 2 larvae/dip^{-1} in mid-August and remained low thereafter in both treatments (Figure 1). However, the variation in the mean number of larvae per dip for marshes assigned to either treatment was large and is attributed to differences in marsh type (one- vs. three-phase marshes: Walton and Workman, unpublished data). Mosquito larvae in the inflows and outflows of the research cells were more concentrated than they were in the vegetation along the long axis of the research cells; larval densities exceeded 100 individuals/dip^{-1} in the inflows and outflows of many of the cells in late June and early July.

Culex tarsalis predominated among the species collected as larvae during this study, comprising more than 50% of the larvae collected on most dates. Two other *Culex* species commonly associated with wastewater, *Cx. stigmatosoma* and *Cx. quinquefasciatus*, were somewhat less common; they comprised 20-40% and 50-20% of the larvae collected, respectively. Three other species were

rarely collected as larvae (< 5% of the total larvae collected on most dates): *Culex erythrothorax*, *Culiseta inornata*, and *Anopheles franciscanus*.

Fish: The stickleback proved to be ineffective as a biological control agent for mosquitoes in the research cells. The abundance of mosquito larvae did not differ significantly between the fish and fishless treatments (Repeated measures ANOVA: $F_{1,6} = 0.26$; $P>0.62$). This result is not surprising because sticklebacks apparently did not survive in the research cells. Sticklebacks were collected in minnow traps (average number per research cell $\pm$ SE: 2.8 ± 1.4) placed in the research cells on July 14, but were never collected again thereafter (Figure 2). We verified in a separate study that sticklebacks will enter baited minnow traps, so we conclude that the trapping method is reliable.

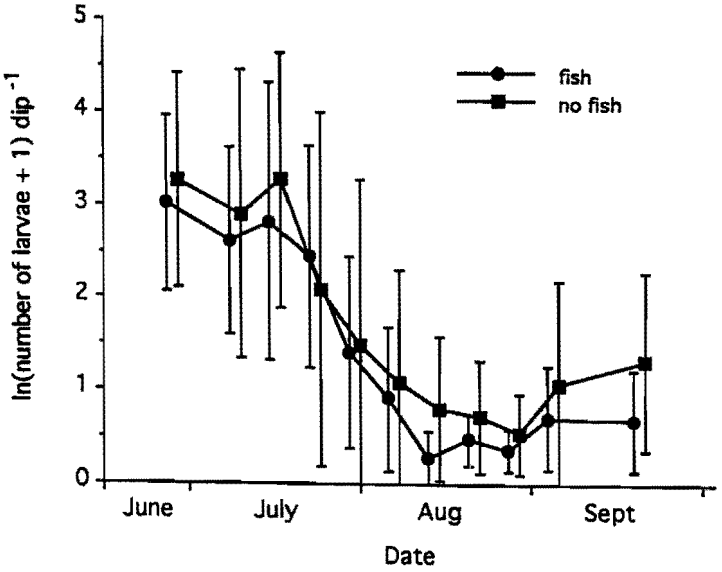

Figure 1. Abundance of *Culex* spp. larvae in research cells without sticklebacks and with sticklebacks stocked at 0.75 kg/ha^{-1} during 1995. Error bars are 1 SE. The data for each sampling date are spread out horizontally to facilitate illustration.

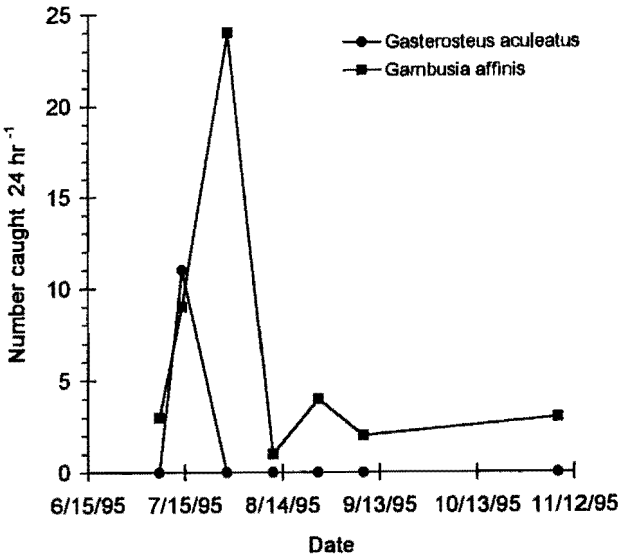

Figure 2. Total number of fish captured in minnow traps in the four research cells stocked with fish during 1995. *Gambusia affinis* was found in one of the research cells that had been stocked with *Gasterosteus aculeatus*.

We suggest that the stickleback's demise in the research cells was probably caused by an environmental stress, such as the low dissolved oxygen concentrations that resulted from large bacterial populations associated with decaying *Scirpus* or from high concentrations of ammonia, rather than lack of food or avoidance of minnow traps. Sticklebacks, particularly juveniles, feed readily on zooplankton (Wooton 1976, Gibson 1980, Hangelin and Vuorinen 1988). Bay (1985) cited evidence that *G. aculeatus* preferred mosquito larvae to ten other food organisms and that a stocking rate of two individuals/m^{-2} was marginally effective against *Culex pipiens* larvae in open water situations. The sticklebacks used in our study fed readily on mosquito larvae in the laboratory and were observed to forage on aggregated larvae in the field immediately after stocking. The abundance of mosquito larvae, other insects, and zooplankton in the research cells should have been sufficient to maintain the sticklebacks.

Based on length-weight data for a representative sample of the stocked fish, the sticklebacks were in reasonably good condition and should have reproduced during the period of this study; it is unlikely that the minnow traps failed to collected *G. aculeatus*. Wet weight increased at a rate slightly less than the cube of length (Figure 3). The exponent for the relationship between the weight and the length of the Mojave River sticklebacks was slightly lower than that of riverine and pond populations of sticklebacks in the United Kingdom (exponent ≅ 3; Pennycuick 1971, Wooton 1976); yet, it is well within the range (2.5 to 4.0) observed for other fish species (Le Cren 1951). Atypical rainfall during the spring severely scoured many of the stickleback habitats in southern California and reduced the overwintering populations of reproductive adult fish. The growth of fish populations dominated by young-of-the-year is expected to be slower than for populations where reproductive individuals are stocked into marshes. Although we collected mostly young-of-the-year, most of the sticklebacks used for this study should have been reproductive by late summer or early autumn. Sticklebacks reach reproductive maturity at approximately 40 mm (TL: total length) in males (Wooton 1976) and, in California, between 38-50 mm SL in females (Baker 1994). Females in an English river have been reported to reach reproductive maturity as small as 25 mm SL (O'Hara and Penczak 1987). When given adequate food and reared at 20°C, *G. aculeatus* completes juvenile development in about four months (Wooton 1976). Maturation rate in natural populations is usually slower than for populations reared under comparatively constant conditions in the laboratory (Wooton 1976). Sticklebacks typically breed from March through July; although, under appropriate conditions, breeding in southern California can occur as early as mid-February and as late as August (Coykendall 1980) or even October (Baker 1994).

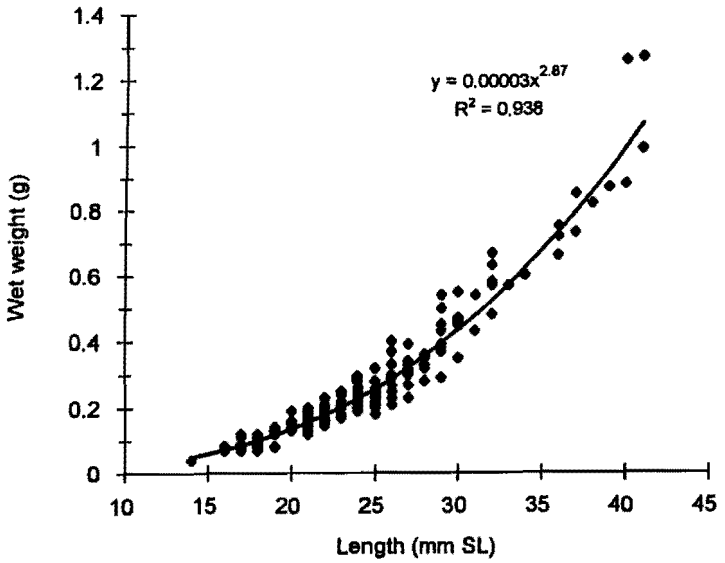

Figure 3. The relationship between weight and standard length of *Gasterosteus aculeatus* from the Mojave River near Victorville, CA. The best-fitting regression line and equation are shown. Y = wet weight in grams. X = standard length in millimeters.

Secondary treated wastewater often has a low oxygen content and high ammonia concentrations; both are toxic to sticklebacks. Dissolved oxygen levels can be depressed even further by the oxidation of ammonia and by bacterial populations. Previous studies carried out by the principal agencies participating in the wetlands project indicated that dissolved oxygen saturation increased from the inflow to the outflow of the research cells (USBR, NBS and EMWD 1994). Conversion of the mean percent saturation to oxygen concentration indicates that, depending on the time of year, dissolved oxygen levels increased by more than four-fold as water moved through the research cells. The minimum oxygen concentration at which sticklebacks can exist is approximately 0.25 to 0.50 mg/l^{-1} (Jones 1964); however, respiratory distress developed rapidly in sticklebacks exposed to dissolved oxygen concentrations below 2 mg/l^{-1} at 20°C (Jones 1952). The research cells were in the third year of operation and *Scirpus californicus* has a triennial cycle in which major die off of the emergent shoots occurs in the third year. Both the natural die off of emergent shoots and water-related stress probably contributed to a massive, vernal die off of *Scirpus* in the research cells which persisted as masses of dead material above the water surface until late November 1995. We hypothesize that the stickleback was unable to maintain populations in these small marsh systems because of the oxygen demand caused by bacterial populations decomposing dead *Scirpus*. Whereas ambient dissolved oxygen concentrations during the first year of operation (1993) should have been sufficient to support sticklebacks, dissolved oxygen concentrations in the water column in 1995 were very low (≤ 0.5 mg/l^{-1}) in regions of the cells.

Ammonia is the major form of nitrogen in reclaimed water and the undissociated hydroxide (NH_4OH) is especially toxic to sticklebacks. The 96 hour median tolerance limit at 15°C for freshwater sticklebacks was 2.1 mg NH_4OH/l^{-1} and tolerance limits were a function of salinity and temperature (Hazel et al. 1971, Wooton 1976). The ratio of NH_4^+ to NH_4OH in water is a function of pH and temperature; as pH increases from 8.0 to 9.5, the ratio of NH_4 : NH_4OH changes from 30:1 to near unity and, in the presence of large quantities of ammonia, it is likely that toxic levels of ammonium hydroxide will be reached (Hutchinson 1975). The average NH_4-N concentration in inlet water was 11.7 mg/l^{-1} and in the outlet water were 5.7 and 13.4 mg/l^{-1} in the three-phase and one-phase wetlands, respectively (1993 data: USBR, NBS and EMWD 1994). Although pH in the research cells does not usually exceed 8.3 (1993 data: USBR, NBS and EMWD 1994), stressful levels of ammonium hydroxide might be reached during periods of high algal production and warm water temperatures.

Mosquitofish were unexpectedly found in research cell 1 on July 7; however, after increasing abruptly in late July, the population remained small for the remainder of the study (Figure 2). In addition, *Gambusia* was collected only at the outflow until early November when mosquitofish were collected in minnow traps at both ends of the research cell. Even though the research cells were dried between autumn 1994 and spring 1995, we conducted two surveys prior to starting our experiment because mosquitofish were stocked into the research cells in 1994. Mosquitofish were not seen nor captured in the two initial surveys. We presume that the mosquitofish observed in research cell 1 originated either from individuals that survived during the period that water was turned off in the research cells or from a population in an adjacent nursery cell for *Scirpus*. Even though mosquitofish are capable of supplementing metabolic requirements by gulping atmospheric oxygen (Odum and Caldwell 1955), the restricted distribution of the mosquitofish in the research cells and the failure to proliferate suggest that conditions were also limiting for *G. affinis*.

It is unlikely that the extent of *Scirpus* die off observed in the research cells during 1995 is representative of the die off that occurs in the natural cycles of the bulrush. The water was turned off to the research cells for an extended period which probably severely stressed the *Scirpus*. Flow rates into the cells after late June were quite variable. This led to fluctuations in water levels and, possibly, inadequate flushing during periods of our study. Multipurpose constructed wetlands designed for large-scale water reclamation (i.e., 10 ha marshes) may function very differently from the small experimental systems studied here. An understanding of how vegetation type and natural cycles affect these systems, and how these factors impact our ability to use biological control agents for mosquitoes, is clearly needed. Our results in the small marshes suggest that the mosquitofish, or another species that is capable of withstanding low dissolved oxygen concentrations and other stressful factors found in constructed wetlands, might be a

better alternative than the stickleback. However, using mosquitofish as a biological control agent for mosquitoes is contrary to the goals of promoting local biodiversity of endemic fishes in these systems. To the extent that the small experimental marshes indicate how the larger ecosystems will function, constructed wetlands have an enormous potential for mosquito production and our options for biological control agents may be limited during periodic die off of the vegetation.

ACKNOWLEDGEMENTS

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AN UNUSUAL INVASION OF A RESIDENTIAL NEIGHBORHOOD BY THE CHEESE SKIPPER, *PIOPHILA CASEI* (DIPTERA: PIOPHILIDAE)

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In the late summer of 1995, the authors conducted an investigation in response to complaints from residents of an Oakland neighborhood (Alameda County, California) concerning large numbers of small flies. The area affected was in a mixed residential-commercial neighborhood.

Three city blocks commencing with the 10900 block of Russet Street were particularly affected by large swarms of flies. Flies noted outside were landing on garbage dumpsters and exposed pet food. Inside the homes, concentrations of flies were found inside the refrigerator and freezer compartments. The infestation extended at least one-eighth of a mile west of this street, indicating a strong flight pattern.

Fly Identification and Infestation Source

Adult flies collected from homes were identified as the Cheese skipper, *Piophila casei* L. This species is generally not classified as a "domestic fly", since they are usually not encountered as a neighborhood nuisance. Complainants who worked in a used-container cleaning and recycling facility adjacent to and downwind from Russet Street suggested that the plant was the source of the problem. Inspection of the recycling plant confirmed that it was a very high source of *P. casei*, the same species found in the homes. This recycling facility had stored various types of "empty" containers stacked in their outdoor yard (Figure 1). These containers were to be cleaned and resold. Included in the stacks were pallets holding plastic 5-gallon pails that contained liquefied, processed, salted egg yolk residue. Adult flies were flying and landing on these particular pallets. Adults, larvae, and pupae were found inside the containers with the solidified egg yolk residue. Larvae and pupae were also found between the stretch wrap and containers. Large numbers of pupae were on the ground under the pallets.

Figure 1. Breeding site of *P. casei* - pallets of salted egg yolk residues in containers stored outdoors at a cleaning and recycling facility.

Cause of the Infestation

The events that led to this infestation were a classic example of improper handling methods for putrescible wastes. The recycling plant was in the process of moving to new premises and no adjustment in operating procedures was instituted to compensate for this new situation. During this transitional period, used containers were being received and stored at the new location. The operator accepted containers with food residues, which included the processed salted egg yolk. Because the cleaning machinery had not been installed, the pallets of product were now being stored for longer periods (over a month) outdoors. The operator had also accepted more product than could be processed in a timely manner.

There were approximately ten pallets of 5-gallon plastic containers inserted into each other. The majority of these containers had food residues. As the egg yolk residue dried and solidified, it became an ideal breeding medium for *P. casei*. Simmons (1927) determined that *P. casei* has a short life cycle, minimally 12 days, and a large reproductive potential - he reared 52,627 skippers from one dried-cured ham - it did not take long for this fly to become a problem.

Control Measures Taken

The pest control company employed by the plant operator had set out fly traps baited for vinegar flies (*Drosophila* spp.). The pest control operator apparently had confused *P. casei* with the vinegar fly. The attractant bait in the traps was changed by the pest control operator when informed of the target pest. Traps are a useful tool for surveys but are totally inadequate for control of large fly infestations. As a temporary measure, a pesticide treatment, such as fogging with a pyrethrum-based insecticide, was considered. Interestingly, there was no pesticide label that directly states that "food" containers could be treated even though these would subsequently be cleaned. The only acceptable and legal pesticide treatment that would control this infestation would be fumigation with hydrogen phosphide or methyl bromide.

