

# PROCEEDINGS AND PAPERS

of the

Sixty-First Annual Conference of the

California Mosquito and Vector Control Association, Inc.

January 24 thru January 27, 1993

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- Pages in Bold Print Indicate Principal Authorship -

# California Mosquito and Vector Control Association, Inc.

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Volume 61

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## CONFERENCE DEDICATION

### DEDICATION OF THE 61ST ANNUAL C.M.V.C.A. CONFERENCE TO JOHN C. COMBS

Gilbert L. Challet

Orange County Vector Control District  
P.O. Box 87  
Santa Ana, California 92702

A year ago we left the Annual Conference with a vision of and a plan of action for legal, legislative and political action that was provided mainly by John Combs. John was our visionary. John died on August 24, 1992. He was many things to this Association and is being awarded at this conference the California Mosquito and Vector Control Association Meritorious Service Award for his work. Only two other people have received this award in the history of the California Mosquito and Vector Control Association (CMVCA). His community and association work is what I want to tell you about now.

John was described in the *Visalia Times-Delta* as being remembered as a man with a passion for Visalia, a strong commitment to his work and a genuine interest in others. John was a native of Visalia, his family having lived there for four generations. His great grandfather built the first house in Visalia. John served Visalia well. He was chairman of the Visalia Historic Preservation Committee, member of the Visalia Affirmative Action Advisory Committee, the Agricultural Industrial Committee for Visalia's General Plan and served on Visalia's Planning Commission since 1983.

I think most of all John will be remembered for helping people. He was a mentor to a number of young people in Visalia. He helped them in their careers and political campaigns. Several have been

city council members in Visalia. Many young people were hired by the District as summer workers so that John could help them.

One of my fond memories of John was his ability to come up with a quotation from a famous person to fit the occasion. I heard one of our politicians the other day give this quote and I thought that it personified John Combs. The quote is from Jack Kemp.

"People don't care how much you know  
until they know how much you care."

John brought to the CMVCA a vision for the future at a time when it was sorely needed. He was a leader, a communicator, an astute politician and a visionary. The future of the CMVCA was, in his vision, as a strongly scientific and politically active Association. We will miss his guidance and vision.

The specific accomplishments of John Combs in California vector control are as follows:

1. He was Executive Director of the CMVCA from July 27, 1984, to January 14, 1991, while he was Manager of the Delta Vector Control District. The difficulty of these additional duties was exacerbated by the office and support for the CMVCA being located in Sacramento while John was employed in Visalia.

2. John called for the first ever retreat for Managers and Trustees to discuss, agree and adopt prioritized goals for the CMVCA. These goals help set the vision of the CMVCA in the future.
3. The employment of a full-time CMVCA Executive Director was made possible by John through the culmination of the setting of goals at the retreat, the increased CMVCA funding through doubling member agency dues and his personal lobbying efforts with the member agencies.
4. He led the establishment of the CMVCA Research Foundation, which he envisioned as a means to facilitate needed research to help member agencies and the CMVCA.
5. John made the arrangements and provided

the CMVCA effort to get in print the monograph entitled *The Epidemiology and Control of Mosquito-Borne Arboviruses in California, 1943-1987* by Professor W.C. Reeves, another recipient of the CMVCA Meritorious Service Award.

6. Lastly, John Combs tempered his vision of the future with the connections from our past. His historical perspective made us stop and realize the accomplishments of the pioneers of our profession and avoid the mistake of repeating past failures.

The thought that John was more active outside of his professional life is an accomplishment that should inspire all of his colleagues and friends.

We, the members of the California Mosquito and Vector Control Association, dedicate the 1993 Annual Conference to John C. Combs.



## SURVEILLANCE FOR ARTHROPOD-BORNE VIRAL ACTIVITY AND DISEASE IN CALIFORNIA DURING 1992

Richard W. Emmons, Michael S. Ascher, Dale V. Dondero, Barryett Enge,  
William K. Reisen<sup>1</sup>, Marilyn M. Milby<sup>1</sup>, Donald A. Eliason<sup>2</sup>, Robert A. Murray<sup>3</sup>,  
Jieyan Lin, Franklin Ennik<sup>4</sup>, Lucia T. Hui<sup>4</sup>, James L. Hardy<sup>1</sup>,  
Sally B. Presser<sup>1</sup>, William C. Reeves<sup>1</sup> and Larry Barrett<sup>3</sup>

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This brief report summarizes arboviral surveillance activities during 1992 and is the 23rd report to the California Mosquito and Vector Control Association (CMVCA) since 1969. The surveillance program involves cooperative efforts by many groups and individuals from local mosquito control agencies; the Arbovirus Research Program at the University of California at Berkeley; the California Mosquito and Vector Control Association; the CMVCA Research Foundation; county and local public health departments; the California Department of Food and Agriculture (CDFA); physicians and veterinarians throughout California; and three units of the California Department of Health Services- the Division of Communicable Disease Control, the Environmental Management Branch and the Viral and Rickettsial Disease Laboratory (VRDL) of the Division of Laboratories.

Announcements about the program and 27 weekly bulletins (April 30 - October 23) were distributed widely during the season to provide detailed surveillance data. Most recipients now receive these via facsimile transmission (FAX) and eventually all recipients of the bulletin should have FAX equipment to receive reports as quickly as possible. Information

is also available on the CMVCA's MosquitoNet computer bulletin board system. In addition to the weekly bulletins, positive findings were telephoned immediately to the agency which submitted the mosquito pools or sentinel chicken sera.

### Equine WEE Case.

One presumptive case of western equine encephalomyelitis (WEE) was detected in an Imperial County horse in early 1992, prior to the usual season.

On March 7, 1992, a three year old quarter horse mare living in Westmoreland, Imperial County, was euthanized following a 24-hour history of fever, paraparesis and obtundation. The carcass was rendered and the animal was not tested for rabies. A single serum sample was tested by the VRDL and was positive for WEE at titers of 1:32 and >1:512 by complement fixation (CF) and indirect fluorescent antibody (IFA), respectively. The horse had been owned by the same person for two years and had not been vaccinated during that period. The horse had travelled in Arizona and Imperial County. The serum was sent to the National Veterinary Services Lab in Ames, Iowa to test for eastern equine encephalomyelitis (EEE) antibody with the idea that the WEE

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antibody could be due to unrecorded immunization, since all equine encephalitis vaccines marketed in the U.S. are bivalent products containing WEE and EEE. The WEE titers were confirmed and the EEE titers were negative. These results are consistent with a natural infection with WEE acquired by this horse in California or Arizona in the early months of 1992.

#### **Human SLE Case #1.**

Two human cases of arboviral encephalitis were recorded in California during the 1992 season. Both cases were contracted within the state and were caused by St. Louis encephalitis (SLE) virus.

A 75-year old female resident of El Monte (Los Angeles County) became ill on September 2, 1992, with fever (103°F), headache, vomiting and disorientation. She was hospitalized on September 7 and discharged from the hospital on September 21 to receive care at home. A blood specimen collected September 12, was tested by a private medical laboratory in Cypress, California, and found to have an IgG titer of 1:128 and IgM titer of >1:320 for SLE by IFA tests. These results were confirmed by the VRDL by the same tests and a supplemental enzyme immunoassay (EIA) test that indicated a recent or current infection with SLE virus. Several attempts to obtain another blood specimen were unsuccessful. The patient had not travelled outside her area of residence for at least three weeks prior to the onset of illness.

#### **Human SLE Case #2.**

A 73-year old female resident of Oxnard (Ventura County) became ill on September 10, 1992, with fever and vomiting. On admission to the hospital, a medical examination revealed signs and symptoms of possible Guillan-Barre syndrome or viral encephalitis. Blood specimens collected 12 days and one month after illness onset and tested by the VRDL showed a rise in IgG antibody to SLE virus (1:1024 to 1:2048, respectively) by IFA. During the same period, her IgM antibody titer dropped from 1:128 to 1:32. An EIA test at the VRDL was interpreted as demonstrating a significant rise in SLE antibodies. The patient had not travelled outside Ventura County during the accepted incubation period for SLE.

#### **Mosquito Pools.**

Tests were done by the VRDL on 2,329 mosquito pools, containing 100,613 mosquitoes

(Table 1). Tests were limited this year to the four major species most important in amplifying SLE, WEE and California encephalitis (CE) viruses. Only one virus isolate was made by the VRDL - WEE from *Culex tarsalis* Coquillett collected in June 17, 1992, at Needles, San Bernardino County. Mosquitoes tested and viruses isolated by the Arbovirus Research Program, School of Public Health, University of California, Berkeley, are shown on Table 2.

#### **Sentinel Chickens.**

Sentinel chickens were located at 137 sites (including six in Nevada), representing most enzootic areas of the state. Serum samples were collected and tested for SLE and WEE antibodies twice a month from most flocks, from April 20 through November by the VRDL, and during the winter period for selected flocks in special study areas by the U.C. Berkeley Arbovirus Research Program. Sero-conversions occurred from June through December for SLE and mostly from June through August for WEE, with only two WEE conversions in the September-October period (Tables 3 and 4).

A study was carried out (reported separately in these Proceedings) on the feasibility of doing blood collections on filter paper, and this will be the routine method for the 1993 surveillance program.

#### **ACKNOWLEDGEMENTS**

We thank Sarah Ball and Atefeh Hosseini for special assistance in the testing of mosquito pools and chicken sera, and in data management. We are also grateful for the help of many other staff members of the Viral and Rickettsial Disease Laboratory, Environmental Management Branch and Communicable Disease Control Division of the California State Department of Health Services; the Arbovirus Field Station and the Arbovirus Research Unit, School of Public Health, University of California, Berkeley; participating local mosquito control agencies; local health departments; the California Department of Food and Agriculture; and physicians and veterinarians who submitted specimens from suspect clinical cases. We especially thank the California Mosquito and Vector Control Association for support needed for laboratory tests.

Table 1. Number of mosquitoes and mosquito pools tested by the VRDL for WEE, SLE and CE viruses during 1992, by mosquito control agency and species.

Agency	<i>Cx. tarsalis</i>		<i>Cx. pipiens</i> complex		<i>Cx. stigmatosoma</i>		<i>Ae. melanimon</i>		Totals	
	mosq.	pools	mosq.	pools	mosq.	pools	mosq.	pools	mosq.	pools
DLTA	572	13	126	3					698	16
EAFB	1,300	26							1,300	26
KERN	3,210	85							3,210	85
LACO	1,914	57	3,229	87	694	25			5,837	169
LAKE	973	20					150	3	1,123	23
LONG	7,464	155	12,721	259	339	8			20,524	422
LOSA	6,616	152	13,702	309	449	18			20,767	479
MADR	431	11	1,393	30			50	1	1,874	42
MERC	525	11							525	11
NWST	5,419	110	2,894	62	225	7			8,538	179
ORCO	1,928	45	7,245	180					9,173	225
SACR	5,697	119					200	4	5,897	123
SANB	1,583	39	11	1					1,594	40
SAND	2,585	71	33	1					2,618	72
SBOV	1,355	36	612	21	535	20			2,502	77
SHAS	372	9	59	1					422	10
SOUE	5,088	116	3,662	91	237	10			8,987	217
SUYA	1,022	32							1,022	32
TRLK	368	8	275	6			84	2	727	16
VENT	1,200	24	175	3					1,375	27
WVAL	800	19	950	19	150	3			1,900	38
<b>Totals</b>	<b>50,422</b>	<b>1,155</b>	<b>47,078</b>	<b>1,073</b>	<b>2,629</b>	<b>91</b>	<b>484</b>	<b>10</b>	<b>100,613</b>	<b>2,329</b>

Table 2. Number of mosquitoes and mosquito pools tested by the Arbovirus Research Laboratory during 1992 from the Coachella Valley and Imperial County study areas.

Species	Coachella Valley			Imperial County		
	mosq.	pools	isolates	mosq.	pools	isolates
<i>Aedes dorsalis</i>	134	6		655	26	
<i>Culex quinquefasciatus</i>	880	22	1 SLE	1,895	46	
<i>Culex tarsalis</i>	34,805	819	6 WEE, 5 SLE	18,117	428	14 WEE, 4 SLE
<i>Culex stigmatosoma</i>	18	1				
<i>Culiseta incidens</i>	4	1				
<i>Culiseta inornata</i>	1,061	41		28	3	
<b>Totals</b>	<b>36,902</b>	<b>890</b>		<b>20,695</b>	<b>503</b>	

Table 3. SLE seropositive chickens during 1992 by location and bimonthly interval.

COUNTY	LOCATION	CITY	END DATE OF INTERVAL								TOT				
			JUN 15 30	JUL 15 30	AUG 15 30	SEP 15 30	OCT 15 30	NOV 15	DEC 15						
IMP.	ARNIAZ	SEELEY		1	3	4		2			10				
IMP.	CHRISTOPHER	EL CENTRO				2					2				
IMP.	GRACEY	SEELEY				4		3			7				
IMP.	KRAMER	SEELEY				1					1				
IMP.	DERRICK	SEELEY				2					2				
L.A.	SANTA FE REC PK	IRWINDALE		1	4						5				
L.A.	SHORELINE RV PARK	LONG BEACH			1						1				
L.A.	EL DORADO PARK	LONG BEACH			1	1	3	1			6				
L.A.	HARBOR LAKES	WILMINGTON		3	4	1	1				9				
L.A.	LABREA TAR PITS	L.A.					1	3	1		5				
L.A.	CAL POLY UNIVERSITY	POMONA		1							1				
L.A.	WEST NEWMARK AVE	MONTEREY PARK		1		1	1				3				
L.A.	404 CITRUS	WEST COVINA			1	2	2				5				
L.A.	SEPULVEDA DAM	ENCINO				1	1				1				
ORANGE	BUENA PARK	IRV., FULLERTON					1				1				
ORANGE	20 RCH DUCK CLUB #1	IRVINE				1		1			2				
ORANGE	20 RCH DUCK CLUB #2	IRVINE				1		1			2				
RVRSIDE	SALTON SEA ST. PARK	N.SHORE	1	1	2	1	1			1	7				
RVRSIDE	GONZALEZ RANCH	THERMAL					2				2				
RVRSIDE	PABLO RANCH	INDIO			1	4	1				6				
RVRSIDE	ADOHR FARMS	MECCA			2	2					4				
RVRSIDE	FERNANDEZ RANCH	N.SHORE				1	5				6				
RVRSIDE	FOLEY RANCH	MECCA						1			1				
RVRSIDE	MACHUCA	MECCA				1					1				
RVRSIDE	DEX-O-TEX	MECCA		1	1						3				
RVRSIDE	JESSUP	VALERIE	4	4	1	1	1				12				
RVRSIDE	PRADO BASIN	CORONA					1				1				
VENTURA	HILL CANYON	THOUSAND OAKS					2				2				
FRESNO	KERMAN	KERMAN						1			1				
KERN	OILDALE	OILDALE				1					1				
KERN	MARICOPA	MARICOPA						1			1				
KERN	WIBLE & HOUGHTON RD	BAKERSFIELD							1		1				
MADERA	HARMON RANCH	CHOWCHILLA						3	1		4				
TULARE	KINGSBURG GC	KINGSBURG			1						1				
CHRCIL	MACARI LANE	FALLON	1								1				
Totals			6	6	5	7	8	20	15	27	9	12	2	1	118

Table 4. WEE seropositive chickens during 1992 by location and bimonthly interval.

COUNTY	LOCATION	CITY	END DATE OF INTERVAL							TOT				
			JUN 15 30	JUL 15 30	AUG 15 30	SEP 15 30	OCT 15 30	NOV 15						
IMP.	ARNIAZ	SEELEY	1	2	2					5				
IMP.	GRACEY	SEELEY		1	1			1		3				
IMP.	KRAMER	SEELEY		5	1					5				
IMP.	CHRISTOPHER	EL CENTRO	1	2						3				
IMP.	RIO BEND RV PARK	SEELEY	1	5	1					7				
L.A.	PALMDALE	PALMDALE			1					1				
L.A.	SEPULVEDA DAM	ENCINO			1					1				
L.A.	LANCASTER	LANCASTER			1					1				
RVRSIDE	MECCA FARMS	MECCA			1					1				
RVRSIDE	FOLEY RANCH	MECCA			1					1				
RVRSIDE	MACHUCA	MECCA	1							1				
RVRSIDE	ADOHR FARMS	MECCA			1					1				
RVRSIDE	JESSUP	VALERIE		1	2	1				4				
RVRSIDE	FERNANDEZ RANCH	N.SHORE	2	1	2	1				2				
RVRSIDE	SALTON SEA ST. PARK	N.SHORE	1	6	1					8				
SAN BER	SEWAGE TREAT. PT.	NEEDLES	1			3				4				
FRESNO	FRIANT ROAD	FRIANT				1				1				
TULARE	KINGSBURG GC	KINGSBURG			1					1				
CHRCIL	MACARI LANE	FALLON		1						1				
Totals			4	11	4	16	2	12	1	0	0	1	0	51

## ARTHROPOD-BORNE ENCEPHALITIS VIRUS SURVEILLANCE IN THE SAN GABRIEL VALLEY, LOS ANGELES COUNTY, DURING 1992

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### ABSTRACT

Arthropod-borne encephalitis virus surveillance in the San Gabriel Valley was conducted in 1992 by the San Gabriel Valley Mosquito Abatement District and the Los Angeles County Department of Health Services Vector-Borne Disease Surveillance and Entomology Programs (VDS). None of the 185 mosquito pools collected in the area contained encephalitis virus. Antibodies to St. Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) viruses were initially found in sera collected from feral birds on April 9 and July 29, respectively. SLE antibody positive sera were obtained from 4 of 5 sentinel chicken flocks throughout the valley; the first positive sera were collected on July 15. One SLE case was reported with the onset of illness on September 2. Mosquito abatement districts and the Los Angeles County Department of Public Works cooperated with VDS to minimize the creation of potential breeding sites.

In 1991, arthropod-borne encephalitis virus activity in the San Gabriel Valley of Los Angeles County was relatively high compared to that of previous years. One case of SLE was reported from a male who was exposed at the Santa Fe Dam Recreational Area (Fujioka et al. 1992). Fourteen of fifteen chickens (93%) from the sentinel flock located at Santa Fe Dam generated antibodies to St. Louis encephalitis (SLE) virus. At approximately the same time, a large population of *Culex tarsalis* Coquillett developed as a result of standing water impounded behind the dam after a water release by the Los Angeles County Department of Public Works. In addition, SLE virus was isolated from one pool of *Culex stigmatosoma* Dyar collected at California State Polytechnic University (Cal Poly) in Pomona.

As a result of this activity, surveillance efforts in 1992 were increased. In addition to the collection of mosquito pools and sentinel chicken sera for

encephalitis virus testing, mosquito populations were closely monitored at Santa Fe Dam and at Cal Poly. A system for testing sera from feral birds was developed as an early warning device. Also, the Los Angeles County Department of Health Services Vector-Borne Disease Surveillance and Entomology Programs (VDS) began working with the Los Angeles County Department of Public Works and mosquito abatement districts (MAD's) within the county to institute communications that would help to avoid the creation of potential breeding sites such as that at Santa Fe Dam in 1991.

### MOSQUITO COLLECTIONS

During the 1992 season, 20,499 mosquitoes were collected in the San Gabriel Valley. Collections included weekly trappings with CDC CO<sub>2</sub>-baited traps

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at the Santa Fe Dam Recreational Area in Irwindale and at California State Polytechnical University (Cal Poly) in Pomona. Mosquito populations in these areas were monitored due to previous viral activity. From the Santa Fe Dam, 7,388 mosquitoes were collected while 3,275 mosquitoes were collected from Cal Poly. The remaining mosquitoes (9,836) were collected at various areas in the San Gabriel Valley.

One hundred eighty-five mosquito pools were submitted for testing to the California State Department of Health Services Viral and Rickettsial Disease Laboratory. These included 96 pools (3,860 individuals) of *Culex quinquefasciatus* Say, 61 pools (1,758 individuals) of *Cx. tarsalis* and 28 pools (805 individuals) of *Cx. stigmatosoma*. There were no isolations of SLE or WEE viruses from these pools despite numerous seroconversions in the sentinel chicken flocks and feral birds.

To help avoid conditions such as those created at Santa Fe Dam in 1991, representatives from VDS, the Department of Public Works, and the various MAD's met in March, 1992, to discuss what could be done to avoid creating potential mosquito breeding sources. A meeting of this kind had never before taken place. It was agreed that the Department of Public Works would inform VDS of any water releases and remove vegetation in the Santa Fe Dam spreading grounds. The removal of vegetation decreased breeding areas and provided easier access for abatement activities. Once advised of the water releases, VDS would, in turn, notify the affected MAD. The MAD could then monitor the water levels, mosquito populations, and if necessary, treat larval populations.

#### SENTINEL CHICKEN SERA COLLECTION

Five flocks of sentinel chickens were located within the San Gabriel Valley during 1992. Flocks of 10 chickens each were located at Cal Poly, Santa Fe Dam and at Eaton Canyon Nature Center in Altadena. Additionally, flocks of five chickens each were placed in the Monterey Park City Yard in Monterey Park and at a residential site in West Covina. The chickens were bled every other week from April to November, 1992.

Antibodies to SLE virus were first detected in two sera collected July 15; one sample was collected from the Monterey Park flock and the other from Santa Fe Dam. Antibodies to SLE virus were then found in sera from flocks at Cal Poly and West

Covina on July 27 and August 11, respectively. Following the initial seroconversions, SLE virus activity within the sentinel chicken flocks continued. The final number of chicken sera positive for SLE virus antibodies include 1 of 10 chickens (10%) from Cal Poly, 3 of 5 (60%) from Monterey Park, 6 of 9 (67%) from Santa Fe Dam, and 5 of 5 (100%) from West Covina. None of the chickens at the Eaton Canyon flock were positive for SLE virus antibodies.

#### FERAL BIRD SERA COLLECTION

To acquire an earlier warning of encephalitis viral activity, sera were obtained from feral birds and tested for antibodies to mosquito-borne viruses. Feral birds in Orange County have consistently shown seroconversions months before positive mosquito pools or chicken seroconversions occurred (Bennett et al. 1990). Four modified crow traps were stationed within the San Gabriel Valley. Once again, Santa Fe Dam and Cal Poly were chosen as locations due to previous SLE virus activity. The other two sites were in Monterey Park and San Gabriel. The traps were placed and constructed to attract house sparrows and house finches (Gruwell et al. 1990). Beginning in March, feral birds were sampled, banded and then released from the traps every other week. The sera were tested by Orange County Vector Control District.

A total of 827 feral bird sera samples were collected. These included 698 from house sparrows (*Passer domesticus*), 123 from house finches (*Carpodacus mexicanus*), 5 from white-crowned sparrows (*Zonotrichia leucophrys*) and 1 from a scrub jay (*Aphelocoma coerulescens*).

The first indication of SLE virus activity occurred April 9 when sera from a house finch from Santa Fe Dam and a house sparrow from Cal Poly were positive for SLE virus antibodies. This was three months prior to the first seroconversion in any sentinel chicken flock (Fig. 1). On the July 29 bleeding, one house sparrow from Cal Poly tested positive for WEE virus antibodies but no further WEE virus activity was found in feral birds, sentinel chickens, or mosquito pools. Saint Louis encephalitis virus activity has continued throughout the year including December, which is the final month addressed in this report. After the initial sera were positive for antibodies to SLE virus on April 9, sera from 28 house sparrows and one house finch were positive for SLE virus antibodies.

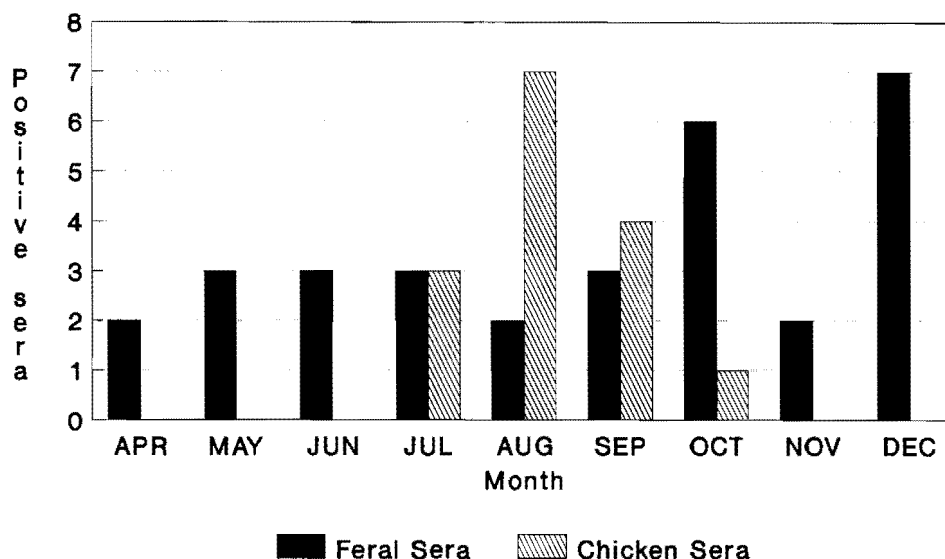


Figure 1. Seroconversions in feral birds and sentinel chickens in the San Gabriel Valley during 1992.

Recaptures were valuable in determining the acceptance of the crow traps as well as providing information regarding the titer retention in the feral birds. Seven of the 13 positives at Cal Poly were recaptures as were seven of 15 from Santa Fe Dam.

An especially interesting house sparrow (band #388) accounted for three of the seven positive recapture sera from Santa Fe Dam. It was originally captured September 23 and had a positive titer of 1:20 to SLE virus. On October 8, it was negative. The bird again tested positive on both October 20 and November 3 with a 1:20 titer. Unfortunately, the bird was not present in the trap during the next two bleedings to provide subsequent data. This may illustrate that antibody titers in the feral birds are present for longer than two weeks.

However, six other birds demonstrated that this may not be the case. During three consecutive bleedings these birds initially tested negative, then positive for SLE virus antibodies, then negative again on the final of the three bleedings. This would establish a titer retention of approximately two weeks.

In addition, four birds other than #388 were SLE antibody positive, then negative and reinfected with SLE virus at a later time. One sparrow of particular interest frequents the Cal Poly trap. It was positive for SLE virus antibodies on its initial capture May 7 with a 1:20 titer. On the June 4 and July 16 bleedings it was negative for antibodies to mosquito-borne viruses. On the July 29 bleeding, it tested

positive for WEE antibodies with a 1:40 titer. It was again negative on the next bleeding August 13.

#### HUMAN ILLNESS

There was one reported case of SLE in the San Gabriel Valley. The patient is a 75-year old female from El Monte with no travel history. She became ill on September 2 with a fever of 103°F, headache, vomiting and disorientation. She was hospitalized September 7 and discharged September 21. Antibodies to SLE virus were found in serum obtained September 11 by a private laboratory and the State Viral and Rickettsial Disease Laboratory. Her date of exposure coincided with a period of increased SLE virus activity in both avian populations.

#### CONCLUSIONS

Interagency cooperation between VDS, Public Works, and the MAD's was very successful in preventing large mosquito populations from forming and reduced the potential for human exposure to mosquito-borne encephalitis viruses.

With our limited data we feel the feral bird testing project was successful and has much potential as an early warning system. However, more data are needed to determine the extent of its effectiveness. We intend to learn whether SLE virus is present year-

round within the San Gabriel Valley and whether a baseline of activity exists and what level of viral activity in the feral bird populations indicates the potential for human illness. More data are also needed to ascertain how long feral birds retain a titer and what role the individual species of birds and virus strain play in the length of retention.

#### ACKNOWLEDGEMENTS

The authors gratefully thank Drs. Jim Webb and John Gruwell, Orange County Vector Control District, for their guidance and training in establishing the feral bird project. A special thanks is extended to Carrie Fogarty, Orange County Vector Control District, for testing the feral bird sera. We also recognize the County of Los Angeles Department of Public Works for their enthusiastic and timely help regarding mosquito breeding site reduction.

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## MOSQUITO-BORNE ENCEPHALITIS SURVEILLANCE IN WESTERN LOS ANGELES COUNTY DURING 1992

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### ABSTRACT

Five hundred and twelve peridomestic bird sera were tested for antibodies to Saint Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) viruses. Seventeen sera tested positive for SLE antibodies. Forty sentinel chickens, dispersed at four locations, were tested semi-monthly for viral activity. Three chickens, located at the La Brea tar pits, seroconverted for SLE antibodies. The total collection of 542 mosquito pools, made up of 23,293 mosquitoes, tested negative for SLE or WEE viruses.

The Los Angeles County West Mosquito Abatement District continued its surveillance program for St. Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) viruses in 1992. The surveillance involved three separate programs: 1. Peridomestic Bird Testing Program to determine the level of infection in the wild bird populations, since peridomestic birds are reservoirs for SLE and WEE viruses in urban areas; 2. Mosquito Testing Program to determine the potential for disease transmission from the reservoir avian population to new hosts such as uninfected birds, horses and humans; 3. Sentinel Chicken Program to detect actual viral transmission by mosquitoes to uninfected hosts such as sentinel chickens.

### MATERIALS AND METHODS

#### Peridomestic Bird Testing Program.

Four Australian Crow traps, one in each location, were used to attract and capture peri-domestic wild birds at the La Brea tar pits, the Playa del Rey salt flats, the Chevron Oil refinery in El Segundo and Charmlee Park in Malibu.

Birds were captured, tagged, bled and released. Disposable, 26 gauge syringes were used to extract approximately 0.1 cc of blood from the birds' jugular vein. The blood samples were poured into test tubes

holding 0.9 cc bovine solution and analyzed with HIA tests for antibodies to SLE and WEE by Carrie Fogarty of the Orange County Vector Control District. Birds were not collected during the months of January or April.

#### Mosquito Testing Program.

Trapping was performed weekly at random locations throughout the District in areas of known mosquito activity and next to wild bird collection traps and sentinel chicken flocks. All specimens were collected with either CDC dry ice-baited traps or modified gravid female traps (Cummings 1992). Generally, trapping was done four nights per week with a total of 390 locations sampled.

Female mosquitoes were anesthetized with triethylamine, sorted to species and pooled into freezer vials. The vials were held on dry ice and shipped to the California Department of Health Service's Viral and Rickettsial Disease Laboratory (VRDL) in Berkeley for testing.

#### Sentinel Chicken Program.

Forty sentinel chickens were placed, ten each, at four locations; the La Brea tar pits, the Las Virgenes Water District Headquarters in Calabasas, the Torrance Mounted Posse Equestrian Center and Charmlee Park in Malibu. The last two sites were locations from which seven mosquito pools tested positive for WEE in

1991 (Kovaltchouk 1992).

Chickens were bled semi-monthly from April 20, 1992, through February 2, 1993, with the California Department of Health Services, Environmental Management Branch's "1991 Revised Protocol for Sentinel Chicken Serum Submission" followed in the preparation of the sera. Blood sera obtained from April 20 to November 3, were tested by the VRDL. Sera obtained after November 3 were tested by the Orange County Vector Control District.

**RESULTS AND DISCUSSION**

Five hundred and twelve (512) birds were captured, tagged, bled and released. The majority of specimens collected and tested were house finches (*Carpodacus mexicanus*). Overall, 17 birds (3.3%) tested positive for SLE: twelve finches, four morning doves (*Zenaida macroura*) and one California towhee (*Pipilo crissalis*). Viral activity was detected during eight of the ten months of testing (Fig. 1). Monthly infection rates ranged from 1.3 to 11.7 percent, supporting the theory that SLE is endemic in the avian population of urban areas of Southern California (Gruwell et al. 1990). Viral activity was detected

almost weekly in avian sera obtained from August to early December (Fig. 2). Infection rates in October, November and December were 9.4%, 8.7% and 10%, respectively. It should be noted however, that sample sizes were small during these months (53, 23 and 20, respectively).