The operator of this facility was instructed to immediately clean the containers or dispose of them. Storing food waste residues not only posed a public health and nuisance condition but was in violation of solid waste management regulations. The operator disposed of the containers. No further complaints were received from residents.

Biology of *Piophila casei*

The Cheese skipper is a cosmopolitan acalypterate muscoid fly. The adult is black with bronzy tints on the thorax, reddish brown eyes and slightly iridescent wings that lie flat over the body when at rest (Figure 2). The adult is one-third to three-fifths the size of the house fly. The systematics of this species is well described by McAlpine (1977).

P. casei is considered a scavenger, preferring high protein foods in advanced stages of decay. The skipper has been reported in ham, bacon, human excrement, dried bones, moist dog hair, overripe cheese and beefsteaks (Simmons 1927). This species is also found in human cadavers, making this fly of importance in forensic entomology (Liu and Greenberg 1989).

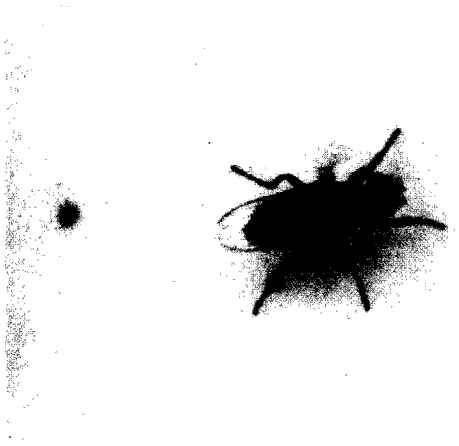

Figure 2. Adult Cheese skipper, *Piophila casei*.

Life cycle development is dependent upon temperature. Eggs are laid on the surface or cracks of a food source and hatch 30-40 hours later. The larvae burrow into the food source and remain there until mature (8-15 days). The larvae then migrate to pupate. The pupal stages last between 7-12 days. The larvae have the ability to "jump" or "spring", giving the skipper its name. The larva curls into a C-shape and attaches its mouth hooks to its posterior end. When muscular tension is created, the hooks are released from the posterior end, hurling the larva 8-10 inches into the air. The ability to jump is well-developed in the third instar larvae, possibly acting as an avoidance mechanism when finding a pupation site. When larvae were photographed, intense natural light elicited strong jumping reactions by the larvae.

Economic and Public Health Importance

The Cheese skipper is a well-known pest of the cheese and meat industry. Most of the literature concerning *P. casei* is related to this species' importance as an economic pest. *P. casei* is a synanthropic species, yet it has rarely been reported as a neighborhood nuisance pest. A literature search indicated one reported case of *P. casei* as being a domestic fly nuisance. This case involved open dumping-grounds in Cairo, Egypt (Daniel et al. 1989).

P. casei can act as a mechanical carrier of pathogens, especially in view of its behavior - active movements, strong and persistent attraction to a food source, attraction to refrigerators and high reproductive potential. *P. casei* is probably the most common species involved in enteric myiasis (Harwood and James 1979). Because the larvae burrow deeply into their food source, it makes their detection difficult. The larvae are resistant to various chemicals and can pass through the digestive tract unharmed. If swallowed, the larvae can cause intestinal irritation, lesions and scarification (Harwood and James 1979). Apparently in the past there were some gourmets that were fond of maggot-infested cheese (Smith 1973). The cheese skipper has been implicated in urinary myiasis (Saleh 1993) and has been found in the nose and chests of human beings (Mallis 1990).

Conclusions and Recommendations

One would imagine that an urban area would have plenty of breeding sources, especially in areas where cheese and dried meats are commonly used food products, privately and in restaurants. One of the authors observed a Cheese skipper during a restaurant inspection and it is suspected that this species may be more prevalent than commonly believed. Awareness that this species is present in our area, that it may become a neighborhood nuisance, and that it may infest retail food products is important because incorrect identification and hence inadequate control may pose undue health implications.

This incident showed that processed, salted egg yolk is a very good breeding medium for *Piophila casei*. Under certain conditions, this species may be involved in widespread infestations. Since this species is present throughout California, Vector Control Officers and Technicians, Environmental Health Specialists and Pest Control Operators should become familiar with its biology, habits and public health significance. This incident also demonstrates the need for surveillance of facilities such as container cleaning and recycling plants to ensure that recyclers employ procedures that will not lead to domestic pest problems.

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PUBLIC RELATIONS AND ITS ROLE IN THE FUTURE SURVIVAL OF LOCAL GOVERNMENTAL AGENCIES

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ABSTRACT

Changes in Alameda County MAD's public relations efforts and the concurrent efforts of surrounding districts resulted in an increased demand for district services. Presented is a summary of service request volume relative to mass media events and ACMAD's ability to provide continued effective service without an increase in staff.

Ralph Heim's keynote presentation on January 22, 1996, underscored the need for all public service agencies to have the best public relations programs possible. With state government, Local Agency Formation Commissions (LAFCO's), the California Constitutional Revision Commission, groups like the Jarvis Tax Payers Association and possibly county governments all clamoring to restructure local government, particularly special districts, these organizations can no longer afford to function as "silent warriors".

The purpose of this paper is to remind everyone of the value of a strong community relations program and to illustrate Alameda County Mosquito Abatement District's (ACMAD's) efforts to change with these turbulent times for local government.

Approximately five years ago ACMAD began to aggressively develop a public relations program to better inform their constituents of the cost effective services available to them. The first step in this process was the creation of the Environmental Specialist position, whose duties included the development and implementation of a public relations and community education program. The second part of the process was incorporation of employee input and shared responsibility for success of the program. Figure 1 illustrates the current structure of ACMAD which has a manager and twelve employees that share in the day-to-day decision making of all aspects of the organization's activities and functions. Allocation of staff and equipment, the type and application of larvicides, creation or evaluation and modification of district programs, employee evaluations, and future planning are examples of those responsibilities shared by all members of the District.

The current community relations program has the following elements:

- Proactive contact with the media (television, radio and newspapers) to inform them of upcoming events and District concerns.
- A school program that involves classroom presentations, supplying educational materials, and participation with school research projects and grants.
- Involvement in County shows, fairs, and regional events.
- Presentations to city councils, chambers of commerce, and community organizations.
- Development of a broad range of informational brochures for distribution to any individual in the county.
- Participation in state, regional and local committees and organizations involved in wetlands research, restoration and preservation.

Figure 2. Illustrates the effects of ACMAD's increased community relations efforts. Although the total number of public relation hours is still less than 1,000 per year, demand for district services (especially delivery of mosquitofish) has shown a significant increase. ACMAD has also found that irrespective of changes in precipitation levels, residents within the county have become more aware of the services available to them. Rapid response (usually within 24 hours of a request for service) has further resulted in the organization being viewed by its constituency as "unusually responsive government". This appears to be counter to the claims of the legislature and those organizations that feel there are too many unresponsive and unaccountable special districts and local governmental agencies.

Figure 3 indicates the weekly service request volume from 1 January 1995 to 31 July 1995. It should be noted that only mass media activities of ACMAD and its neighboring Districts are highlighted while other public contact events have been ignored. Throughout the time period there was a steady volume of mass media contact. However, the most significant service request volume occurred shortly after major flood events for the region which contributed to heightened public concern. Figures 4 and 5 provide a detailed breakdown of the service request volume by day for February and April, both of which were the season's highest peaks. An important feature to note is the cumulative effect of the other North Coastal Region Districts media activities on ACMAD's service request volume. It became quite apparent that the public relations efforts of neighboring Districts did increase public awareness and demand for ACMAD's services especially when a mass media forum (i.e., television or radio) was used.

Although ACMAD is still developing its public relations program, the resultant increased demand for the District's services has also had effects on other District functions. Increased public awareness has resulted in a need for increased efficiency to meet the demand and still provide the same level of service and freedom from pestiferous mosquitoes that existed in prior years. All of this has occurred without an increase in the number of field staff which has meant an increased workload for technicians. To compensate, there have been changes in how office staff function. For example, they now regularly participate in routine field operations such as handling service requests, performing mosquito source inspections and treatments, and maintenance and repair of

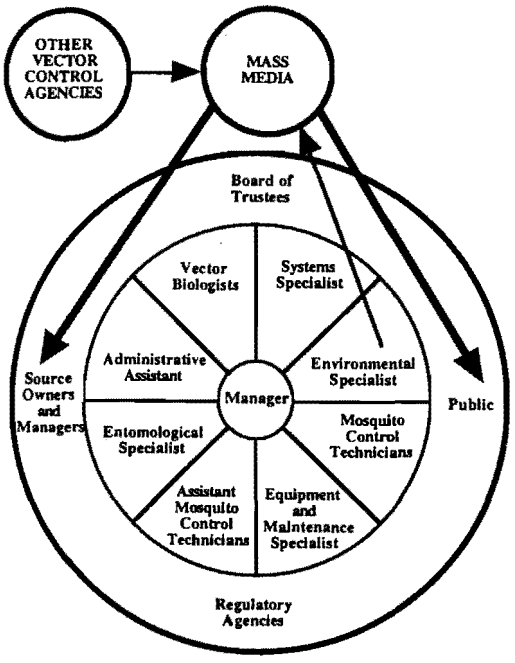

Figure 1. ACMAD organizational structure and relationships.

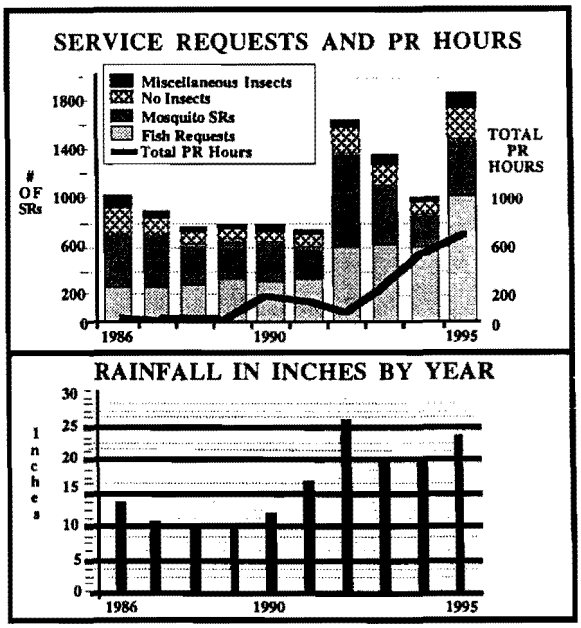

Figure 2. Service request volume by year and by type for 1986-1995. Total rainfall data and PR hours are included for comparison.

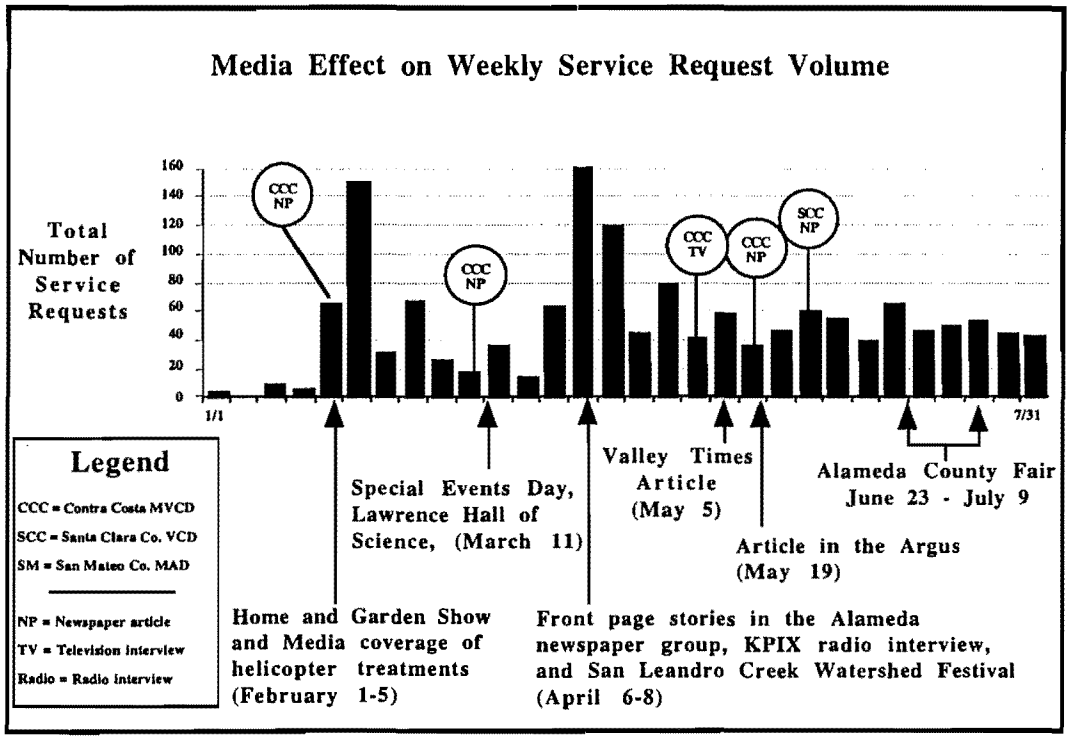

Figure 3. Relationship of mass media events to weekly service rates for January 1, 1995 to July 31, 1995.

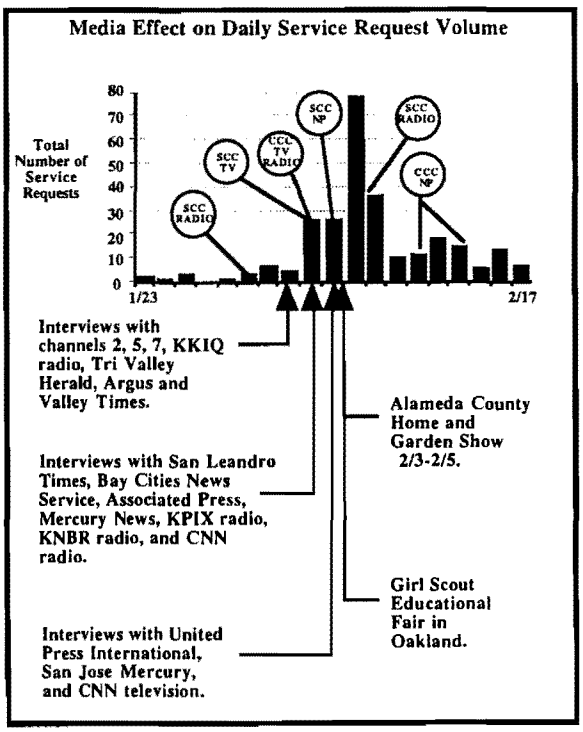

Figure 4. Daily service request rates for January 23 to February 17, 1995.

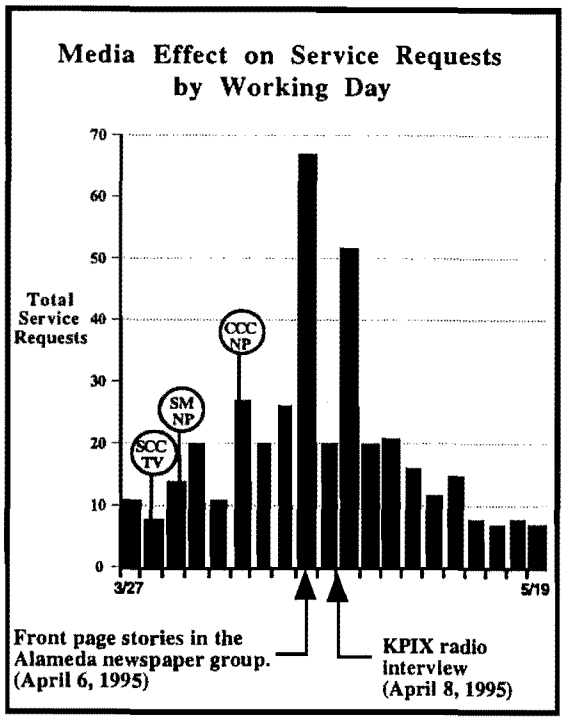

Figure 5. Daily service request volume from March 27 to May 19, 1995.

equipment. The change in job functions has also necessitated the need for increased communication amongst all individuals within the organization. Improved communication has also led to a better understanding of the relationships between all organizational members and the tasks they are required to complete.