The sentinel chicken flocks placed in Torrance, Calabasas and Malibu demonstrated no viral activity. The La Brea tar pits flock, however, detected positive SLE antibodies in September and October. Three chickens, 37.5% of the flock (2 chickens were stolen on May 11), became infected (Fig. 3) on September 22 (1 hen) and October 5 (2 hens). Viral activity observed in the sentinel chickens suggest an infection period of early to mid-September. The La Brea tar pits is a major source of *Culex tarsalis* Coquillett mosquitoes with rain water and landscape irrigation water draining into the natural lake and the many small ponds. Vegetation growing in the lake reduces the efficacy of mosquito suppression efforts. Although a vegetation management program has been implemented by the Los Angeles County Natural History Museum, some mosquitoes still escape the District's control program. Trapping for mosquitoes at the tar pits was generally done weekly with a total of 49 mosquito pools, made up of 2,044 mosquitoes, submitted for testing (Fig. 4).

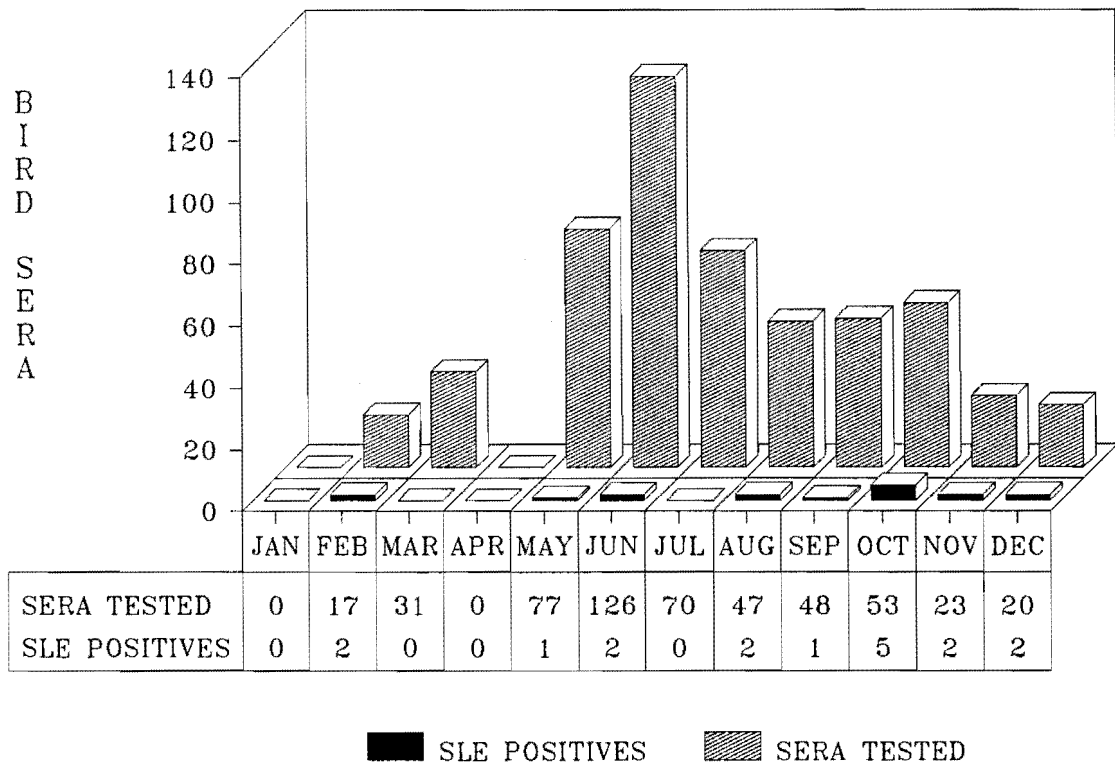


Figure 1. Monthly results of 1992 peridomestic bird testing program, indicating SLE positive sera and sera tested.

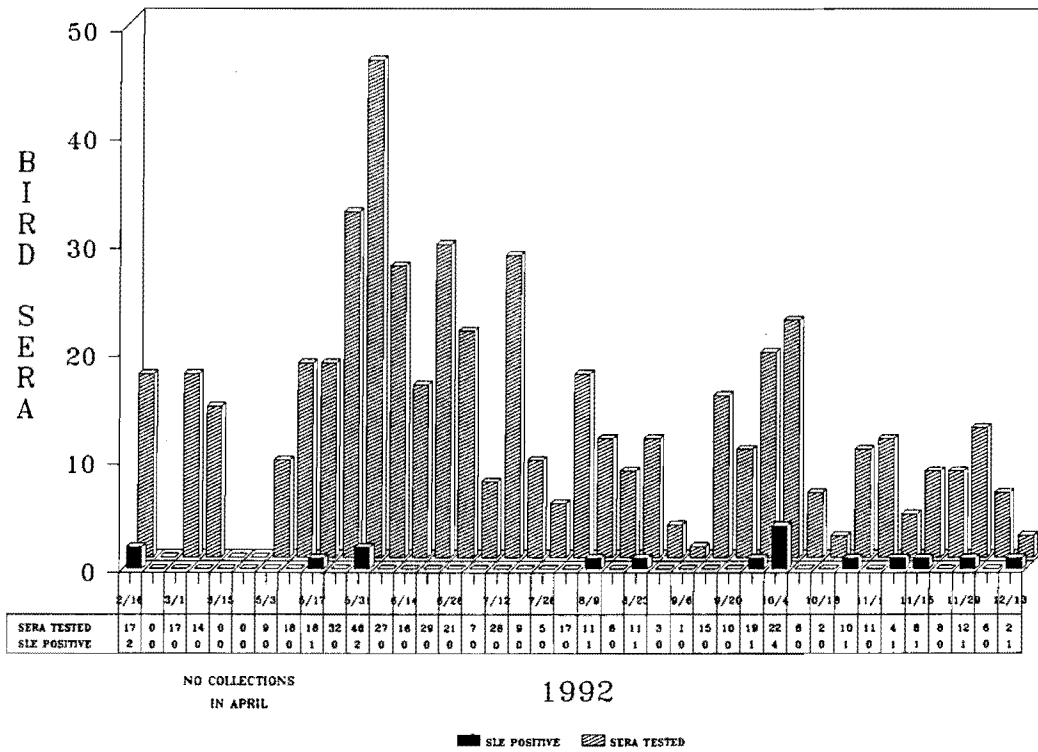


Figure 2. Weekly results of peridomestic bird testing program, indicating SLE positive sera and sera tested.

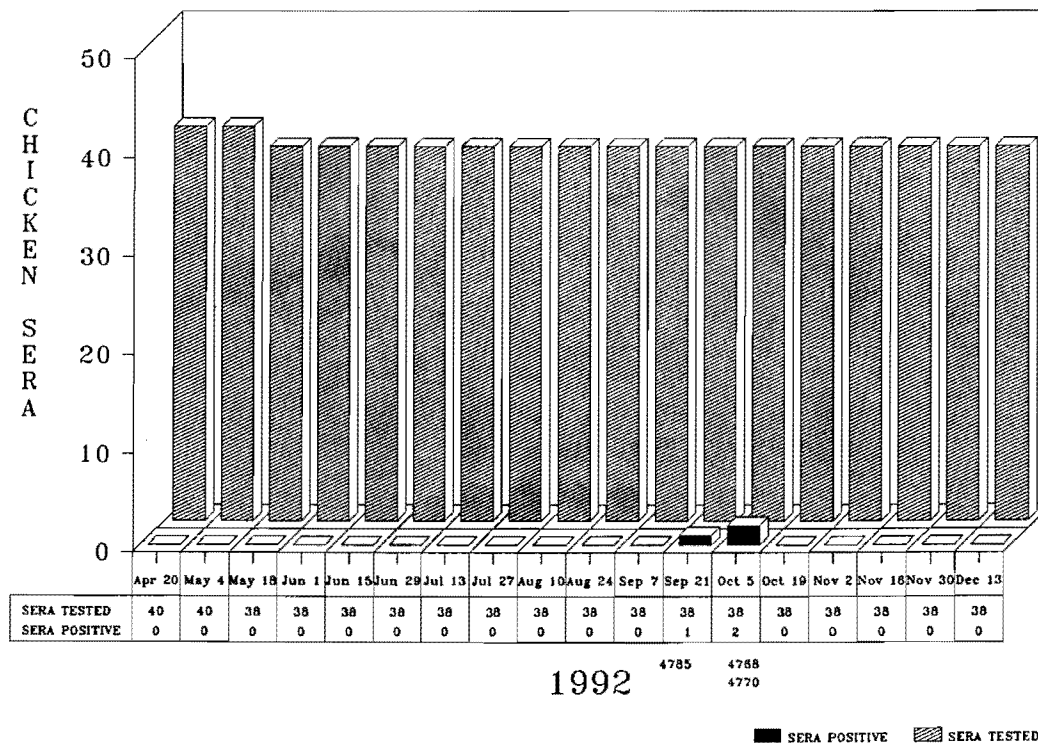


Figure 3. Results of 1992 sentinel chicken program, indicating three SLE seroconversions.

None tested positive.

Only four peridomestic birds (none from the tar pits) were captured and tested in early to mid-September. Correlating the period of infection in the chickens with viral activity in the wild bird population is, thus, not feasible. However, as noted earlier, 4.7% of the wild birds seroconverted for SLE during the month of August.

Throughout the District, 23,293 mosquitoes were collected, sorted and pooled with 542 pools sent to the VRDL for testing (Fig. 5). These were comprised of 16,125 *Culex quinquefasciatus* Say, 6,622 *Cx. tarsalis* and 526 *Culex stigmatosoma* Dyar. Most *Cx. quinquefasciatus* were collected with oviposition traps and were gravid. No pool tested positive. It should be noted that neighboring districts and agencies involved with arbovirus surveillance also experienced a high incidence of sentinel chicken and avian seroconversions (Brisco et al. 1993), (J. Hazelrigg, SEMAD, personal communication), (Bennett et al. 1993). In addition, two human cases of SLE were diagnosed in Los Angeles and Ventura Counties in August and September.

Statewide, 14,009 chicken sera were tested with 51 seroconverting for WEE and 118 for SLE. Yet, amidst all this viral activity, none of the 2,329 mosquito pools tested statewide for arboviruses were positive for

SLE and only one was positive for WEE.

**ACKNOWLEDGEMENTS**

The authors express their gratitude to James P. Webb for his support and expertise. Special thanks is offered to Carrie Fogarty for her hard work in testing the bird sera with thanks also extended to Gilbert Challet and the Orange County Vector Control District for their cooperation.

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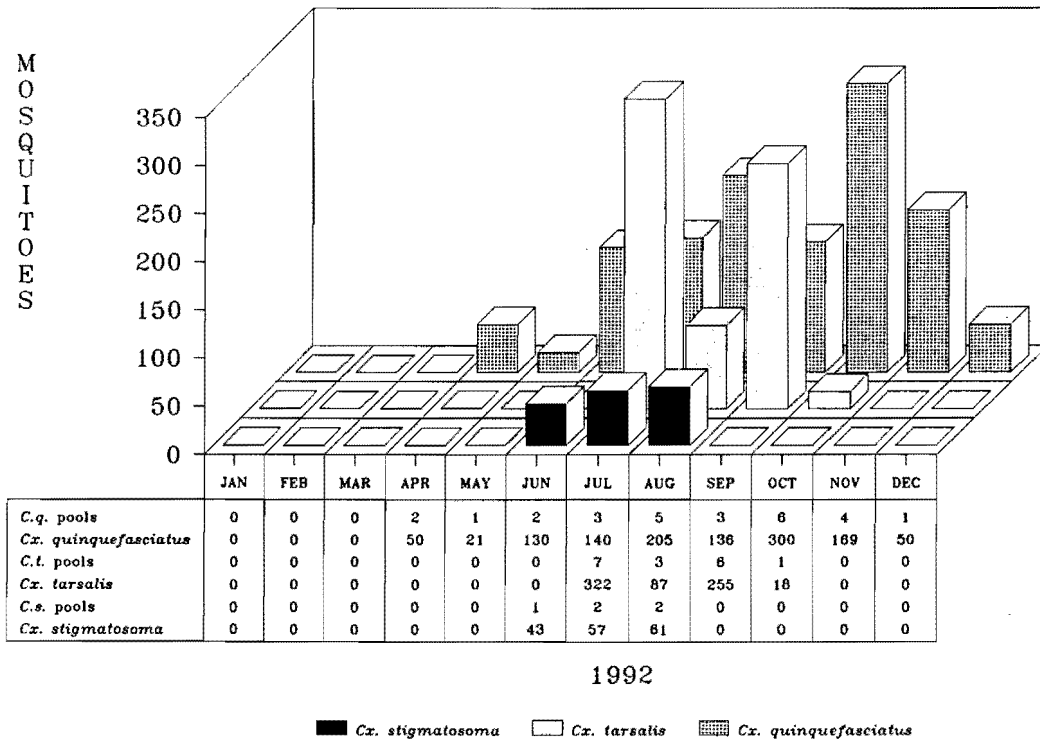


Figure 4. Results of 1992 La Brea tar pit surveillance, indicating collected mosquitoes and pools submitted for testing.

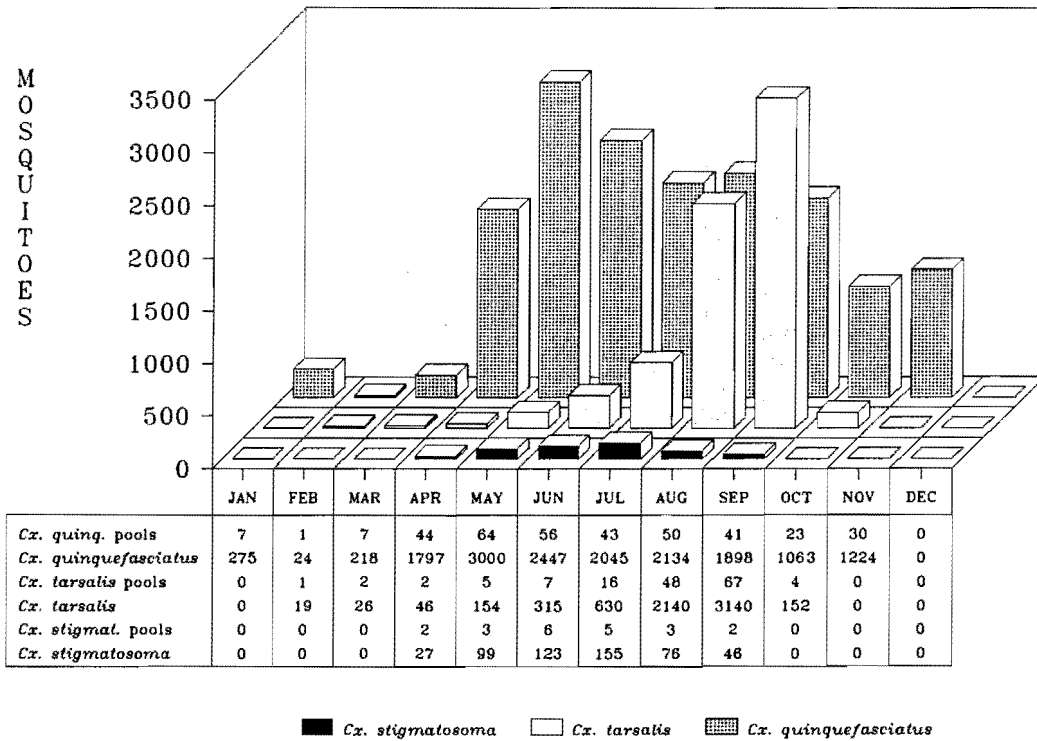


Figure 5. Results of 1992 mosquito testing program, indicating collected mosquitoes and pools submitted for testing.

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## EVALUATION OF MOSQUITO AND ARBOVIRUS ACTIVITY IN ORANGE COUNTY DURING 1992

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### ABSTRACT

The Orange County Vector Control District continued its surveillance of mosquito and arbovirus activity throughout 1992 by collecting blood samples from wild birds and sentinel chickens as well as collecting adult mosquitoes from CDC CO<sub>2</sub>-baited, ovipositional and stable traps. There were no positive mosquito pools or human cases in Orange County during 1992. Chicken seroconversions included 2 of 15 chickens bled on September 9 from a sentinel flock in Irvine; 1 of 5 bled on September 23 from a stable trap in Fullerton and 1 of 5 bled on October 6 from a stable trap in Irvine. Wild birds were found SLE-positive every month of the year with house sparrows accounted for the majority of the positives; ranging from a high in April (6.8%) to additional highs in September (8.8%) and October (18.8%). *Culex quinquefasciatus* was the most common mosquito collected except for rural areas of Irvine, where *Cx. tarsalis* was predominant. Stable traps were more efficient than CDC CO<sub>2</sub>-baited traps in attracting host-seeking *Cx. quinquefasciatus*, but made little difference with regards to *Cx. tarsalis*. Gravid *Cx. quinquefasciatus* were collected from both suburban and rural sites with the most productive site (reaching 460 per trap-night in May) being a backyard source in Anaheim.

In 1992, the Orange County Vector Control District (OCVCD) continued its mosquito and encephalitis virus surveillance throughout the year. Mosquitoes were collected at ten permanent sites throughout the county (Bennett et al. 1990) utilizing 19 CDC CO<sub>2</sub>-baited traps as well as five modified Reiter gravid female ovipositional traps (Cummings 1992). One of the 19 CDC CO<sub>2</sub>-baited traps was discontinued in August at the Carbon Canyon locality. Additionally, blood-fed female mosquitoes were collected inside three stable traps (Magoon 1935) containing five white leghorn chickens each.

A total of 9,949 mosquitoes were collected from which 251 pools were submitted to the California State Department of Health Services' Viral and Rickettsial Diseases Laboratory at Berkeley for virus testing (Table 1). The collections included 206 pools of *Culex quinquefasciatus* Say and 45 pools of *Culex tarsalis* Coquillett. None of these pools tested

positive for St. Louis encephalitis (SLE) or western equine encephalomyelitis (WEE) viruses.

Sentinel chicken flocks used during 1992 included one large flock (25 chickens) at the 20 Ranch Duck Club in Irvine and three mini-flocks (5 chickens each) held in stable traps in Fullerton, Buena Park and the 20 Ranch Duck Club. While there were no sentinel chicken seroconversions for SLE or WEE in Orange County during 1991, sentinel chicken seroconversions for SLE virus this year included 2 of 15 chickens bled on September 9 from the 20 Ranch Duck Club (now the San Joaquin Wildlife Sanctuary); 1 of 5 chickens bled on September 23 from the Fullerton College stable trap and 1 of 5 chickens bled on October 6 from the 20 Ranch Duck Club stable trap. It should be noted, however, that five pools of mosquitoes (168 blood-engorged *Cx. quinquefasciatus*) collected during September 1-23 from the Fullerton College stable trap

Table 1. Number of mosquito pools submitted for SLE and WEE virus testing by species and trap type from Orange County during 1992.

Species	Stable traps	Oviposition traps	CDC traps	Total pools
<i>Culex quinquefasciatus</i>	131	57	18	206
<i>Culex tarsalis</i>	27	-	18	45
Totals	158	57	36	251

and 14 pools (10 consisting of 456 *Cx. quinquefasciatus* and 4 consisting of 138 *Cx. tarsalis*) collected between September 15 and October 6 from the 20 Ranch Duck Club stable trap all tested negative.

No human cases of SLE or WEE were reported from Orange County in 1992. However, there was one SLE case from El Monte in neighboring Los Angeles County (onset September 2) and one from Ventura County.

Wild bird sera were tested for SLE and WEE antibodies by the OCVCD at the laboratories of the Orange County Health Department. Nine modified Australian Crow traps (McClure 1984) were used in 1992 to trap a total of 12,223 birds from which 3,663 blood samples were taken and tested (Table 2). The crow trap at the Canyon Carbon locality was discontinued in August because of vandalism.

The overall percentage of SLE-positive house sparrows for the year was 3.43%, while house finches and were found SLE-positive at 1.34% (4.9 and 0.95%, respectively in 1991). SLE-positive birds (house sparrows and finches combined) were found during every month of the year (Fig. 1) with the highest SLE-positive activity (15.4% positive) observed in October (5.5% positive in September 1991). However, the only locality sampled in

October was Central Park in Huntington Beach (26 birds). An increase in small bird seroconversions for SLE occurred in May prior to the chicken seroconversions and the SLE case in the Los Angeles basin. House sparrows accounted for the majority of seroconversions (Fig. 2) and from August to September increased from 1 to 9%, reaching a high of 18.8% in October (3 of 16 birds). Positives in sparrows were also high in January (6%) and April (7%). Three sites, Central Park in Huntington Beach, a residential site three kilometers from the park, and Fullerton College, produced the majority of SLE-positive sparrows in 1992 (Figs. 3, 4 and 5). Two of these sites, the Huntington Beach locals, were also high in SLE-positive sparrows during 1990 and 1991.

*Culex quinquefasciatus* was the most common host-seeking mosquito at both Huntington Beach sites, persisting in low numbers through October and November while *Cx. tarsalis* disappeared by the end of August. As in previous years, this trend repeated itself at most other localities in Orange County during 1992. At the Central Park site, *Culex tarsalis* had the highest counts during April at 32 females per trap-night (60 females per trap-night in 1991) while *Cx. quinquefasciatus* was highest during June and July at 10 females per trap-night (45 females per trap-night in August of 1991). The highest percentage of SLE-

Table 2. Small bird seroconversions for SLE and WEE antibodies in Orange County during 1992.

Species	No. positive		No. bloods sampled	% positive	
	SLE	WEE		SLE	WEE
House Sparrow	54	0	1,573	3.43	-
House Finch	28	0	2,086	1.34	-
White-Crowned Sparrow	0	0	4	-	-
Totals	82	0	3,663	2.23	-

positive sparrows at these sites occurred in October (18.8% positive). At the Huntington Beach residential site, the highest percentage of SLE-positives occurred in January (18%), April (14%), and May (8%). Highest counts for both *Cx. tarsalis* and *Cx. quinquefasciatus* were in April and May (23-34 females per trap-night).

The highest rate of SLE-positives at Fullerton College occurred in September (20 and 22%) at the same time (Week 37) a stable trap chicken seroconverted for SLE (Fig. 5). At this site, the predominant mosquito collected from CDC CO<sub>2</sub>-baited, gravid female and stable traps throughout the year was *Cx. quinquefasciatus*. However, mosquito data from the stable trap show a small peak of *Cx. tarsalis* activity (9 females per trap-night) three weeks prior to the chicken seroconversion. *Culex quinquefasciatus* activity at the same time ranged from 10-24 females per trap-night (Fig. 6). Many of the birds trapped at this site (978) were also banded with individual numbers, so any previously negative bird that became SLE- or WEE-positive during a two week period would be detected and visa versa. Although these data have not yet been completely analyzed, banded sparrow and finches from both Huntington Beach sites, Fullerton College, and Irvine have displayed fluctuating antibody titers during the year.

*Culex tarsalis* was more abundant in rural or suburban areas such as the San Joaquin Freshwater Marsh and 20 Ranch Duck Club (San Joaquin Wildlife Sanctuary), both in Irvine (Figs. 7 and 8). Populations of *Cx. tarsalis* in the marsh were highest in April (240 females per trap-night), May (525 females per trap-night), and mid-June (175 females per trap-night). In the marsh, mosquito population peaks normally occur during this time of year, but may have been enhanced by the lack of insecticidal spraying for a two week period in May when equipment broke down (highest count in 1991 was 155 females per trap-night in June). *Culex quinquefasciatus* was not very abundant in the marsh and only reached 20 females per trap-night in May (25 females per trap-night in October 1991). Host-seeking *Cx. tarsalis* activity (as measured by CDC CO<sub>2</sub>-baited traps) at 20 Ranch Duck Club (Fig. 8) mirrored that at the San Joaquin Marsh between April and October. At this site, the seasonal occurrence of the highest activity counts were virtually the same as in 1991, but the actual counts were different. The highest counts in 1991 were in July (25-28 females per trap-night) and a second peak was observed in

August at 20 females per trap-night. During 1992, however, the counts rarely got below 30 females per trap-night all year and the highest occurred from May to June at 100-200 females per trap-night. Numbers of host-seeking *Cx. quinquefasciatus* caught in CDC CO<sub>2</sub>-baited traps at 20 Ranch Duck Club were also higher during 1992, reaching 70-110 females per trap-night in May (55 females per trap-night in September 1991).

During 1992, mosquitoes were recovered from all stable traps each day for four days a week (Monday through Thursday) and summed for a weekly total number collected. At 20 Ranch Duck Club, the stable trap collected good numbers of both *Cx. tarsalis* and *Cx. quinquefasciatus* (Fig. 9). However, it proved to be much more efficient than the CDC CO<sub>2</sub>-baited trap (hanging from the stable trap) at collecting large numbers of *Cx. quinquefasciatus* (Fig. 10). In contrast, the number of *Cx. tarsalis* collected from both traps was approximately the same (Fig. 11). At the peak of activity, between May and June, *Cx. quinquefasciatus* ranged from 100-265 females per trap-night (80-220 females per trap-night from August to October 1991). A peak of 130 females per trap-night did occur in late August in 1992, declining sharply in September to 20 females per trap-night only to reach a last peak of 60 females per trap-night in October. *Culex tarsalis* collected from the stable trap ranged from 80-190 females per trap-night in May and declined to 5-12 per trap-night in September (23-30 females per trap-night in June and July 1991). Both mosquito species were present in low numbers in September prior to the stable trap chicken seroconversion.

Throughout the year, gravid female *Cx. quinquefasciatus* were obtained from modified Reiter ovipositional traps at both suburban and rural sites. Gravid female mosquito activity at 20 Ranch Duck Club in Irvine and a residential site six kilometers away were similar except for June and July when *Cx. quinquefasciatus* numbers dropped from 80 gravid females per trap-night to almost zero at 20 Ranch Duck Club while they remained at 50-125 per trap-night at the residential site. Overall, the residential site was more productive than the rural site throughout most of the year, reaching a high of 225 gravid females per trap-night in May, a time when the rural site was peaking at only 80 gravid females per trap-night. In 1991, the peak activity at the rural site occurred from September to November (90-185 per trap-night) with very little activity in May.

Host-seeking *Cx. quinquefasciatus* at the Irvine



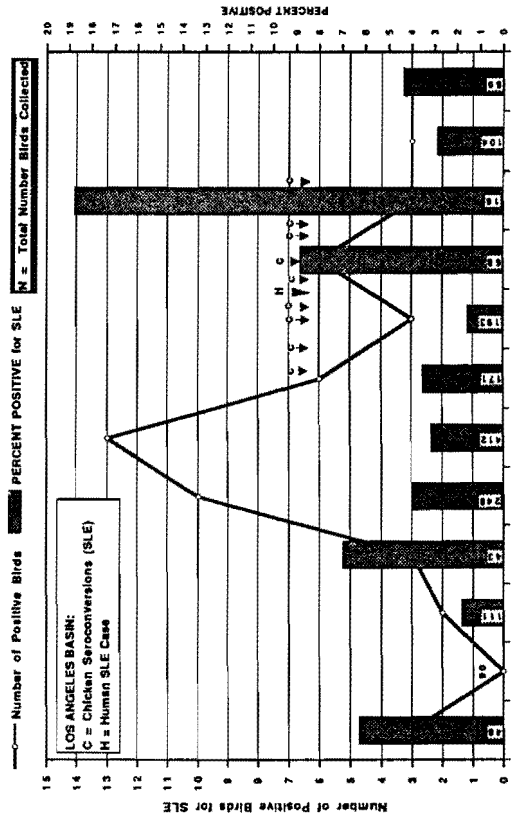


Figure 1. SLE virus activity in the Los Angeles basin and wild bird seroconversions in Orange County during 1992.

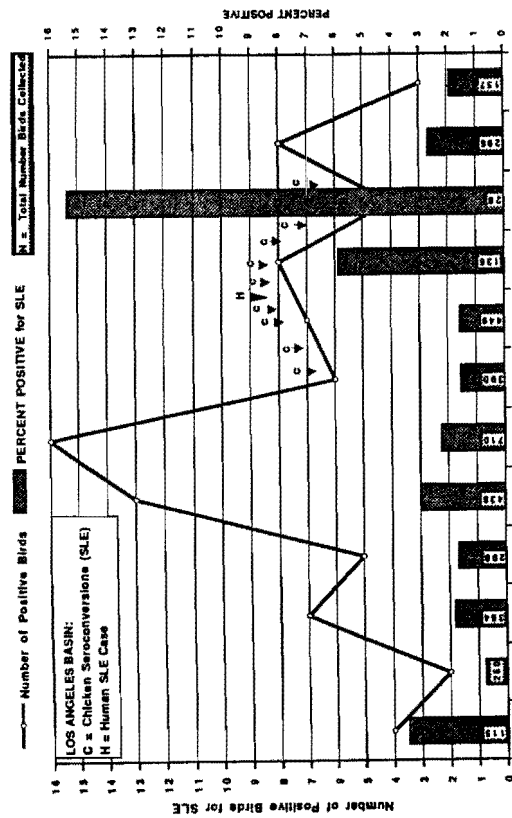


Figure 2. SLE virus activity in the Los Angeles basin and house sparrow seroconversions in Orange County during 1992.

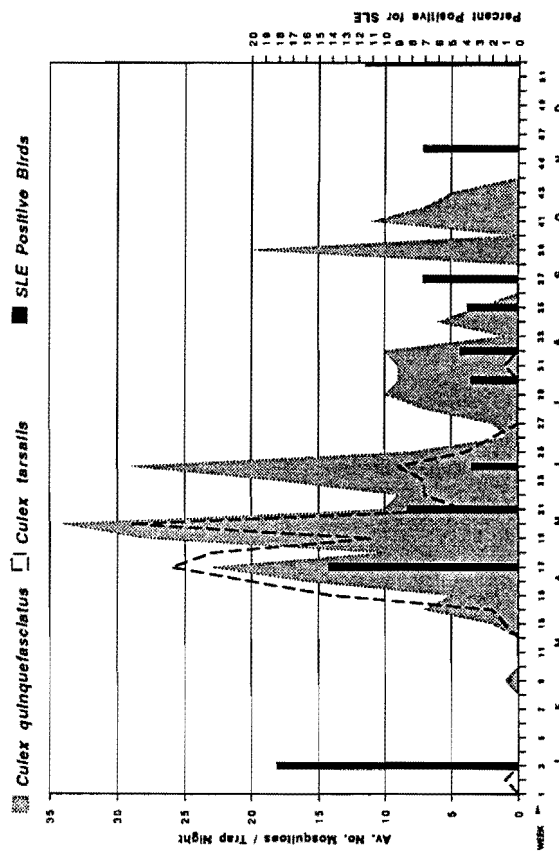


Figure 3. Host-seeking mosquito and SLE activity in house sparrows from Central Park, Huntington Beach during 1992.