In the final analysis, the survival of special districts will hinge on how aware their constituency is of who their local government is and what services they provide. Heightened awareness brings with it increased demands for service and an accompanying workload change. The ultimate task is for local government to adapt, with the limited funds and staff available, to their public's rising demand for service.

ACKNOWLEDGEMENTS

The authors thank Kriss Costa, Santa Clara County Vector Control District; Elayne Azevedo, Contra Costa County Mosquito and Vector Control District and Judy Robinson, San Mateo County Mosquito Abatement District for providing information on their agencies' public relations activities. We also thank Vinay Davis and William Hamersky for reviewing this manuscript.

MOSQUITO ABATEMENT ON PRIVATE PROPERTY THROUGH DUE DILIGENCE

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ABSTRACT

Property owners who have vacated their homes, and left their pools filled and unserviced have created perfect environments for mosquito breeding. In 1995, the Coachella Valley Mosquito and Vector Control District issued sixty-seven mosquito abatement notices. Unfortunately, some property owners leave no forwarding addresses, which makes it very difficult for vector control technicians to contact the owner(s) so they may request permission to enter the property for sampling and treatment purposes. The constitutional right of privacy overrides the California Health and Safety Code on entering property; we must use the process known as "Due Diligence" and make every effort to locate the property owner(s). This win/win situation has been very successful (sixty-one abatement notices have been resolved) in the Coachella Valley Mosquito and Vector Control District's efforts to eliminate potential breeding sources.

Pursuant to the Health and Safety Code Section 2270, *et seq.*, of the State of California, a Mosquito and Vector Control District is empowered to abate a condition which is deemed a nuisance. In the past, we have abated nuisances on vacant property without first obtaining consent. The Constitutional right of privacy outweighs the right of entry permitted by the California Health and Safety Code. It is because of this right of privacy, according to our legal counsel, that we implemented a policy to attempt contact with a property owner. The process is called "Due Diligence."

Before a court will issue an inspection warrant, the inspecting district must submit a declaration which convinces the court that all possible means of obtaining consent from the property owner and occupant have been exhausted.

The process for abatement through Due Diligence includes the following: accessibility, confirmation of property owner (mail to address, A.P.N., legal owner), abatement notification (certified mail), postal office (forwarding address, documentation), posting property (date, time, initial posting), register of voters (D.O.B., last known city of registration), Department of Motor Vehicles, declaration to the court of due diligence requesting warrant to access a property.

Receiving A Service Request

When a complaint is received about a possible breeding source on private property. The *first obvious step* is to verify and locate the address for inspection purposes. Upon arrival at the suspected property, attempt to get acknowledgment from the occupant(s) of your presence. If you get no reply from ringing the door bell or knocking, walk around to the rear area and announce yourself. Check for any signs that could help you in determining suspicions of vacancy such as neglected landscaping, dead shrubbery, excessive build up of newspapers and mail, posted disconnection notices from utility companies, or any Realtor signs. If you do not make contact, leave an informational brochure along with your business card and a short note to give you a call when possible. Next, try again some other day at a different time of the day. If the homeowner or occupant is home, introduce yourself and explain to them that you are doing a survey of the neighborhood because of complaints about mosquitoes in the area and that you noticed their swimming pool, while inspecting a neighboring home, and you would like to offer them a free property inspection to point out possible breeding sources. By being very cordial, you remove any suspicions that neighbors have made complaints

directed towards them, and you also keep the owner from becoming angry with you. Never state the name of the caller who complained as this may cause problems.

Accessibility

If the exterior of the property is not fenced or locked and does not have a *no trespassing* warning posted, you may enter, and inspect the property, to confirm that a nuisance or possible breeding source does exist. If breeding is occurring, take a sample for identification and records. In the event that the property is locked try to contact a neighboring property owner for permission to view the problem source from an accessible point. Usually the caller of the complaint will be more than happy to help you out in this area. Viewing may be done by using the neighbors patio or sun deck as a vantage point. This is legal, however climbing walls or using a ladder is considered a breech of privacy. After confirming that a possible breeding source does exist, leave an informational brochure dealing with mosquito control along with a business card in an area where these will most likely be seen in the event the owner returns to inspect their property.

Confirmation Of The Property Owner

Determine the Assessor's Parcel Number (APN) for the property address, legal owner's name and "mail to" address. Verifying the property owner can be done through the use of your local county tax assessor, county or city code enforcement, or through a title company usually at no charge (it would be very beneficial to secure a contact and form a relationship with one of these sources). Most code enforcement departments are more than willing to aid in any way they can, and will often allow you the use of their information systems. There are various ways to retrieve the information of legal ownership whether it be through an on-line network such as Data Quick®, or through a Micro Fiche data system. Both of these systems may be purchased, and I am sure a few districts are already set up with one of these systems or something similar. There is also an on-line access to the Department of Motor Vehicles, as wall as telephone access by an issued code number obtainable by completing the required application forms. Verification of the property owner is very important, because the responsibility for the reduction of breeding sources on private property lies solely with this/these person(s).

Abatement Notification

Prepare an official Abatement Notice with a cover letter explaining the existing problem(s). Request that the District be contacted concerning these matters. Include legal ownership, i.e., persons, bank, trustee, etc., the property address, and the assessor parcel number. The notification should include all services which your district will provide to aid in correcting the problem(s) as well as the responsibilities of the property owner(s). Send the notification via certified mail to the forwarding address. This will ensure a record of your attempt to notify the owner which will be necessary should your have to submit a declaration to the court in proving diligence. If the "mail to" address is the same as the property address, indicate "forwarding address requested" on the envelope below the return address (if property is obviously vacant). Allow enough time, usually 15-30 days for the property owner to either reply to the letter or take corrective action before a follow up inspection of the property.

Posting Of Property

When posting a copy of the abatement notice at a subject property it is preferred that the notice be placed in a highly visible area. You should initial, date, and note the time of posting. Use a sheet protector to hinder moisture from damaging the notice, and post the notice out of direct sunlight to prevent bleaching. Posting should be done when no reply had been received within ten to fifteen days, or following a "return to sender" of a mailed notification. When granted a warrant to enter a property, posting must be done 24 hours prior to acting on the warrant. When posting a property, care should be taken to avoid any unnecessary damage to the structure through the use of staples; Scotch tape or push pins tend to work best.

Registrar Of Voters

When an abatement notice is returned without a forwarding address, another attempt towards locating the owner would be to contact the Registrar of Voters. Under elections code 608(g) a governmental agency is privileged to certain information for a small fee. Unfortunately there have been some recent changes in the law, which limits the information that can be obtained. You will only be able to acquire the city the person last registered, and most importantly the date of birth of the subject if they registered to vote. Section 2277

of the Health and Safety Code requires written notice to the property owner of the existence of a nuisance. It is for this purpose that the information you seek can be obtained from the voter registration records. You will receive the information requested in approximately ten working days, and you will most likely be able to work out some type of payment account with the voters registration rather than on a per request fee.

Department of Motor Vehicles

In order to obtain information over the telephone from the D.M.V., as I stated earlier you must first apply for an access code from the department. You will need to contact the main branch in Sacramento, and request an application form INF 1130/1 (Rev. 6/93), or you can submit the proper forms to request information by mail. Once you have a date of birth from the Registrar of Voters, the Department of Motor Vehicles will be better able to pin point any known forwarding address or information on the subject for you. You might also want to make note of the license plate number of any vehicles parked at the subject property. The more information you can accumulate, the better your chances are of locating the owner through the Department of Motor Vehicles, which I have found to be highly successful.

Correspondence

If contact is made to the district concerning the abatement notice by the legal owner or trustee of the property, invite them to take advantage of the services which your district provides and any suggestion that could benefit the owner in correcting the problem. Explain to them, their responsibility as the property owner, that you will return to verify that corrections have been made, and give any further assistance that you might be able to provide. Allow 5-10 working days before a follow up inspection of the property to confirm that corrective action has been taken. In the event that the owner does not comply with the request of the district's recommendation for corrective action, a second and final abatement notice in conjunction with a cover letter stating any previous correspondence to correct the problem should be sent, again by certified mail, to the owner stating their responsibilities. An additional 10 working days should be allowed for the owner to conform to the district's request. Should the property owner fail to perform corrective measures, then the district will act on the second notice, requiring the property owner to appear at a scheduled board meeting. A hearing will be held to allow the opportunity for the property owner to present their reasons for not taking corrective action. The final decision will be determined by the district's board of trustees.

SUMMARY

Property owners need to take responsibility for abating mosquito problems. Our approach to using legal abatement has been successful in our first year. The steps listed above will continue to be used so we can provide "permanent" control instead of relying on constant visits by technicians to treat the source.

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AFRICANIZED HONEY BEES "THEY'RE HERE!!"

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ABSTRACT

The Africanized Honey Bee was first detected in Riverside County, California in October 1994. The Coachella Valley Mosquito and Vector Control District has been preparing for the arrival of AHB by: 1) media awareness, 2) responding to bee swarm calls, 3) public education, and 4) courtesy inspections.

By here I mean the Africanized Honey Bees have arrived in our county and our district. The Africanized Honey Bees have been detected in four locations in Riverside County and 16 locations in Imperial County. The first find of Africanized Honey Bees was in October 1994 at the Chuckwalla State Prison, 60 miles east of our District. The third find was along the northeast portion of the Salton Sea in the Coachella Valley in September 1995 in a trap suspended from a tamarisk tree that was operated by biologists of the Agricultural Commissioner. This site is approximately 20 miles from where our District Offices are located. Personnel from the California Department of Food and Agriculture (CDFA) and the Riverside County Agricultural Commissioner's Office have been trapping for Africanized Honey Bees and continue to monitor the situation. A combined total of 78 traps is utilized in their surveillance program.

The Africanized Honey Bee is a concern for the district because of the climate and visitors that are drawn to this area. The Coachella Valley is a desert with 80+ golf courses which attract tourists to the area. The temperature during January can range from the lows in the mid-40s to highs in the mid-80s. Bee swarming activity occurs primarily in the spring, but can occur in the fall and winter depending on the weather.

The Coachella Valley Mosquito and Vector Control District has been preparing for the arrival of the Africanized Honey Bee in a number of ways: 1) media awareness, 2) responding to bee swarm calls, 3) public education, and 4) courtesy inspections.

Media Awareness. The local media (newspapers and television) have been very helpful to the District in informing the public with eight television appearances and seven newspaper articles. We have made ourselves available to the media and the public to supply them with information, brochures and the District's policy in dealing with the Africanized Honey Bee (AHB). Public awareness has been conducted in both English and Spanish to inform them on the do's and don'ts with regard to the Africanized Honey Bees. A brochure on animal and livestock in regards to the AHB was produced by the District, using Orange County's brochure as a guideline. A gardening/landscape brochure is being developed by the Mosquito and Vector Control Association of California, co-edited by Mindy Franklin, the District's bee specialist, to address industries that are at high risk - gardeners and utility companies (i.e., water and power).

Responding To Bee Swarm Calls. District personnel are capable of responding seven days a week, 24 hours a day by the use of pagers. During business hours, calls related to bees are referred to Mindy Franklin. For off hours and weekends, the AHB phone number is on our business answering machine. The District responds to bee swarm calls only on public property. When bee swarms are located on private property, the caller is referred to a list of structural pest control companies with AHB training. Entry on private property is to give advice and reduce hysteria. Sometimes a resident just needs to know that the swarm is not going to attack them.

A truck equipped with a power sprayer, hand

cans, ladder, Mpede insecticidal soap, bee suits, and collecting equipment is used to control bees for a service request. Once the bees are eliminated, a remnant trap is placed on site to catch any bees that may have been missed, and a sample is taken for identification by our staff. Our personnel have been trained to identify AHBs by Dr. Eldon Reeves, a Riverside County entomologist, and by a CDFA entomologist. The training is also utilized by assisting pest control companies in identification of suspected AHBs. If the sample is suspicious, the specimens are sent to Sacramento for positive identification.

European Honey Bees are vital to the agricultural industry for pollination of various crops. According to the Riverside County Crop Report, the agricultural industry generated five million dollars of income to the County in 1994. By not controlling bees on private property, we lessen the demise of the European Honey Bee in the area. People usually wait for a swarm to leave when they have to pay a pest control company.

Public Education. Africanized Honey Bee presentations are given to government employees, schools, service clubs, hotels, golf course, and landscape personnel. These presentations are conducted in English and/or Spanish. The District also participates in a County Fair which runs for 10 days. Last year, approximately 12,000 people received information from the District (one-third were children).

The District's bee specialist is contacting the Valley's city offices to place a display at their locations about AHBs. The local libraries will also be contacted. The Riverside County Superintendent of Schools has developed an Africanized Honey Bee curriculum to be taught in schools - an interactive CD-Rom for grades 1-12.

Courtesy Inspections. On-site visits are made by District personnel to residences, condominiums, and businesses. The District personnel review ways the owners can bee proof their property by exclusion and debris removal. Brochures are also given to the owners at this time. Mindy Franklin is working full-time on this project along with doing presentations and displays. She has trained other staff members to conduct these inspections. Our goal is to eventually have the technicians do all the courtesy inspections. Thirty to 50 inspections are anticipated per month.

SUMMARY

1). Public Education/Media awareness on AHB. Facts not Fiction. The brochures are used to educate and inform the public with the facts. We have had over 100 calls for information and expect a lot more this year.

2). Bee proofing structures - we can't stop the bees but through courtesy inspections, we can reduce the risk of stinging attacks.

3). Responding to bee swarm calls.

By following the criteria and goals set by our District, we believe informed residents, tourists, and businesses will learn to adapt to their new neighbor, the Africanized Honey Bee!

ACKNOWLEDGEMENTS

The authors gratefully thank the staff members of Coachella Valley Mosquito and Vector Control District, especially Mindy Franklin, for their valuable work. We would also like to thank the Riverside County Agricultural Commissioner's Office and the California Department of Food and Agriculture for their surveillance.

1996 Proc. Mosq. and Vector Control Assoc. of CA 64:114-115

"HONEST, WARDEN! A BOBCAT® MADE THESE TRAILS"

Steve Mulligan

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Within the boundaries of the Consolidated Mosquito Abatement District in the heart of California flow the Kings and San Joaquin Rivers. Along these two rivers, riparian areas form a greenbelt of diverse vegetation creating a source of natural beauty and a reservoir for wildlife. Adjacent riparian corridors, as with many California rivers, have been greatly impacted and reduced. Historically these corridors were wide expanses covering broad floodplains. Today, the corridors are restricted to narrow bands along channeled, flood-controlled rivers. Agricultural and urban development encroach upon what remains. These remnants serve as valuable preserves for diversity of plant and animal species.

Unfortunately, included in that faunal diversity is an abundance of mosquito species. Dense vegetative growth in seepage and overflow areas provide ideal breeding sites and harborage for 17 species of mosquitoes. One of the most common mosquitoes found in these riparian areas is the encephalitis mosquito *Culex tarsalis*. Also plentiful in this habitat are passerine birds that can serve as reservoirs for St. Louis encephalitis and western equine encephalomyelitis viruses. These viruses, which are vectored by mosquitoes, can cause serious illness in man and other animals. Because of the continual increase in numbers of people living adjacent to these riparian areas, effective mosquito control and virus surveillance is critical.

Access to mosquito breeding sources in riparian habitat is often very difficult because of dense vegetative growth. To address this problem, the District attempted to establish and maintain trails into riparian breeding sources along the Kings and San Joaquin Rivers. In doing so, however, the District discovered that approval to undertake such work was required from the California Department of Fish and Game.