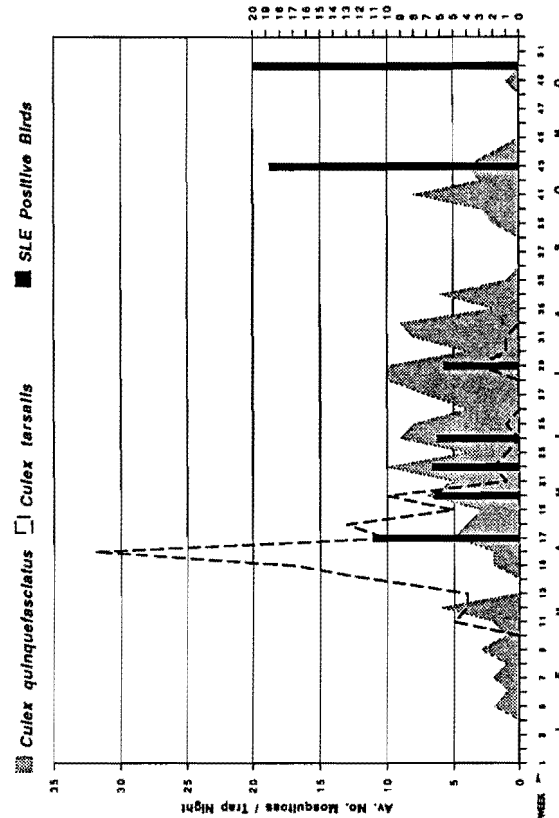


Figure 4. Host-seeking mosquito and SLE activity in house sparrows from a residence in Huntington Beach during 1992.

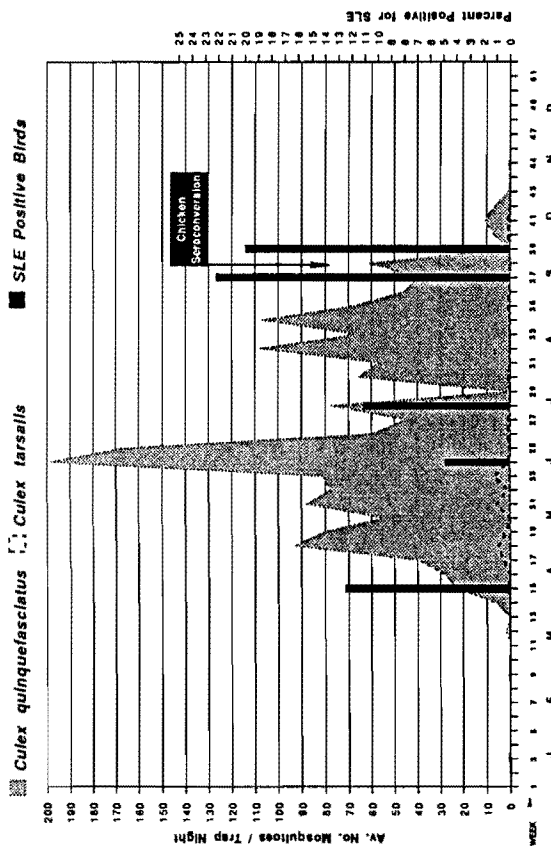


Figure 5. Host-seeking mosquito (CDC trap) and SLE activity in house sparrows from Fullerton College during 1992.

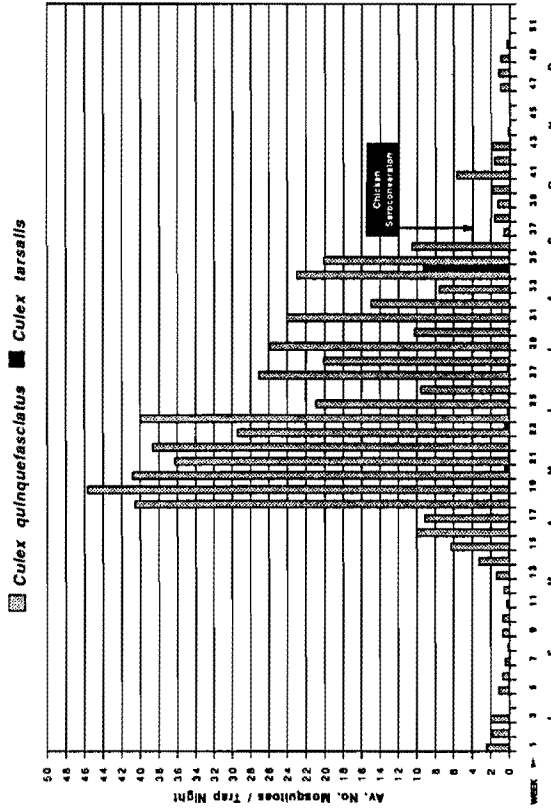


Figure 6. Host-seeking mosquito (stable trap) activity from Fullerton College in Fullerton during 1992.

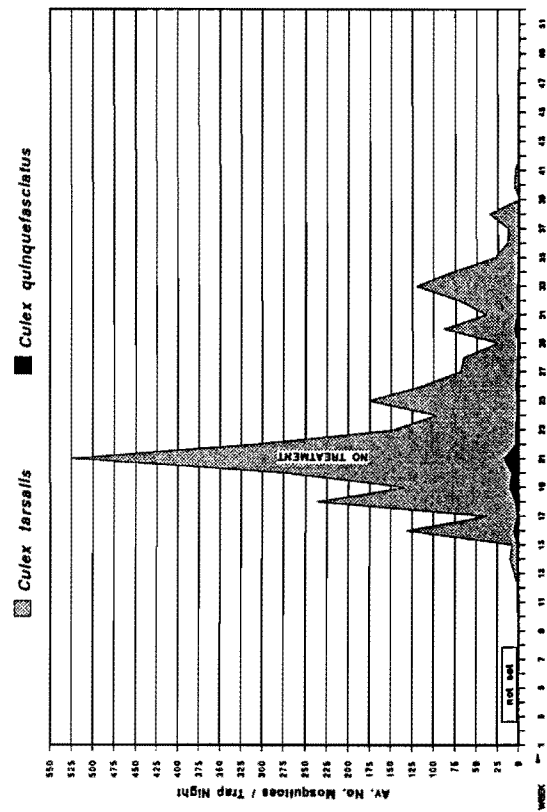


Figure 7. Host-seeking mosquito (CDC traps) activity from the San Joaquin Freshwater Marsh in Irvine during 1992.

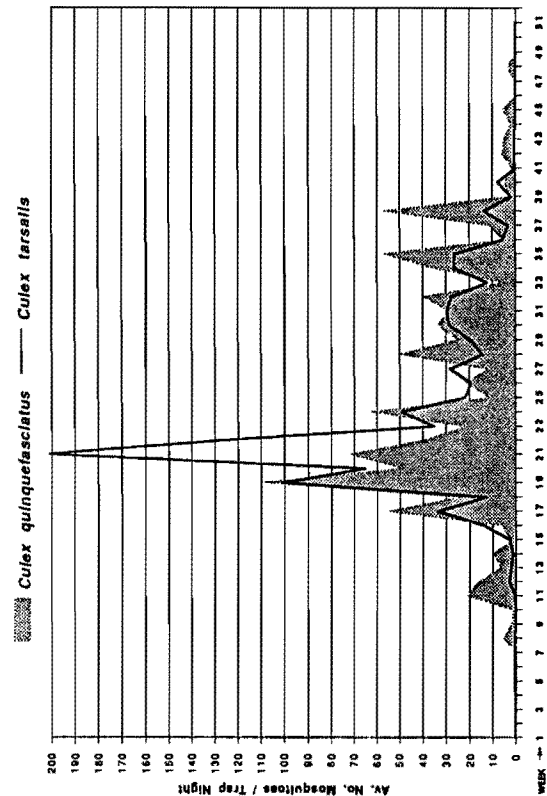


Figure 8. Host-seeking mosquito (CDC trap) activity from the 20 Ranch Duck Club in Irvine during 1992.

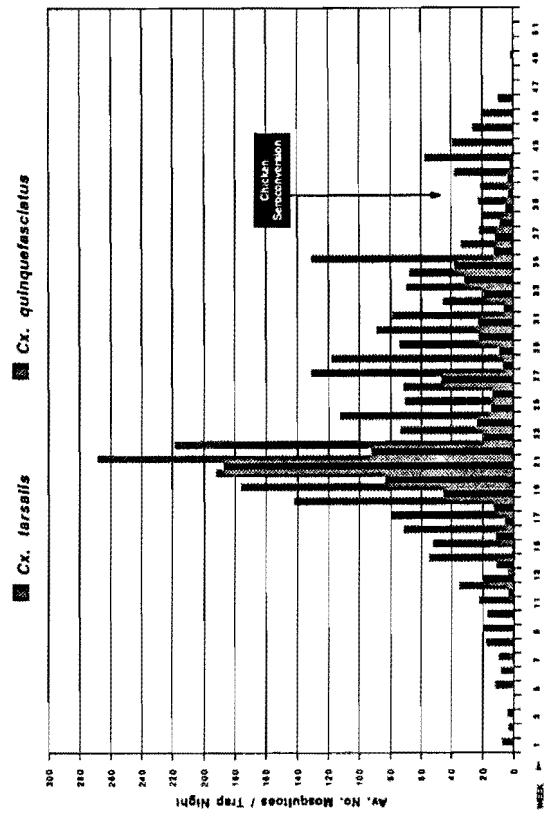


Figure 9. Host-seeking mosquito (stable trap) activity at the 20 Ranch Duck Club in Irvine during 1992.

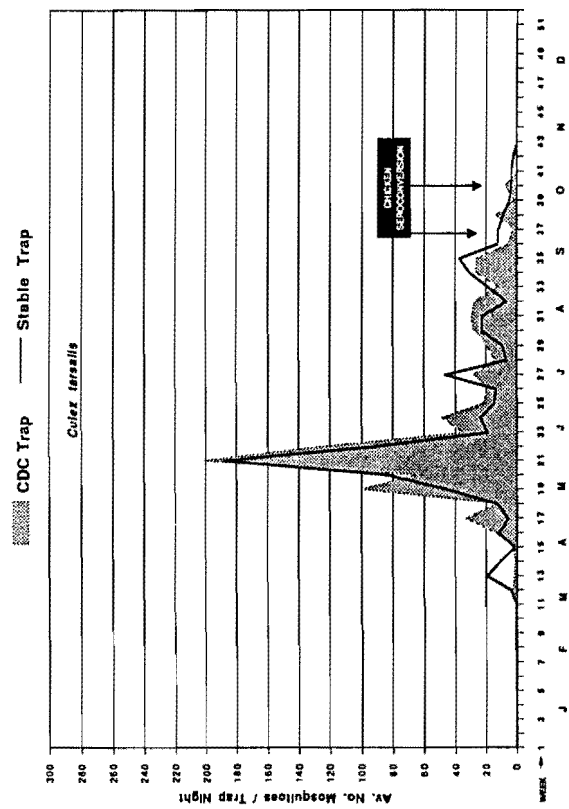


Figure 11. Host-seeking *Cx. tarsalis* (stable and CDC traps) activity from the 20 Ranch Duck Club during 1992.

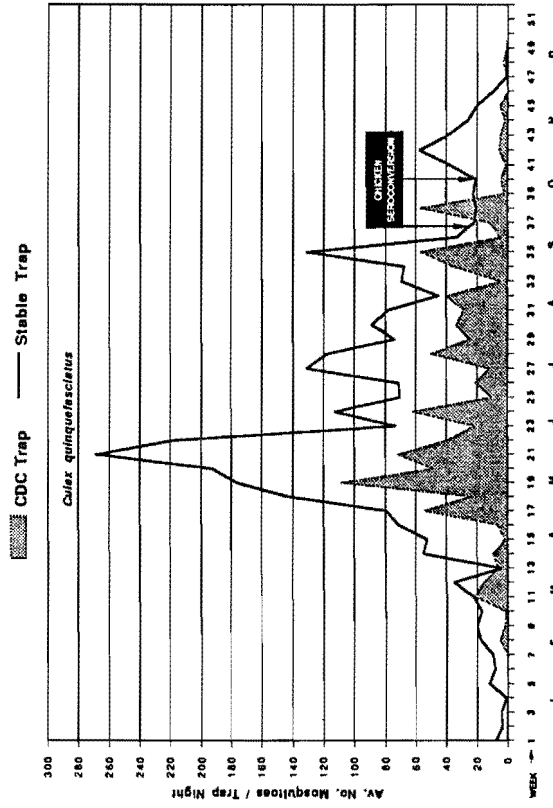


Figure 10. Host-seeking *Cx. quinquefasciatus* (stable and CDC traps) activity from the 20 Ranch Duck Club during 1992.

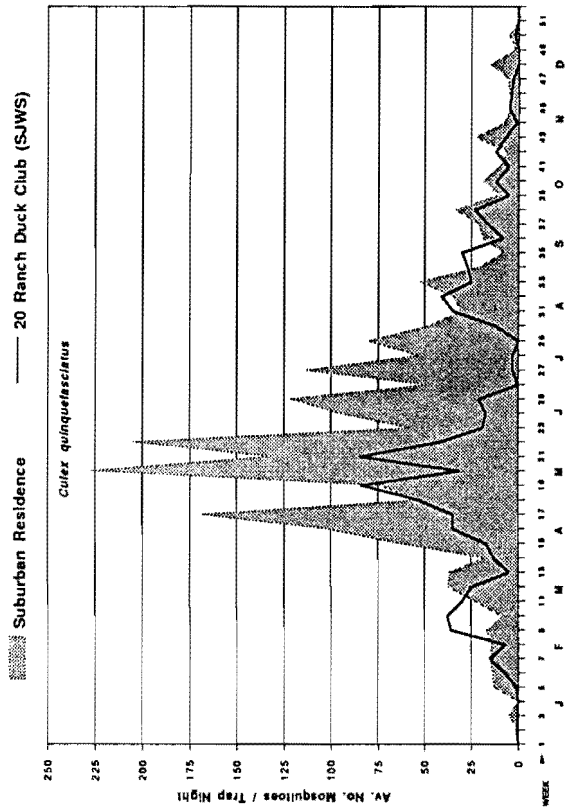


Figure 12. Gravid mosquito (modified Reiter ovipositional trap) activity in Irvine during 1992.

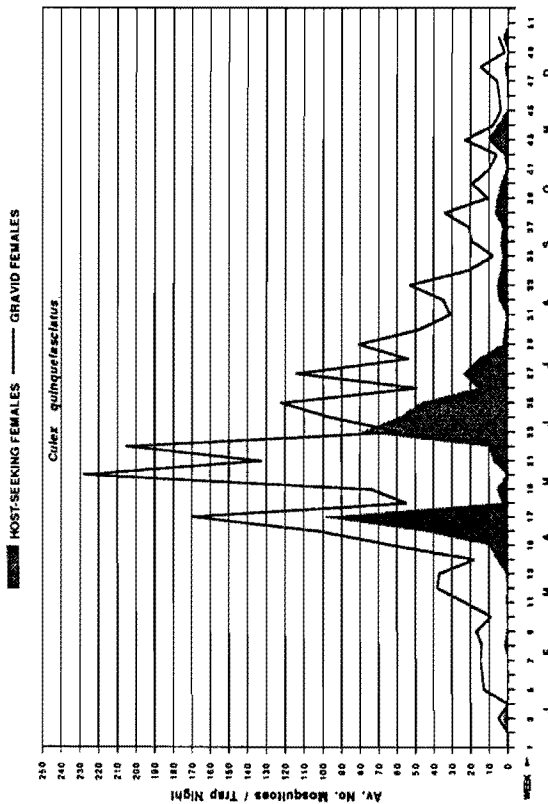


Figure 13. Mosquito (CDC and ovipositional traps) activity from a suburban residence in Irvine during 1992.

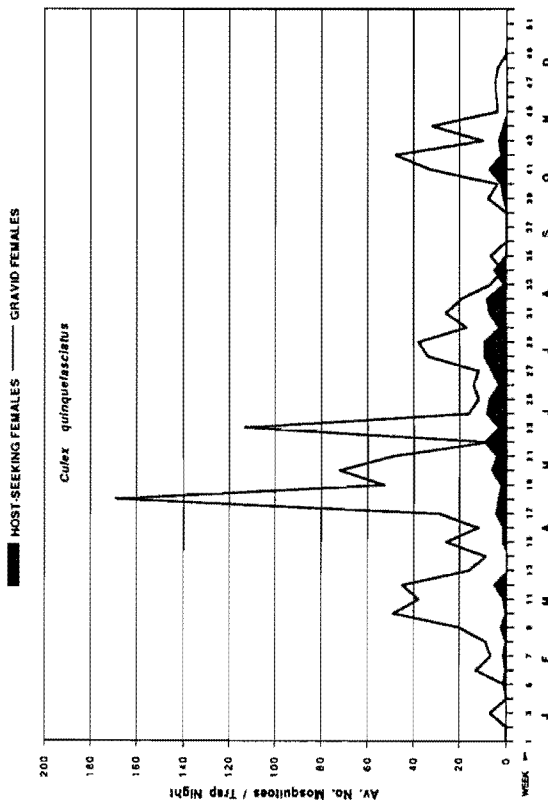


Figure 14. Mosquito (CDC and ovipositional traps) activity from Central Park in Huntington Beach during 1992.

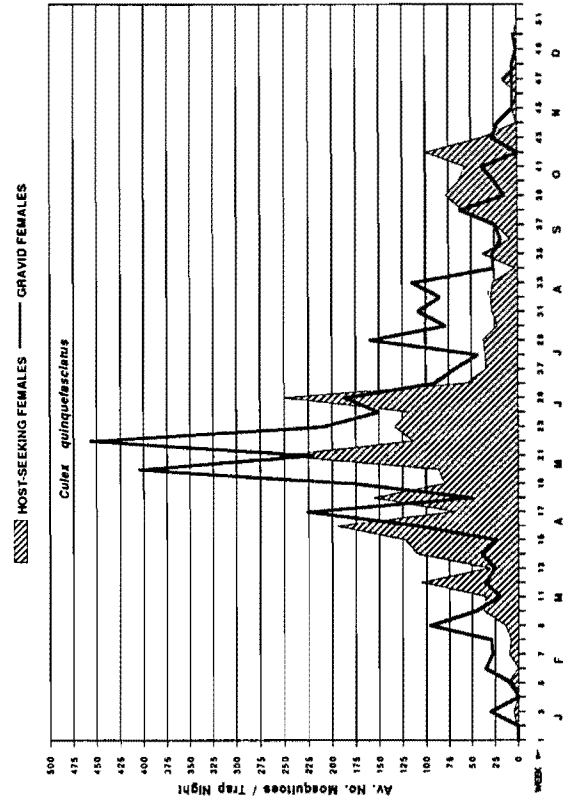


Figure 15. Mosquito (CDC and ovipositional traps) activity from a suburban residence in Anaheim during 1992.

residential site were very sporadic when compared to gravid females (Fig. 13), although they did reach 100 females per trap-night in April and 80 females per trap-night in June (only 10 females per trap-night in June 1991).

During 1992, ovipositing female *Cx. quinquefasciatus* at Central Park in Huntington Beach (Fig. 14) increased considerably from 1991 when there was very little activity from March to May and a burst of activity from October through December (50-160 gravid females per trap-night). Very few host-seeking female *Cx. quinquefasciatus* were taken during the same time in 1992.

The most productive site for gravid *Cx. quinquefasciatus* in 1992 was a residence near Modjeska Park in Anaheim (Fig. 15). Approximately 100 gravid females per trap-night were being taken as early as February. These continued to increase to 460 gravid females per trap-night in late May, and gradually decreased through the fall and winter. The pattern was almost identical in 1991 except peak abundance in June was 670 gravid females per trap-night. During 1992, host-seeking *Cx. quinquefasciatus* at this Anaheim site were more abundant than in 1991 and ranged from 200-260 per trap-night during April to June (100-160 per trap-night from April to June 1991). *Culex tarsalis* were present at this site in lower numbers (10 or fewer females per trap-night from March to September).

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## MOSQUITO ABUNDANCE AND ARBOVIRAL ACTIVITY IN SAN BERNARDINO COUNTY DURING 1992

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### ABSTRACT

Of the 9,996 mosquitoes collected in New Jersey light traps and dry ice (CO<sub>2</sub>)-baited traps in San Bernardino County during 1992, *Cx. tarsalis* (55.0%), *Cs. inornata* (14.4%), *Ae. vexans* (13.2%) and *An. franciscanus* (13.0%) dominated in the desert region while *Cx. tarsalis* (42.1%), *Cx. quinquefasciatus* (21.3%) and *Cx. stigmatosoma* (20.1%) were predominant in the valley region. Mosquito activity in the desert region was lower in the rural habitats (8.1%) than the suburban (27.4%) and urban sites (64.5%). In the valley region, however, more mosquitoes were found at the rural sites (55.1%) than the other two habitats. Mosquito populations peaked in May and November in the desert region and during June in the valley region.

Of the 117 mosquito pools submitted for virus determination, one pool of *Cx. tarsalis* collected in Needles tested positive for western equine encephalomyelitis (WEE) virus. Also, 4 of the 10 sentinel chickens in the Needles flock seroconverted to WEE. No chicken sera or mosquito pools collected in the valley region showed any virus activity.

As part of the California encephalitis virus surveillance (EVS) program, the San Bernardino County Vector Control Program has carried out EVS and other mosquito control activities in both the valley and desert areas of San Bernardino County for several years. Geographically, the county consists of three distinct regions; the desert, mountain and valley regions. Demographically, the valley region houses over 80% of the nearly 1.4 million county population with the remaining scattered over various parts of the desert and mountain regions. Historically, cases of both Saint Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) have been reported in the desert and valley regions from time to time.

After experiencing 26 human cases of SLE in southern California during 1984, the only human case of encephalitis (SLE) in California during 1987 was reported from San Bernardino (Emmons et al. 1988). Of the two cases reported statewide in 1988, one was from the same San Bernardino site (Emmons et al. 1989). During the same period, both SLE and WEE

virus activities were reported in the desert region, especially Needles, and adjoining areas along the Colorado River. Due to periodic incidences of encephalitis, mosquito control and EVS activities have been routinely carried out in the desert and valley regions of this county. Data generated in routine EVS activities are appraised here in relation to the population dynamics of adult mosquitoes and arboviral activity in San Bernardino County during 1992.

### MATERIALS AND METHODS

General EVS procedures as described by Mian and Prochaska (1990) were continued 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 region, the traps

were stationed at seven locations; Yucaipa Regional Park, Loma Linda, Colton, San Bernardino, Fontana, Ontario and Upland. These traps were roughly divided into two trap sites each in suburban and rural environments and three sites in urban 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 weekly in all traps were counted and identified to species and sex with Adult Mosquito Occurrence Reports submitted to the California Department of Health Services.

#### Arboviral Activity in Adult Female Mosquitoes.

Arboviral activity in local mosquito populations was monitored in both the desert and valley regions using dry ice (CO<sub>2</sub>)-baited traps to collect host-seeking adult female mosquitoes. Eight or more such traps were operated on a bi-weekly (valley region) or monthly (desert region) basis.

Female mosquitoes collected overnight were anesthetized using triethylamine (TEA), counted, identified to species and sex, and then pooled by species with 10-50 adults per each labelled vial. All pools (vials) were stored in 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.

#### Arboviral Activity in Sentinel Chicken Flocks.

Both wild and domestic birds are known to play a significant role in the epidemiology of mosquito-borne encephalitides by acting as reservoir hosts for the encephalitis virus(es). Therefore, one sentinel flock consisting of 10 white leghorn chickens was maintained in both the valley and desert regions. The valley flock was stationed near a horse ranch at the northeastern corner of Meridian Avenue and Olive Street in the City of Colton. This site is near the area of the SLE cases in 1987 and 1988. The desert flock was maintained at the sewage treatment facility in the city of Needles. New Jersey light traps were regularly operated at both flock sites. Blood serum samples from all sentinel chickens, taken on pre-determined dates during the mosquito season, were sent to the VRDL for detection of arboviral activity.

## RESULTS AND DISCUSSION

Of the 9,996 mosquitoes collected in New

Jersey light traps and CO<sub>2</sub>-baited traps at various sites in the county during 1992, the most abundant culicine species in the desert region was *Culex tarsalis* Coquillett, representing 55.0% of all collected mosquitoes (Table 1). In this region, *Culiseta inornata* Williston was the second most abundant species (14.4%) followed by *Aedes vexans* Meigen (13.2%), *Anopheles franciscanus* McCracken (13.0%) and *Culex quinquefasciatus* Say (4.2%). Other species totaling <1.0% each of the total included *Culex erythrothorax* Dyar and *Culex stigmatosoma* Dyar. Earlier studies in this area, indicated *Cx. tarsalis* as most abundant, comprising as much as 72%, 62%, 86%, 55.4% and 69.8% of the mosquitoes collected in 1986 and 1987 (Reisen et al. 1988), 1989, 1990 and 1991 (Mian and Prochaska 1990, 1991 and 1992), respectively.

In the valley region, mosquito composition by species was *Cx. tarsalis* (42.1%), *Cx. quinquefasciatus* (21.3%), *Cx. stigmatosoma* (20.1%), *Culiseta incidens* (Thompson) (9.4%), *An. franciscanus* (4.4%), and *Cs. inornata* (1.7%), with *Aedes increpitus* Dyar, and *Cx. erythrothorax* each comprising <1.0% of the total. In the Chino area of this valley region, the three culicine species in order of their relative abundance have previously been reported to be *Cx. quinquefasciatus*, *Cx. stigmatosoma* and *Cx. tarsalis* (Pfundner 1988). The Chino area is composed of various agricultural biotopes including, but not limited to, dairy farming which provide ideal habitats for the breeding of mosquito species in the aforementioned order.

Based on New Jersey light trap data (Table 2), mosquito activity was greatest in the desert region at

Table 1. Mosquito composition from all traps in San Bernardino County during 1992. Total collected mosquitoes were 6,375 and 3,621 in the desert and valley regions, respectively.

Species	% Composition	
	Desert	Valley
<i>Aedes increpitus</i>	0.0	0.9
<i>Aedes vexans</i>	13.2	0.0
<i>Anopheles franciscanus</i>	13.0	4.4
<i>Culex erythrothorax</i>	0.1	<0.1
<i>Culex quinquefasciatus</i>	4.2	21.3
<i>Culex stigmatosoma</i>	0.1	20.1
<i>Culex tarsalis</i>	55.0	42.1
<i>Culiseta incidens</i>	0.0	9.4
<i>Culiseta inornata</i>	14.4	1.7

urban sites (64.5%) followed by suburban (27.4%) and rural sites (8.1%). Both the suburban and urban sites were closer to mosquito breeding sources (treated sewage water basins and golf course ponds) than the rural sites. In contrast to the desert region, mosquitoes were found in higher numbers in both rural (55.1%) and suburban (36.4%) habitats than at urban sites (8.4%) of the valley region. This distribution pattern could be attributed to the proximity of trap sites to mosquito breeding habitats ranging from domestic or residential swimming pools to flood control structures in the urban and suburban habitats, or to seepage water in ponds, ground depressions and irrigation ditches in cultivated crops by the Colorado River in rural areas.

Table 2. Distribution of mosquitoes collected in New Jersey light traps operated throughout San Bernardino County during 1992. Total collected mosquitoes were 3,113 and 3,831 in the desert and valley regions, respectively.

Trap location	% Mosquitoes/trap-night	
	Desert	Valley
Rural	8.1	55.1
Suburban	27.4	36.4
Urban	64.5	8.5

Mosquitoes from the desert region showed population peaks in all habitats during May and November (Table 3). Two small peaks of mosquito activity also occurred during February and July. High spring and fall mosquito population levels in various habitats resulted in intensified control efforts employing different larvicides such as larvicidal oil (Golden Bear 1356), methoprene (Altosid® briquets) or *Bacillus thuringiensis* var. *israelensis* (Bactimos® pellets), depending on the breeding site.

*Culex tarsalis* was predominant in New Jersey light traps during the spring and summer months, whereas *Cs. inornata* prevailed during the fall and winter months (Table 4). *Culex tarsalis* was similarly the most abundant species in the CO<sub>2</sub>-baited traps of this region during April through October.

In the valley region, mosquito populations peaked in the rural sites during June. Populations in the urban and suburban sites remained <1.0 and <2.0 mosquitoes/trap-night, respectively, during the season (Table 3). Mosquito activity at these habitats during April through October was mainly due to *Cx. stigmat-*

*osoma*, *Cx. tarsalis*, and *Cx. quinquefasciatus* (Table 5). Dry ice-baited traps, however, presented a slightly different pattern of mosquito abundance for this time with *Cx. tarsalis* most abundant followed by *Cx. stigmatosoma* and *Cx. quinquefasciatus* (Table 5).

In our arbovirus surveillance studies, 117 mosquito pools (40 from the desert and 77 from the valley region) were submitted to VRDL for testing. One pool of *Cx. tarsalis* collected on June 17 in Needles tested positive for WEE. During the same month, on June 30, one sentinel chicken seroconverted to WEE. Later, on August 25, three more seroconversions were found in the Needles flock. Upon detection of the virus activity, the area was posted with encephalitis warning signs and area residents were advised on preventative measures through newspaper press releases. At the same time, source reduction and larvicidal activities were intensified.

Unlike the desert region, no valley region mosquito samples or chicken sera showed virus activity.

#### ACKNOWLEDGEMENTS

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Table 3. Seasonal abundance by habitat of mosquitoes collected in New Jersey light traps from the desert and valley regions of San Bernardino County during 1992.

	Month	Mosquitoes/trap-night			Mean
		Urban	Suburban	Rural	
<b>DESERT REGION</b>	January	3.4	5.4	0.9	3.2
	February	0.0	15.5	1.5	5.7
	March	6.2	5.6	0.5	4.1
	April	9.1	1.5	0.4	3.7
	May	15.6	2.6	2.5	6.9
	June	5.5	0.1	0.5	2.0
	July	16.2	0.0	0.3	5.5
	August	1.7	0.0	0.5	0.7
	September	0.5	0.1	0.1	0.2
	October	1.4	1.5	0.8	1.2
	November	4.8	18.6	1.1	8.2
	December	3.7	6.1	0.1	3.3
<b>VALLEY REGION</b>	April	0.9	1.4	1.3	1.2
	May	0.3	0.4	3.3	1.3
	June	0.4	1.1	5.0	2.2
	July	0.1	0.7	2.0	0.9
	August	0.2	1.4	1.9	1.2
	September	0.1	1.6	1.2	0.6
	October	<0.1	0.0	1.5	0.5
	November	<0.1	0.0	0.8	0.3
December	<0.1	0.0	0.1	0.1	

Table 4. Seasonal abundance of mosquito species collected in New Jersey light traps and CO<sub>2</sub>-baited traps from the desert region of San Bernardino County during 1992. Total collected mosquitoes were 3,113 and 3,262 in the New Jersey and CO<sub>2</sub>-baited traps, respectively.

Month	Mosquitoes/trap-night by species						
	<i>Ae. vex.</i>	<i>An. fran.</i>	<i>Cx. eryth.</i>	<i>Cx. quinq.</i>	<i>Cx. stig.</i>	<i>Cx. tars.</i>	<i>Cs. inorn.</i>
<b>N.J. LIGHT TRAPS</b>							
January	0.0	0.0	0.0	<0.1	0.0	0.9	2.2
February	0.0	0.0	0.0	0.0	0.0	3.0	5.5
March	0.0	0.0	<0.1	0.2	<0.1	2.5	1.2
April	0.0	0.0	<0.1	0.7	<0.1	2.8	0.1
May	0.0	0.0	0.0	0.5	0.0	5.9	0.0
June	0.0	<0.1	0.0	0.5	0.0	1.5	0.0
July	<0.1	0.1	0.0	0.1	0.0	8.1	0.0
August	0.0	0.0	0.0	0.2	0.0	0.6	0.0
September	0.0	<0.1	0.0	0.0	0.0	0.2	0.0
October	0.1	<0.1	0.0	<0.1	<0.1	0.1	0.8
November	<0.1	0.0	0.0	0.0	0.0	0.1	7.9
December	<0.1	0.0	0.0	0.0	0.0	0.1	4.2
<b>CO<sub>2</sub>-BAITED TRAPS</b>							
April	1.5	0.0	0.0	1.8	0.0	42.0	0.1
May	5.3	0.0	0.0	0.4	0.0	52.3	0.0
June	0.0	0.0	0.0	0.9	0.0	6.3	0.0
July	0.3	0.0	0.0	0.7	0.0	123.1	0.0
August	1.4	0.0	0.0	0.0	0.0	3.6	0.0
September *	--	--	--	--	--	--	--
October	110.7	116.0	0.0	0.1	0.0	3.4	0.9

\* No data available.