Authority to oversee human activity in riparian areas is codified in Sections 1600-1607 of the California Fish and Game Code. These regulations affect all individuals as well as state and local government agencies whose activities or projects might substantially "divert, obstruct or change the natural flow or bed, channel or bank or any river, stream or lake...in which there is at any time an existing fish or wildlife resource or from which these resources derive benefit..." (Code Sec. 1601). Riparian habitat, by definition, occurs along banks of rivers and streams and wildlife, by nature, inhabit and derive benefit from such habitat. Thus, any modification to riparian areas, such as establishment and maintenance of trails by District personnel, comes under the purview of Fish and Game for review and approval. The regulations also make provision for the negotiation of agreement or memoranda of understanding (MOU) to allow for approval of ongoing projects and routine maintenance activities without continual notification and review.

In 1991, the District signed a current MOU with Fish and Game that allows the District to open access-ways to any river or stream within the District boundaries for the inspection and abatement of mosquitoes. It permits the clearing of vegetation and debris from access-ways using hand tools, including chain saws, but places stipulations on the size of trees that can be removed. With the MOU in hand, the District sent work crews into riparian areas to cut trails into and through mosquito breeding sources. Chain saws and machetes were used to clear willows, vines and bamboo thickets. The work resulted in better access to routinely treated mosquito sources and opened up areas previously inaccessible for surveillance and control. Major trail work was carried out during the winter off-season, but actual work time was limited by a short off-season, bad weather, holidays and other projects. In addition, creating the trails by hand proved to be an arduous and time consuming task. The development of new trails was slowed because original trails had to be

re-cleared each year. In the fall of 1994, the District began to look for a more efficient means to establish and maintain trails. At this point came the idea of using a small skid steer loader.

A compact and highly maneuverable front end loader, the skid steer is widely used commercially for various jobs including swimming pool excavation and construction site work. Its value is enhanced in confined areas because of its short turning ability and responsiveness. To determine the utility of this type of machine in riparian areas, the District arranged for a field demonstration of a Bobcat® loader (Melroe Company). The test site chosen was densely vegetated and represented severe conditions, but performance of the Bobcat® exceeded expectations. The machine had plenty of power and pushed through the vegetation with relative ease. The local Fish and Game warden was also present to observe and evaluate the environmental impact of the Bobcat®. After watching the demonstration, the warden was satisfied with the Bobcat® and its intended use and wrote an addendum to the MOU allowing its use to be added under the existing agreement. To insure that trails continue to meet Fish and Game approval, the District maintains communication and cooperation with the local warden.

In January 1995, the District purchased a 736 Bobcat® loader with a 46 HP diesel engine and a 66-inch toothed bucket. Later, metal "Tire Crawler" tracks were purchased to install over the tires for added traction, especially in wet and muddy conditions. Also, several modifications were made to the Bobcat®. A heavy gauge, metal mesh door was placed on the front of the cab. Some areas of the cab frame were beefed up and protruding fixtures were removed. These changes were made to improve the safety and durability of the Bobcat® when crashing through heavily wooded vegetation.

The District now uses the Bobcat® for virtually all trail development and maintenance work, as it is an effective and acceptable means to accomplish these goals. Appropriate amounts of vegetation can be removed, leaving trails surrounded with sufficient cover. Currently, the program consists of the Bobcat® and operator supported by a 1 to 2 man crew, who help direct the work, assist with chain saws and service the machine. Use of the Bobcat® allows smaller crews to clear more access-ways and will enable crews to maintain all sites every year. It has proven to be a versatile machine, as the District continues to discover new capabilities and uses.

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PRELIMINARY RESULTS OF A TICK SURVEILLANCE PROGRAM IN SELECTED AREAS OF SAN JOAQUIN COUNTY

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ABSTRACT

Ticks were collected in parks and obtained from veterinarians in San Joaquin County, CA. Parks included in the survey were located along the Stanislaus and Mokelumne Rivers. One other park was located on Corral Hollow Road southeast of the city of Tracy. *Dermacentor occidentalis* Marx, *D. variabilis* (Say) and *Ixodes pacificus* Cooley and Kohls were collected in the parks. No specimens of *I. pacificus* were collected along the Stanislaus River. Ticks submitted by veterinarians included the three species collected in the parks as well as specimens of *Otobius megnini* (Duges) and *Rhipicephalus sanguineus* (Latreille).

A tick survey was conducted in order to determine if the principal vector of Lyme disease in the western United States, *Ixodes pacificus* (Burgdorfer et al. 1985), was present in San Joaquin County, California. Surveillance of a limited number of parks located along the Mokelumne and Stanislaus Rivers began in 1992. Collection sites in the parks were vegetated with brush and grass and had a high level of public access. Sites selected along the Mokelumne River included East Bay Municipal Utility District Park (Camanche Dam below the fish ladder), the Bowmen's Club (west of Highway 88 bridge) and Stillman Magee Park. Sites along the Stanislaus River included the McHenry Recreation Area (near McHenry Avenue bridge), the Ripon Recreation Area at the Highway 99 bridge, the Mohler Recreation Area located near Mohler Road west of the city of Ripon, Caswell Memorial State Park and Jacob Myers Park. The Carnegie State Vehicular Recreation Area in the Carnegie Hills was also included in the survey.

In addition to park surveillance, tick specimens were accepted from local veterinarians beginning in 1994. Local veterinarians were contacted and asked to submit ticks. Veterinarians who participated in the program were provided with information concerning ticks and tick-borne diseases.

MATERIALS AND METHODS

Surveillance in local parks was accomplished by flagging and visually locating ticks on vegetation. Ticks were easily found on twigs, grass tips and leaves. Flags used for tick collecting were made of polyester flannel approximately two square feet in size and attached to a four-foot handle. As specimens were collected, they were placed in small plastic containers, labeled, and returned to the laboratory for identification and storage.

A mailing kit was sent to each veterinarian in San Joaquin County. The kit contained blank reporting forms, containers for specimen storage and shipment, self-addressed return envelopes and a form letter. The letter outlined the surveillance program and requested the veterinarian's name and address, the date and location of the collection, the host animal and whether the tick might have originated outside San Joaquin County. After identification of submitted specimens, a copy of the completed form was returned to the veterinarian along with information on ticks and tick-borne diseases.

RESULTS AND DISCUSSION

Ticks were found at survey sites along both the Stanislaus and Mokelumne Rivers and at the Carnegie State Vehicular Recreation Area. Ticks collected at sites along the Mokelumne River included *D. occidentalis*, *D. variabilis* and *I. pacificus* at Camanche Dam and *D. occidentalis* and *D. variabilis* at the Bowmen's Club. No ticks were found at Stillman Magee Park. Ticks collected at sites located along the Stanislaus River included *D. occidentalis* and *D. variabilis* at McHenry Recreation Area and *D. variabilis* at Ripon Recreation Area, Mohler Recreation Area and Caswell Memorial State Park. No ticks were collected at Jocob Meyer Park. Specimens of *D. occidentalis*, *D. variabilis*, *I. pacificus*, *Otobius maegnini* and *Rhipicephalus sanguineus* were collected in the Carnegie Hills at the Carnegie State Vehicular Recreation Area.

Ticks submitted by veterinarians included all of the above mentioned species.

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FIELD TRIALS OF VECTOLEX GRANULES (*BACILLUS SPHAERICUS*) IN SOUTHERN CALIFORNIA DAIRY WASTEWATER LAGOONS

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ABSTRACT

Field evaluation of the VectoLex-G (Abbott Laboratories) granular formulation of *Bacillus sphaericus* against larval populations of *Culex* mosquitoes was conducted during the summer of 1995 in southern California at ten dairy wastewater lagoon sites. The VectoLex-G (strain 2362, 50 *Bs* ITU/mg) was tested at five and ten pounds per acre, applied to ten organically enriched ponds at different dairies ranging in size from 0.25 to 1.8 acres in order to determine: 1) duration of field persistence, 2) most effective rate for control, and 3) effectiveness against various species. Pond environments showed great variation and so did results. Generally, larval populations declined markedly after the initial treatment at both dosages, and were reduced for one to three weeks.

In the summer of 1995, Abbott Laboratories received a Research Authorization to test VectoLex-G, already marketed in other states, in organically enriched ponds in California. A representative from Abbott approached the University of California at Riverside to assist in developing a protocol for the trials and to conduct separate laboratory evaluations (Mulla and Rodchaeron 1996). This report describes the cooperative field trials conducted by the Northwest Mosquito and Vector Control District (NWMVCD) in Riverside County and West Valley Vector Control District (WVVCD) in neighboring San Bernardino County.

Both agencies chose five ponds, at different dairies, but all within a 10-mile radius in the inland Chino-Corona Valley of southern California, home to densely confined dairy production operations in an agricultural preserve bordered by suburban residential tracts. The dairies impound runoff and wastewater from milk barn operations in earth-bermed lagoons or pasture checks. The combination of high organic load, rafting solids and vegetation, and daytime temperatures surpassing 90° F (32° C) in summer and fall result in the rapid production of high densities of mosquitoes, primarily *Culex quinquefasciatus* Say and *Cx. stigmatosoma* Dyar; sometimes hundreds per dip.

The traditional chemical means of obtaining residual control has been through the use of organophosphates, but because of resistance problems, larval reduction in the last decade has consisted of frequent re-treatments with a larvicidal oil, combined with vegetation and water management.

Bacillus sphaericus Neide (*B.s.*) is a commonly occurring bacterium found in soil and aquatic environments throughout the world. It produces a protein δ-endotoxin upon sporulation, which has a mode of action very similar to *B. thuringiensis* var. *israelensis* de Barjac, as it affects the larvae of mosquitoes when ingested. *Culex* species are described as being the most sensitive to *B.s.*, followed by *Anopheles*, then *Aedes*, but it is virtually non-toxic to black flies.

Larval mortality begins within hours of treatment, and previous controlled testing has shown *B.s.* to persist for 2-4 weeks, reportedly undergoing limited recycling within larval mosquitoes in certain organically rich environments.

The VectoLex-G granular formulation (50 *B.*

[1] Present address: Santa Cruz Mosquito and Vector Control District, 640 Capitola Road, Santa Cruz, CA 95060.

[2] West Valley Vector Control District, 13355 Elliot Avenue, Chino, CA 91710.

sphaericus ITU/mg), 10/14 mesh corn cob in 40 pound bags, is labeled for use in permanent mosquito breeding sites which are organically enriched.

MATERIALS AND METHODS

NWMVCD used the 10 lb/acre application rate and WVVCD used 5 lb/acre, which was applied by ground application equipment; NWMVCD using a Maruyama backpack blower.

Pond selection criteria was based on a history of frequent breeding and breeding at time of application. Five ponds were chosen at each agency ranging in size from 0.25 to 1.8 acres. Ponds were re-measured and the granule blower was calibrated. As the swath width of the blower was about 25 feet, the perimeter of the larger ponds received more granules than the center.

On August 15, a pre-treatment count was taken and the VectoLex applied at the NWMVCD ponds. Sampling protocol called for post-treatment sampling intervals of 3, 7, 14, 21, 28 days for as long as acceptable control persisted, with re-treatment as necessary.

Twenty dips were taken per pond using a 350 ml dipper with five dips each from four areas representative of the highest breeding in each pond. An alcohol squirt bottle was used to wash down the contents of five dips in a mesh bag (used as a concentrator) into each sample bottle. Therefore a composite of four samples, each concentrated from five dips, were collected from each of the five ponds.

In the laboratory, an aliquot from each sample was placed in a gridded counting dish and examined microscopically. Ten late instar larvae were identified to species from each of the twenty samples on each sampling date. A count of each developmental stage was tabulated. Sample data was then averaged for each age group per date and all but egg rafts graphed to show a trend.

RESULTS AND DISCUSSION

Each pond was unique in its situational factors, therefore results varied in response to treatment. Factors such as water level, age of water, presence or absence of vegetation, and re-enrichment with new effluent varied from pond to pond, and had effect upon breeding density and proportion of mosquito species.

The absence of pupae was determined to be the factor most indicative of control (Mulla and Rodchaeroen 1996). In the NWMVCD dairies (Dairies A-E) treated at 10 lb/acre on August 15, Dairy A received aged water from other ponds, contained tumbleweeds, and supported *Cx. tarsalis* Coquillett. It had initial larval counts of 38 ($1^{st}/2^{nds}$) and 60 ($3^{rd}/4^{ths}$) per dip, but pupal counts were less than two throughout the 5-week trial (Figure 1). Larval numbers dropped to almost zero following treatment (trt), then early instars spiked to 29 per dip at the week 2 sampling but declined by wk 3.

Dairy B received periodic influxes of effluent slurry, had floating manure and vegetation mats, and supported *Cx. quinquefasciatus*, *Cx. stigmatosoma* and *Cx. tarsalis*. Initial dip counts of 106 ($3^{rd}/4^{ths}$) dropped to five one-week post-trt. The pond was re-treated at wk 2, had renewed breeding after wk 3, and was treated with larviciding oil at wk 5 (Figure 2).

Dairy C received new slurry and had floating mats, many egg rafts and initial counts of 104 pupae/dip. (Figure 3). At 72 hours post-tx, pupae were at three per dip but increased to 51 per dip at wk 2 when the pond was re-treated. Pupae dropped to three per dip by wk 3 but rose to 40 by wk 5.

The environment and breeding profile of Dairy D was similar (Figure 4). It was breeding *Cx. quinquefasciatus* and *Cx. stigmatosoma* at a ratio of approximately 2:1. Sampling at 72 hours post-trt showed early and late instars reduced reduced by about 92%, from 776 per dip to 51 for $3^{rd}/4^{ths}$. Pupal counts at wk 1 were down 86%, from an average of 99 per dip. At week 2, late instars had rebounded to 40% of pre-trt, though pupae remained at 23% of pre-trt values. The pond was re-treated at the end of wk 2 due to the increase of larvae (>300/dip). Late instars again dropped to 4% of pre-trt levels, while pupae were at 8% of pre-trt. By week 5, pupal numbers were 74% of pre-trt and larviciding oil was applied, terminating the test for that pond.

Dairy E was a push-up pond with aging water, low breeding and declining water level, though its profile was not dissimilar (Figure 5).

At the WVVCD ponds (Dairies 1-5), treated at 5 lb/acre on August 22, numbers of larvae were monitored and sampled for a longer period. Dairies 1, 4 and 5 showed similar profiles (Figures 6, 7 and 8 respectively). Larvae were identified as primarily *Cx. quinquefasciatus* with some *Cx. stigmatosoma*. Dairy 1 received water from other ponds, the other two received effluent and had floatage.

Suppression appeared rapid following treatment with a minor re-bound at 3 weeks; a re-trt; a population spike at six weeks at Dairies 1 and 5; followed by a second re-trt.

Dairies 2 and 3 showed anomalies. Dairy 2 breeding was controlled following treatment but there was a large burst of breeding at wk 7 that subsided prior to re-trt (Figure 9). The pond was relatively clean with a stable water level. Dairy 3 had a surge of breeding at wk 3 that did not respond typically to re-trt until a third re-trt at wk 5, then breeding increased following at re-trt at wk 8 (Figure 10). The pond was small with heavy floatage.

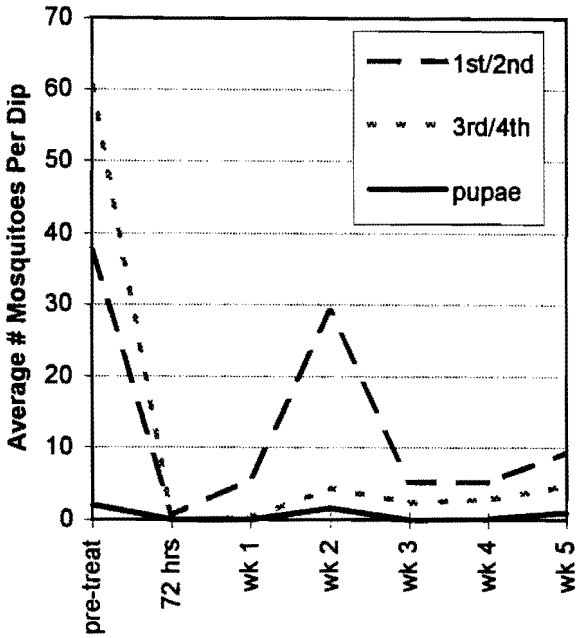

Figure 1. Dairy A, Corona, 1.3 acres. Treated August 15, 1995 @ 10 lb/A.