Table 5. Seasonal abundance of mosquito species collected in New Jersey light traps and CO<sub>2</sub>-baited traps from the valley region of San Bernardino County during 1992. Total collected mosquitoes were 718 and 2,903 in the New Jersey and CO<sub>2</sub>-baited traps, respectively.

Month	Mosquitoes/trap-night by species							
	<i>Ae. increp.</i>	<i>An. fran.</i>	<i>Cx. eryth.</i>	<i>Cx. quinq.</i>	<i>Cx. stig.</i>	<i>Cx. tars.</i>	<i>Cs. incid.</i>	<i>Cs. inorn.</i>
<b>N.J LIGHT TRAPS</b>								
April	<0.1	0.0	0.0	0.1	0.2	0.2	<0.1	0.2
May	0.0	0.0	0.0	0.3	0.4	0.2	0.1	<0.1
June	0.0	0.2	<0.1	0.1	0.4	0.2	<0.1	0.0
July	0.0	0.2	<0.1	<0.1	0.1	0.2	<0.1	<0.1
August	0.0	0.3	0.0	0.1	0.2	0.2	<0.1	0.0
September	0.0	0.1	0.0	0.2	0.1	0.1	<0.1	0.0
October	0.0	0.1	0.0	0.1	<0.1	0.1	0.0	0.2
November	0.0	0.0	0.0	<0.1	<0.1	<0.1	0.0	0.1
December	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<0.1
<b>CO2-BAITED TRAPS</b>								
April	4.2	0.0	0.0	4.9	11.4	27.3	7.0	0.1
May	0.0	0.0	0.0	10.2	30.2	34.4	4.6	0.2
June	0.0	0.0	0.0	6.8	2.8	11.8	5.3	0.0
July	0.0	0.0	0.0	7.0	7.0	9.9	4.4	0.0
August	0.0	0.0	0.0	10.2	5.4	4.4	2.0	0.0
September	0.0	0.0	0.0	9.5	3.0	23.2	0.9	0.0
October	0.0	0.0	0.0	4.0	2.3	3.9	3.1	0.2

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## ENCEPHALITIS VIRUS ACTIVITY IN THE COACHELLA VALLEY DURING 1992<sup>1</sup>

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### ABSTRACT

Seasonal and geographical distributions of mosquito abundance and encephalitis virus activity were monitored during 1992 at 18 sites throughout the Coachella Valley. *Culex tarsalis* abundance increased during spring at saline marshes along the Salton Sea, decreased during midsummer and then increased again during fall in association with the flooding of duck ponds. Encephalitis virus activity was detected first at saline marsh and duck club habitats along the Salton Sea in the southern portions of the valley and then dispersed northwest along the Whitewater Channel as far north as Indio. These temporal and spatial patterns of virus activity observed during 1992 were consistent with those observed during 1991. Encephalitis cases were not recognized during either year, despite virus transmission in close proximity to humans.

Saint Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) viruses have been active in the Coachella Valley during six of the last eight years. Sampling has been intensified progressively from a single site near the town of Mecca in 1986 to 18 sites scattered throughout the valley in 1992. This enhanced surveillance has increased the sensitivity of virus detection and delineated seasonal and spatial patterns of transmission. The patterns observed during 1991 (Reisen et al. 1992) indicated that the failure to detect virus activity in previous years may have been the result of low and/or spatially discontinuous activity which was not detectable at the limited number of widely spaced sample sites.

The purpose of the present research was to extend our 1991 findings pertaining to the seasonal

patterns of encephalitis virus activity and to relate these findings to vector abundance and landscape ecology.

### MATERIALS AND METHODS

Saint Louis encephalitis and western equine encephalomyelitis virus activity was monitored biweekly at 18 study sites during 1992 by bleeding flocks of ten sentinel white leghorn chickens to detect seroconversions (Fig. 1). Positive birds were replaced after a confirmatory bleed. *Culex tarsalis* Coquillett abundance was monitored at two fixed standards at each study site using CDC-style traps baited with dry ice. Collections were averaged to diminish trap position effects. Up to 10 pools of 50 *Cx. tarsalis*

<sup>1</sup> A paper fully detailing this study will be submitted for publication in the Journal of Medical Entomology.

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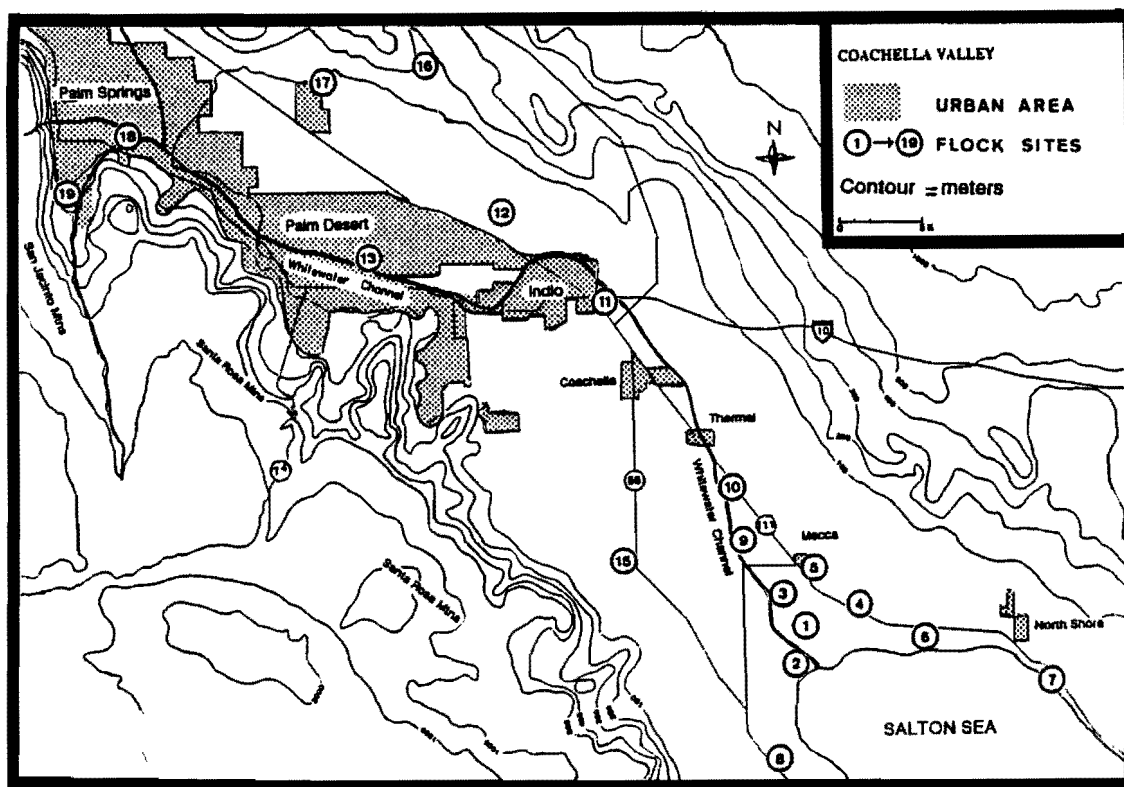


Figure 1. Map of the Coachella Valley showing the distribution of 18 study sites during 1992.

females from each site were tested for viral infection using a plaque assay with Vero cells for virus isolation and an in situ enzyme immunoassay (EIA) for virus identification. Other potential vector mosquitoes, including 134 *Aedes dorsalis* (Meigen), 880 *Culex quinquefasciatus* Say, and 1,061 *Culiseta inornata* (Williston) were tested for virus when *Cx. tarsalis* was absent or in very low numbers.

Sample sites used during 1991 (Reisen et al. 1992) were continued during 1992 (Fig. 1), with the exception of site 14 which had shown little virus activity and low *Cx. tarsalis* abundance during the past five years. Four new sites were added in the northern region of the valley. Sites 18 and 19 were situated in heavily populated Palm Springs, whereas site 17 was located at a horse stable in the arid eastern region. Site 16, at the Thousand Palms Oasis, was selected because of its potential to concentrate vertebrate and invertebrate hosts in an isolated foothill environment.

## RESULTS

### Vector Abundance.

*Culex tarsalis* abundance in 1992 followed a bimodal pattern similar to 1991 and previous years (Fig. 2). Similar to 1991, sites were placed into one of three groups (A, B or C) based on seasonality and abundance. Group A (sites 7 and 8) and Group B (sites 1-6, 9 and 10) were situated around the northern shore of the Salton Sea and northwest up the Whitewater Channel as far as Thermal. The differences in abundance within this region appeared to be related to the proximity of trap sites to breeding habitats; however, a careful mapping of breeding sites and their temporal patterns coupled with studies of adult dispersal will be required to elucidate factors responsible for among-site differences. *Culex tarsalis* abundance at Group C (sites 11-13 and 16-19), although still showing a bimodal trend, remained at very low levels throughout 1992. In general, abundance was greatest in proximity to the shoreline of the Salton Sea and associated duck clubs, and

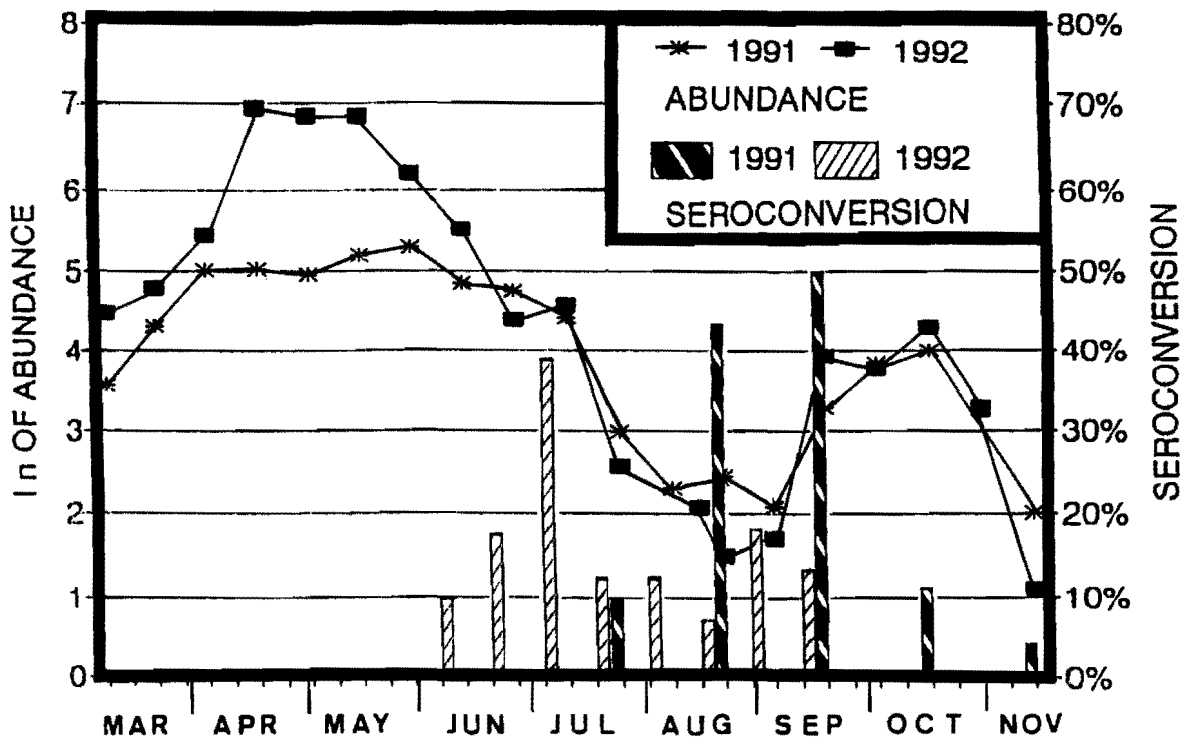


Figure 2. *Culex tarsalis* abundance at CO<sub>2</sub> traps and percentage of sentinel chickens seroconverting to SLE in the Coachella Valley during 1991 and 1992.

declined northward.

**SLE Virus Activity.**

Three SLE virus transmissions to sentinel chickens were detected during the winter of 1991-1992: site 2 in December 1991 and sites 6 and 12 in January 1992. These seroconversions seemed to be associated with a continuation of SLE virus activity during the fall of 1991, because there was a gap of more than five months before SLE virus was detected again in June 1992.

SLE virus transmission, measured by chicken seroconversions, conformed spatially to the geographic pattern of mosquito abundance, being highest near the Salton Sea and then declining northward. One exception was the Pablo Ranch in a citrus orchard habitat (site 12, Fig. 1), which was disjunct from the remaining SLE virus positive sites in the lower valley. Intervening sites 10 and 11 showed no virus activity. The overall seasonal distribution of SLE virus included sites 1-9, 12 and 15.

SLE virus was first detected on June 1 at site 8, but then by-passed sites in the central region to appear at site 7 on June 15 and site 2 on June 29.

Virus activity did not spread to site 12 until 10 August. Final transmission to sentinel chickens was detected on December 15 at site 7. Five SLE positive *Cx. tarsalis* pools were collected from four separate sites (2, 6, 8 and 15); chickens did not seroconvert at site 15. One positive pool of *Cx. quinquefasciatus*, the first since 1986, was collected at site 12 on August 8, during a time when the first sentinels became infected at this site; the remaining mosquito species tested were negative. Virus activity was found to be temporally discontinuous at some sites, but continuously active in the Coachella Valley.

**WEE Virus Activity.**

WEE virus seroconversions in sentinel chickens were limited to sites in the southeastern portion of the valley and were restricted geographically when compared with 1991. The final distribution of WEE virus included seroconversions at sites 1 and 3-8, plus positive mosquito pools at sites 9, 10 and 12.

WEE virus was first detected on February 27 at site 9 in a *Cx. tarsalis* pool; however, seroconversions in sentinel chickens did not occur until June 1 at sites 5 and 6. Seroconversions followed at site 7

on June 15 and site 8 on June 29. The last seroconversions were detected on August 25 at sites 3 and 4, and the last virus positive pool was collected on October 14 at site 2. Six positive *Cx. tarsalis* pools were found, four of which were collected at sites 2, 9 and 10, where chickens remained seronegative.

### DISCUSSION

The temporal and spatial discontinuity of SLE and WEE virus distributions may have been the result of low level activity during 1992. Although WEE virus activity was too low to allow a meaningful comparison with 1991, SLE virus activity was sufficiently active. Because sentinel chickens were bled at 2-week intervals in 1992 and at 4-week intervals in 1991, the temporal data requires some explanation. The onset of SLE virus activity at Group A sites began at least three weeks earlier and at much higher levels in 1992 than in 1991. Activity was bimodal in 1992, declining in August, but then increasing again in September, whereas activity in 1991 was unimodal with a single increase during August and September. Group B data from the duck club region showed a similar, but more disparate relationship. Here, the onset in 1992 was at least seven weeks earlier than 1991, but late season activity was much below that of 1991.

Similar to 1991, virus activity in 1992 was asynchronous temporally with mosquito abundance. SLE virus activity exhibited a bimodal pattern, increasing initially after the decline of *Cx. tarsalis* abundance in May, declining concurrent with abundance in July, forming a second peak in September somewhat before the fall rise in *Cx. tarsalis* abundance, and then disappearing around the middle of October as fall vector abundance reached its seasonal maximum.

In summary, the level of virus activity as measured by positive mosquito pools and sentinel chicken seroconversions was less in 1992 than 1991. However, the spatial distribution of SLE and WEE virus activity was similar in both years, although

slightly restricted during 1992. Because WEE virus activity in 1992, with the exception of site 7, was barely detectable, its geographical distribution was discontinuous. In 1991, both SLE virus and WEE virus were first detected near salt marshes along the margin of the Salton Sea; however, in 1992 WEE virus transmission appeared first simultaneously at sites 5 and 6, whereas SLE virus, in accordance with 1991, appeared first at site 7. Generally the activity of both viruses moved from the Salton Sea northward to the towns of Mecca, Thermal, and Indio. Although virus activity has been detected repeatedly in and around these towns, human cases were not reported by the Encephalitis Virus Surveillance Program during either 1991 or 1992. Planned research during 1993 will attempt to determine the role of these arboviruses as a source of clinical and sub-clinical illness in the southern Coachella Valley.

### ACKNOWLEDGMENTS

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## EVALUATION OF NEW METHODS FOR SAMPLING SENTINEL CHICKENS FOR ANTIBODIES TO WEE AND SLE VIRUSES <sup>1</sup>

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### ABSTRACT

A new method of bleeding sentinel chickens was evaluated in 1992 using experimentally and naturally infected birds. Several drops of whole blood from a small lancet prick of the comb apex were absorbed onto filter paper strips, air-dried, mailed to the laboratory and stored at room temperature. Blood spots were eluted overnight and the eluate compared against sera collected by jugular puncture using the same enzyme immunoassay. Time after infection until a diagnostic rise in antibody titer was similar for both samples for WEE virus; however, detection of a diagnostic rise in antibody to SLE virus was delayed 2 days with the blood spot method. Overall, >99% of 461 paired samples from sentinel chickens gave identical results using both bleeding methods; only 2 of 34 WEE and 1 of 38 SLE samples were positive by the jugular method and negative by the blood spot method. There were no false positives. Based on these results, the blood spot method will be used for sentinel chicken bleeding during 1993 in the statewide Encephalitis Virus Surveillance Program.

### BACKGROUND

The California statewide Encephalitis Virus Surveillance (EVS) program utilizes white leghorn laying hens as sentinels to monitor the enzootic transmission of western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses. Over the past few years the Arbovirus Research Program of the University of California, the Viral and Rickettsial Disease Laboratory (VRDL) of the California Department of Health Services, and the California Mosquito and Vector Control Association (CMVCA) have attempted to improve the sensitivity and rapidity of the EVS program so that control agencies have sufficient time to prevent the

amplification of encephalitis viruses to levels where they are transmitted tangentially to humans.

Previously, sentinel chickens were bled every four weeks by jugular venipuncture, and the sera tested for IgG antibodies using an enzyme immunoassay (EIA). In 1992 detection time was shortened by bleeding the sentinels every two weeks. However, processing still required centrifugation to separate the sera and shipment with refrigeration, either cooled by "blue ice" or frozen on dry ice. Sera had to be shipped before Wednesday to ensure arrival at the VRDL during the work week. In practice, specimens frequently were shipped the following week, thereby delaying testing by one week.

The present report summarizes new research

<sup>1</sup> A paper fully detailing this study will be submitted for publication in the Journal of the American Mosquito Control Association.

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aimed at facilitating the bleeding of sentinel chickens and at reducing the time from sentinel infection to antibody detection.

### NEW BLEEDING METHOD

Enzyme Immunoassays for antibodies to a variety of viruses are performed routinely at the VRDL on specimens of dried blood collected on Whatman No. 1® filter paper. The purpose of our research was to develop and evaluate a blood spot collection system that could be used to detect antibodies to WEE and SLE viruses in the blood of sentinel chickens.

#### Sample Collection.

Bird band numbers were stamped onto filter paper strips (0.5 x 3.0 inches) using a sequential number stamp. The apex or tip of the chicken's comb was punctured with a standard blood collection lancet. The wound was allowed to bleed until a drop of blood formed. The pre-numbered strip of filter paper then was touched to the droplet and the blood absorbed onto the paper. Sufficient blood was collected in this fashion until a 0.75 inch portion of the 0.5 x 3.0 inches filter paper strip was soaked thoroughly with blood. This size sample was necessary to enable the laboratory to make at least two standard-sized punches from the paper strip. Special care was taken to prevent contamination among strips and to make sure the strips were thoroughly air dried before packaging. For mailing, strips from each flock then were stapled onto a 5.0 x 7.0 inches index card, upon which was written the date and the flock location. The dried blood samples then were placed into a plastic bag, the data sheets attached with a paper clip, and placed with the other flocks into an envelope and mailed to the VRDL for testing.

### EVALUATION

#### Experimental Infections.

Laboratory and field research was conducted during 1992 to evaluate the new blood collection procedure and to determine the feasibility of modifying the on-line EIA to detect IgM antibody. Groups of five adult white leghorn hens were inoculated subcutaneously in the neck with 4.3 log<sub>10</sub> plaque forming units (PFU) of WEE virus (BFS 1703 strain) and 3.2 and 4.1 log<sub>10</sub> PFU of SLE virus (BFS

1750 strain) per 0.1 ml. An additional bird was inoculated with saline and added to each cage as a control. All birds were bled daily from the jugular vein for seven days to detect viremia using a plaque assay in Vero cell culture. Sera were collected by jugular venipuncture on alternate days for 18 days, weekly for four weeks and then biweekly for twelve weeks. Sera were separated by centrifugation, stored at -70°C, and later tested by EIA for IgG and IgM antibodies and by indirect fluorescent antibody (IFA) assay. Concurrently with serum samples, whole blood was collected on filter paper, air dried and stored at room temperature. At a later date, a punch from each paper was placed into a well of a 96 well plate, the blood eluted into 200 µl of buffered saline and the eluate tested for IgG antibody by the standard EIA.

The peripheral viremia for all birds infected with WEE virus remained <1.6 log<sub>10</sub> PFU per 0.1 ml of blood for days 1-7 post infection. This viremia was too low to infect all but the most susceptible *Culex tarsalis* Coquillett. Chickens infected with SLE virus did not produce a detectable viremia. Therefore, adult chickens were considered safe for use as sentinels near human populations.

IgM antibodies to WEE virus were detected in sera from 4 of 5 hens on day 6 post infection; all hens were IgM positive by day 8. Four of 5 birds were positive on day 10 by both serum and blood spot IgG EIA's and by the IFA test. All infected birds were positive by all assays by day 10 post infection. All sera positive for WEE antibody by EIA was positive concurrently by IFA assay.

In the SLE virus experiment, serum from birds inoculated with 3.2 log<sub>10</sub> PFU became IgM positive between days 4 and 14 post infection; 8 to 0 days before becoming positive for IgG antibody. Birds were positive for SLE IgG antibodies by the serum EIA 4 to 0 days before becoming positive by the EIA performed on blood spot samples and by the IFA assay. All birds inoculated with SLE virus were positive by all tests by day 16. Similarly, when inoculated with 4.1 log<sub>10</sub> all 5 birds became positive for IgM antibody on day 8, for serum IgG antibody by day 10 and for IFA and blood spot IgG antibodies by day 14.

Therefore, the time from infection to antibody detection in adult chickens by the blood spot and serum IgG EIA's was estimated experimentally to be about 10 days for WEE and 14 days for SLE viruses. Because of the slight delay in IFA compared with the serum IgG EIA, birds recently positive for SLE virus



by IgG EIA would not necessarily be confirmed by an IFA test on the same sample. These data emphasized the importance of confirmatory second bleeds for SLE positive birds.

Control birds in all groups remained negative for all tests, indicating that there was no bird to bird transmission within the cages through food, water, or pecking.

Results with IgM EIA on serum samples were encouraging; however, this test did not work well with antibody eluted from whole blood spotted onto filter paper. Therefore, the IgG EIA was used for the field evaluation during 1992 and for the EVS program starting in 1993.

#### Field Evaluations.

During 1992, three sentinel flocks each in the Imperial and Coachella Valleys were bled concurrently using the standard jugular venipuncture method and the new blood spot method described above. Sera from jugular samples were tested by IgG EIA as part of the EVS program; positive birds were rebled and then replaced. At a later date, blood spot samples were tested for antibodies to WEE and SLE viruses using the same IgG EIA.

Overall, there was >99% (n = 461) agreement between the IgG EIA performed on sera and blood spot samples. Three chickens were positive for both WEE and SLE antibodies by both tests. Two of 34 and 1 of 38 serum samples positive for antibodies to WEE and SLE viruses, respectively, were missed by testing blood spot samples. There were no false positives. The three false negatives had EIA optical

density values just below the cut-off. Because serum IgG was detected before blood spot IgG in some experimentally infected birds and because all positive birds were replaced, false negatives presumably occurred in birds recently infected and sampled before the 10 and 14 day period necessary for the diagnostic antibody rise to WEE and SLE viruses, respectively.

#### BENEFITS OF NEW BLEEDING METHOD

We anticipate that using the blood spot method will increase the rapidity of antibody detection by reducing field processing time (Table 1). Blood spot samples do not need to be centrifuged, shortening processing time by one day. Shipping time also can be reduced by one day by using overnight mail instead of ground transport by a shipping service. Conversely, laboratory testing time may be increased by one day, because punches from the blood spots may have to be eluted overnight to ensure the recovery of sufficient antibody from the paper. As before, the EIA can be run in one day, with positives confirmed the following day using IFA. Therefore, if bloods are collected on Monday and mailed the same day by overnight mail, they will be received in the laboratory by Tuesday morning. If the plates are set up the same day, the EIA could be performed on Wednesday and positives confirmed by IFA on Thursday. Bleeding, shipping, processing and reporting thus would take four or five days.

Currently using the jugular venipuncture

Table 1. Time in days from virus infection until WEE and SLE virus antibody detection by EIA and confirmation by IFA in jugular sera and whole blood spotted onto filter paper.

	Time (days)			
	Sera		Blood-spot	
	WEE	SLE	WEE	SLE
Antibody rise	10	14	10	14
Bleeding/processing	2	2	1	1
Shipment/handling	2	2	1	1
EIA + IFA	2	2	3	3
Total processing*	9-10	9-10	5	5
Biweekly bleed range**	20-34	24-38	15-29	19-33
Monthly bleed range**	20-48	24-52	15-43	19-47

\* Presumes samples were taken on Monday and includes delays due to weekends.

\*\* Range in days after chicken was bitten by an infective mosquito until antibodies are first detected. Upper limit presumes the chicken was infected when bled, but antibody titer was below the threshold for a positive EIA result.

method, bloods collected on Monday are centrifuged on Monday afternoon or Tuesday morning, frozen, and then shipped to the VRDL on Tuesday. Shipments are received on Thursday and then saved for processing on the following Monday. The EIA takes the same one day, with the confirmatory IFA performed the following day. Therefore, overall bleeding, shipping and processing time actually is 9-10 days. With a biweekly bleeding schedule and 10-14 days required for a detectable antibody rise in all sentinels, the time from infection to detection can be reduced by about five days for both viruses. Bleeding once a month greatly delays detection time to as long as 43 or 47 days after sentinel infection, even using the new method.

The blood spot method may save CMVCA agencies as much as \$27,000 per year in the cost of bleeding supplies and shipping expenses. For a flock of 10 hens, bleeding supplies cost about \$6.00 per bleed for the jugular venipuncture method compared to \$0.80 per bleed for the blood spot method. Considering dry ice and shipment charges based on current costs in Kern County, each bleed costs about \$20 (admittedly shipping costs per flock will decrease with the number of flocks included in each shipment). During 1993 there will be a projected 1,422 flock bleeds (i.e., number of flocks of 10 chickens each x number of times bled) at a possible maximum cost of \$29,506 for shipping and supplies. Comparable calculations for the blood spot method would be only \$2,403 using regular mail or \$16,623 by overnight express mail. Not included in these calculations were

the labor required to prepare the needles and vacutainers and to centrifuge and process the blood specimens. If preparation time for vacutainers and filter papers is considered to be roughly similar, centrifugation and decanting sera would require an additional 30 min per flock. If the average salary is considered to be around \$8.00 per hour for technical staff, the new method provides an additional salary savings of \$5,688 for the EVS program.

In summary, the blood spot method should increase the rapidity of antibody detection, while facilitating the field processing of specimens without losing sensitivity or specificity. We feel this new method will facilitate sentinel bleeding during the 1993 season and may convince those agencies still bleeding monthly to bleed biweekly.

#### ACKNOWLEDGMENTS

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## BIONOMICS OF *CULEX TARSALIS* IN THE COACHELLA AND IMPERIAL VALLEYS

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### ABSTRACT

Population dynamics and bionomics of host-seeking *Culex tarsalis* was investigated at a farm near the Salton Sea in southeastern Coachella Valley and near a heron rookery along the New River in Imperial Valley. Both western equine encephalomyelitis and St. Louis encephalitis viruses were active during periods of the year when host-seeking *Cx. tarsalis* were smallest in size and least likely to survive more than a single gonotrophic cycle. The number of host-seeking parous females was greatest during the spring and fall, before and after peak virus activity.

The purpose of the present research was to relate temporal changes in the bionomics of *Culex tarsalis* Coquillett to seasonal changes in western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) virus activity in the Coachella and Imperial Valleys of southeastern California. This project extended the earlier studies of Nelson (1971) and others, and was particularly important, because both viruses are active during midsummer when vector abundance is reduced, and then decline in activity during fall, even though temperatures are still warm and *Cx. tarsalis* females increase in abundance (Reisen et al. 1992). The present interim report summarizes data collected from March 1991 through November 1992. We plan to complete observations on host-seeking female *Cx. tarsalis* during the winter of 1992-1993, when we will supplement our findings with comparative data on resting females.

### MATERIALS AND METHODS

#### Study Areas.

Two study areas were selected in southeastern California: Adohr Farms near the town of Mecca in Riverside County and Rio Bend near Seeley in Imperial

County. Both areas historically have supported elevated *Cx. tarsalis* abundance and WEE and SLE virus activity. Representative of the southeastern Coachella Valley, Adohr Farms consists of undeveloped alkaline desert intermixed with date orchards and duck ponds. The Rio Bend area of the New River meanders through dense Tamarisk stands within a bottom area eroded into the surrounding farm land where flood-irrigated alfalfa and sudan grass are grown. Mosquitoes were trapped along the river bottom near a small lake supporting a rookery for a variety of water birds, especially cattle egrets.

#### Mosquito Collection and Processing.

Host-seeking female mosquitoes were collected biweekly from March 1991 to November 1992 using 3-4 CO<sub>2</sub>-baited traps operated at each site from dusk to dawn. Mosquitoes were returned to the laboratory alive where they were anesthetized, sorted to species and counted. Up to 50 *Cx. tarsalis* females from each collection were frozen at -70°C for later dissection. The remaining *Cx. tarsalis* were sorted into ≤10 pools of 50 females each, frozen at -70°C and later tested for WEE and SLE viruses using an in situ enzyme immunoassay. A flock of 10 sentinel white leghorn chickens was maintained at each site and bled monthly at Rio Bend

<sup>1</sup> A final version of this interim summary will be submitted for publication to the Journal of Medical Entomology.

into  $\leq 10$  pools of 50 females each, frozen at  $-70^{\circ}\text{C}$  and later tested for WEE and SLE viruses using an in situ enzyme immunoassay. A flock of 10 sentinel white leghorn chickens was maintained at each site and bled monthly at Rio Bend and biweekly at Adohr Farms to detect seroconversions to WEE and SLE viruses. Seropositive or dead chickens were replaced.

Up to 50 *Cx. tarsalis* females from each collection were dissected. The length of one wing was measured as an index of body size. The ovaries were excised, and one examined intact to determine parity from the degree of coiling of the tracheoles. The second ovary was disrupted, the primary follicles scored into Christophers' developmental stages, and the number of dilatations counted. The remnants including the intact diverticula were tested for the presence of fructose using the cold anthrone reaction. The autogeny status of *Cx. tarsalis* was determined at monthly intervals for females collected as pupae from breeding sites located near Adohr Farms.

## RESULTS AND DISCUSSION

### Bionomics of Host-Seeking *Cx. tarsalis* Females.

Overall, 3,306 host-seeking *Cx. tarsalis* females from Rio Bend and Adohr Farms were dissected. Most females (90%) had ovaries at Christophers' Stage I or I-II; only 1.3% exhibited maturation beyond Stage II and may have taken a previous blood meal. The parity rate at Adohr Farms (53%,  $n = 1,638$ ) was greater than at Rio Bend (41%,  $n = 1,668$ ). Only 6% of all females exhibited more than one dilatation; however, it bears mentioning that scoring dilatations in *Culex* may underestimate the number of older females (Reisen et al. 1986). Most host-seeking females (78%) with primary follicles at Stage I were parous; those with primary follicles at Stages I-II (39%) or II (1%) with detectable yolk were less likely to be parous.

Few host-seeking females (7%) had sacculate dilatations indicating recent oviposition. In agreement with the parity rates, more females at Adohr Farms (10%) than at Rio Bend (4%) had sacculate dilatations. These data indicated that most parous females probably refed the night after oviposition.

Despite major differences in topography and vegetation, about 40% of host-seeking females at both Adohr and Rio Bend were fructose positive.

There was no difference in the mean wing length of females trapped at Adohr Farms and Rio Bend. The mean wing length of host-seeking females pooled

over sites increased as a function of physiological age: 0 dilatations, wing length = 3.281 mm ( $n = 1,757$ ), 1 = 3.345 (1,354), 2 = 3.372 (187), and 3 = 3.415 mm (8). Therefore, larger females collected during winter also survived to oviposit more frequently than smaller females collected during summer. At Adohr Farms, the wing length of females collected as pupae and scored for autogeny status was compared with the wing length of concurrently collected host-seeking females during three months. Most emerging females (80%,  $n = 138$ ) were autogenous and significantly larger (3.51 mm,  $n = 110$ ) than concurrently emerging anautogenous females (3.22 mm,  $n = 28$ ). The wings of females emerging from field-collected pupae were longer than those of host-seeking females, which were not significantly different from anautogenous females emerging from field-collected pupae. Therefore, parous females which were comprised of both autogenous females host-seeking for the first time and anautogenous females seeking their second blood meal were larger than host-seeking nulliparous females comprised solely of anautogenous females.

At Rio Bend, parity increased to  $>40\%$  during midsummer, but then declined markedly to  $<20\%$  in December concurrently with a decrease in host-seeking abundance. Parity then increased markedly to  $>40\%$  during January-February, but then decreased again to  $<20\%$  during March, in conjunction with an increase in abundance. The actual number of parous host-seeking females was  $>200$  per trap-night during spring in both years and during the fall of 1992. Host-seeking female wing length was shortest ( $<3.25$  mm) and the fructose positivity rate was lowest ( $<40\%$ ) during late summer of both years. The fructose positivity rate increased during winter ( $>95\%$ ) concurrent with the increase in the parity rate at this time.

Comparable abundance and parity rate patterns were observed at Adohr Farms, except that a marked autumnal increase in abundance accompanied the flooding of duck ponds. The fall increase in the parity rate during 1992 (86%) accompanied a major increase in the autogeny rate of pupae collected from duck ponds at that time (76%). Autogeny rates were lowest (0%) during December 1991. Similar to Rio Bend, females were smallest (wing length  $<3.25$  mm) and less likely to have imbibed fructose ( $<40\%$ ) when collected during late summer.

### Relationship to Arbovirus Activity.

At Rio Bend, virus positive mosquito pools were

collected from July through September (1991: 18 WEE, 16 SLE, n = 1,731 females tested; 1992: 10 WEE, 2 SLE, n = 1,692) when the parity rate was >40%. However, the number of host-seeking parous females per trap night was greatest during May-June and September. Failure to detect virus during population increases was not just a statistical phenomenon related to limiting the numbers of pools tested to 10 per sample, because seroconversions in sentinel chickens also were not detected during this same time period. Seroconversions to WEE virus occurred during both years, whereas chickens did not seroconvert to SLE virus at Rio Bend during the summer of 1992.

Neither virus was detected in *Cx. tarsalis* females collected at Adohr Farms (1991, n = 3,657 females tested for virus; 1992, n = 5,194), even though sentinel chickens seroconverted to both viruses during July to September of both years (1991: 17 WEE and 11 SLE positive chickens; 1992: 1 WEE, 4 SLE). The percentage of new seroconversions varied independently of both the parity rate and the number of host-seeking parous females. Interestingly, seroconversions were detected during both years when the autogeny rate was high. This was unexpected because autogeny delays blood feeding in *Cx. tarsalis* and has been postulated as one factor responsible for suppressing arbovirus transmission.

As with most field research, our on-going study seems to have raised more questions than it has answered. Some points requiring further investigation include:

1. Why were both WEE and SLE viruses undetectable during spring when the number of parous host-seeking females collected per night increased?
2. Virus activity was restricted to midsummer when host-seeking *Cx. tarsalis* females were smallest. Does this imply that small-sized females were more efficient vectors of virus than larger females collected during fall and winter?
3. Virus activity declined markedly during late fall when the abundance of host-seeking females decreased, even though larvae were abundant throughout winter. This decrease in host-seeking abundance did not appear to be related to a simple decline in the recruitment rate, because

the parity rate decreased rather than increased during this period. Do *Cx. tarsalis* females emerging during late-fall enter a transient reproductive arrest which is rapidly terminated in late December?

4. Was the decrease in virus activity during periods of elevated vector abundance related to avian host-avoidance behavior diverting host-seeking *Cx. tarsalis* to mammalian hosts and thereby decreasing transmission efficiency?
5. Although scoring dilatations in *Culex* is controversial, our data indicated that only 6% of host-seeking females may have taken more than one previous blood meal. During the cooler months of the year, virus replication rates in mosquitoes may be too slow to be transmitted by 1-parous females. Does this thermodynamic interaction limit the period of virus transmission to the hot midsummer months?
6. Is horizontal virus transmission essentially independent of vector bionomics and dependent on the immune status of the avian host population?

We hope our on-going and planned research may be able address some of these yet unanswered questions.

#### ACKNOWLEDGMENTS

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## BIOGEOGRAPHY OF THE *Aedes (Ochlerotatus) communis* SPECIES COMPLEX IN THE WESTERN UNITED STATES <sup>1</sup>

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### ABSTRACT

It has recently been demonstrated that *Aedes communis* (DeGeer) represents a complex of cryptic species, at least three of which, *Aedes tahoensis* Dyar, *Aedes nevadensis* Chapman and Barr, and *Aedes communis* (DeGeer), are found in the Western United States. These three species are essentially indistinguishable on a morphological basis, and therefore, little is known regarding the geographic range of each species. Since members of this complex have been implicated as vectors of Jamestown Canyon (JC) virus in California and elsewhere, it is important to delimit the distribution of individual species in order to clarify their viral associations.

Specimens of *Aedes communis* (*sensu lato*) were collected as larvae or pupae at several sites in the Sierra Nevada, Cascades, and Klamath Range in California, as well as the Cascades in Oregon and Washington, the Ruby Mountains in Nevada, and the Wasatch Range in Utah. Living specimens were returned to the laboratory at U.C. Davis where they were reared to adults, identified as *Ae. communis s.l.*, sexed, and frozen at -80°C for arbovirus testing and electrophoretic studies. In addition, voucher specimens of larval and pupal exuviae and pinned adults were retained from each collection whenever possible. Frozen specimens were thawed, homogenized in individual microfuge tubes, and identified to species using polyacrylamide electro-

phoresis and histochemical staining to examine five diagnostic allozyme loci. Starch gel electrophoresis was used to examine loci these plus ten additional allozyme loci to investigate geographic variation within species.

Specimens from the Sierra Nevada, southern Cascades, and Trinity Alps in California were all determined to be *Aedes tahoensis*. Specimens from Oregon, Washington, Nevada, and Utah were identified as *Aedes nevadensis*. Based on our collections and previous literature, it appears these two species are allopatric, with *Aedes tahoensis* restricted to the high mountains of California and *Aedes nevadensis* found in the northern Cascades, Ruby Mountains, Wasatch and Bitterroot Ranges.

Although genetic distances among *Ae. tahoensis* populations were small, both species exhibited geographic variation in allozyme frequencies among populations. Intraspecific genetic distances were correlated with geographic distance between collecting sites in a linear manner, consistent with a "stepping stone" model. This suggests that each population along a mountain chain exchanges genes only with its nearest neighbors and therefore exhibit limited migration and dispersal ability. Since the dispersal range of these species is limited, arboviruses associated with them may be patchily distributed among populations.

<sup>1</sup>A manuscript fully reporting the results of this study has been submitted for publication in *Mosquito Systematics*.

## DEVELOPMENTAL RATES OF IMMATURE MOUNTAIN MOSQUITOES

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### ABSTRACT

Estimates of larval population parameters of mountain *Aedes* were collected because of extensive documentation that adult mosquito abundance and accompanying disease outbreaks may be related to developmental rates and survivorship of the immature stages. Both native and sentinel populations of two mosquito species, *Aedes cataphylla* Dyar and *Aedes schizopinax* Dyar, were monitored from the time they hatched until adult emergence with daily notation of larval numbers and life stage.

Days of stage duration were estimated to be 8 for 1st, 7 for 2nd, 13 for 3rd and 12 for 4th instar larvae in native *Ae. schizopinax*. Instar durations in sentinel *Ae. schizopinax* larvae were considerably different from natives with estimated durations in days of 13.3 for 1st, 4.8 for 2nd, 16.4 for 3rd and 5.9 for 4th instars. Pupae took the same time (9 days before eclosion) in both natives and sentinels. In contrast, *Ae. cataphylla* sentinel larvae exhibited a trend of shortening instar duration as time progressed; 1st instar duration was 9.2 days, 2nd was 9.6, 3rd was 7.2, 4th was 4.2 and pupa was 4.4 days. The overall developmental rate for immature *Ae. cataphylla* was faster at 34.6 days compared to 49.4 days

for *Aedes schizopinax*.

Habitat reduction due to drying and subsequent mosquito concentration produced sampling errors which prevented calculation of life table parameters for *Ae. cataphylla*. However, an estimation of the survivorship curve for *Ae. schizopinax* was produced and from that, stage-specific life table data was generated. It was found that the greatest mortality among native *Ae. schizopinax* larvae occurred in the 3rd instar and pupal stages. *Aedes schizopinax* immature survivorship (overall survival from 1st instar larvae to pupae) for native populations was estimated to be 7.5% in comparison to immature survival estimates of 32% for *Ae. schizopinax* sentinel larvae.

These data are suggestive of two things. First, application efficiency might be increased by treatment after the 3rd instar larval stage to allow natural mortality factors to augment control efforts and second, identification of predators or pathogens responsible for the differential mortality observed between native and sentinel groups could contribute favorably to future mosquito control efforts in snow pool habitats.



## MARK-RELEASE-RECAPTURE OF *Aedes dorsalis* IN CONTRA COSTA COUNTY

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### ABSTRACT

Two mark-release-recapture studies were conducted to determine the flight range and direction of *Aedes dorsalis* (Meigen), a pestiferous salt marsh mosquito. The studies were conducted along Suisun Bay in northern Contra Costa and southern Solano Counties, California, in July and September, 1992.

In July, approximately 12,260 *Ae. dorsalis* females were released from three Contra Costa County release points on one date. Mosquitoes were marked with a site-specific color of fluorescent dust. Forty CDC style CO<sub>2</sub>-baited traps were set over an 8 km (5 mi) radius for 12 consecutive nights. Six of the 40 traps were placed along the northern shore of Suisun Bay in Solano County with the remainder along the southern shore of the bay in Contra Costa County. Thirteen mosquitoes were recaptured in Contra Costa County and none in Solano County (recapture rate = 0.1%). Maximum recorded dispersal was 5.8 km (3.3 mi); this mosquito was recaptured five days post-release and had flown to the northwest, into the prevailing winds. In general, however, the predominant flight direction was to the northeast,

with the prevailing winds. The last mosquito was recaptured seven days post-release.

In September, approximately 24,070 *Ae. dorsalis* females were released from one Contra Costa County site on three dates. Mosquitoes were marked with a date-specific color of fluorescent dust. Thirty traps were set over a 2.4 km (1.5 mi) radius for 16 consecutive nights. Ten of these traps were set in Solano County, 1.6 to 2.4 km (1.0 to 1.5 mi) from the release point. Two hundred and ninety mosquitoes were recaptured in Contra Costa County and none in Solano County (recapture rate = 1.2%). Maximum recorded dispersal was the same as the trapping radius (2.4 km). The average distance dispersed was 1.0 km (0.64 mi) and the predominant flight direction was to the east, with the prevailing winds. The last mosquito was recaptured 15 days post-release, 1.2 km (0.75 mi) from the release point.

Based on these studies, most *Ae. dorsalis* females do not disperse more than 1.6 km (1.0 mi) from their larval source and fly primarily in the direction of the prevailing winds.

## HOW DO MALE *ANOPHELES FREEBORNI* FUEL SWARMING FLIGHT?

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### ABSTRACT

The objective of this study was to determine, in the field, the energy budgets of swarming male *Anopheles freeborni* Aitken. In particular, we established the energy source(s) used to fuel swarming flight, the amount of energy invested in this activity, and when sugar-feeding occurs.

Methods involved sampling *An. freeborni* males while resting in the morning and late afternoon, others as they began to swarm, and still others 20-25 minutes into swarming. To determine the exact amounts of lipids, carbohydrates and glycogen each individual male was crushed in 0.2 ml of a 2% sodium sulfate solution before 1.5 ml of a chloroform:methanol (1:2) solution were added to the homogenate and centrifuged. After centrifugation, the supernatant was divided into two parts - one for the lipid assay and the other for sugar assay. The precipitate was retained for glycogen analysis.

The first precipitate was used for lipid analysis by evaporating to dryness, adding 0.2 ml of sulfuric acid and heating the solution for ten minutes. Following heating, 4.8 ml of vanillin reagent were added and absorbance was read at 525 nm on a spectrophotometer after ten minutes.

Sugars were determined by evaporating the second supernatant to approximately 0.1 ml. Fructose, the most common nectar sugar, was determined by adding anthrone reagent and incubating at room temperature for 45 minutes before reading at 625 nm. This sample was then heated for 15 minutes to determine the concentration of non-fructose sugars (glucose and trehalose) and again read at 625 nm. Glycogen was also verified by the hot anthrone test. Anthrone was added to the precipitate and heated for 15 minutes and read at 625 nm.

Our results indicate that lipids were not used to fuel flight but may be used in resting metabolism. Stored sugars (glucose and trehalose) and glycogen are the primary sources of energy for swarming flight. During the 20-25 minutes of swarming, 0.09 cal/mosquito, derived from sugar, and 0.05 cal/mosquito, derived from glycogen, were consumed. Sugar-feeding occurs sometime during the night after swarming is concluded. Nectar sugars are, therefore, not available to fuel flight. On average, swarming consumes over 50% of the fuel reserves available to male *An. freeborni*. Accordingly, the ability of an individual to find and metabolize nectar sources will greatly affect reproductive success.

## INTERACTION OF CHEMICAL AND VISUAL CUES IN *CULEX TARSALIS* OVIPOSITION<sup>1</sup>

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### ABSTRACT

Populations of mosquitoes which are vectors of arboviral diseases are often monitored with traps baited with host or oviposition-site attractants. Oviposition attractants are most useful because they attract primarily gravid blood-fed mosquitoes which may have become infected with virus from feeding on a diseased host. However, few oviposition attractants have been identified. Furthermore, the interactions between chemical and physical cues which mediate oviposition are poorly understood. A detailed study of *Culex tarsalis* Coquillett oviposition behavior has been initiated. In the present study, we tested the oviposition responses of gravid *Cx. tarsalis* females to water treated with fermented Bermuda grass infusions, or fractions thereof, and/or black dye.

Bioassays were conducted in an environmentally controlled chamber maintained at  $26\pm 1^\circ\text{C}$ ,  $50\pm 10\%$  RH, with a 12L:10D photcycle provided by overhead florescent lights and one-hour dawn and dusk periods provided by a single 60W incandescent light bulb. Bioassays were conducted in rectangular cages (30 x 30 x 45 cm) constructed from PVC plastic frames covered with white muslin fabric. Depending on the availability of gravid females, four or eight cages were used for bioassays simultaneously. Gravid females (20/cage) were used 7-10 days after blood-feeding on chickens, and were given free access to 10% sugar water and raisins until one hour before bioassays, unless otherwise specified. Oviposition cups were clear glass (6 cm diameter x 6 cm high) which were filled with 100 ml of the aqueous tests solutions. Test solutions consisted of distilled water dyed with a drop of India ink (Faber-

Castell brand), or various dilutions of Bermuda grass infusions prepared according to Reiter (1983) or distilled water control. Treatments were tested in two-choice tests. Gravid females were allowed to oviposit overnight for a 15-hour period including dusk and dawn, unless otherwise specified. Bioassays were evaluated by counting the number of egg rafts laid in response to the various treatments and results were statistically analyzed by paired t-tests.

Gravid *Cx. tarsalis* females were stimulated to oviposit by all concentrations of the Bermuda grass (BG) infusions tested (0.8, 4, 10, 20 and 100%). Mosquitoes were also stimulated to oviposit by the dyed water. In combination, dye and BG infusions acted synergistically. However, responses varied with light levels during the dark cycle, when mosquitoes normally oviposit, and with feeding status. With gravid females denied access to sugar water for one hour before bioassay, and complete darkness during the bioassay period, BG infusion was preferred over dye, while dye was preferred over BG infusion under continuous dim light conditions (4W bulb). Under natural light conditions, with mosquitoes starved for a one-hour period prior to bioassay, responses to dye and BG infusions were not significantly different. However, the response of mosquitoes which had been denied sugar water for 24 hours was somewhat different; under natural light conditions, 24-hour starved mosquitoes laid significantly more egg rafts in the BG infusions than in the dyed water. Furthermore, examination of the egg rafts for the degree of tanning (proportional to age) demonstrated that 1-hour starved mosquitoes laid most of their egg

<sup>1</sup> A manuscript fully summarizing this study will be submitted to the Journal of the American Mosquito Control Association.

rafts around dawn, towards the end of the bioassay period, whereas 24-hour starved mosquitoes laid most of their egg rafts around dusk at the beginning of the bioassay period.

We conclude that gravid *Cx. tarsalis* use either chemical or visual cues, or both, to locate oviposition sites, depending on ambient light levels. Furthermore, subtle shifts in the response to oviposition cues occur, depending on the feeding status of the mosquitoes. The timing of oviposition bouts is also affected by feeding status, with sugar-starved mosquitoes favoring oviposition early in the dark cycle, while recently sugar-fed mosquitoes

oviposited preferentially late in the dark cycle.

With the simple bioassays used, it was not possible to determine whether the BG infusion contained oviposition attractants acting over a distance, or oviposition stimulants which released oviposition behavior on contact with the treated water, or both.

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## FIELD EVALUATION OF *CULEX* OVIPOSITION ATTRACTANTS ISOLATED FROM BERMUDA GRASS INFUSIONS

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The selection of an oviposition site is an important part of the life history of mosquitoes. The attractiveness of a particular oviposition site to gravid females is dependent upon a number of physiochemical factors. Previous studies have shown that various chemical compounds and visual cues may serve as oviposition stimulants and/or attractants for *Culex quinquefasciatus* Say, a common mosquito species which breeds in a wide variety of polluted water habitats. Millar et al. (1992) identified a five-component mixture (phenol, 4-methylphenol, 4-ethylphenol, indole and 3-methylindole) from an infusion of Bermuda grass, *Cynodon dactylon* (Linnaeus), which was shown in the laboratory to be attractive to ovipositing *Cx. quinquefasciatus*. In laboratory studies, 3-methylindole, a tryptophan degradation product, was shown to be the most attractive component and was attractive to ovipositing females at concentrations as low as ten parts per trillion (Millar et al. 1992). Other laboratory experiments showed that the chemical attractant mixture acted synergistically with visual cues in attracting gravid *Cx. quinquefasciatus* (Beehler et al. 1993). Our objective was to evaluate the attractiveness of these mixtures to ovipositing *Culex* mosquitoes under field conditions.

The first experiment was conducted on the premises of the Orange County Vector Control District (OCVCD) in Garden Grove. Four pairs of black plastic tubs (38 x 28 x 19 cm) were placed along a transect over four nights. Each pair of tubs was separated from the others by approximately 25 meters and the tubs in each pair were one meter apart. The tubs contained either eight liters of tap water or test solutions made up of eight liters of tap water containing a mixture of 4-methylphenol (1 mg), 4-ethylphenol (8 mg), phenol (19 mg), indole (1 mg) and 3-methylindole (6 mg). The chemical compon-

ents of the attractant mixture were present in the same ratio as those isolated from Bermuda grass infusion (Millar et al. 1992). After four consecutive nights, the egg rafts deposited in the tubs were counted and removed. The number of rafts recovered from the tubs containing the attractant mixture were compared to the number of egg rafts in the control tubs. This experiment was repeated three times for a total of twelve replicates.

Tubs which contained the attractant mixture collected significantly more egg rafts, receiving a mean of 1.6 rafts/trap-night, than controls which received a mean of 0.3 rafts/trap-night (paired t-test,  $P < 0.05$ ). Subsamples of egg rafts were collected, returned to the laboratory, reared individually to the fourth instar larval stage and all identified as *Cx. quinquefasciatus*.

The second experiment was also conducted at the OCVCD using gravid female mosquito traps which collect adult mosquitoes rather than the tubs which collect only egg rafts. Three pairs of gravid female mosquito traps (Reiter 1983) set on top of black plastic tubs were placed along a transect as described above. In each pair, one trap contained the attractant mixture in tap water (eight liters per trap) and the other, a control, contained only tap water. Traps were placed two hours before sunset and adult mosquitoes were collected from the traps the following morning, frozen and identified. Traps were set one night per week for six weeks (total of 18 replicates). The number of *Cx. quinquefasciatus* females collected were transformed to square roots ( $\sqrt{x}$ ) and treatments were compared to controls using multiple regression methods.

Gravid female traps containing attractant mixture collected significantly more *Cx. quinquefasciatus* adults than did untreated controls ( $P < 0.001$ ). During the six-week study period, the treated traps

collected approximately 2-3 times as many gravid *Cx. quinquefasciatus* as untreated controls. The treated traps also collected some *Culex tarsalis* Coquillett females (< 1.0% of the total) and several midges (*Chironomus* sp.).

Two field experiments were conducted to determine the attractancy to ovipositing *Culex* of 3-methylindole alone (the major attractant in the five-component mixture) using experimental ponds at the University of California-Riverside Aquatic and Vector Control Research Facility in Riverside. Six wooden-sided experimental ponds (27 m<sup>2</sup>) were flooded to a mean depth of 30 cm using canal water and the level was maintained using a float valve system. Three days post-flooding, three randomly selected ponds containing no egg rafts were treated with 5 g of 3-methylindole (0.6 mg/l pond water) in 100 ml of ethanol. The other three ponds, which also contained no egg rafts, were left untreated as controls. The following morning, a transect (5.5 m x 14 cm) along the south and west rail of each pond was examined for the presence of egg rafts. The experiment was repeated for a total of six treated and six untreated ponds.

Treated ponds received significantly more egg rafts than did the untreated controls (ANOVA,  $P < 0.05$ ). Ponds treated with 3-methylindole received a mean of 9.2 rafts/transect while a mean of 1.2 rafts/transect was sampled from the control ponds. Subsamples of egg rafts were reared individually in the laboratory to the fourth instar larval stage and identified as *Cx. quinquefasciatus* (47%) and *Culex stigmatosoma* Dyar (53%).

In summary, these studies show that the attractant mixture identified by Millar et al. (1992) was attractive to ovipositing *Cx. quinquefasciatus* in

the field. The most attractive component of the mixture, 3-methylindole, was shown to be attractive for both *Cx. quinquefasciatus* and *Cx. stigmatosoma*. Further work is needed to develop and standardize *Culex* attractants for arbovirus surveillance. In the future, these attractants may be integrated into overall control programs for mosquitoes.

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# SEASONAL ABUNDANCE AND DIVERSITY OF AQUATIC MIDGES IN COUNTRY CLUB LAKES IN THE COACHELLA VALLEY

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In the family Chironomidae there are currently recognized 10 subfamilies and 24 tribes (Ashe 1983), of which 7 subfamilies and 14 tribes with at least 2,000 species occur in the Nearctic region.

Midge larvae occur in all types of aquatic and semiaquatic habitats and to a lesser extent in brackish or marine waters, semi-terrestrial or terrestrial habitats. Midges, when emerging in large numbers, can cause a severe nuisance problem to residents around lakes, ponds and channels. They can cause considerable economic loss to the tourism industry. These aspects are discussed in detail by several authors (Mulla 1974, Ali and Mulla 1979, Ali 1991).

The problem of nuisance aquatic midges has been on the increase in the Coachella Valley of Southern California. This is especially critical in the cove cities area where country clubs and resorts have developed many ponds and lakes, which are potential breeding sites for midge larvae. Therefore, we conducted studies at four sites in the Coachella Valley with the purpose of elucidating the relationships between physical and chemical factors and midge abundance and to determine the seasonal population trends of midge larvae and genera.

## RESEARCH SITES AND METHODS

Our research sites were three country clubs (Portola Country Club, Lake Mirage Country Club and Mission Hills Country Club) and one public golf course (Palm Lakes Golf Course). The main reason to include these four sites in our studies were:

- type of water in the lakes

- a) tertiary effluent (Portola and Palm Lakes)
- b) well water (Lake Mirage and Mission Hills)
- physical features of the lakes
- age of the lakes
- weed and algae control program
- presence of fish species

At each of the four sites, we sampled two lakes with surface areas from 1-2 acres and depths of 3-8 feet. The big lake at Lake Mirage C.C. differed, being about 25 acres and 4-28 feet deep.

At Portola Country Club we sampled two lakes, No. 1 and No. 2, of seven in that country club. All lakes were lined with a heavy plastic layer, covered with about a 2-foot thick mixture of sand and detritus, and had concrete-lined banks. Tertiary water was used in all other lakes and in lake No. 2 during June, July and August. The rest of the year, lake No. 2 is filled with a mixture of well and tertiary effluent water from the nearby Palm Desert Sewer Plant which is operated by the Coachella Valley Water District.

The other site with a tertiary effluent water supply was Palm Lakes Golf Course. The main difference between these two sites was in the physical features of the lakes. At Palm Lakes G.C., the lakes have natural drainage without a plastic liner and concrete banks.

The lakes at Lake Mirage and Mission Hills Country Clubs were supplied with well water only. The big lake at Lake Mirage C.C. had concrete banks while the rest of the lakes had natural drainage without plastic or concrete layers on the bottom or sides.

Forty-one samples were taken bi-weekly for one

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year from along the shore with a long handled scoop or from a boat with an Ekman dredge. Larvae were separated from the bottom materials by the procedures described by Mulla et al. (1971).

In most cases, larvae were identified to genus. Some individuals were reared and identified to species by associating larval and pupal exuviae with adult males, using keys for Chironomidae of the Holarctic region (Wiederholm 1983, 1986, 1989).

Water samples were also taken bi-weekly from each lake. We estimated amounts of ammonia, ammonium, nitrate, nitrite and phosphate in the laboratory using a dr-er/2 Hach spectrophotometer. On each site, the following factors were measured: dissolved oxygen, water temperature, salinity, conductivity and pH, using a portable conductivity meter by Hanna Instruments (model HI 8033) and an Orion dissolved oxygen meter (model 820).

## RESULTS AND DISCUSSIONS

Substantially higher amounts of ammonia, ammonium, nitrate, nitrite and phosphate were recorded in the lakes supplied with tertiary effluent water, which at the same time supported a higher number of midge larvae.

At Portola C.C. midge densities averaged 300 larvae per sample, which equates to 50 million larvae per acre. We also recorded extremes of over 2,000 larvae per sample equating to 300 million midge larvae per acre.

In lake No. 1 at this site, dominant midge genera were *Chironomus* Meigen and *Tanytus* Meigen from October through May, *Cryptochironomus* Kieffer from October through April, and *Polypedilum* Kieffer from June through September. Specimens from the genera *Procladius* Skuse and *Goeldichironomus* Fittkau were also recorded in low numbers in this lake.

In lake No. 2 also at this site, *Cryptochironomus* represented 60% of the total larvae from December to March, *Einfeldia* Kieffer dominated from March through May, and *Polypedilum* from June through September, when lakes were supplied 100% with tertiary effluent water. Genera *Chironomus* and *Procladius* were recorded in low numbers during the winter months and *Goeldichironomus* was recorded in low numbers during the summer months.

As mentioned above, lakes at the other site with a tertiary effluent water supply, Palm Lakes G.C., have natural drainage and exhibit different midge

larval compositions than the lakes at Portola C.C. The dominant genera in these Palm Lakes G.C. lakes were *Tanytus* Wulp (present the entire year, constituting more than 50%, but less in August), *Tanytus* (October through March), *Procladius* (February to May) and *Polypedilum* (70% of the total midge larvae in August only).

The two other sites we sampled, Lake Mirage C.C. and Mission Hills C.C., have lakes supplied with well water. In Lake Mirage Country Club we sampled a large lake designated "big lake" and a smaller lake, designated "pond". Besides differences in size and depth, big lake has been under a microbial control program for weed and algae control for over two years. Samples from this site averaged 25 midge larvae. The microbial program probably reduced the food source for larvae, which may account for the low numbers of larvae found in the big lake. The average number of midge larvae per sample exceeded 200 in the pond with the most abundant genera being *Chironomus* (October to March), *Procladius* (March to June) and *Tanytus* (during summer and fall months). Other genera, such as *Polypedilum*, *Ablabesmyia* Johannsen, *Glyptotendipes* Kieffer, and *Cryptochironomus* were also encountered, but in low numbers.

At Mission Hills Country Club we began intensive research on two lakes in January, 1992. One of these lakes, designated "new lake", was completely reconstructed and refilled with water in the middle of November, 1991. Nine genera were recorded from this lake, including *Chironomus* and *Procladius* from January to March and *Ablabesmyia* from April through August. Specimens from five other genera, *Dicrotendipes* Kieffer, *Paratendipes* Thienemann and Bause, *Tanytus*, *Cricotopus* Wulp and *Cryptochironomus*, were also present year round, but not in high numbers.

The other lake we sampled at Mission Hills C.C., designated as "old lake", was about 15 years old. It was composed of two basins. The upper basin mainly produced *Chironomus* and *Tanytus* from January to May and *Polypedilum* in July and August while the lower basin produced *Ablabesmyia*, *Paratendipes*, *Goeldichironomus*, *Cryptochironomus* and *Cricotopus* midge larvae with *Psectrocladius* Kieffer being also present, but in low numbers.