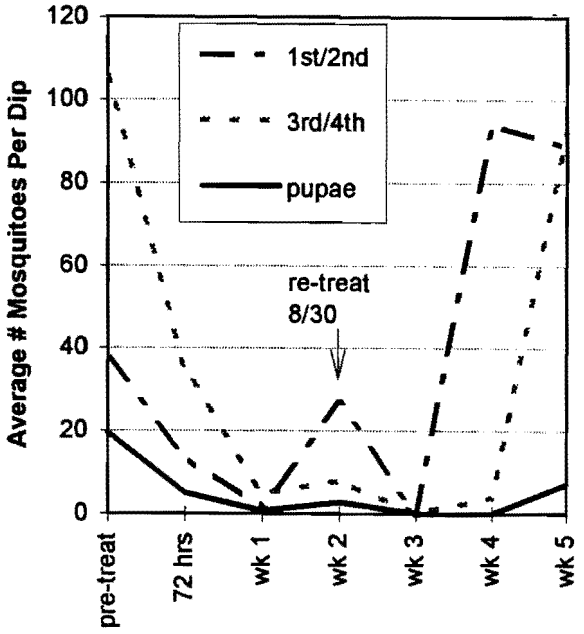

Figure 2. Dairy B, Corona, 1.4 acres, Treated August 15, 1995 @ 10 lb/A.

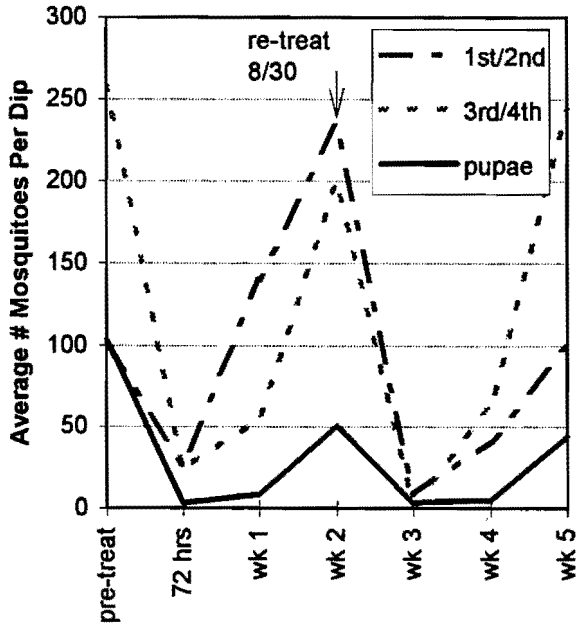

Figure 3. Dairy C, Corona, 1.1 acres. Treated August 15, 1995 @ 10 lb/A.

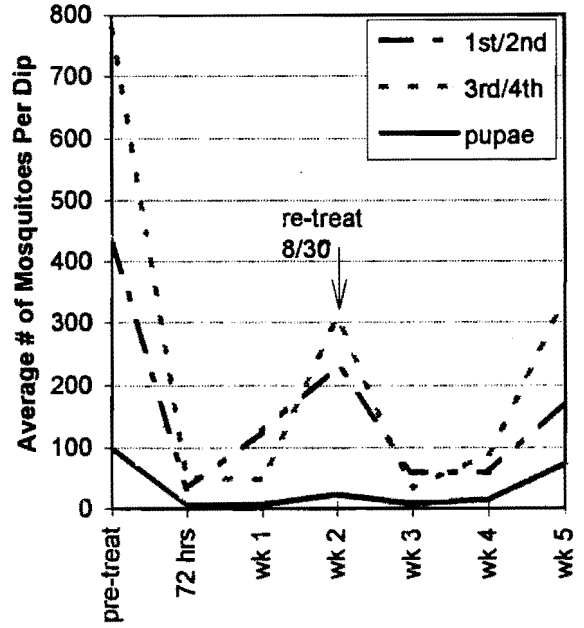

Figure 4. Dairy D, Corona, 1.8 acres. Treated August 15, 1995 @ 10 lb/A.

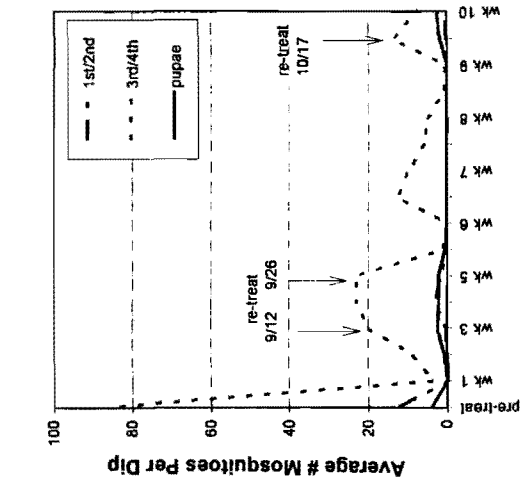

Figure 7. Dairy 4, Chino, 0.25 acres. Treated August 22, 1995 @ 5 lb/A.

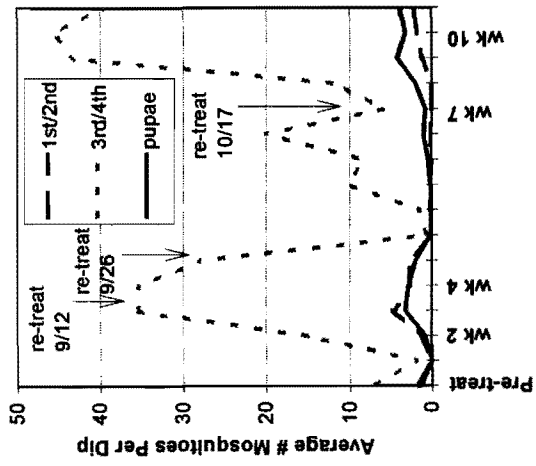

Figure 10. Dairy 3, Chino, 0.25 acres. Treated August 22, 1995 @ 5 lb/A.

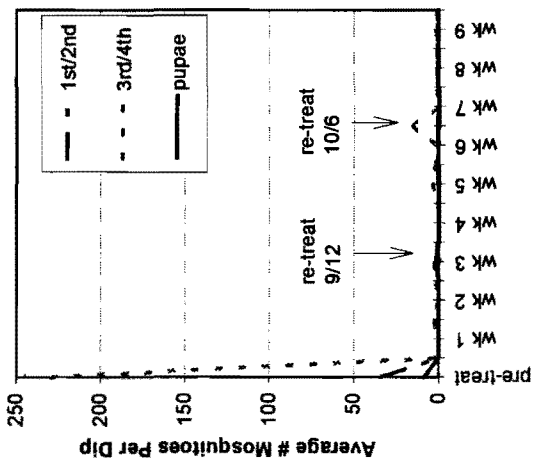

Figure 6. Dairy 1, Chino, 1.8 acres. Treated August 22, 1995 @ 5 lb/A.

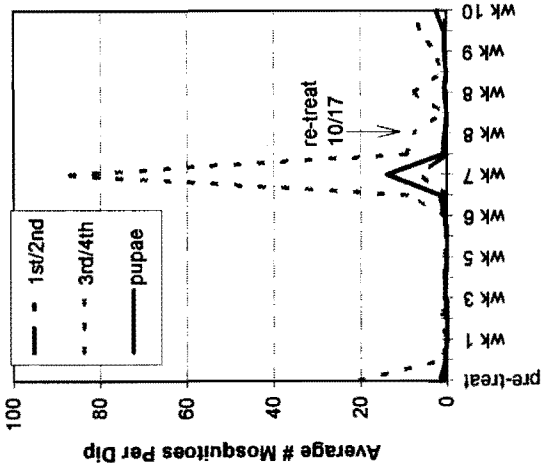

Figure 9. Dairy 2, Chino, 0.5 acres. Treated August 22, 1995 @ 5 lb/A.

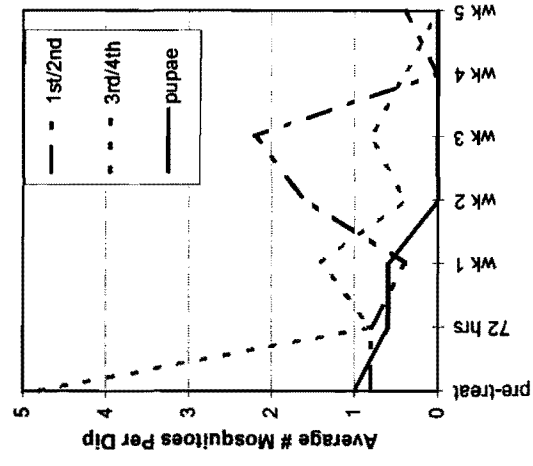

Figure 5. Dairy E., Corona, 0.6 acres. Treated August 15, 1995 @ 10 lb/A.

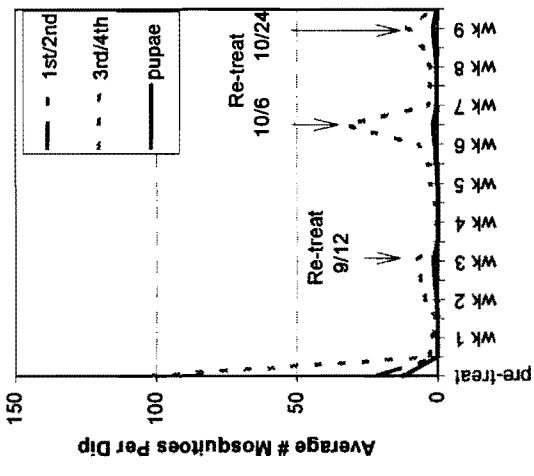

Figure 8. Dairy 5, Chino, 0.25 acres. Treated August 22, 1995 @ 5 lb/A.

CONCLUSIONS

As factors varied among ponds and no control was used, no statistical analysis could be employed. While the results described were representative and supportive of control, there were a few anomalies.

No obvious control differences among the 5 lb/acre and the 10 lb/acre treated ponds were noted; nor differences in species susceptibility.

Floating mats of manure and vegetation may reduce control as they could hinder penetration of granules into the water layer. Mosquito larvae tend to harbor in an among these mats.

It was somewhat noticeable that those ponds that received fresh input of new organic slurry were prone to a resurgence of breeding (Ponds A, E, 1 and 5). Beehler and Mulla (1995) have demonstrated that ponds receiving new highly organic slurry are more attractive to oviposition than ponds that do not. Therefore, the use of VectoLex-G in ponds with frequent new organic input or fluctuating water levels should be closely monitored. It has been recommended that this product be used in rotation with other larvicides to prolong the development of resistance. Historically, resistance has been problematic in this type of breeding source.

Overall, in our field tests, VectoLex-G appeared to provide adequate to good control of *Culex* mosquito larvae at 5 to 10 lb/acre for one to three weeks in dairy ponds with high organic loads.

From 1996, *Bacillus sphaericus* is being marketed as VectoLex-CG.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Jeffrey Beehler (Northwest Mosquito and Vector Control District) for his advice and review, Dr. Mir Mulla and fellow researchers at the University of California at Riverside, and Dr. Brian Melin of Abbott Laboratories. Gratitude also to John Deck of Santa Cruz MVCD for his help with the graphs.

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PLAGUE SEROLOGY OF A CALIFORNIA GROUND SQUIRREL, *SPERMOPHILUS BEECHEYI*, POPULATION IN THE SAN JACINTO MOUNTAINS, RIVERSIDE COUNTY, CALIFORNIA; MAY TO OCTOBER, 1995

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ABSTRACT

Serological samples were obtained from a population of the California Ground Squirrel, *Spermophilus beecheyi*, at an enzootic plague focus in southern California from May to October, 1995. The study was initiated because it appeared that there maybe at least partial "resistance" to plague, as evidenced by a long history of high antibody titers and lack of observed sick or dead animals. Coincidentally, the proposed study area, Stone Creek Campground, was experiencing an explosive epizootic when the study commenced. Consequently, of the 98 serological samples from 38 marked and released adult *S. beecheyi*, 71 samples derived from 30 individuals provided seropositive titers for *Yersinia pestis*. Twenty-seven of the 38 animals provided two to six serial samples, with titers ranging from zero to 1:8192. Changes in antibody titers over time are demonstrated through graphics on frequency distribution, as well as, individual squirrels. Thirty-one juveniles were also marked and released, providing 43 serosamples all of which were negative. Only three of 22 adults with titers from 1:256 to 1:8192 during the year had titers that dropped below 1:256. Conversely, of the 19 individuals showing titers of 1:128 or less, 16 never exceeded a titer of 1:128.

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EFFICACY OF *BACILLUS SPHAERICUS* AT THE SACRAMENTO REGIONAL WASTEWATER TREATMENT PLANT DEMONSTRATION WETLANDS

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ABSTRACT

Field trials with *Bacillus sphaericus* strain 2362 (VectoLex G) demonstrated that it was effective in controlling mosquito larvae in a secondary effluent demonstration wetlands. Immatures of *Cx. tarsalis, Cx. erythrothorax* and *Cx. pipiens* were susceptible to the microbial insecticide. Residual activity was also noted in the post-treatment monitoring of the wetlands.

The mosquito larvicidal properties of *Bacillus sphaericus* Neide were first recognized by Kellen and Myers (1964) from a collection of moribund *Culiseta incidens* (Thomson) larvae obtained from rock pools at Big Creek in Fresno County, California. Although the *B. sphaericus* K strain proved to be an ineffective biocontrol agent, its discovery led to a world wide search for viable strains that were efficacious to immature mosquitoes. Several toxic strains were isolated and tested for development over time. Field trials with strains 1593 and 2362 showed that both were most effective in controlling *Culex* larvae (Yap 1990).

Laboratory trials by Mulligan et al. (1980) with strain 1593 demonstrated that activity was reduced in the presence of suspended solids from waste effluent and exposure to sunlight. Increased temperatures conversely had a positive correlation with microbial activity which is in agreement with the findings of Mulla et al. (1990) in testing strain 2362 and Wraight et al. (1987) with strain 1593. Mulla et al. (1990) also noted that there was an inverse relationship between larval density and reduction of the immature population.

The recycling ability of *B. sphaericus* in larval cadavers has been demonstrated by many researchers including Davidson et al. (1975), Ramoska and Hopkins (1981), Davidson et al. (1984) and Becker et al. (1995). This ability to multiply vegetatively in the cadavers may be a potential source to infect larvae hatching from newly laid egg rafts (Des Rochers and Garcia 1984) and thus explain the residual activity noted in some field trials. The range of residual activity of *B. sphaericus* strain 2362 in field trials containing suspended organic materials varied from nil (Mulla et al. 1984a) in experimental ponds containing polluted water, 14-21 days in dairy wastewater lagoons (Mulla et al. 1988), to 60 days in sewage tanks (Hornby et al. 1984). The residual activity or lack of may be correlated to the affinity of *B. sphaericus* to attach to the type of sediments present (Yousten et al. 1992). They hypothesized that *B. sphaericus* may be resuspended by turbulence in the water (wind action, animal activity) if not strongly bound to the sediments.

With this information in mind, it was decided that trials of a granular formulation of strain 2362, VectoLex G, would be conducted at the Sacramento Regional Wastewater Treatment Plant (SRWTP) Demonstration Wetlands when it became available for experimental use by Abbott Laboratories in August of 1995. After 2.5 years of sampling at the SRWTP demonstration wetlands, the personnel at SRWTP and the Sacramento/Yolo MVCD were looking for a better means of controlling mosquito larvae occurring in secondary effluent treatment cells. Weekly treatments were being made on cells that were producing mosquitoes to maintain the larval population below the 0.1 larva per dip criteria which was the established threshold.

SITE DESCRIPTION

The Sacramento Regional Wastewater Treatment Plant demonstration wetlands is located ca. one-half mile east of Franklin Boulevard and one and a quarter miles north of Simms Road in T. 7 N R. 5 E S. 17. The demonstration wetlands was created as an experimental project using vegetation as a means to remove heavy metals from treated secondary effluent.