It is important to mention that in this section of the lake in May, submerged vegetation became visible as filamentous branches of *Najas* pondweed, reached the water surface. At the same time, the Coachella Valley Mosquito Abatement District began



to receive complaints from the residents at Mission Hills Country Club. Visual observations confirmed that the onshore midge population was much higher than the larval numbers indicated. It appeared that genera such as *Psectrocladius*, *Goeldichironomus* and *Cricotopus*, collected as adults around the lake, were poorly sampled by the scoop because they were incidentally shaken loose as the scoop passed through the vegetation on the way to the bottom.

Fifteen midge genera were recorded between the four sites in the Coachella Valley. During the study period, five of them, *Chironomus*, *Tanypus*, *Procladius*, *Cryptochironomus* (except August) and *Tanytarsus*, were present throughout the year in different proportions (Table 1).

In the warmer months, generally beginning in March, the genera *Ablabesmyia*, *Paratendipes*, *Dicrotendipes*, *Einfeldia* and *Cricotopus* were present, but not abundant, while the genera *Goeldichironomus*, *Microspectra* and *Glyptotendipes* were overall relatively scarce (Table 1).

In the lakes supplied with tertiary effluent water, the genera *Chironomus*, *Tanytarsus*, *Tanypus* and *Cryptochironomus* were predominant in the winter and spring, replaced by *Polypedilum* in most of the summer months. In the cooler months of 1991 and

1992, *Chironomus* was the main source of nuisance, with 300 to 1,000 larvae per sample. Gradually its numbers declined in the summer to 10-200 larvae per sample, coinciding with higher numbers of *Polypedilum* larvae, which reached over 2,000 larvae per sample.

It is important to point out that the main breeding site for *Tanytarsus* larvae were lakes at Palm Lakes Golf Course while it was not recorded in the lakes at Portola Country Club. It is also evident that the lakes supplied with well water generally supported a lower number of midge larvae, but they had a much more diverse composition of fauna.

As in the lakes with tertiary effluent water, well water-supplied lakes were abundant in *Chironomus* which composed 20-80% of the total larval collection from October to May in 1991-92. At the same time, *Tanypus* and *Procladius* composed 10-70% of the total collections depending on the month and genus.

In the spring and summer, the genera *Ablabesmyia* and *Paratendipes* were recorded but not abundant, while the genera *Cricotopus*, *Dicrotendipes*, *Glyptotendipes*, *Goeldichironomus* and *Tanypus* were scarce. The genus *Psectrocladius* was recorded in such low numbers throughout the study that it is not

Table 1. Monthly composition by genus of aquatic midge larvae (in percentage of total collection) from two lakes at each of four sites in the Coachella Valley during 1991-92.

Genera	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
<i>Chironomus</i>	56	55	60	51	50	45	36	21	5	10	7	20
<i>Tanypus</i>	20	18	15	15	10	10	10	15	5	5	5	4
<i>Procladius</i>	2	1	5	10	10	14	18	10	5	5	2	1
<i>Cryptochironomus</i>	2	6	7	14	17	8	5	7	7	20		2
<i>Polypedilum</i>									35	50	71	52
<i>Tanytarsus</i>	17	17	13	10	10	10	12	17	20	5	3	10
<i>Ablabesmyia</i>						2	5	3	5	3	10	4
<i>Cricotopus</i>				1	2	4		8				
<i>Dicrotendipes</i>						5	2					
<i>Paratendipes</i>					1	2	2	5	5	1	1	1
<i>Einfeldia</i>							5	8	6			
<i>Goeldichironomus</i>							2	3				
<i>Microspectra</i>	3	3					3	3				
<i>Glyptotendipes</i>										2		

presented in Table 1. Some genera like *Goeldichironomus*, *Psectrocladius* and *Cricotopus* were scarce in benthic studies because their larvae favor floating and submerged vegetation which was not sampled.

It is important to realize that significant emergence of midges may arise from substrates other than sand or mud, such as submerged vegetation and algal mats. The role of these niches in the overall production of midges is not known at this time.

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## ROOF RAT SURVEILLANCE AND CONTROL IN THE URBAN ENVIRONMENTS OF SAN BERNARDINO COUNTY DURING 1992

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### ABSTRACT

Based on environmental manipulation, surveillance and control of the roof rat, *Rattus rattus*, were carried out in the urban environment of the San Bernardino Valley during 1992. Of the 888 total service requests received from residents, 862 were provided with educational information over the phone or through literature (874 rat brochures distributed). During the period, technicians inspected 833 residential premises and 470 sanitary sewers and used 15.50 pounds of bromadiolone and 124.02 pounds of diphacinone in rat control measures. Also, 1,272 glue traps and 98 live traps were employed to control roof rat infestations in urban residential areas.

The roof rat, *Rattus rattus* L., is the one commensal rodent species which accounts for approximately 32% of all vector complaints received by the San Bernardino County Vector Control Program (SBCVCP) from the urban areas of San Bernardino County (Mian 1989). Weighing 0.5-0.63 lbs., the adult roof rat has a combined head and body length of 6.0-8.5 inches and a tail of 7-10 inches in length (Ebeling 1978). It has a pointed muzzle and large, prominent ears. With a life expectancy of 9-12 months, it reaches sexual maturity in 3-5 months and has 6 litters per year with an average of 6-8 young per litter. The roof rat is omnivorous and is known to consume more vegetable matter (vegetables, fruits, nuts and grains) than meat or garbage. Being a good climber, the roof rat has the ability to move from trees to rooftops and can climb vertical pipes up to 6 inches in diameter. In addition to its ability to move along telephone and utility lines, the roof rat is known to swim long distances under water.

Due to its great versatility and omnivorous behavior, the roof rat has adapted well to the urban human milieu and has thus increased the chances of disease transmission to both humans and animals. Diseases transmitted by roof rats through

ectoparasites, urine, feces or bites include plague (Salmon and Gorenzel 1981), murine typhus (Pratt and Wilson 1962), rat mite dermatitis (Ebeling 1960), leptospirosis (Eagle 1948), listeriosis and amoebic dysentery (Ebeling 1978), salmonellosis (Scott 1959), ratbite fever (Scott 1966) and trichinosis (Storer 1968). In addition to being either a direct or indirect vector of the aforementioned diseases, the roof rat has been known to cause considerable economic damage to building structures and agricultural concerns.

In view of its importance as both a vector or host to vectors of several diseases and as a destructive pest, the SBCVCP carries out a continuing roof rat surveillance and control program based on the concept of environmental manipulation of roof rat infestations (Zudunowski 1980). The present report provides data on the surveillance and environmental management of roof rat infestations in the San Bernardino Valley area of San Bernardino County during 1992.

### MATERIALS AND METHODS

Based on the environmental manipulation

concept, ongoing roof rat surveillance and control operations are carried out in the urban environments of San Bernardino Valley. This region includes areas from Upland in the west to Yucaipa in the east. The environmental manipulation is essentially based on four components or measures: sanitation, exclusion, suppression and continued maintenance.

#### **Sanitation.**

Proper sanitation, both indoor and outdoor, plays a pivotal role in denying roof rats food and shelter. Sanitation measures that can help manage roof rats include: 1. Proper storage of garbage in rodent-proof containers; 2. Proper storage of fruits, vegetables and grains; 3. Proper management and containment of bird and pet food; and 4. Elimination of snails as a potential food source for rats.

Another important sanitary measure for roof rat control in urban residential areas is the manipulation or elimination of rodent harborage sites such as improperly maintained woodpiles, waste lumber or scrap metal piles including abandoned motor vehicles, heavy vegetation ground cover of Algerian ivy or elephant grasses, untrimmed or unbanded palm trees and unpruned or unthinned trees overhanging buildings.

#### **Exclusion.**

Rat proofing of buildings has proven to be very effective in excluding roof rats from entering residential homes and commercial buildings. Rat proofing measures include: 1. Patching holes in walls and outbuildings; 2. Screening crawl holes, foundation vents and attic vents with 1/4 inch mesh wire screen; 3. Weatherstripping garage doors and replacing broken door sidings; and 4. Trimming trees to a minimum of three feet away from buildings, roofs or utility wires.

#### **Suppression.**

Roof rat population suppression is carried out by killing the roof rats through the use of chemical poison baits or mechanically trapping and killing them with glue traps or live traps. The poison baits used in these operations included two second generation anticoagulant rodenticides, bromadiolone and diphacinone, which are imbedded in paraffin blocks for ease of handling and safety reasons.

#### **Continued Maintenance.**

Last, but not least, the continued maintenance phase of the environmental manipulation concept is

the ongoing, reinforcement or reenactment of part or all of the aforementioned components or measures. Most of the data reported here for 1992 were generated almost entirely in this phase. Education of the homeowners through phone advice, roof rat literature and premise inspection has been the driving force of this program. In case(s) of non-compliance to laws under the unified environmental health code, enforcement activity ended up, in most cases, with a notice of violation of legal property owners.

## **RESULTS AND DISCUSSION**

During 1992 our surveillance and control program received 888 service requests for roof rat problems (Table 1). As was expected, the high numbers of requests during the fall months, especially September and October, was due to the ingress of rats into residential areas, in part caused by the lack of food and the onset of cold outdoor temperatures. These requests were responded to promptly and appropriately, i.e., through education, site inspection and control measures. In our educational service, 862 of the requests (97.1% of the total received) were advised over the phone and were also provided with 874 educational pamphlets on roof rat management through environmental manipulation. During premise inspections of the 888 requests, each lasting on the average approximately one hour, homeowners were given a written Premise Correction Notice. Each Premise Correction Notice (example and explanation of which is given by Zdunowski 1980) gives detailed advice and explanation of both external and internal structural corrections to be made as well as potential backyard sources of rat harborage and proliferation sites to be eliminated.

In addition to the service requests, and as part of our continued maintenance measures, 470 sanitary sewers were inspected for roof rat infestations, mainly during the first half of the year. A small number of the sewers with rat activity (<1.0%) were poison-baited effectively.

In roof rat management, both chemical and non-chemical agents/devices were employed (Table 1). Chemical rodenticides used in this program included 15.50 pounds of bromadiolone and 124.02 pounds of diphacinone in the form of paraffin bait blocks. Of the non-chemical devices, 1,272 glue traps and 98 live traps were used to control roof rat infestations in residential areas.

Table 1. Roof rat surveillance and control program summary for the San Bernardino Valley during 1992.

Month	Service requests	Educational services		Inspections		Chemical control		Mechanical control	
		Phone advice	Pamphlets distributed	Residential premises	Sanitary sewers	Bromadiolone (lb.)	Diphacinone (lb.)	Glue traps	Live traps
January	45	45	45	43	168	0.00	7.75	102	10
February	45	42	45	39	150	0.00	10.00	84	3
March	36	36	45	35	0	1.00	8.25	66	2
April	43	43	42	42	25	3.50	15.38	94	7
May	61	61	61	56	88	7.00	10.50	64	6
June	73	72	80	71	39	0.00	5.00	38	15
July	90	84	78	84	0	0.00	5.00	112	6
August	85	81	83	71	0	0.00	10.00	140	8
September	144	136	143	132	0	0.00	13.88	214	33
October	110	110	119	108	0	2.00	19.63	158	5
November	95	92	81	92	0	1.00	12.38	142	3
December	61	60	52	60	0	1.00	6.25	58	0
Totals	888	862	874	833	470	15.50	124.02	1,272	98

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## ECTOPARASITES ASSOCIATED WITH ROOF RATS IN KERN COUNTY

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### INTRODUCTION

The ecology of rodent vector-borne diseases in California has been altered dramatically as a consequence of urbanization and fortuitous introductions of Old World commensal (murine) rodents harboring ectoparasites and associated disease agents. Historical introductions of the roof rat (*Rattus rattus* Linnaeus) and Norway rat (*Rattus norvegicus* Berkenhout) into our port cities of Los Angeles and San Francisco resulted in flea-borne outbreaks of plague and murine typhus (Zinsser 1937, Kartman 1970, Traub et al. 1978). Today, increased awareness and extensive control efforts have virtually eliminated rat-borne plague and typhus from the San Francisco Bay area and Los Angeles Basin. However, in the interim, the roof rat more than the Norway rat has rapidly dispersed throughout the state and currently represents one of the leading sources of vertebrate vector complaints to local vector control agencies.

The possibility of commensal rodent disease transmission in California still remains a potential threat to residents if environmental changes occur in a manner that reestablishes historical patterns of transmission. Previously low-risk areas like the arid southern San Joaquin Valley may now support disease transmission by commensal rodent ectoparasites. Urbanization and agriculture have significantly altered the ecology of the region from a predominately arid desert environ to an artificial mesic environ maintained by extensive irrigation of landscape and agricultural vegetation. Considering the emerging prospects for disease transmission by commensal rodent ectoparasites in the southern San Joaquin Valley, the Kern Mosquito and Vector Control District (KMVCD) initiated studies to determine what ectoparasites were associated with roof rats infesting urban and suburban Bakersfield.

### MATERIALS AND METHODS

#### Ectoparasite Study Areas.

Ectoparasite were sampled from roof rats live-trapped at the sites of routinely solicited service requests within the greater Bakersfield area. The greater Bakersfield area was subdivided into three contiguous areas based upon geography, home architecture, landscaping, and sanitation: 1) southwest Bakersfield, 2) central and east Bakersfield (includes southern neighborhoods), and 3) Oildale and northeast Bakersfield. Southwest Bakersfield contains upper to middle income dwellings with slab foundations, abundant landscaping, and minimal sanitation problems (i.e., open/damaged garbage receptacles and backyard refuse). Central and east Bakersfield, by comparison, include predominately middle to lower income dwellings with conventional raised foundations, abundant to sparse landscaping, and variable sanitation problems. Oildale and northeast Bakersfield contain a mixture of conventional and slab-founded, middle to low income dwellings, abundant to sparse landscaping and variable sanitation problems.

#### Trapping and Processing Rats for Ectoparasites.

Roof rats were live-trapped at private residences as part of our routine control service. Traps were baited with a mixture of peanut butter and rolled oats and occasionally supplemented with citrus and other fresh fruits to enhanced attractancy. Trapped rats were picked up the day following the night of capture and returned to the KMVCD rodent facility for processing. Individual rats were humanely euthanized with CO<sub>2</sub> gas in the live-trap over an enamel pan and then "brushed" in the same pan to maximize the recovery of any ectoparasites that may be present. Ectoparasites were collected by an artist's brush and

transferred to vials containing 70% ethanol as a preservative. All samples were examined by binocular stereo-microscope at 25 to 100X. Ectoparasites obtained from individual rats were identified to species and counted.

**RESULTS**

A total of 313 roof rats live-trapped from the three regions comprising the greater Bakersfield area were examined for ectoparasites. Among the sample of 313 individual roof rats, only 120 (38%) were found infested with the tropical rat mite [*Ornithonyssus bacoti* (Hirst)] (Fig. 1). Other than the tropical rat mite, no other ectoparasite species (e.g., fleas, ticks, etc.) were found associated with the roof rats we examined.

The number of roof rats examined from each area varied, however the tropical rat mite infestation rate was relatively uniform throughout the greater Bakersfield area ranging from a high of 43% (36/120) among rats from central and east Bakersfield, followed by Oildale and northeast Bakersfield at 38% (52/188) and southwest Bakersfield at 34% (32/125).

Infestation densities or the number of tropical rat mites recovered from individual roof rats averaged approximately nine mites per infested animal (Fig. 2). Infestation densities among individual rats and areas

did not vary significantly. Tropical rat mite infestation densities ranged from an average of six mites per rat among the roof rats trapped in southwest Bakersfield to an average of eleven mites per rat among the roof rats examined from central and east Bakersfield.

**DISCUSSION**

The relatively high tropical rat mite infestation rate associated with roof rats in the greater Bakersfield area (38%) suggests that environmental conditions within our service area contribute significantly to the survival and persistence of tropical rat mite populations. Tropical rat mites require elevated humidities and protective shelter in conjunction with abundant murine rodent host populations for optimal reproduction and survival. Throughout the greater Bakersfield area, extensive landscaping characteristic of typical neighborhoods is largely maintained by routine irrigation and sprinkling. This practice has created ideal environmental conditions for harboring both rats and mites that is further enhanced by an overall mild climatic regimen. Therefore, in the process of transforming significant portions of the once prevalent desert environ of the southern San Joaquin Valley into a moist urban/mixed-agriculture environ, a stable association has been created between

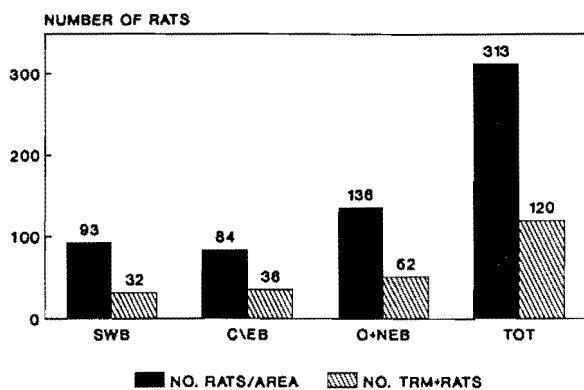


Figure 1. Tropical rat mite (TRM) infestation rates among roof rats sampled from three areas of greater Bakersfield; SWB = southwest Bakersfield, C+EB = central and east Bakersfield, O+NEB = Oildale and northeast Bakersfield, and TOT = total for all three areas. Numbers above bars represent the number of roof rats examined (solid) and roof rats infested with TRMs (hatched).

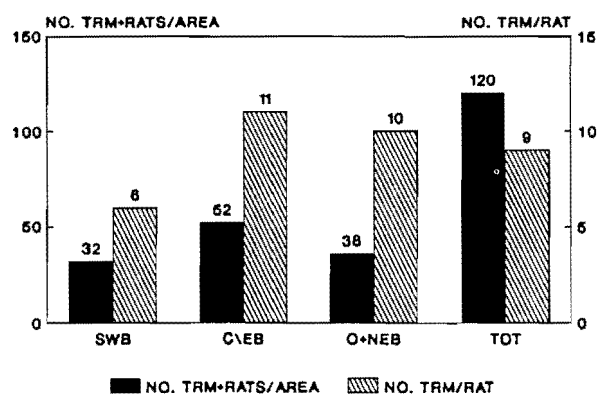


Figure 2. Tropical rat mite (TRM) infestation densities per infested roof rat in each of the three areas of greater Bakersfield; SWB = southwest Bakersfield, C+EB = central and east Bakersfield, O+NEB = Oildale and northeast Bakersfield, TOT = total for all 3 areas. Numbers above bars represent the number of roof rats examined with TRMs (solid) and the average number of TRMs per infested roof rat (hatched).

introduced roof rats and Tropical rat mites.

Historically, the tropical rat mite has not been incriminated as a vector of arthropod-borne diseases to humans (Keh 1957). The bite of the mite is reportedly painful and produces an itching hemorrhagic lesion and dermatosis at the site of the bite in sensitive individuals (Harwood and James 1969). In the laboratory, the tropical rat mite has been experimentally infected with plague [*Yersenia pestis* (Lehmann and Neumann)] (Yamada 1932), murine typhus (*Rickettsia mooseri* Monteiro) (Dove and Shelmire 1932), and rickettsialpox (*Rickettsia akari*) (Philip and Hughes 1948). Transmission of murine typhus and plague occurs as a flea-borne phenomenon, and rickettsialpox cases usually involve transmission by the house mouse mite [*Liponyssoides* (= *Allodermanyssus*) *sanguineus* (Hirst)] (Meehan 1984). As a consequence, the probability of tropical rat mite-borne disease transmission in California is extremely low and presents no immediate threat to residents. However, existing environmental conditions in the southern portion of the state are certainly supportive of mite-borne disease transmission in the event of a fortuitous introduction.

#### ACKNOWLEDGEMENTS

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## BORRELIAL SPIROCHETES FOUND IN TWO SPECIES OF TICKS INFESTING SHADOW CHIPMUNKS AND CHICKAREES

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### ABSTRACT

Butte County has a history of human disease caused by infections with a variety of tick-borne spirochetes belonging to the genus *Borrelia*. A survey of ticks found infesting rodents captured in campgrounds was conducted. Two Chickarees and six Shadow Chipmunks were found to have ticks infesting them. The ticks were larval and nymphal stages of *Dermacentor variabilis* and nymphal and adult stages of *Ixodes angustus*. Four nymphal *D. variabilis* and one adult *I. angustus* showed fluorescent spirochetes when tested by IFA using monoclonal antibody H9724. Fluorescent spirochetes were not observed in samples tested with monoclonal antibody H5332.

The high elevations of the Sierra Nevada mountain range offers some of the most scenic vistas of California. These magnificent areas attract thousands of people each year who take advantage of the many campgrounds which are located in the Sierra Nevada. Camping and associated recreational activities create a unique setting in terms of transmission of zoonotic diseases.

Camping seems to bring together many conditions which would favor the transmission of a zoonotic disease. First, there is a clustering of susceptible individuals (campers) in a small area, who can come in close contact with tame rodents and their ectoparasites. Next, there are several species of sciurid rodents commonly found inhabiting campgrounds which can serve as reservoirs for a variety of zoonotic diseases (Hull 1968). Campgrounds also provide food and water for rodent populations, which are able to respond by growing to abnormally high levels (Marsh et al. 1981). Finally, populations of vectors such as fleas and ticks, which are associated with rodents, are also enhanced. It is the combination of these factors which would favor the transmission of a zoonotic disease.

The Butte County Mosquito Abatement District has been conducting surveillance for zoonotic

diseases in campgrounds because of the increased potential for transmission. The primary focus of attention has been on sylvatic plague. However, Butte County has a history of human disease caused by infections with a variety of spirochetes belonging to the genus *Borrelia* (Monsen et al. 1990). As a result, a survey of ticks found infesting rodents captured in campgrounds was conducted, with the results included in this report.

### MATERIALS AND METHODS

#### Collection Sites.

Collections were made in Butte County from three campgrounds located approximately 16 kilometers (10 miles) apart. The sites surveyed included, United States Forest Service campgrounds at Cherry Hill and West Branch and a Pacific Gas and Electric campground located at Philbrook Reservoir. Elevation at the sites ranged between 1,160 meters (4,600 ft) and 1,260 meters (5,000 ft). A mixed conifer habitat type predominated in each of the campgrounds. Successional stage in development consists of very large trees generally over 15 meters (50 ft) in height. Important tree species includes

Incense Cedar (*Calocedrus decurrens*), Red Fir (*Abies magnifica*), Sugar Pine (*Pinus lambertiana*), and Douglas Fir (*Pseudotsuga menziesii*). Generally, the ground cover is sparse with many decaying logs and large boulders present.

#### Rodent Sampling.

Rodents were trapped in Sherman and National live traps baited with peanut butter and rolled oats. Traps were placed 10 to 20 meters apart and left overnight. Each campground was sampled once in the fall of 1992.

Rodents captured were anesthetized with ether at the collection site, assigned a number, vital measurements were taken, and infesting ticks removed. After recovery, each animal was released at the point of capture.

#### Tick Testing.

After identification, infection of individual ticks was determined by dissection and analysis of midgut diverticula using an Indirect Fluorescent Antibody (IFA) technique (Steere et al. 1983). In this method, midgut samples from adult ticks and nymphs were smeared onto 2 sections of a slide. Smears in section 1 were assayed with *Borrelia*-specific monoclonal antibody H9724 (Barbour et al. 1986). Midgut smears in section 2 were assayed with *Borrelia burgdorferi*-specific monoclonal antibody H5332 (Barbour et al. 1983).

Tick larvae of *Dermacentor variabilis* (Say)

were also tested by IFA. However, due to their small size, each larva was smeared whole. Larval smears were tested only with monoclonal antibody H9724.

## RESULTS

A total of 94 rodents representing 5 species, was collected and examined for ticks (Table 1). Of the 5 species collected, 2 were found to have ticks infesting them; 2 Chickarees (*Tamiasciurus douglasii*) and 6 Shadow Chipmunks (*Tamias senex*) were found to have ticks infesting them.

Six nymphal *D. variabilis* were removed from the Chickarees (Table 1), while 6 larval and 36 nymphal *D. variabilis* ticks were taken from the Shadow Chipmunks. One individual chipmunk was heavily infested, having 6 larvae and 13 nymphal *D. variabilis* ticks. Additionally, 4 nymphal and 2 adult female *Ixodes angustus* ticks were found on three chipmunks.

All of the ticks found infesting the chipmunks and chickarees were tested by IFA (Table 1). Of the 42 *D. variabilis* nymphs tested, 4 (10%) showed fluorescent spirochetes when viewed under epifluorescent illumination using monoclonal antibody H9724. One adult female *I. angustus* also exhibited fluorescent spirochetes using monoclonal antibody H9724. Spirochetes were not found fluorescing in any of the samples tested with monoclonal antibody H5332.

Table 1. Tick species and stage collected from all rodents captured at three Butte County campgrounds during the fall of 1992. Included are the results of ticks tested by IFA for *Borrelia*- and *B. burgdorferi*-specific monoclonal antibodies H9724 and H5332, respectively.

Rodent species	Number collected	Tick species	Stage	Number collected	Positive	
					H9724 (%)	H5332 (%)
<i>Tamiasciurus douglasii</i>	2	<i>Dermacentor variabilis</i>	Nymph	6	2 (33)	0 (0)
<i>Tamias senex</i>	19	<i>Dermacentor variabilis</i>	Larva	6	0 (0)	-
			Nymph	36	2 (6)	0 (0)
		<i>Ixodes angustus</i>	Nymph	4	0 (0)	0 (0)
			Female	2	1 (50)	0 (0)
<i>Neotoma cinerea</i>	3	none	none			
<i>Peromyscus maniculatus</i>	64	none	none			
<i>Spermophilus lateralis</i>	6	none	none			

Positive ticks were collected from each of the three campgrounds. Positive ticks included: two of four nymphal *D. variabilis* and one of two female *I. angustus* taken from shadow chipmunks collected at the West Branch campground, one of two nymphal *D. variabilis* from chickarees collected at the Philbrook campground, and one of four nymphal *D. variabilis* from chickarees trapped at the Cherry Hill campground.

Overall, the positive spirochetes collected from the ticks in this survey appeared morphologically dissimilar when compared to samples of *Borrelia burgdorferi* (Figs. 1 and 2). When magnified at 400X, the positive spirochetes appeared shorter and more tightly coiled than *B. burgdorferi* viewed at the same magnification.

### DISCUSSION

The appearance and fluorescence of the spirochetes using monoclonal antibody H9724 in the tissues of ticks collected from campgrounds may

signal potentially serious implications. Fluorescence with a *Borrelia*-specific monoclonal antibody would suggest that these spirochetes may be a species of *Borrelia*. While the lack of fluorescence in the assays using H5332 indicates that these spirochetes are not *B. burgdorferi*.

There are several species of *Borrelia* which are pathogens, often causing severe manifestations in humans. Burgdorfer et al. (1982) were able to associate *B. burgdorferi* with the clinical symptoms known as Lyme disease. Lane et al. (1985) implicated *B. coriaceae* with epizootic bovine abortion and numerous researchers have investigated the role of *B. hermsii*, *B. parkeri*, *B. recurrentis* and *B. turicatae* in relapsing fever. The collection of infected ticks from each of the three campgrounds would also suggest a widespread distribution.

The presence of the spirochetes in *D. variabilis* and *I. angustus* is also a concern. *Dermacentor variabilis* feeds on humans and is considered the most important vector of rocky mountain spotted fever (Furman and Loomis 1984). *Ixodes angustus* has also been reported to bite man (Furman and

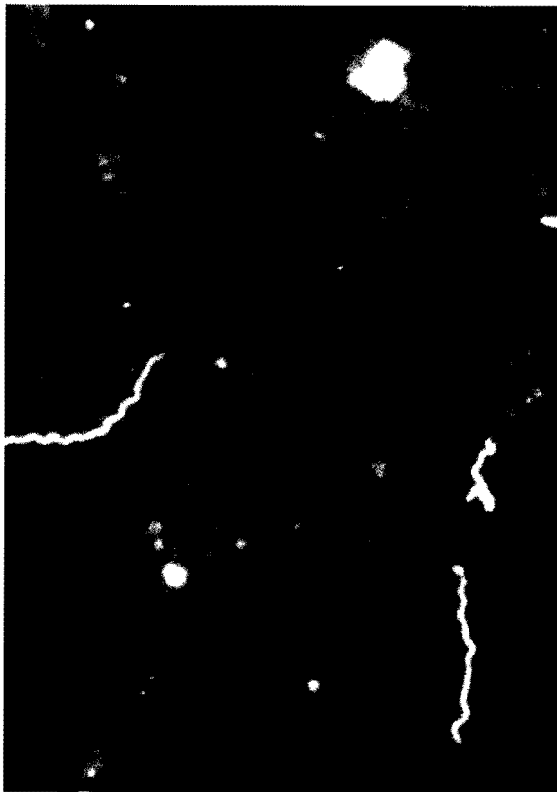


Figure 1. IFA view of *B. burgdorferi* spirochetes (1,000X) in an *Ixodes pacificus* midgut smear using monoclonal antibody H5332.

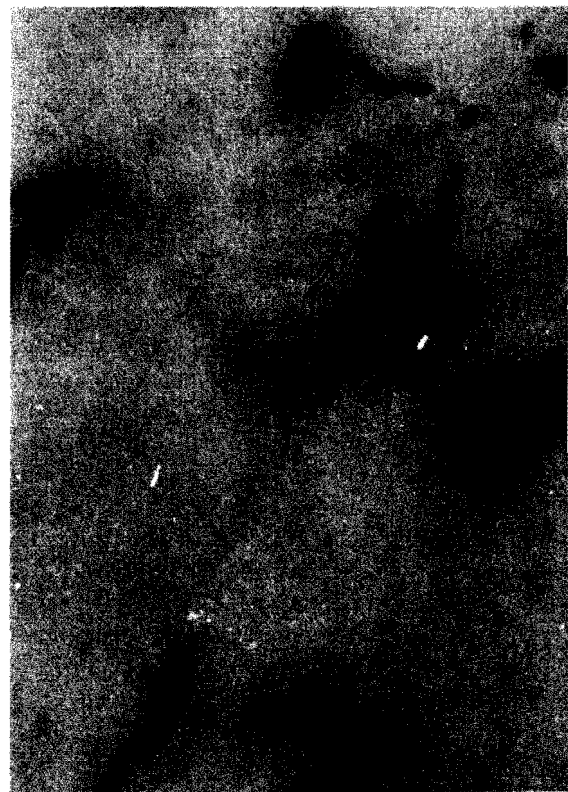


Figure 2. IFA view of *Borrelia* spirochetes (1,000X) in an *Ixodes angustus* midgut smear using monoclonal antibody H9724.

Loomis 1984). The competence of these Ixodid ticks to vector borrelial spirochetes has not been fully evaluated. Despite the paucity of such data, it appears these ticks can be used as indicators of spirochetosis of the host and of a campground in general.

The finding of borrelial spirochetes in sciurid rodents is not novel. Porter et al. (1932) were able to identify spirochetes from the blood and organs of chipmunks and squirrels collected in the immediate vicinity of cabins where human cases of tick-borne relapsing fever were recorded. In many instances, human cases of relapsing fever occur when normal tick/rodent feeding is disrupted.

In a campground, usual tick host selection can be disrupted when rodent hosts are not available. Rodent populations are susceptible to epizootics. Heavy predation or rodent control programs may also cause a reduction in the number of hosts available to ticks. Such an event could intensify the number of tick-human contacts in a campground which would seem to facilitate the transmission of a tick-borne spirochete.