The wetlands is comprised of 16.5 acres of cells with a two-acre habitat cell located on the west end. One million gallons per day of secondary effluent is disinfected using UV light and distributed to 10, 1.5-acre treatment cells in this experimental project. An additional 1.5-acre cell (Cell 5) serves as an experimental control and receives groundwater fertilized with liquid ammonia. Cell 6 is an overland flow design with the inlet end of each half cell being independent and flowing northward to the discharge point. The treatment cells are bow shaped and constructed in two halves, A and B. The inflow half cell is designated as B and the discharge half cell is designated as A. The half cells (A and B) of each respective cell are connected by an underground pipe on the south ends for water transfer between the two half cells. The numerical sequence begins from the west end, with the cell furthest west being half cells 1A and 1B, respectively, etc. until half cell 11B on the east end. The half cells were designed with three mosquitofish overwintering/holding areas that are located at both ends and the midpoint of the half cells except for cell 6 which is the overland flow cell with holding areas at the north ends and midpoints of each half cell. The overwintering areas were three feet deep and essentially devoid of emergent vegetation except along the margins of the cells (Nolte and Associates, Inc. and Jones and Stokes Associates, Inc. 1995). The water depth within the vegetative stand is approximately six inches (Nolte and Associates 1992).

The cells assigned as test plots were 4 and 7 with half cell 10A serving as a control. SRWTP was conducting a field trial on half cell 10B to determine if running a sprinkler system throughout the night was effective in repelling gravid mosquitoes seeking an oviposition site. Cell 4 is a recycle cell whereby the secondary effluent from the discharge point is mixed with inflow at a 1:1 ratio, cells 7 and 10 are plug flow cells with equal inflow and drainage (Nolte and Associates, Inc. and Jones and Stokes Associates, Inc. 1995). The water levels remained constant in the test plots during the trials except for cell 10 which was drained for maintenance purposes after the October 20 samples were taken.

Vegetation within the wetland cells consists of a mix of common tule, *Scirpus acutus* Muhl, and cattail, *Typha latifolia* L. As this was the third year of the project, the cells in which the field trials were conducted had a dense canopy and were thatched in certain areas of the cells. There were also open water areas from foot paths and uprooted plants with the vegetative stands which aided in the penetration of the insecticide granules to the water surface. Open water surfaces of the cells were covered with duckweed, *Lemna minor* L., by varying degrees. The density of duckweed along the sampling margins ranged from light as in cell 4 to very dense as was found in cell 10. The duckweed density determined the amount of processing time required for the samples brought back to the laboratory for larval counts and identification.

The predominant mosquito species throughout the sampling period was *Culex tarsalis* Coquillett, followed by *Culex erythrothorax* Dyar and *Culex pipiens* L. respectively. An *Anopheles freeborni* Aitken larva was sampled once in cell 4 on August 21 and was the only time that any *Anopheles* larvae were found.

MATERIALS AND METHODS

For sampling purposes, each cell was divided into four quadrats to differentiate between north and south ends of each half cell. Twelve dips were taken per quadrat resulting in 24 dips taken per half cell and 48 dips per cell. Dips were taken with a 250 ml plastic dipper and concentrated in a gussey bucket having a fine mesh nylon screen. The concentrated sample from a quadrat was then back flushed with 75% isopropyl alcohol into a pint Mason jar for sorting back at the laboratory. The amount of duckweed obtained in the samples necessitated processing of the samples in aliquots under a dissecting microscope at 10x magnification. The larvae were sorted, indentified and quantified by instars.

The sampling regimen followed a pre-treatment, 3, 7, 14, 21, etc. post-treatment schedule until it was determined that control was no longer effective. A break from protocol occurred during sampling of half cell 10B that was treated on September 19. The

post-treatment sampling regimen was modified to days 3, 10, 17, 24 and 31 which coordinated with the sampling dates for cells 4, 7, and half cell 10A. As *B. sphaericus* is a slow acting insecticide and may demonstrate residual activity in this situation, percent reduction of the immature population for the day 3 post-treatment count was based on the presence of third and fourth instars (Mulla et al. 1990). The cells were not re-treated until the majority of the immature population sampled were late fourth instars or pupae.

Treatment of the cells was done with a Green Machine® back pack blower. Each half cell was 0.75 acre in area and was individually treated with 7.5 pounds of VectoLex G yielding an application rate of 10 pounds of granules per acre. There was no variation in the application rate for all of the trials conducted. At the calibrated rate of output, each half cell was encircled twice during an application for greater uniformity of coverage.

Limited water chemistry analyses were performed on September 15, 1995 prior to treating cell 7 and half cell 10A. Water quality measurements were also taken for half cell 10B on September 19. A Corning Checkmate M90 probe was used to measure water temperature, conductivity, total dissolved solids and dissolved oxygen. A Digi/sense probe was used to measure pH.

FIELD TRIALS AND RESULTS

Pre-treatment sampling was instigated on August 9, 1995 on cells 4, 7, and half cell 10A. The average number of larvae per dip during the initial sampling was 0.08 for cell 4, 1.33 for cell 7, and 5.33 for half cell 10A. Based on the 0.1 larva per dip threshold, cell 4 was not treated, cell 7 was treated and half cell 10A was left as a control. Fifteen pounds of VectoLex G (7.5 lb./half cell) were applied to cell 7 on August 11, 1995. Post-treatment samples taken three days later showed a decrease in the average number of larvae per dip for all monitored plots. The seven day post-treatment counts on August 18 had a slight number increase in cells 4 and 7 whereas half cell 10A increased 2.7x the pre-treatment count. We were then requested to treat half cell 10A by SRWTP personnel so cell 4 became the control cell and was never productive throughout the test period.

Larval counts remained low throughout this trial period for cell 7. There was only one pupa found among the larval instars for weeks 2 and 3 post-treatment so monitoring was continued. The majority of larvae were primarily third and fourth instars in the week 4 sample so the cell was resampled five days later (September 13). Late fourths and pupae constituted 34.8% of the population (Figure 1) so the September 13 sampling served as the pre-treatment sample for Trial 2.

Post-treatment sampling of half cell 10A demonstrated a typical population census of alternating low and high numbers that occurs with many *B. sphaericus* treatments. The week 3 post-treatment sampling showed an increase in population numbers and with the majority being late instars (Figure 1). As with cell 7, half cell 10A was sampled five days later on September 13 for confirmation that the insecticide was no longer effective and served as the pre-treatment count for Trial 2.

Trial 2 began on September 13, but cell 7 and half cell 10A were not treated until September 15 (Figure 2). The day 3 post-treatment sampling showed a reduction of 100% and 96% for cell 7 and half cell 10A respectively. Larval numbers remained low and did not recover to pre-treatment levels throughout the sampling period for cell 7. Larval population numbers did not begin to rise until week 3 post-treatment for half cell 10A. There was a dramatic increase in numbers in the fourth week (10/13/95), but the majority of larvae were second instars (68-2nds, 10-3rds, 2-4ths; N = 80). No pupae were sampled throughout the trial period. Sampling was terminated on October 27 as no larvae were found in cell 7 and half cell 10A was drained for maintenance purposes.

Trial 3 began with the pre-treatment sampling of half cell 10B on September 19 after termination of the sprinkler deterrent test by SRWTP. A 93% reduction in numbers was noted on the day 3 post-treatment count. A gradual increase in population numbers was noted from the second week of sampling. Fourth instar larvae were not collected until the October 20 (day 31) sampling period (Figure 3). Monitoring was terminated when half cell 10B was also drained for maintenance purposes.

Water quality measurements were performed for Trials 2 and 3. Sampling locations were approximately 50 feet from the inlet and outlet ends of the cells. The results are shown in Table 1.

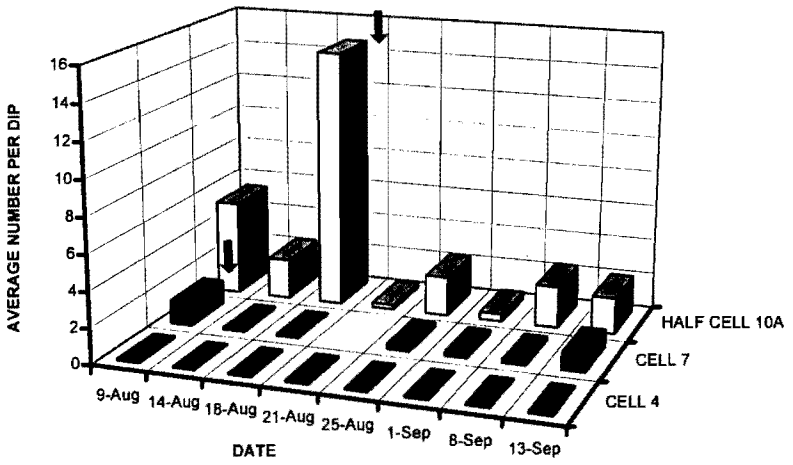

Figure 1. Pre- and post-treatment counts for cells 4, 7 and half cell 10A. Arrows indicate treatment dates (August 11 for cell 7; August 18 for half cell 10A).

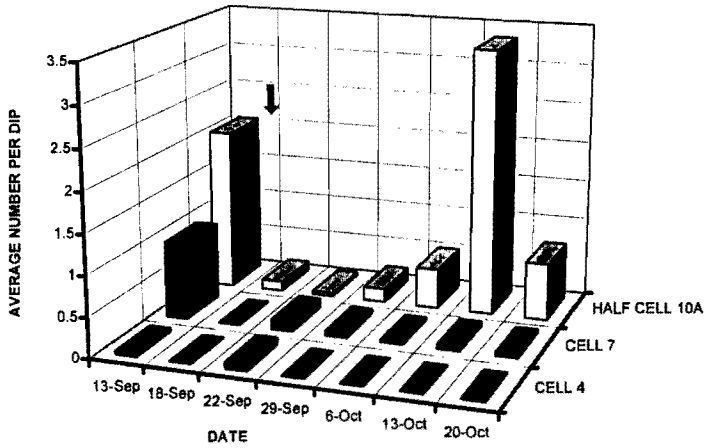

Figure 2. Pre- and post-treatment counts for cells 4, 7 and half cell 10A. Arrow indicates treatment date (September 15).

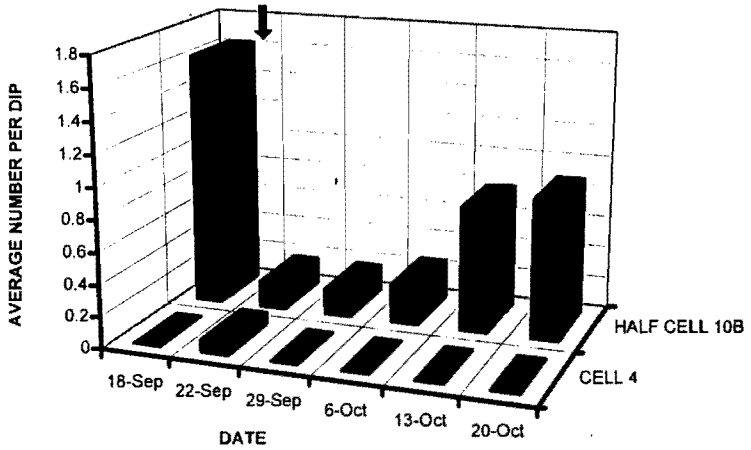

Figure 3. Pre- and post-treatment counts for cell 4 and half cell 10B. Arrow indicates treatment date (September 19).

Table 1. Water chemistry data from cells treated with VectoLex G. Measurements taken on September 15, 1995 for cell 7 (A and B) and half cell 10A; September 19 for half cell 10B.

	7A	7B	10A	10B
Temp. (°C)	18.7	20.7	18.7	32.1
pH	7.78	7.37	7.38	7.27
Conductivity (μohms)	643	643	650	573
TDS (mg/l)	317	318	328	286
DO (mg/l)	0.7	0.5	0.7	0.4

DISCUSSION

Environmental, physical, and biological factors may have played a key role in the control and residual activity that was observed. The water inflow at approximately 70 gallons per minute per cell followed the path of least resistance which was along the vegetation free margins of the cells. The basically static water condition in the interior of the vegetative stand with an average depth of six inches may have been favorable for the recycling of *B. sphaericus*. Measured temperatures of the secondary effluent was adequate in promoting infectivity to the mosquito larvae and the amount of total dissolved solids within the water column did not seem to be a detrimental factor in nullifying the efficacy of the product.

Decomposing cadavers may have been trapped within the filamentous algae near the water surface thereby facilitating spore dispersal within the feeding column and lower numbers of gravid female mosquitoes from the background population late in the season may have contributed to the seemingly prolonged residual activity noted during the September 15 and 19 trials.

ACKNOWLEDGEMENTS

The authors wish to thank Chuck Williams and his staff at the Sacramento Regional Wastewater Treatment Plant for their cooperation and in providing the test plots. We also thank Dr. Brian Melin and Abbott Laboratories for providing the experimental use permit and the VectoLex G which made this project possible.

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MONITORING POTENCY OF *BACILLUS THURINGIENSIS* serotype H-14 LIQUID FORMULATIONS

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Formulations of *Bacillus thuringiensis* serotype H-14 (*B.t.i.*) De Barjac that are commercially available constitute a blend of fermented batches with differing quantities of the bacterial active ingredient. Each batch that is produced is bioassayed to determine the potency of the active ingredient. Batches with lower potencies are mixed in proportion with higher batches to produce the final commercial product that approximates the stated International Toxic Units (I.T.U.) on the insecticide label. Every commercial batch is then assigned a Lot Number as a means of quality control. This blending results in commercial formulations with varying potencies among manufacturers and different Lot Numbers from a manufacturer.

This operational note covers a method of comparing the relative potency of *B.t.i.* liquid formulations.

MATERIALS AND METHODS

Before taking an aliquot from the insecticide container, the contents should be well agitated to obtain a uniform suspension (this may require rolling the 5- or 30-gallon container back and forth on the ground). A 5-ml pipette is used to draw 2-3 ml of the *B.t.i.* suspension which is then transferred to a sample vial. The vial is capped and labeled with the manufacturer's name and Lot Number for reference.

A simple way to compare product potencies is to perform a larval bioassay along the guidelines of Gillies and Womeldorf (1968). Twenty fourth instar larvae are placed in a bioassay cup containing 100 ml of dechlorinated tap water with a minimum of three replicates per concentration. As the actual International Toxic Units are unknown, the bioassay is treated as a field dosage rate. The dosage rates for a bioassay using dechlorinated tap water are much less than the actual field label rates as the bioassay is being performed under optimum conditions.

The surface area at the 100 ml water level in the bioassay cup has to be determined; in this case by using the formula for determining the area of a circle. The area calculated in square inches is converted to square feet and finally to part of an acre. Using the Sweetheart® 3 oz. ice cream and food cups, No. S303 (Sweetheart® Products Group), the surface area at the 100 ml meniscus is approximately 9.04^{-7} acre.

Once the surface area is known, the amount of liquid formulation required for a predetermined field dosage rate can be calculated. The range of concentrations used is 0.025 to 0.25 pt./acre (Table 1). A dosage lower than 0.025 pt./acre could be used if zero percent mortality is desired at the low end of the treatment range. Multiplying the surface area by the field dosage rates gives the amount of insecticide needed to achieve those dosage rates. The use of a low field dosage rate for the initial calculation makes it easier to dispense the finished formulation through the bioassay series using a 1 ml in 1/100 pipette. In this case, multiplying 9.04^{-7} acre by 0.05 pt./acre yields 4.5^{-8} pint per test cup at the 0.05 pt./acre dosage rate. Converting 4.5^{-8} pint to microliters yields the equivalency of 0.0213 microliter of *B.t.i.* per test cup at the 0.05 pt./acre dosage rate. Therefore, selecting 0.1 ml of stock solution to be pipetted to contain 0.0213 µl of actual commercial preparation, the dose series is then determined. If 0.1 ml of stock solution is equivalent to the 0.05 pt./acre field dosage rate, then 0.2 ml of stock equals 0.10 pt. acre, and so on down the line.

The final step is to determine the amount of mixture required to perform the bioassay. This is done by totaling the amount of stock solution dispensed and the number of replicates per dose. Always make more stock solution than is required to

Table 1. Amount of stock solution pipetted with equivalent *B.t.i.* formulation used and field dosage rates.

Stock solution (ml)	*B.t.i.* formulation (µl)*	Field dosage rate equivalency (pt./acre)
0.05	0.0106	0.025
0.10	0.0213	0.05
0.20	0.0427	0.10
0.30	0.0639	0.15
0.40	0.0852	0.20
0.50	0.1065	0.25
0	0	0 (Control)

* Rounded off to the nearest ten-thousandth.

prevent pipetting of air during the final dose treatments. Twenty milliliters of finished formulation is more than sufficient to do the bioassay using the recommended concentration range with three replicates per dose. Agitate the liquid *B.t.i.* commercial formulation in the sample vial before drawing into a micropipette. The stock solution is made by adding 4.26 µl of the commercial formulation to be tested to 20 ml of dechlorinated tap water in a beaker and swirling the contents to obtain a uniform mixture. The addition of 4.26 µl of commercial formulation to 20 ml water yields a mixture of 0.213 µl of the commercial formulation per ml or 0.0213 µl per 0.1 ml of stock solution.

The stock solution should always be swirled prior to pipetting to ensure a uniform mixture is being used. Tilt the beaker and pipette from the middle of the stock solution before dispensing the required solution amounts to the bioassay cups.

After all the test cups have been treated (except for controls), the cups are covered with a cafeteria tray to keep the larvae from being disturbed by external activity. Mortality counts are taken 24 hours post-treatment and duly recorded and filed for comparison with other similar formulations.

DISCUSSION

The results obtained from this bioassay methodology is limited in that it can only be used to compare other liquid formulations. It may be useful in testing the potency of a similar formulation that has been in storage and unused for a number of years in comparison with fresh stock.

Observed larval mortality in the test cups is not synonymous with actual field mortality as the laboratory bioassay is performed under optimum conditions. Differences in field water quality and the presence of other filter feeders which may affect the outcome is not being tested. Neither does the observed mortality serve as a baseline for performing susceptibility bioassays as the actual International Toxic Units of the stock solution remain unknown.

The most important factor in working with any type of suspension is agitation. Whether dealing with wettable powders or microencapsulated products, agitation is essential to obtain a uniform mixture prior to taking an aliquot or during the test procedure to ensure that the tested sample remains true to the packaged product.

ACKNOWLEDGEMENTS

Thanks goes to Tom Ehrke of Sacramento/Yolo MVCD who notified me about each new shipment of *B.t.i.* that was delivered and helped agitate the *B.t.i.* formulations in the 30-gallon containers and to Mac Thompson of CDHS-VBDS who reviewed this article.

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POPULATION DYNAMICS OF MOSQUITO VECTORS ASSOCIATED WITH STONE LAKES WILDLIFE REFUGE

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ABSTRACT

A mark-release-recapture study of *Culex tarsalis* was conducted on Stone Lakes Wildlife Refuge in order to determine whether mosquito populations and western equine encephalomyelitis activity associated with a developing wetland habitat might impact surrounding communities. Mosquito populations and virus activity were also monitored and compared with data from a previous study. The results indicated a flight range sufficient to reach nearby inhabited areas as well as considerable virus transmission.

There has been a trend toward wetland development for the express purpose of attracting and providing habitat for migrating waterfowl and other wildlife in California. This is one of the overall goals of the recently established Stone Lakes Wildlife Refuge (SLWR). The refuge will one day extend from Upper Beach Lake at its northern boundary to Twin Cities Road as its southern boundary. When implemented, restoration of this property will involve conversion of approximately 18,000 acres of dry grassland and irrigated pastures to permanent and seasonal marshes, vernal pools and riparian woodlands. Unfortunately, this practice has resulted in the production of enormous numbers of mosquitoes on other refuges (Garcia and Des Rochers 1984) and studies have correlated increases in mosquito density with disease transmission (Olson et al. 1979). Furthermore, wildlife refuges have the potential to provide habitat that is favorable to both the host and vector species. Isolations of western equine encephalomyelitis (WEE) have been made from *Aedes melanimon* Dyar and black-tailed jack rabbits at Gray Lodge Wildlife Refuge as well as from *Culex tarsalis* Coquillett and finches (Hardy 1987).

Casual sampling of mosquitoes in the Stone Lakes area revealed the presence of a number of potential pest and vector species such as: *Culex tarsalis*, *Cx. pipiens* L., *Cx. erythrothorax* Dyar, *Culiseta inornata* (Williston), *Anopheles freeborni* Aitken, *An. franciscanus* McCracken, *Ae. melanimon* and *Ae. vexans* (Meigen) (Stan Wright pers. comm.). Wildlife surveys included potential primary and secondary hosts like: house finch, American goldfinch, black-tailed jack rabbit, mourning dove and other passerine species (E.A. Engineering, Science and Technology. Stone Lake Resource Analysis 1989-90). The question of whether the above factors would be present in adequate numbers and interact with one another to produce a pest and disease problem on this refuge provided impetus for the first year of research on Stone Lakes.

Our data suggested that the refuge may serve as a significant source of western equine encephalomyelitis (WEE) virus and its primary vector, *Cx. tarsalis* (Dritz et al. 1993). In view of this, and the fact that SLWR is located in close proximity to residential communities, a mark-release-recapture (MRR) study was conducted in the second year of research to determine whether mosquito populations originating on the refuge pose any concern to humans and animals in surrounding areas. Those results plus data on mosquito abundance, diversity and WEE transmission are reported herein.

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MATERIALS AND METHODS

Two separate flight studies were planned, the first utilizing wild caught adult mosquitoes and the second consisting of colony reared, known age mosquitoes. *Cx. tarsalis* was selected because it is a known WEE vector and it made up 54.9% of the species captured in EVS traps (Figure 1). A total of 10 dry ice-baited EVS traps were placed on SLWR near each of the sentinel chicken flocks (Figure 2) to collect material for the first release. However, due to insufficient numbers, we decided to utilize collection sites in Sacramento and Yolo Counties where *Cx. tarsalis* densities were higher. A total of five CDC and 18 EVS traps baited with dry ice were run for two nights. Collections were returned to the laboratory daily and sorted by sex and species into 3.8 liter cartons for estimation of numbers (Dow et al. 1965). Following removal of a sub-sample for laboratory longevity studies and dissection, adults were counted, transported to the refuge, marked with florescent dust and released. The cartons were allowed to remain in the field overnight and collected the following morning at which time any remaining adults were subtracted from the release total.

Nineteen dry ice-baited EVS traps were placed on and around the refuge with the release site at the center for the recapture portion of the study. Because dispersal was greater than expected, two additional traps were added on the third day for a total of 21 traps. Collections were conducted daily for ten days. Following the release, trap contents were examined under a black light for the presence of marked individuals and the number, distance and direction were tabulated. All mosquitoes were identified to species and number and sex were recorded.

Standard EVS trapping was conducted on a weekly basis before and after the release utilizing ten traps placed in the same locations used in our previous study (Dritz et al. 1993). In addition, ten flocks of ten sentinel white leghorn chickens (Figure 2) were bled bimonthly for antibodies to WEE.

RESULTS AND DISCUSSION

A total of 10,845 female *Cx. tarsalis* were marked. Of these, 1,611 remained in the cartons and were counted as dead resulting in an actual total release of 8,474 mosquitoes. Marked individuals were collected in traps located at both the 0.5 and 1.0 km points on the first trap night. Maximum dispersal was at least 3 km and was achieved by the third day, suggesting a flight range of 1.0 km per day. It was suspected that dispersal distance could have been greater but reduced longevity of the adult population prevented confirmation of this hypothesis. Sub-samples of mosquitoes from the release group which were held in the laboratory at room temperature with sugar wicks and under wet paper towels died six days into the release and it is

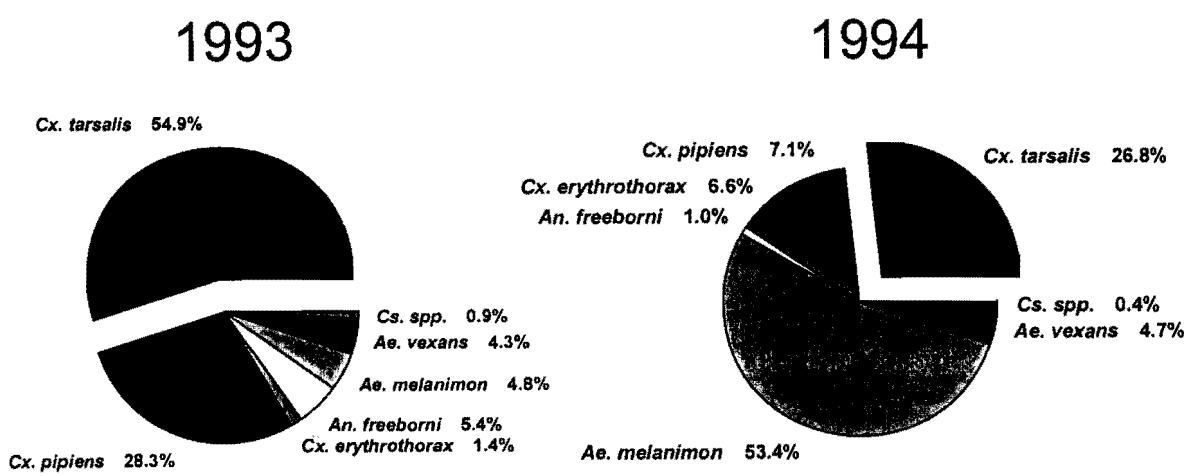

Figure 1. Pooled collection results from dry ice-baited EVS traps operated from June 28 to September 20, 1993 and June 21 to September 22, 1994 on and adjacent to Stone Lakes Wildlife Refuge, Sacramento, CA.

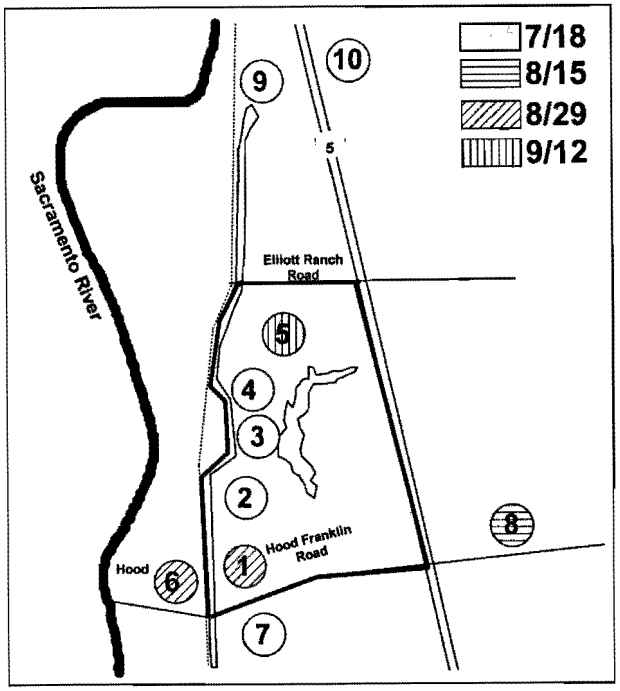

Figure 2. Map of the study area around Stone Lakes Wildlife Refuge illustrating perimeter, sentinel chicken flock locations and spatial and temporal progression of seroconversions to western equine encephalomyelitis.

possible field longevity was similar as no marked individuals were recovered after that time. Attempts to retrieve marked specimens which might have blood fed and been resting on and around the refuge also failed.

Dissections of a sub-sample of 46 individuals from the release population using the tracheal skein method (Detinova 1962) showed all to be parous, indicating mosquitoes were older at the time of release and further supporting the idea that death may have occurred before maximum flight range was achieved. Despite these potential drawbacks, it was concluded that even with a 3 km maximum flight range, mosquitoes originating in the center of Stone Lakes Wildlife Refuge could easily impact the communities of Hood, Merrit Island, Freeport and planned developments on the eastern side of Interstate 5.

An examination of the data on the composition and numbers of native mosquito populations revealed why there was difficulty obtaining sufficient *Cx. tarsalis* specimens for the MRR on site. Although similar numbers of adult mosquitoes were collected overall in 1994 (n = 7,532) as compared to 1993 (n = 6,920), the species composition was markedly different. The relative percentages (Figure 1) illustrate a shift in principal species from *Cx. tarsalis* to *Ae. melanimon*. It is interesting to note however, that *Ae. melanimon* is also a vector of WEE and comprised over half the mosquito population in 1994 during which there was a significant amount of virus transmission detected at Stone Lakes for the second consecutive year (Figure 2). Unfortunately, no samples of this species were collected for WEE testing so any relationship to virus activity remains unconfirmed. In contrast, a comparison of peak host-seeking activity of *Cx. tarsalis* with the date of the first WEE isolation from a mosquito pool (Figure 3) suggests an association between mosquito population density and virus for this species.

The second release was to be composed of laboratory reared specimens of three strains of Cx. tarsalis (wild caught, Breckenridge 80 and Kaiser) to compare survival and flight range in the field. Although a cohort of over 5,900 mosquitoes was successfully reared, the study had to be canceled after sentinel chicken seroconversions necessitated adulticiding operations on the refuge.

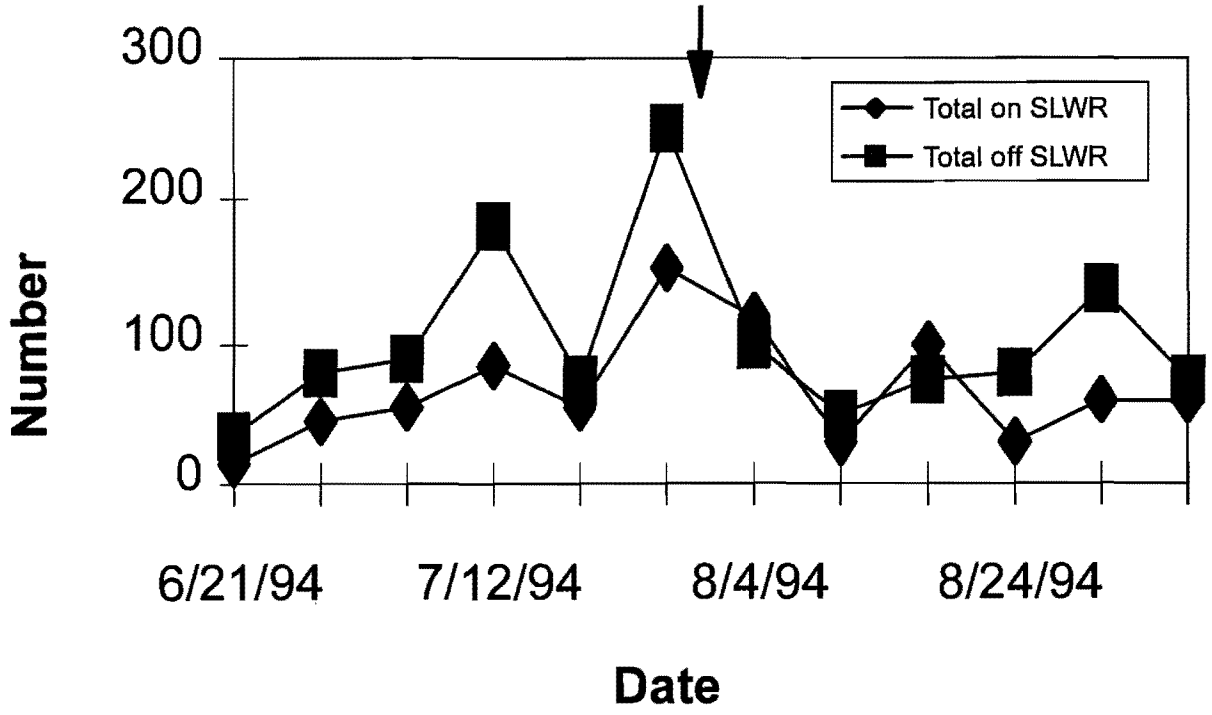

Figure 3. Carbon dioxide trap collections of *Culex tarsalis* illustrating the relationship between peak mosquito populations and first virus isolation (arrow) from a mosquito pool associated with the Stone Lakes Wildlife Refuge.