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## POPULATION DYNAMICS OF MUSCOID FLIES AT THREE COMMERCIAL POULTRY RANCHES AND ASSOCIATED FLY COMPLAINTS IN SAN BERNARDINO COUNTY DURING 1992

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### ABSTRACT

Muscoid fly population dynamic studies were carried out using sticky tapes at three commercial poultry ranches in San Bernardino County during 1992. At the Fontana and Redlands ranches *Fannia canicularis* was the most abundant species (68.1 and 55.7%), followed by *Musca domestica* (26.5 and 31.8%) and *Fannia femoralis* (2.8 and 10.9%) while *M. domestica* (72.6%) and *F. canicularis* (27.0%) dominated at the Yucaipa ranch. Small numbers (<0.1%) of *Muscina stabulans*, *Ophyra leucostoma* and *Phaenicia sericata* were found at all ranches. Spatial distribution of these flies around the poultry houses varied both within and between ranches. Seasonally, *F. canicularis* prevailed earlier in the season with population peaks noted in April and May in Fontana and Redlands and May and June in Yucaipa. *Musca domestica*, a warm season species, was prevalent in the summer and early fall. Residents complained more often about *F. canicularis* than *M. domestica* early in the season. Using weekly *F. canicularis* data in relation to residents complaints, the thresholds of fly tolerance by residents and resulting action levels were determined separately for each ranch during January through June, 1992.

Muscoid flies constitute an important group of cyclorrhaphan vectors that includes both biting and nonbiting species. Of the nonbiting species, the cosmopolitan house fly, *Musca domestica* L., is known to transmit several diseases such as diarrhea, dysentery, cholera, typhoid, poliomyelitis, yaws, anthrax and tularemia and the little house fly, *Fannia canicularis* L., is a persistent nuisance pest in many parts of California (West 1951, Meyer et al. 1987). Among biting muscoid species, the tsetse fly, *Glossina* spp., is an established vector of both animal (nagana disease) and human (sleeping sickness) trypanosomiasis in many parts of Africa. The stable fly, *Stomoxys calcitrans* (L.), and hornfly, *Haematobia irritans* (L.), are known to suck blood from animals and sometimes humans (Harwood and James 1979, Kettle 1984).

The muscoid flies are also referred to as synanthropic flies or filth breeding flies. The phenomenon of synanthropy or zoophily, an ecological association of flies with humans or animals, along with notes on a number of these flies has been reviewed by Mulla and Mian (1991). As filth breeders, a great majority of flies breed in a variety of animal wastes in dairies, horse stables, dog kennels, poultry ranches and in human excrement (Greenberg 1971). The proximity of breeding sites of these flies to human habitations undoubtedly creates health problems, ranging from nuisance to disease transmission.

High population growth and relatively low land prices in southern California during the past decade have led to significant shifts in planning and development policies in favor of urbanization encroachment upon agricultural zoned rural areas.

Whereas this policy change, urbanization over ruralization, might have helped the real estate industry, it certainly has exposed the intolerance of new residents in and adjacent to agricultural biotopes to muscoid flies, a product of many agricultural industry operations.

Owing to the low tolerance of residents to flies in certain municipalities, it became necessary to study and reevaluate adult fly population levels against fly complaints by residents. In monitoring adult fly populations, several sampling methods have been used by various researchers. These include fly grids (Scudder 1949), attractant baited jug-traps (Burg and Axtell 1984), sticky fly tapes (Anderson and Poorbaugh 1964, Legner et al. 1973, Meyer et al. 1987) and sticky cards (Hogsette et al. 1993). Using sticky fly tapes, a study was undertaken to monitor muscoid fly population dynamics around three commercial poultry ranches (one each in Fontana, Redlands and Yucaipa) and to ascertain and establish tolerance thresholds of residents to fly nuisance utilizing citizen complaints from each city during 1992.

## MATERIALS AND METHODS

A study was conducted to monitor the population dynamics of muscoid flies around commercial poultry ranches in both the east and west ends of the San Bernardino Valley. One ranch each was studied in the city of Fontana in the west and the cities of Redlands and Yucaipa in the east end. At each study site four 4 x 56 cm sticky fly tapes (Aeraxon Fly Catcher™, Roxide International Inc., New Rochelle, N.Y.) were hung at about eye-level (approx. 1.6 m) at the periphery of each ranch. The location of traps at each ranch was as follows:

### Fontana Ranch.

Trap #1 was located inside the old office building at the east side of the ranch. Trap #2 was hung from a fruitless mulberry tree on the east, whereas trap #3 was placed inside the dead bird bin on the south side. Trap #4 was hung from a *Ligustrum* sp. plant on the west side of the ranch.

### Redlands Ranch.

Traps #1, 2 and 3 were hung from the rafters on the east, north and west side of the ranch, respectively. Trap #4 was hung under the eave of a barn on the south side of the ranch. Unlike traps 1, 2 and 3, which were right next to the chicken houses,

trap #4 was some 100 m away from the nearest chicken house. The ranch which is situated on the outskirts of the city of Redlands, is surrounded to the east and south by citrus trees, to the north by the Santa Ana River (wash) and to the west by a junk yard and open field. The nearest residential housing track is about 150 m to the west of the ranch.

### Yucaipa Ranch.

Trap #1 was hung from a rafter on the west side of the ranch. Trap #2, 3 and 4 were hung on the north, east and south side of the ranch, respectively. This ranch is situated in the foothills and is surrounded by an open field to the north, east and south. On the west side there are some residences almost across the street from the ranch.

Most of the traps were exchanged with new ones weekly during the study period with each trap brought in to the laboratory and carefully examined under a lighted binocular microscope to determine total fly numbers and identifications to genera and species. To correlate fly population levels in each study area with citizen complaints, complaints were retrieved from our daily service request database.

Complaints were examined against weekly fly population levels over the period of January through June, 1992. The thresholds of fly tolerance by residents, enforcement action and adulticidal applications, especially for *F. canicularis*, were calculated accordingly. The threshold of fly tolerance was calculated as the number of flies/tape/week sufficient to result in the first complaint of the season. The threshold of enforcement action including but not limited to ranch inspections was fly population levels sufficient to cause 1.5-3.0 complaints/week. Population levels of flies resulting in >3.0 complaints necessitated adulticidal sprays to quell fly nuisance.

All temperature and rainfall data used in these studies were obtained through the courtesy of the San Bernardino Sun and the San Bernardino County Flood Control Department, Hydrology Section.

## RESULTS AND DISCUSSION

Based on the total number of flies collected on sticky tapes, the fly composition by species varied from ranch to ranch (Table 1). At both the Fontana and Redlands ranches, *F. canicularis* was the most predominant (68.1 and 55.7%) followed by *M.*

Table 1. Percent composition of fly fauna (%) and actual numbers of flies collected (N) on sticky tapes at three commercial poultry ranches in San Bernardino County during 1992.

Species	Fontana		Redlands		Yucaipa	
	%	N	%	N	%	N
<i>Fannia canicularis</i>	68.1	9,233	55.7	633	27.0	14,507
<i>Fannia femoralis</i>	2.8	382	10.9	124	6.3	147
<i>Musca domestica</i>	26.5	3,597	31.8	362	72.6	38,998
<i>Muscina stabulans</i>	0.4	56	1.0	11	<0.1	12
<i>Ophyra leucostoma</i>	0.3	44	0.4	5	0.1	73
<i>Phaenicia sericata</i>	1.9	260	0.2	2	0.0	0
Totals	100	13,572	100	1,137	100	53,737

*domestica* (26.5 and 31.8%) and *Fannia femoralis* (Stein) (2.8 and 10.9%), respectively. Other species collected in small numbers included *Muscina stabulans* (Fallen), *Ophyra leucostoma* (Wiedemen) and *Phaenicia sericata* (Meigan). At the Yucaipa ranch, however, *M. domestica* was the most dominant species (72.6%), followed by *F. canicularis* (27.0%) with *F. femoralis*, *M. stabulans* and *O. leucostoma* accounting for <1.0% of the total collections.

Apart from flies, other arthropods caught on sticky tapes at the Fontana ranch included Diptera - 1 Chloropidae, 8,297 Sphaeroceridae, 86 Mycetophilidae, 3 Cecidomyiidae; Hymenoptera - 460 Pteromalidae/Braconidae; Coleoptera - 2 Tenebrionidae; Lepidoptera - 2,323 Tineidae and Dermaptera - 1 Furficulidae. In the Redlands tapes, there were 4 chloropids, 3,226 sphaerocerids, 39 mycetophilids, 47 tineid moths, 57 tenebrionids, 1 sylvanid, 17 pteromalids, 1 aphid, and 13 termites (winged). The Yucaipa tapes had 1,380 sphaerocerids, 26 cecidomyiids, 2 pteromalids, 1 earwig and 1 spider.

Local spatial distribution of flies varied at each of the three ranches (Table 2). At the Fontana ranch, the trap operated at the north side of the poultry house collected the highest number of flies followed by traps located to the south, east and west of the ranch in that order. At the Redlands ranch, traps operated on the east side had the highest number of flies followed by traps located on the north, south and west. At the Yucaipa ranch, however, the trap on the west side had the highest number of flies followed by traps operated on the north, east and south sides. This pattern of fly distribution was expected because each ranch differed by location and surroundings. The pattern also clearly shows that at least one trap on each of the four sides (north, east, south and west)

should be operated in order to have representative samples in fly distribution surveys.

*Musca domestica* and *F. canicularis* were the dominant species collected on sticky tapes during the 12 month study period. At the Fontana ranch, *F. canicularis* appeared early in the season with a population peak occurring in May (Table 3). This was in part due to prevailing daytime temperatures of 70-80°F and over 13 in. of rainfall during January through May. Also, residents' complaints were higher during February through May, necessitating increased ranch inspections and adulticidal sprays in fly affected residential neighborhoods.

Unlike *F. canicularis*, *M. domestica* was more predominant in the summer and early fall with peak populations found in September and October. At the Redlands ranch *F. canicularis* also showed a population peak in April which resulted in the highest number of residents' complaints (Table 3). *Musca domestica* populations peaked in September. Unlike the Fontana and Redlands ranches, the Yucaipa ranch showed fly population peaks of *F. canicularis* in May and June drawing the highest number (48) of residents complaints (Table 3). Heavy rainfall and optimum temperatures were partly responsible for this heavy population build up of *F. canicularis* that resulted in increased ranch inspections and enforcement activity coupled with adulticidal sprays in affected neighborhoods.

*Fannia canicularis* was the one species that aroused most citizen complaints early in the season. On the other hand, *M. domestica*, although showing a higher population peak than that of *F. canicularis* in the later part of the season, did not result in higher numbers of complaints. This might be due to the fact that residents became more tolerant to flies later in

Table 2. Total number of muscoid flies (N) and percentage each site is of the total (%) at three commercial poultry ranches in San Bernardino County during 1992.

Ranch	North		East		South		West		Total	
	N	%	N	%	N	%	N	%	N	%
Fontana	6,671	49.1	1,704	12.6	4,609	34.0	588	4.3	13,572	100
Redlands	237	20.8	487	42.8	212	18.7	201	17.7	1,137	100
Yucaipa	15,148	28.2	9,876	18.4	9,558	17.8	19,155	35.6	53,737	100

Table 3. Monthly fly populations, residents' complaints and meteorological data at each of the three commercial poultry ranch study sites in San Bernardino County during 1992. Fly population numbers indicate mean number of flies from four sticky tapes.

Month	<i>Musca domestica</i>	<i>Fannia canicularis</i>	Other* species	Total flies	Residents' complaints	Temperature (°F)		Rainfall (in.)
						Max.	Min.	
<b>FONTANA</b>								
Jan	299	125	11	435	1	70	50	2.11
Feb	22	332	60	414	20	70	50	6.14
Mar	7	534	445	986	9	63	47	4.81
Apr	17	1,901	48	1,966	10	77	54	0.06
May	43	2,933	29	3,005	5	78	56	0.05
Jun	308	1,631	31	1,970	3	83	58	0.00
Jul	210	744	99	1,053	1	90	65	0.04
Aug	294	374	61	729	1	96	69	0.00
Sep	990	282	52	1,324	0	95	66	0.00
Oct	1,042	138	29	1,209	0	81	58	0.62
Nov	344	100	3	447	0	74	54	0.00
Dec	20	1	9	30	0	63	44	5.70
<b>REDLANDS</b>								
Jan	1	7	89	97	0	63	43	2.76
Feb	10	25	17	52	2	63	46	4.89
Mar	11	81	36	128	3	62	47	3.99
Apr	1	189	6	196	12	79	51	0.00
May	12	147	4	163	3	84	56	0.43
Jun	30	124	4	158	2	88	59	0.00
Jul	74	58	0	132	0	93	64	0.64
Aug	72	16	0	88	0	97	68	0.00
Sep	145	10	0	155	1	92	65	0.00
Oct	92	20	0	112	1	79	57	0.88
Nov	104	8	0	112	1	66	48	0.00
Dec	49	30	7	86	0	56	40	4.94
<b>YUCAIPA</b>								
Jan	129	60	8	197	0	63	43	2.88
Feb	76	156	4	236	4	63	46	6.47
Mar	36	727	160	923	9	62	47	5.83
Apr	0	640	37	677	5	79	51	0.00
May	24	3,868	18	3,910	48	84	56	1.21
Jun	4,113	6,368	1	10,480	11	88	59	0.00
Jul	5,503	1,987	4	7,494	0	93	64	0.70
Aug	13,985	187	0	14,172	8	97	68	0.00
Sep	8,106	208	0	8,314	1	92	65	0.00
Oct	3,316	322	0	3,438	2	79	57	1.16
Nov	3,597	524	0	4,121	1	66	48	0.00
Dec	633	123	8	764	1	56	40	4.96

\* Other species includes *Fannia femoralis*, *Muscina stabulans*, *Ophyra leucostoma*, and *Phaenicia sericata*.



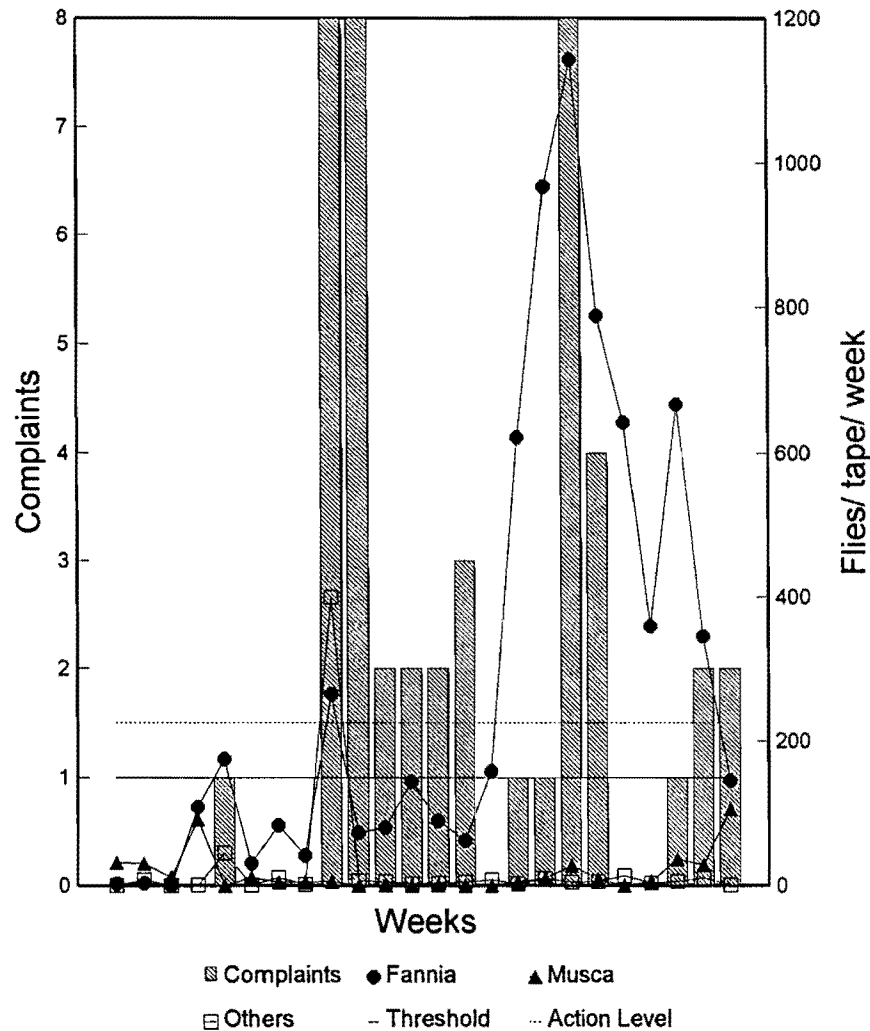


Figure 1. Weekly fly population at the Fontana poultry ranch site and residents' complaints from the City of Fontana during January through June, 1992.

the season. Moreover, the flying movement of *M. domestica*, which differs from the zigzag dancing and hovering of *F. canicularis*, may be another reason residents became less tolerant to the latter species.

Weekly fly population data in relation to residents' complaints during January through June 1992 provided different thresholds of fly tolerance, enforcement action and adulticidal sprays at each poultry ranch. At the Fontana ranch, the threshold of tolerance for *F. canicularis* was 149 flies/tape/week (Fig.1). The threshold at which enforcement action began was 225 flies/tape/week. On a weekly 3-complaint or higher basis, the threshold for carrying out community-wide adulticidal sprays was 449 flies/tape/week. Compared to the Fontana ranch, the threshold figures at the Redlands ranch were quite

smaller (Fig. 2). The threshold of early season tolerance for *F. canicularis* estimated at 1-complaint basis was 13 flies/tape/week. Similarly, the threshold for enforcement action and adulticidal application was 20 and 40 flies/tape/week, respectively. In the Yucaipa study, the three thresholds (i.e., fly tolerance, enforcement and adulticidal activity) were the highest at 155, 232 and 476 flies/tape/week, respectively (Fig. 3). At the Yucaipa ranch enforcement activity also picked up during weeks 9-12. The sudden drop in *F. canicularis* population and simultaneously sharp increase in *M. domestica* population during weeks 13-15, were due to a combination of factors such as little or no precipitation accompanied by about 18°F rise in daily temperatures. High populations of *F. canicularis*, resulting in three or more complaints/

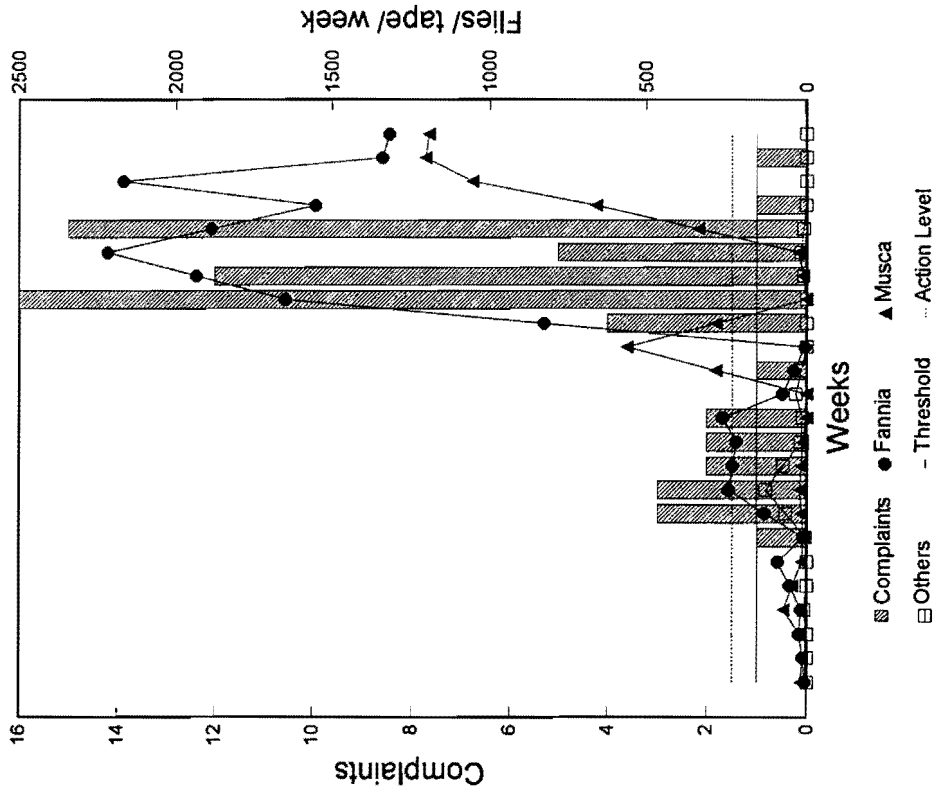


Figure 2. Weekly fly population at the Redlands poultry ranch site and residents' complaints from the City of Redlands during January through June, 1992.

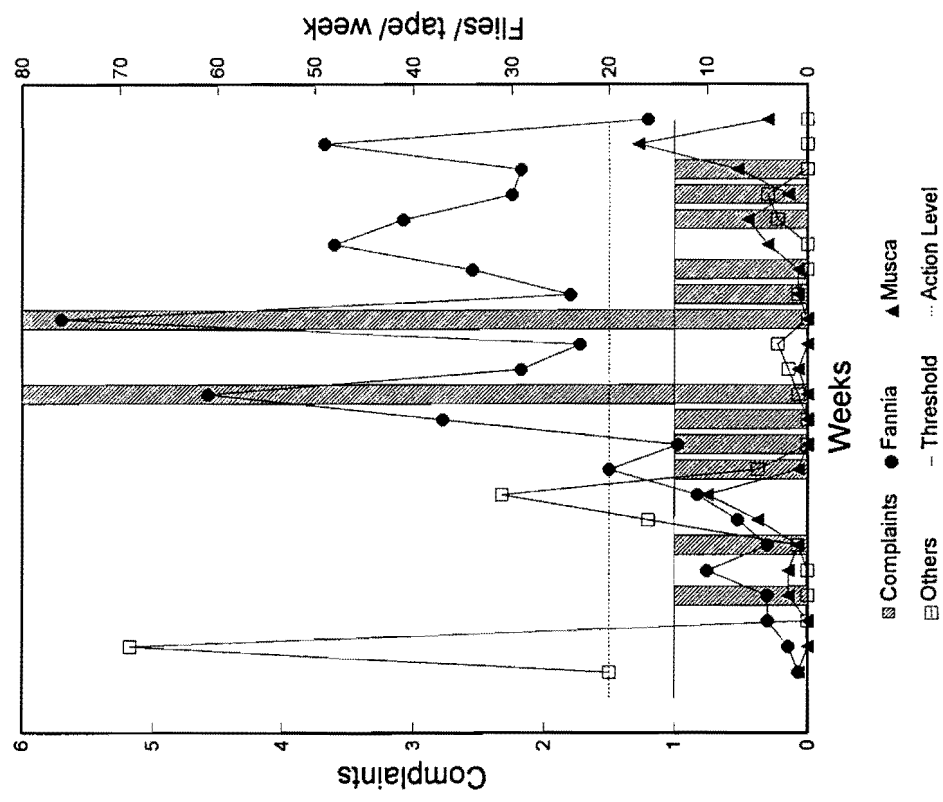


Figure 3. Weekly fly population at the Yucaipa poultry ranch site and residents' complaints from the City of Yucaipa during January through June, 1992.

week in later weeks, necessitated several early morning adulticidal sprays.

The presented data are part of a three year study. At the end of the third year, hopefully, these data will provide more meaningful information that will be utilized in developing fly surveillance and control models as part of our routine vector control operational protocol.

Finally, it is important to point out that the complaints used in establishing various thresholds were assumed to be due to fly breeding at the poultry ranches. In establishing these thresholds, however, other possible fly sources such as domestic garbage, rotten fruit/vegetable matter, compost, pet droppings, animal manure, etc. have to be ruled out before relating a complaint to fly breeding at a commercial poultry ranch. Other factors such as the distance between a complaint source and that of fly breeding, prevailing temperature, humidity, precipitation, sky cover, wind speed and direction need to be taken into consideration prior to establishing a relationship between fly breeding at poultry ranches and resident complaints. None-the-less, the aim of this study has been to gather factual information on the subject matter and not to incriminate commercial poultry establishments as fly production sources.

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## HIPPELATES EYE GNAT ATTRACTANT AND TRAP DESIGN

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### ABSTRACT

Present control strategies for *Hippelates* eye gnats in the Coachella Valley involve placing a blend of a solid attractant formulation and toxicant sugar bait on moist ground of agricultural fields or golf courses to lure and kill persistent and annoying adult eye gnats. In this study, various commercially available traps, primarily developed for use against other insects, were baited with a similar liquid attractant formulation and evaluated for attractancy against adult eye gnats. None of these traps were found to be effective. Therefore, traps were constructed from plastic containers of various sizes and shapes and evaluated. Two of these fabricated traps, a collar trap and a keg trap, were very effective in trapping eye gnats and another (a soda pop bottle trap) was moderately effective.

*Hippelates* eye gnats (Diptera: Chloropidae) are found in parts of California, Arizona and the southern United States. The adults are non-biting but are noted for being attracted to man, becoming an annoying pest with their constant buzzing around the head and exposed body parts. *Hippelates collusor* (Townsend), the primary pestiferous eye gnat species in the Coachella Valley of southern California, has been a problem since agriculture was introduced into that valley. Immature stages develop in cultivated irrigated sandy soils and are thus closely associated with farming practices (Burgess 1951). They have been incriminated as a mechanical vector of the bacterial eye disease, conjunctivitis, commonly known as "pink eye". Increased incidence of this disease may occur in primary and preschool children when adult gnat populations reach high numbers (Dawson 1960).

During the course of research in developing eye gnat control technologies with attractant baits, chemicals present in putrefied aqueous whole egg suspension (a known eye gnat attractant) were isolated, identified and evaluated for eye gnat attractancy. A combination of some of these

chemicals proved highly attractive to synanthropic flies and eye gnats (Hwang and Mulla 1973, Hwang et al. 1975, Mulla et al. 1973). The product derived from blending these attractant chemicals was designated Synthetic Fly Attractant®. Synthetic Fly Attractant (SFA) can be formulated as either a solid attractant (on dry carrier material) or as an aqueous suspension liquid attractant, using water as the carrier material (Mulla et al. 1976).

### MATERIALS AND METHODS

Attractants were prepared in the laboratory from reagents obtained from chemical supply companies, agricultural fertilizer companies, or fish canneries. All tests were carried out in the field against field populations of *H. collusor*.

#### Attractancy Longevity of Solid Attractant.

To study attractancy longevity of solid attractant bait, 4.0 grams of solid attractant were placed on damp sand (200 grams Coachella Valley fine sand, 40 ml water) in an 8 oz. Dixie Cup (no. 2168, James

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River Corp.). Each cup (five replicated cups per day) was placed on a seeper unit (a water reservoir with cord wick embedded in the cup sand) to maintain sand dampness. Cups were set daily and aged in a greenhouse at 84-88°F. On testing day, all cups were moved to the field for assessment. A cup of fresh solid attractant was evaluated along with the aged cups as a standard for comparison. One half gram of sugar scatter bait containing the carbamate insecticide, methomyl, was sprinkled on top of the attractant composition in each cup for toxic effect. Field assessment was carried out using a rotary rod olfactometer placed in a date garden (Mulla et al. 1990). After 2-3 hours of assessment, the cups were returned to the laboratory where the dead gnats in each cup were counted. To determine significant differences, the data were subjected to ANOVA and Duncan's Multiple Range test, using log (n+1) data transformation at 0.05 level of significance (LeClerq 1957).

#### Attractancy Longevity of Liquid Attractant.

To study attractancy longevity of the liquid attractant, 400 ml of liquid attractant was placed in a collar trap for aging in a date garden. The collar trap is made from two quart-sized plastic jars joined together by a collar with holes for gnat entry (Fig. 1). The lower jar of the trap containing the liquid attractant is covered with a 50-mesh brass strainer cloth to exclude gnats. The upper jar containing an inverted 50-mesh screen cone to funnel gnats into the jar and prevent their exiting was removed daily and returned to the laboratory for counting while the lower jar remained exposed in the date garden throughout the study. The upper jar was reset each morning and removed four hours later to coincide with prime gnat activity period (7:00 - 11:00 a.m.).

Each day a fresh cup of damp sand containing solid attractant and toxic sugar scatter bait in a 2:1 ratio as described above, was set as a matched pair with the liquid attractant collar trap on a separate two foot high stake ten feet away for comparison purposes. Each of these sets were replicated four times and separated by at least 60 feet. Gnats in the cups and the upper jars of the traps were returned to the laboratory for counting. Test results were compared statistically by matched pair t-tests using a MacIntosh IIsi computer and the Statview 512+ software program.

#### Evaluation of Various Trap Designs.

All evaluated traps were either commercially

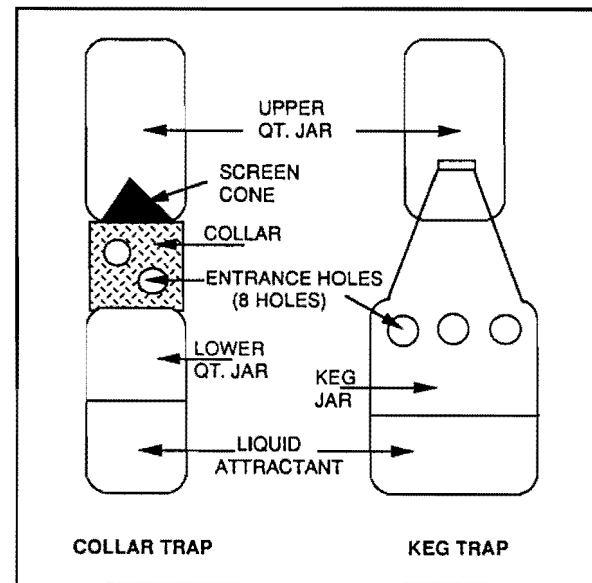


Figure 1. Designs of the two best laboratory-built traps evaluated.

available traps designed for collecting other pests (e.g., houseflies, wasps or yellow jackets) or were constructed in the laboratory from various sizes of plastic containers, jars or bottles.

Evaluation of traps took place under field conditions in a date garden against field populations of eye gnats. Testing was accomplished by setting traps in matched pairs using the same technique as used in studying the attractancy longevity of liquid attractant. In trap evaluation studies, 200 ml of fresh liquid attractant were used per trap, where possible. Some commercially made traps were small and a lesser amount of attractant had to be used. Traps and cups were collected at the end of the tests, returned to the laboratory for counting and the results were analyzed for statistical differences in the same manner as with the results of the longevity studies using liquid attractant.

## RESULTS AND DISCUSSION

#### Aging of Solid and Liquid Attractants.

Although chemically composed of slightly different materials (Table 1), the solid and liquid attractants are remarkably similar in their manner of attractancy. Formulation of the solid attractant is obtained by blending the required attractant chemicals (in the proper amounts) and mixing them with a solid

Table 1. Chemical composition of solid and liquid formulations of Synthetic Fly Attractant (SFA).

Chemical	Solid	Liquid
	(%)	(%)
Trimethylamine hydrochloride	2.5	-
Trimethylamine (25% solution)	-	1.2
Ammonium sulfate	40.0	-
Ammonium hydroxide (29% solution)	-	0.8
Indole	0.25	0.01
<i>n</i> -Butyric acid	2.0	1.0
Linoleic acid	1.0	0.3
Fishmeal (carrier material)	27.125	-
Almond hull (carrier material)	27.125	-
Water (carrier material)	-	96.7
Acetic acid (adjust to pH 6.0)	-	as needed
1% methomyl sugar scatter bait for 2:1 or 3:1 attractant to bait ratio	as needed	-

carrier material. Adding a toxic sugar scatter bait produces a solid attractant "bait". Gnats are drawn to this bait by the attractant and die within a few seconds after contacting or feeding on the toxic sugar scatter bait.