It is evident from the data that adult mosquito movements can easily exceed the boundaries of Stone Lakes Wildlife Refuge even from a central location. This is of particular concern because SLWR is located near an urban area and on a major transportation corridor around which further residential and commercial developments are planned. Considering the fact that past studies have demonstrated a strong relationship between increased water and greater pest and disease problems (Reeves 1968), the probability is great that further development of the wetland habitats both on SLWR and elsewhere will significantly impact the operations of mosquito abatement districts and public health agencies. In view of that, further research will be required on currently utilized control materials to elucidate non-target impacts so that concurrent protection of public health, quality of life and restoration of wetland habitats can be achieved.

ACKNOWLEDGEMENTS

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Descriptive and Operational Statistics from Member Agencies for 1995

The following tables summarize the various statistics and operational activities of the member agencies of the Mosquito and Vector Control Association of California. The information was collected by the Association's Chemical Control Committee.

Use of Aircraft in California Mosquito Control in 1995

Aerial Application of Liquids

Agency	AircraftType	Owned Aircraft				Contracted Aircraft			
		Hours	$/Hour	Acres	$/Acre	Hours	$/Hour	Acres	$/Acre
Alameda County MAD	Hiller Helicopter					2.10	600.00	400	3.40
Butte County MVCD	Gruman Ag Cat	138.26	277.00	40,614	0.94				
East Side MAD	Cessna AG TR	74.80	970.00	15,090	4.81				
Fresno Westside MAD	Piper PA	164.24	342.15	14,565	3.66				
Kern MVCD	Thrush T-34	254.00	700.00	40,845	4.20				
Marin/Sonoma MVCD	Hiller Helicopter					13.60	600.00	3,963	2.06
Merced County MAD	Cessna 188-B	161.40	338.61	39,511	1.38				
Napa County MAD	Helicopter					11.00	550.00	1,348	4.50
No. Salinas Valley MAD	Bell Jet Ranger					37.20	850.00	4,043	7.82
Sacramento/Yolo MVCD	Fixed Wing					190.10	425.00	57,404	1.69
San Joaquin County MVCD	Helicopter					72.00	600.00	18,403	4.50
San Mateo County MAD	Helicopter					7.90	600.00	3,960	7.39
Santa Clara County VCD	Helicopter					6.00	600.00	905	6.36
Santa Cruz MVCD	Helicopter					5.20	900.00	330	14.18
Solano County MAD	Helicopter							694	12.72
Sutter-Yuba MVCD	Cessna Ag Husky					84.00	300.00	93,933	0.40
Tulare MAD	PA 235	72.00	340.00	2,600	8.65				
West Valley VCD	Helicopter					58.00	400.00	4,000	10.00

Aerial Application of Granules

Agency	Aircraft type	OwnedAircraft				Contracted Aircraft			
		Hours	$/Hour	Acres	$/Acre	Hours	$/Hour	Acres	$/Acre
Butte County MVCD	Gruman Ag Cat	0.75	277.00	195	1.06				
Lake County VCD	Weatherly							2,407	4.75
Sacramento/Yolo MVCD	Fixed wing					24.50	425.00	3,579	3.12
San Joaquin County MVCD	Helicopter					8.00	600.00	750	6.40
Santa Cruz MVCD	Helicopter					1.00	900.00	30	30.00
Solano County MAD	Helicopter							1,813	3.50

Source Reduction and Public Information Efforts in 1995

	Source Reduction			Public Information Efforts							
	Direct*	Indirect**		Classrms	Talks	Media	Exhibits or fairs		Interviews given		
Agency	Acres	Acres	Sources	visited	given	events	Number	Days	Television	Radio	Newspaper
Coastal											
Alameda County MAD	380		1	9	2	5	8	12	11	6	23
Contra Costa MVCD	300			26	13	0	14	30	3	2	10
Marin-Sonoma MVCD			57	223	6	0	3	6	3	5	8
Napa County MAD	313										1
N. Salinas Valley MAD				190	10	1	3	30	3	1	2
San Mateo County MAD		38	2	12			8	16		1	2
Santa Clara County VCD	250		20	13	45	1	12	20	7	3	10
Santa Cruz MVCD				4	3				1	1	5
Solano County MAD					2		1	3	2	1	2
Subtotal	**1,243**	**38**	**80**	**477**	**81**	**7**	**49**	**117**	**30**	**20**	**63**
Sacramento Valley											
Burney Basin MAD											
Butte County MVCD				60	20		2	7	5	8	3
Colusa MAD				2	1			1		1	5
El Dorado Co. V.C.-CSA3				2	4		2	3		2	4
Lake County MAD	100			16	4					2	2
Pine Grove MAD					1						1
Sacramento-Yolo MVCD	92	32	1	196	1	4	10	15	1	1	2
Shasta MVCD	100			10	9	2	1	15	4	2	18
Sutter-Yuba MVCD				5	2	1			1	4	5
Tehama County MVCD		15	1	2	1	1	1	10	1		3
Subtotal	**292**	**47**	**2**	**293**	**43**	**8**	**16**	**51**	**12**	**20**	**43**
North San Joaquin											
Merced County MAD	233	2	2	101			4	18	***744		2
San Joaquin County MVCD	905			10	3		2	8	2	1	5
Subtotal	**1,138**	**2**	**2**	**111**	**3**	**0**	**6**	**26**	**746**	**1**	**7**
South San Joaquin											
Coalinga-Huron MAD											
Consolidated MAD				12	4	1	2	16	4		2
Fresno MVCD	150			14		1	2	20	3	1	1
Fresno Westside MAD				1			1	5			
Kern MVCD		200	17	3	3	1	0		3	3	2
Madera Co. MVCD	2,000	640	22			1			1	2	3
Tulare MAD	15										1
West Side MVCD	10	40	1	6	2		2	4			
Subtotal	**2,175**	**880**	**40**	**36**	**9**	**4**	**7**	**45**	**11**	**6**	**9**
Southern											
Antelope Valley MVCD			36				1	20			1
Carpinteria/Goleta Vy VCD	1,000		150		3	1					1
Coachella Valley MVCD		10	71	4	12	1	2	50	7	4	6
Compton Creek MAD		5	50	15	3	1	1	2	1		2
Greater L.A. Co. VCD VCD	45	10	151	170	7		11	51	9	5	10
Moorpark MAD		5	8	1			1	4			3
Northwest MVCD	2,000	1,000	165	5	10						25
Orange County VCD			275	247	25	2	2	21			6
Owens Valley MAP	300						1	20	2	5	2
San Bernardino Co. VCP	110	21	1,396	58			2	16	3	3	10
San Gabriel Valley MAD				6	15		9	23	6	1	13
West Valley VCD		5	9	2	1		3		1		2
Subtotal	**3,455**	**1,056**	**2,311**	**508**	**76**	**5**	**33**	**207**	**29**	**18**	**81**
TOTAL	**8,303**	**2,023**	**2,435**	**1,425**	**212**	**24**	**111**	**446**	**828**	**65**	**203**

*Accomplished directly by the agency

** Accomplished indirectly through the use of request, notices or proceedings

*** Cable TV Public Information Announcement running 31 days, 24 times per day

Use of Mosquitofish in 1995

Agency	No. of Sources	Number of Acres Stocked with Mosquitofish					Total Acres
		Rice	Pasture	Ditches	WL Refuges	Other	
Coastal Region							
Alameda County MAD	2,631						
Contra Costa MVCD	164						
Marin/Sonoma MVCD	444						
Napa County MAD	204			60			60
No. Salinas Valley MAD	235					97	97
San Mateo County MAD	203						
Santa Clara County VCD	335					25	25
Santa Cruz MVCD	17					3	3
Solano County MAD	60					1,100	1,100
Subtotal	**4,293**	**0**	**0**	**60**	**0**	**1,225**	**1,285**
Sacramento Valley Region							
Burney Basin MAD	10					850	850
Butte County MVCD	2,190				1,265	278	1,543
Colusa MAD	200			80	800		880
Lake County VCD	35	800		6			806
Pine Grove MAD	15	2,000		10			2,010
Sacramento/Yolo MVCD	2,532	5,204		1,163		1,369	7,736
Shasta MVCD	350			100			650
Sutter-Yuba MVCD	114	5,447	7	136	40	282	5,912
Tehama County MVCD	40	2,000		20		40	2,060
Subtotal	**5,486**	**15,451**	**7**	**1,515**	**2,105**	**2,819**	**22,447**
North San Joaquin Region							
Merced County MAD	12						
San Joaquin County MVCD	100	1,500		100	2,000		3,600
Subtotal	**112**	**1,500**	**0**	**100**	**2,000**	**0**	**3,600**
South San Joaquin Region							
Consolidated MAD	85		18	19		1,523	1,560
Fresno MVCD	648				300	150	450
Fresno Westside MAD	6	5,482	1				5,483
Kern MVCD	4,690			25		3,000	3,025
Madera Co. MVCD	166		800	600			1,400
Tulare MAD	12						
West Side MVCD	50		200	50		1,500	1,750
Subtotal	**5,657**	**5,482**	**1,019**	**694**	**300**	**6,173**	**13,668**
Southern Region							
Antelope Valley MVCD	17			6		125	131
Carpinteria MAD	26					50	50
Goleta Valley VCD	100					200	200
Coachella Valley MVCD	41						
Compton Creek MAD	12			4		1	
Greater L.A. Co. VCD	853			15		7	22
Moorpark MAD	100		4	7			11
Northwest MVCD	230			2		272	274
Orange County VCD	1,422					189	189
Owens Valley MAD	25		200	50			250
San Bernardino Co. VCP	270			1		2	3
San Gabriel Valley MAD	91					10	10
West Valley VCD	678						5
Subtotal	**3,865**	**0**	**204**	**85**	**0**	**855**	**1,144**
TOTAL	**16,226**	**22,433**	**1,026**	**2,369**	**4,405**	**10,217**	**41,005**

Insecticides used for Control of Mosquito Larvae in 1995

AGENCY	GB-1111*	Bti Liq.*	Bti Gran.	Bti Pel./Br.	Bti Tech.	Malathion	Methoprene	Dimilin	Pyrethrin
Coastal Region									
Alameda County MAD	3,183	62	2,980				10		
Alameda County VCSD									
Contra Costa MVCD	6,358	13	6				29	1	
Marin-Sonoma MVCD	1,741	1,175	9,279				8		
Napa County MAD	571	183			224		8		
N. Salinas Valley MAD	3,206	493		87			3		
San Mateo County MAD	2,408	56					101		
Santa Clara County VCD		264					34		
Santa Cruz MVCD	1,567	7	449				5		
Solano County MAD	1,281	186	3,683		1		32		
Sub total	**20,315**	**2,439**	**16,397**	**87**	**225**	**0**	**230**	**1**	**0**
Sacramento Valley Region									
Burney Basin MAD	38		4,463						
Butte County MVCD	1,290	1,574					2		
Colusa MAD	55								
Durham MAD									
El Dorado Co. V.C.-CSA3		5	64						
Glenn County MVCD									
Lake County MAD	6	1	24,070				1		
Pine Grove MAD		4							
Sacramento-Yolo MVCD	13,307	8,661	420				471		
Shasta MVCD	404	74	6,590				5		
Sutter-Yuba MVCD**	1,261	2,959					22	9	
Tehama County MVCD	375	17							
Sub total	**16,736**	**13,295**	**35,607**	**0**	**0**	**0**	**501**	**9**	**0**
No. San Joaquin Valley Region									
East Side MAD	13,262	17				225	39		
Merced County MAD	9,465	278					393		
San Joaquin County MVCD	18,846	1,893	10,850				244		
Turlock MAD	16,287	166					50	17	
Sub total	**57,860**	**2,354**	**10,850**	**0**	**0**	**225**	**726**	**17**	**0**
So. San Joaquin Valley Region									
Coalinga-Huron MAD									
Consolidated MAD	9,895	212			183		162		
Delano MAD	80	69					1		
Delta VCD	9,454	175	585	29	43		67		
Fresno MVCD	3,887	35	310		71		32		
Fresno Westside MAD	473	1,308					59		
Kern MVCD	40,227	8,046			10	4	169		
Kings MAD	35,760	26					51		
Madera County MVCD	17,000	424					25		
Tulare MAD	29,545						27		
West Side MVCD	7,252	2,829	95				1		20
Sub total	**153,573**	**13,124**	**990**	**29**	**307**	**4**	**594**	**0**	**20**
Southern California Region									
Antelope Valley MVCD	111	6					1	80	22
Carpinteria MAD-Goleta Vy VCD	100	3			23		100		
Coachella Valley MVCD	598	453	9,960				9		
Compton Creek MAD	48			76					
Greater L.A. County VCD	9,117	718	640				122	405	
Los Angeles Co. W. VCD	116	55	104	1,419			8		5
Moorpark MAD	39		181	1					
Northwest MVCD	6,806	6	1,431				2		
Orange County VCD	4,854		50,795	6,920			772		
Owens Valley MAP	877				757		4		Trace
San Bernardino Co. VCP	1	5		197			2		4
San Gabriel Valley MAD	310	9	190				1		
West Valley VCD	5,149			1		4	4		Trace
Sub total	**28,126**	**1,255**	**63,301**	**8,614**	**780**	**4**	**1,025**	**485**	**31**
Total	**276,610**	**32,467**	**127,145**	**8,730**	**1,312**	**233**	**3,076**	**512**	**51**

*Usage in gallons, others are in pounds. **Sutter-Yuba MVCD used 34.59 lbs. of chlopyrifos granules. West Valley VCD used 6.2 lbs. of chlorpyrifos
Combined use of various other larvicides was less than 500 pounds among all agencies reporting.

Insecticides for Control of Mosquito Adults in 1995*

AGENCY	Malathion	Pyrethrin	Permethrin	Resmethrin	Dursban	Baygon	Tempo 20WP
Coastal Region							
Alameda County MAD	NR						
Alameda County VCSD	NR						
Contra Costa MVCD		Trace					
Marin-Sonoma MVCD		31		Trace			
Napa County MAD		19		1			
N. Salinas Valley MAD		Trace					
San Mateo County MAD		1					
Santa Clara County VCD				NR			
Santa Cruz MVCD		Trace					
Solano County MAD		44	Trace				
Subtotal	**0**	**83**	**0**	**1**	**0**	**0**	**0**
Sacramento Valley Region							
Burney Basin MAD			51	1			
Butte County MVCD	2,471	188	18	13	6	23	
Colusa MAD	10,161	80	70	3			
Durham MAD		8					
El Dorado Co. V.C.-CSA3		Trace					
Glenn County MVCD	1,019	7	198				
Lake County MAD	17	27	21				
Pine Grove MAD		5	34				
Sacramento-Yolo MVCD	103	163					
Shasta MVCD	432	30	37	14			
Sutter-Yuba MVCD	6,802	141	198				26
Tehama County MVCD	327	4	4				
Subtotal	**21,332**	**653**	**595**	**31**	**6**	**23**	**26**
No. San Joaquin Valley Region							
Merced County MAD	134	119					
East Side MAD	7,190	71					
San Joaquin County MVCD	1,302	265	9	122			
Turlock MAD	240	58					
Subtotal	**8,866**	**513**	**9**	**122**	**0**	**0**	**0**
So. San Joaquin Valley Region							
Coalinga-Huron MAD	NR						
Consolidated MAD	67	8		3			
Delano MAD				Trace			
Delta VCD		1					
Fresno MVCD		Trace					
Fresno Westside MAD		1		3			
Kern MVCD		22		3			
Kings MAD	8,608	7	27				
Madera County MVCD		43					
Tulare MAD		7					
West Side MVCD	NR						
Subtotal	**8,675**	**89**	**27**	**9**	**0**	**0**	**0**
Southern California Region							
Antelope Valley MVCD	NR						
Carpinteria MAD-Goleta Vy VCD	NR						
Coachella Valley MVCD		48					
Compton Creek MAD	NR						
Greater L.A. County VCD	NR						
Los Angeles Co. W. VCD	NR						
Moorpark MAD	NR						
Northwest MVCD			23	6			
Orange County VCD		Trace		145			
Owens Valley MAP		38		30			
San Bernardino Co. VCP				3			
San Gabriel Valley MAD	NR						
West Valley VCD		Trace					
Subtotal	**0**	**86**	**7**	**180**	**0**	**0**	**0**
Total	**38,873**	**1,424**	**638**	**343**	**6**	**23**	**26**

*Pounds of active ingredient

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