Liquid attractant contains chemicals similar to that of the solid attractant. However, there are some differences, principally in the sources of trimethylamine and ammonia. The liquid attractant contains acetic acid to adjust the pH to 6.0 and lacks the carrier material (fishmeal and almond hull) of the solid formulation, using water as its major carrier material instead. Both formulations have *n*-butyric acid and linoleic acid, but in differing amounts.

The two attractant formulations differ in other characteristic as well. The solid attractant bait must be placed on damp ground or substrate to activate and sustain the attractive odors; no attractive odors are generated if applied to dry ground. Applicationally, solid attractant bait is applied in small piles (0-4 grams) on damp ground of recently irrigated fields or golf courses. Occasionally, problems arise with this type of use on golf courses when the solid attractant bait is washed away by the nightly irrigation of fairways.

Attractancy efficiency of the solid formulation decreases with time (Table 2). In two experiments, activity decreased by 30% on day one to a 70% reduction by day three. However, agricultural fields in the Coachella Valley usually become surface-dry within 72 hours, deactivating the solid attractant

Table 2. Attractancy longevity of the solid formulation of SFA over a three day period. Collection numbers indicate the means of five replicates for each of two tests. Means followed by the same letters are not significantly different from each other in ANOVA and Duncan's Multiple Range tests at the 0.05 levels. Attractancy Index = number of gnats trapped with aged SFA ÷ number of gnats trapped with fresh SFA.

Age (days)	Gnats/cup		Attractancy index	
	1	2	1	2
0 (fresh)	137 a	148 a	1.0	1.0
1	98 a	110 b	0.7	0.7
2	48 b	47 c	0.4	0.3
3	42 b	40 c	0.3	0.3

anyway. There is no limit to the number of piles of solid attractant that can be dispensed in a field, the more piles applied, the more effective. Once the solid attractant bait is applied, no servicing of the piles is required. When application of attractant bait follows the irrigation cycle, the bait can be dispensed in a logical manner, thus covering an entire block of trees or a golf course without difficulty.

Liquid attractant, on the other hand, is not attractive if placed directly in contact with the ground. It is most effective when confined in traps. Being liquid, no damp substrate is needed to activate attractant odors. Nor are traps placed on golf courses containing liquid attractant washed away by nightly fairway irrigation. When liquid attractant is used in traps, gnats are killed in the collection jar or by drowning in the liquid attractant itself. With trapping, there is no need for a toxic killing agent.

Attractancy of the liquid attractant remained constant over seven days of exposure. Over that period, no significant daily loss was noted when compared to fresh standard solid attractant (Table 3). Over a week's time, aged liquid attractant attracted daily gnat numbers that were 1.2 - 2.1 times greater than in the cups of fresh standard solid attractant.

Each attractant has advantages and disadvantages. The solid formulation, at the present time, may be best suited for most agricultural situations since large areas of agricultural land exist in the Coachella Valley and tens of thousands of liquid formulation traps would have to be deployed to significantly impact eye gnat populations. Liquid formulation would be best suited for placement in traps on golf courses. However, a trap for use in a large scale control program, using liquid attractant needed to be developed since none was available

Table 3. Attractancy longevity of the liquid formulation of SFA over a seven day period. Collection numbers indicate the means of four replicates for each matched pair tests of the aged liquid SFA in a trap and fresh solid SFA in a cup of damp sand. Attractancy Index = number of gnats trapped with aged liquid SFA + number of gnats trapped with fresh solid SFA. Significance levels indicate results of matched-pair t-tests.

Liquid SFA		Solid SFA		Attractancy Index	Significance level
Age (days)	Gnats/trap	Age (days)	Gnats/cup		
0 (fresh)	198	0 (fresh)	200	0.9	0.45
1	394	0 (fresh)	188	2.1	0.02
2	374	0 (fresh)	267	1.4	0.09
3	322	0 (fresh)	215	1.5	0.04
4	209	0 (fresh)	139	1.5	0.01
7	602	0 (fresh)	502	1.2	0.07

when these studies were initiated.

**Evaluation of Trap Designs.**

In seeking an efficient eye gnat collection trap, various commercially available traps, which were primarily developed for collecting houseflies, wasps or yellow jackets, were evaluated (Table 4). The method of evaluation was to compare each trap, in matched pair t-tests, to a standard solid attractant in cups of damp sand.

No commercially available trap attracted gnats in large enough numbers to be considered as an effective eye gnat trap. The best of these traps captured a little less than 25% of the standard solid attractant. Some of the commercial traps were modified (as with the Fermone Gotchu Trap), resulting in somewhat increased productivity. Other traps (results not presented) were modified too,

resulting in exceedingly large-sized traps. These would have been unsuitable for use in a large scale trapping program.

Along with the evaluation of commercially available traps, traps of various shapes and designs were constructed in the laboratory and evaluated (Table 5). All of the listed laboratory-fabricated traps captured numbers of eye gnats as good as, or significantly better than, the standard solid attractant bait. (0.8 - 3.7 times better). Most of these traps incorporated a two jar design that took advantage of the gnats' negative geotropism behavior of trying to escape capture by moving upward, and getting trapped in the upper jar. Laboratory-fabricated traps that were not as effective at capturing large numbers of gnats are not presented or will not be discussed.

With the collar traps (Fig. 1), entry hole size made a difference. A collar with 17 mm holes (Table

Table 4. Evaluation of commercially-available house fly, wasp, and yellow jacket traps for collecting eye gnats using liquid SFA. Collection numbers indicate the means of four replicates for each matched pair tests of the liquid SFA in a particular trap type and solid SFA in a cup of damp sand. Attractancy Index = number of gnats trapped with liquid SFA + number of gnats trapped with solid SFA. Significance levels indicate results of matched-pair t-tests.

Liquid SFA		Solid SFA	Attractancy Index	Significance level
Trap type	Gnats/trap	Gnats/cup		
Seabright "Yellowjacket Inn"	8	156	0.05	<0.01
Sure Fire Yellowjacket Trap	1	221	0.00	-
Donut-shaped Wasp Trap (tinted)	64	276	0.23	0.01
Fermone Gotchu Fly Trap	48	672	0.07	0.01
Modified Gotchu Fly Trap (hood removed - test 1)	65	268	0.24	0.06
Modified Gotchu Fly Trap (hood removed - test 2)	34	341	0.10	<0.01
Donut-shaped Wasp Trap (clear plastic)	28	341	0.08	<0.01

5, test 3; mean no. of 730 gnats/trap) had an index of 3.7 ( $p = 0.03$  significance). The collar trap with 11 mm holes (Table 5, test 1; mean no. of 484 gnats/trap) had an index of 1.8 ( $p = 0.01$  significance). The ability of the collar trap with the larger holes to collect higher numbers of gnats may be due to more attractive odors escaping through the larger holes or the larger holes may facilitate easier entry of eye gnats.

The "white" collar trap (Table 5, test 1) attracted significantly more gnats (mean no. of 484 gnats/trap) than standard solid attractant (mean of 268 gnats/trap); a difference of 1.8 times. A "clear" collar trap (Table 5, test 2) attracted 1.5 times more gnats (mean of 302 gnats/trap) than the fresh standard solid attractant (mean of 201 gnats/trap); a significant difference at the  $p = 0.08$  level. Both collar traps captured gnat numbers which indicate a rather effective eye gnat trap. Further testing between the clear and white collar traps might elucidate any real difference between these two collars.

A keg bottle (Fig. 1) was another design evaluated as an eye gnat collection trap. When a keg bottle trap with 11 mm entry holes was compared to the standard solid attractant bait (Table 1, test 4), the keg trap captured a mean of 470 gnats/trap, 1.9 times more than the 247 gnats/ trap captured by the standard solid attractant bait (significance at 0.05 level). A keg trap containing 17 mm entry holes (Table 5, test 5) captured a mean of 354 gnats/trap while the standard solid attractant collected a mean of 186 gnats/trap; the liquid attractant trap outperformed the solid attractant cup by 1.9 times (at significance

level of 0.01). Either of these keg traps appear to be excellent eye gnat traps.

Both the collar and keg traps are constructed from two containers. A one container trap was also constructed from a one liter soda pop bottle by placing four holes in its side above the mid-line. The cap was left on to trap gnats inside. The soda pop bottle trap (Table 5, test 6) captured a mean of 194 gnats/ trap; not significantly different ( $p = 0.46$ ) from the 188 gnats/trap collected by the standard solid attractant bait.

The next one container trap evaluated was constructed by placing four holes in the side of a two liter soda pop bottle (Table 5, test 7). This trap captured a mean of 311 gnats/trap; not significantly different ( $p = 0.34$ ) from the 317 gnats/ trap collected by the standard solid attractant bait.

A third one container trap was also constructed from a two liter soda pop bottle. However, this trap had eight entry holes in the side instead of the four holes of the previous traps (Table 5, test 8). This eight hole trap captured a mean of 153 gnats/trap; not significantly different ( $p = 0.22$ ) from the 189 gnats/ trap collected by the standard solid attractant bait.

From all the laboratory-constructed traps evaluated, two stand out as being significantly more effective than the standard cup of solid attractant bait on damp sand; considered to be an excellent attractant bait for *Hippelates* eye gnats. These are the collar trap and the keg trap. Most tests using these traps attracted gnat numbers that were either significantly more or equal to the solid attractant bait

Table 5. Evaluation of laboratory-fabricated traps for collecting eye gnats using liquid SFA. Collection numbers indicate the means of four replicates for each matched pair tests of the liquid SFA in a particular trap type and solid SFA in a cup of damp sand. Attractancy Index = number of gnats trapped with liquid SFA ÷ number of gnats trapped with solid SFA. Significance levels indicate results of matched-pair t-tests.

Test no.	Liquid SFA		Gnats/trap	Solid SFA	Attractancy Index	Significance level
	Trap description	(Holes; Hole dia.)		Gnats/cup		
1	White collar	(8; 11 mm)	484	269	1.8	0.01
2	Clear collar	(8; 11 mm)	302	201	1.5	0.08
3	White collar	(8; 17 mm)	730	197	3.7	0.03
4	Keg bottle	(8; 11 mm)	470	247	1.9	0.05
5	Keg bottle	(8; 17 mm)	354	186	1.9	0.01
6	1-Liter soda pop bottle	(4; 18 mm)	194	188	1.0	0.46
7	2-Liter soada pop bottle	(4; 18 mm)	311	317	0.9	0.34
8	2-Liter soda pop bottle	(8; 18 mm)	153	189	0.8	0.22



on damp sand in a cup.

Another group or style of laboratory-constructed traps that captured large numbers of gnats were fabricated from one or two liter plastic soda pop bottles. These attracted and captured gnat numbers that were comparable to the standard solid attractant bait in a cup.

Whichever design is chosen for mass production and subsequent use in a control program, the actual trap selected will be evaluated on more than efficiency or retention of captured gnats. Since thousands of traps would be deployed, cost of each trap will be a major consideration. Ease of handling and servicing of the trap is important, since hundreds must be deployed daily and operators will not have time to adjust each trap. The trap has to be limited in size, since large numbers have to be carried to the field by individual operators for deployment. An finally, the difficulty of cleaning the trap, once it is retired at the end of the gnat season, may also be of some concern.

#### ACKNOWLEDGEMENTS

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## FIELD INTRODUCTIONS AND ESTABLISHMENT OF THE TADPOLE SHRIMP, *TRIOPS LONGICAUDATUS*, A BIOLOGICAL CONTROL AGENT OF MOSQUITOES <sup>1</sup>

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### ABSTRACT

Tadpole shrimp (*Triops longicaudatus* LeConte) are potential biological control agents for some mosquitoes which develop in temporary aquatic habitats. They are effective larval predators and their presence deters mosquito oviposition. Several life history traits of tadpole shrimp (TPS) accord them considerable advantages for use as biological control agents. Eggs begin hatching twelve hours after hydration and this early hatch in combination with rapid growth rate results in temporal overlap with their mosquito prey. Tadpole shrimp mature and oviposit as early as six days after flooding and may lay more than 1,000 eggs in their lifetime. Finally, TPS lay desiccation-resistant eggs that are able to remain dormant in soil for months to years.

Use of *T. longicaudatus* in practical biological control programs depends in part on their ability to establish permanent populations in appropriate habitats. Introductions of both lab-reared and field-collected eggs as well as gravid adults were made in Imperial Valley ponds. Establishment and persistence of TPS in these ponds was monitored over five floodings from Fall, 1991 to Summer, 1992. Tadpole shrimp colonized and persist in 94% of these ponds (n=16). In one pond where TPS did not establish, no initial hatch was noted. All three methods of introduction were successful, although the hardy egg stage is the preferred mode of distribution for mosquito control programs. Steady population increases occurred in 3 ponds throughout this study, but densities in the remaining ponds was variable.

Knowledge of hatch rates in the field are

important because they will influence initial stocking rates and augmentation decisions. Hatch rates during the initial flood of lab-reared eggs (N=1,135-2,353/pond) ranged from 13.7-19.4% (mean=15.3%), significantly higher than the hatch rate of eggs confined in floating cages which averaged 12.1% ( $G_T = 77.9$ ,  $p < .001$ ). Therefore, floating sentinel cages underestimate the hatch rates actually seen in the field, but still may be useful for rough estimates. Hatch rates of field-collected eggs in floating cages had a maximum mean hatch rate of 16.4% during the initial flood. This is probably an overestimation because eggshells from hatches in earlier flooding may remain in the soil. The relatively low hatch rates observed here are partly due to the installment hatching which occurs in this species. Upon future floodings, some further proportion of the eggs remaining from the first inundation hatch. In this study, however, high water temperatures (>37°C) also adversely affected hatching during the initial flooding.

A great deal of variance in adult shrimp density in these experiments was observed, however, population sizes should continue to increase with more numerous floodings. Factors likely to affect population sizes include initial stocking rates, number of floodings, water temperature, and predation by birds. In spite of adverse conditions during some flooding periods, TPS were still able to persist throughout the duration of this study. Given the ease of transferring TPS into previously unoccupied habitats, large scale introductions may be used in mosquito control programs.

<sup>1</sup> A manuscript summarizing this study has been submitted to Biological Control: Theory and Application in Pest Management.

## LIMNOLOGICAL EVALUATION OF SIX WETLAND SITES

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Freshwater habitats contain some of the most ancient and more complex ecological systems existing today. They are the most productive systems in the world biome and supply the basal trophic support for most terrestrial systems. Freshwater systems are also among the most vulnerable and today many have become threatened.

Until very recently, the fundamental importance of the wetland habitat to the survival of life was not recognized. Wetlands were considered a hindrance to progress and, as such, were systematically transformed to useable agricultural, commercial or urban lands. In California, it is estimated that over 90% of wetlands present 200 years ago have been transformed to land use (Dahl and Johnson 1991).

In an effort to reverse this trend, federal and state wildlife protection agencies are promoting and supporting the restoration of historic waterfowl flyways in California through the construction of created vernal pools, marshes, duck clubs, shallow ponds and lakes. These new habitats are necessarily being constructed without certain understanding of how they function and evolve. Time may be necessary to establish a diverse and balanced ecological community. Fretwell (1987) states that the diversity of wetland systems can be explained by the complexities and dynamics of the flow of nutrients and biomass through the food chain.

The purpose of this investigation was to make bio-diversity and density observations and comparisons of several created wetlands constructed with various ecological strategies and at various successional stages and to compare them with an ancient natural wetland habitat. A second goal was to observe mosquito productivity in each of these wetland sites and identify the physical and biological characters contributing to their success.

### METHODS AND MATERIALS

Twelve monthly collections, each consisting of twenty-four samples, were objectively taken at each of six different wetland sites for one year. Each of the sites was sampled in the same manner at roughly the same time in the morning and consisted of an "A" collection of five dips using a standard 250 ml dipper and a "B" collection using a single sweep with an aquatic D-net. In addition, a single grab from the wetland bottom substrate was collected each month using a Peterson's dredge. These collections were returned to the lab where they were backwashed through a sand-mud sieve with 5.6 mm, 2.0 mm and 300  $\mu$ m screens and then preserved in 85% isopropyl alcohol for later examination.

Conductivity, total dissolved solids, dissolved oxygen, pH and temperature were collected from each site using a Corning M90 portable microprocessor probe and sensor. Samples for water chemistry were collected just before leaving the site and lab-analyzed using HACH DR-EL water analysis kits. Deep water samples were collected using a LaMontte water sampling device. Depth was determined using a sounding lead and calibrated line.

All collected specimens in each sample were counted and identified to at least the genus level with representatives of each species preserved and stored in a reference collection. Identifications were made using the following keys: Darby 1962, Merritt and Cummins 1984, Pennak 1989, Thorp and Covich 1991, Usinger 1956 and Ward and Whipple 1959. Confirmation of most identifications was made at various institutions and museums.

### Morphometrics.

The six sites consisted of five created wetlands and an ancient oxbow lake (representing a natural wetland habitat). The youngest sampled site was Oxbow Lake, a constructed wetland in the Lighthouse

Mitigation Project near the Sacramento River, about 4.7 km northeast of Sacramento in Yolo County. The lake was first flooded just prior to the initial sampling using a combination of well water, Sacramento River water and erosional drainage from a previous agricultural site. The lake has a surface area of 4.5 ha and a depth of one meter. There was virtually no submergent or emergent vegetation on the lake during the sample period.

The West Davis Pond site, a mitigation wetland for an adjacent urban development in the city of Davis, had been completed two years before the start of sampling. The water source for this site is local urban runoff (storm drain and lawn irrigation) from adjacent developments. The pond has a surface area of 2.0 hectares. The basin gradually slopes to a depth of 1.5 meters. Emergent vegetation consist of rush and tules along the shore margins.

The Bushy Lake site, created during the construction of Interstate 80 adjacent to the California State Exposition grounds, is an unmanaged lake and adjacent marsh of approximately 30 years of age at the start of sampling. Water sources include urban runoff and infrequent winter flooding of the American River. The lake has a maximum measured depth of 2.1 m and a surface area of 7.4 hectares. Emergent vegetation consists of water primrose and duckweed.

The Gibson Lake site was created over forty years ago by the damming of a small creek and was originally used for livestock irrigation. Today the lake resides within a county park and has vegetation and basin management. The lake has a maximum measured depth of 1.2 m and 7.5 ha of surface area. Water primrose, duckweed, cattails and bulrushes occur along the shore margins.

The McCormack's Duck Club Pond site is one of a series of duck hunting ponds located in the Yolo bypass. The site has been annually flooded for decades, receiving water from irrigation ditches in November and drained in June. The pond has a maximum depth of 0.6 m and covers an area of 31.2 hectares. Water grass, arrowhead and bulrushes occur throughout the site.

The last site, North Stone Lake, was the only natural wetland sampled in our investigation. It is an ancient oxbow lake of the Sacramento River with an estimated age at over 1,000 years. The lake's water comes from a complex of drains from Upper and Lower Beach Lake, Morrison Creek and from a barrow slough that carries water from local runoff. The lake has a maximum measured depth of 2.7 m and covers an area of 69.7 hectares. Water primrose

and cattails make up the majority of emergent vegetation. This lake acted as a standard for comparison.

## RESULTS AND DISCUSSION

The physicochemical data collected during the year roughly separated these sites into two wetland habitat types. The North Stone Lake and Gibson Lake sites have purer, cleaner water with higher concentrations of dissolved oxygen, slightly alkaline pH levels and low levels of dissolved solids, organic nutrients and pollutants. The other four wetland sites show indications of higher concentrations of free organic nutrients. Bushy Lake, West Davis Pond and McCormack's Duck Club Pond have low levels of nitrate and nitrites. The extremely high ferrous concentration at West Davis Pond and McCormack's Duck Club Pond indicates organic decomposition which with lower dissolved oxygen, higher carbon dioxide and near neutral pH contribute to change the usually nonsoluble ferric complex into solution. The high levels of dissolved solids at McCormack's Duck Club Pond, West Davis Pond and Oxbow Lake sites also indicates a richness in dissolved minerals and organics. These sites can be described as organically polluted.

The community structure of the wetland habitat defines the relationships and productivity of the biotic system (Table 1). Primary consumers, near the base of the trophic pyramid, are the most abundant invertebrates in the aquatic habitat. Their density and diversity can be indicative of organic load and stability of the aquatic system.

A primary consumer indicator of clean unpolluted water is the amphipod *Hyalella azteca*. Among the six sites sampled, amphipod density was greatest at the North Stone Lake site which averaged over 210 individuals per liter and peaked in density during the month of July. Bushy Lake and Gibson Lake also had high average densities of amphipods at 157 and 21 individuals per liter, respectively. Oxbow Lake and West Davis Pond did not support populations of amphipods. In contrast, McCormack's Duck Club Pond had a small amphipod population after flooding, which tapered off to near absent before drying in July, giving the site an average low of 6.5 individuals per liter sampled.

Cladocera are an extremely important primary consumer in wetland habitats. They are well suited for the dynamic seasonal environment as they can

Table 1. Macrofaunal community characteristics for the six wetland sites.

Parameters	North Stone Lake	Gibson Ranch Lake	Bushy Lake	Mc's Duck Club	West Davis Pond	Oxbow Lake
Diversity i.e. #genera/site	114	47	46	91	35	21
% Littoral zone genera	46	34	36	57	50	48
% Limnetic zone genera	27	22	30	20	21	22
% Aufwuchs genera	4	4	2	2	0	4
%Benthic zone genera	22	40	32	21	29	26
Density i.e. #individuals/liter	311.5	370.3	537.9	607.3	49.4	11.3
Planktonic density based on #s of Cladocera/liter	111.4	296.1	173.6	334.3	6.1	0.9
Nektonic density based on #s of Dytiscids/liter	1.2	0.1	0.2	0.9	0.1	0.1
Neustonic density based on #s of Collembola/liter	0.9	0.5	0.5	0.3	2.6	0.1
Benthic density based on #s of gastropods/liter	5.0	0.9	19.3	6.2	2.8	4.9

resist drying and freezing in the form of a diapausing embryo ephippium. Once water is available, they can rapidly increase their population using parthenogenic reproduction.

Cladocera density was highest at McCormack's Duck Club Pond and lowest at Oxbow Lake with 334.3 and 0.9 individuals per liter, respectively, with North Stone Lake between the two at 111.4 individuals per liter (Table 1). Cladocera diversity was far greater at North Stone Lake with nine different genera collected and total numbers evenly distributed among the various genera. McCormack's Duck Club Pond, on the other hand, had lower diversity with only two genera collected - the majority of individuals comprised of one species.

A simple way to analyze organic levels and community structure using wetland invertebrates is to compare the relative abundances (presence or absence) of specific pollution-tolerant and intolerant invertebrate species (Table 2). Each site was

compared for the number of species out of a selected ten which occurred in each class as defined by Beck's Biotic Index (Shaw 1988). Class I species are intolerant of organic pollutants, while Class III species are tolerant of organic pollutants. According to the Index, an unpolluted habitat will include representatives from all the major groups and polluted habitats will consist mostly of pollution-tolerant species. From this perspective, North Stone Lake and Gibson Lake are considered pollution-free as these sites have the greatest diversity, including many intolerant species. Bushy Lake and Oxbow Lake are near neutral, having some intolerant and tolerant species. The remaining sites, West Davis Pond and McCormack's Duck Club Pond, have a high pollution index as their invertebrate community was comprised mostly of pollution-tolerant species.

In the pollution-tolerant category of Beck's Index, most species are Diptera. The seasonality and density of pollution-tolerant Diptera show that much

Table 2. Relative organic pollution based on the Beck's Biotic Index of ten intolerant and ten tolerant invertebrate species.

Parameter	North Stone Lake	Gibson Ranch Lake	Bushy Lake	Mc's Duck Club	West Davis Pond	Oxbow Lake
Numbers of Class I genera present	9	6	5	3	3	4
Numbers of Class III genera present	7	3	4	10	10	6
Index - smaller numbers represent less organic pollutants	-2	-3	-1	7	7	2

CLASS I - INTOLERANT		CLASS III - TOLERANT	
Trichoptera <i>Limnophilus</i>	Pelecypoda <i>Corbicula</i>	Gastropoda <i>Physella</i>	Ephydriidae <i>Notiphila</i>
Ephemeroptera <i>Baetis</i> <i>Callibaetis</i> <i>Siphonurus</i>	Bryozoa <i>Plumertella</i>	Oligochaeta <i>Aeolosoma</i>	Psychodidae <i>Telmatoxenus</i>
Amphipoda <i>Hyalella</i>	Coelenterata <i>Chlorohydra</i>	Chaoboridae <i>Eucorethra</i>	Tabanidae <i>Tabanus</i>
	Gastrotricha <i>Chaetonotus</i>	Chironomidae <i>Chironomus</i>	Syrphidae <i>Eristalis</i>
	Ostracoda <i>Candona</i>	Tipulidae <i>Erioptera</i>	Ceratopogonidae <i>Bezzia</i>

of their population numbers was contributed to an eruption occurring in the fall, directly following the flooding at the McCormack's Duck Club Pond (Fig. 1). All the other sites had population increases in the spring. McCormack's Duck Club Pond's fall peak consisted mostly of syrphids and ephydriids. McCormack's Duck Club Pond also had the highest recorded peak of chironomids (Fig. 2) - 80% more individual chironomids per liter than North Stone Lake but 25% fewer genera. Among the six wetland sites, North Stone Lake had the greatest chironomid diversity with fourteen genera representing predaceous, herbivorous, detritivorous and filter feeder species.

Gastropod populations can impact chironomids significantly as they are competitors for many of the same resources (Danell and Sjoberg 1982). In sites where gastropods were abundant, chironomids were relatively moderate or low when compared to sites where gastropods were less abundant. This was most

apparent in Bushy Lake which had a greater diversity and significantly more gastropods, averaging three times more per liter, than did McCormack's Duck Club Pond. In contrast, the number of chironomids at the gastropod-poor McCormack's Duck Club Pond was nearly four times that which occurred at the gastropod-rich Bushy Lake site. Gastropods populations are limited at the McCormack's Duck Club Pond by the seasonal drying of the site.

Mosquito larvae can be dominant primary consumers in seasonal wetlands where predators and competitors are few or absent and where there are high levels of organic material in suspension. Mosquitoes are best adapted to new or unstable aquatic systems where the normal trophic systems are dysfunctional or absent (Meyer et al. 1982). They rapidly utilize the free organics and can complete their development in just a few days.

Mosquito larval abundance and diversity was greatest at McCormack's Duck Club Pond with four

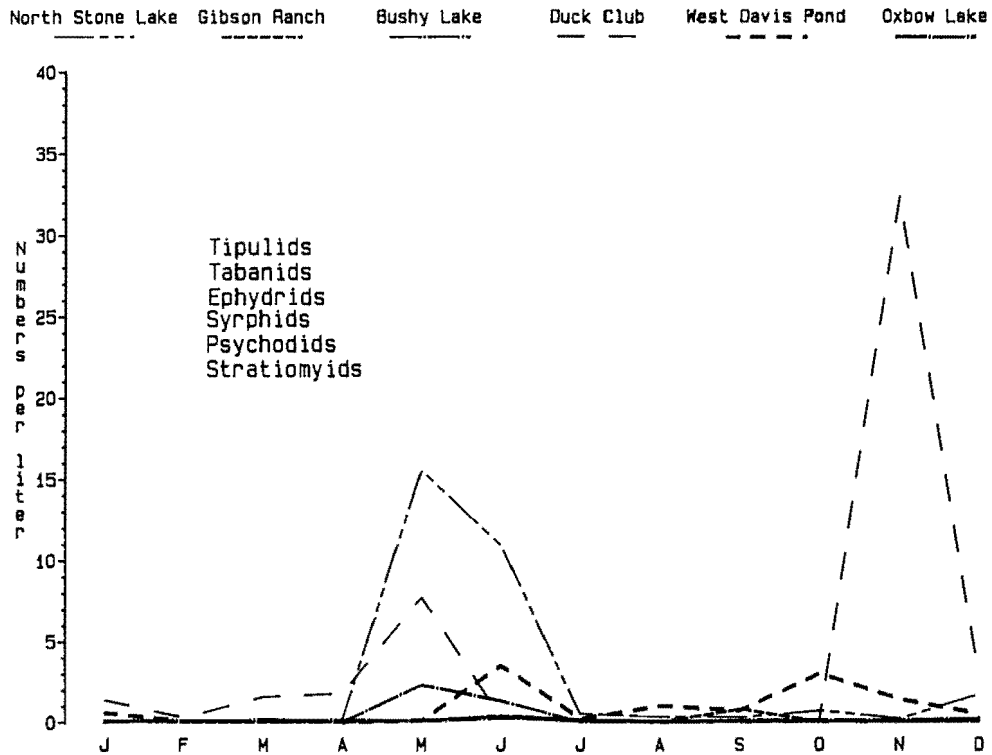


Figure 1. Density of the pollution-tolerant Diptera at the six wetland sites.

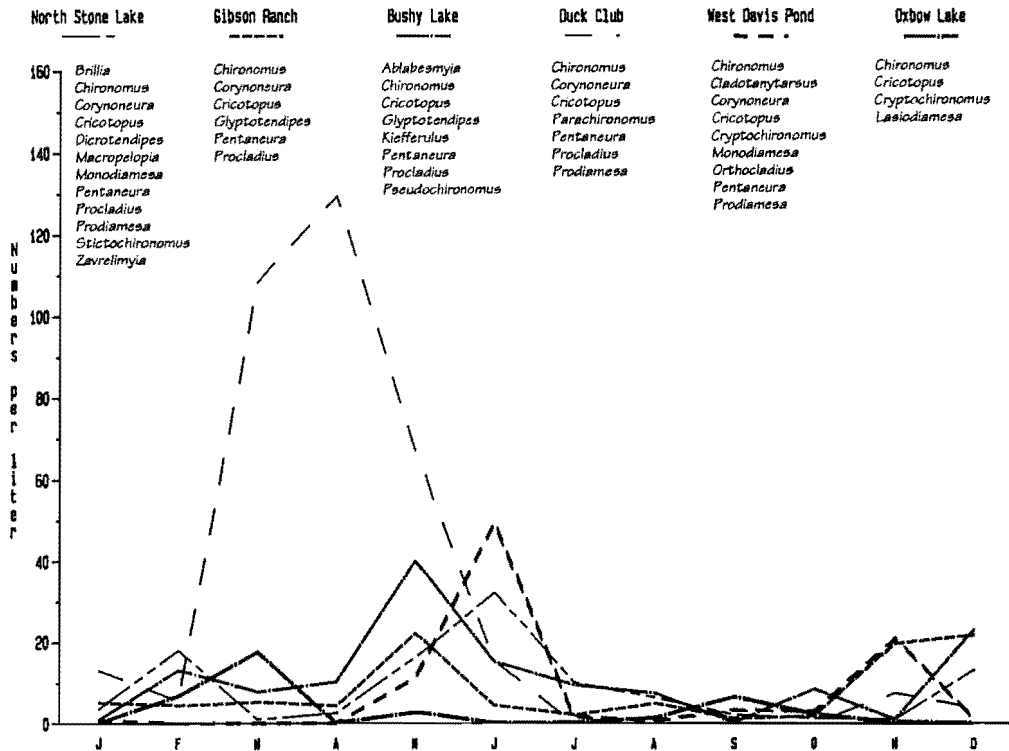


Figure 2. Chironomidae density and diversity at the six wetland sites.

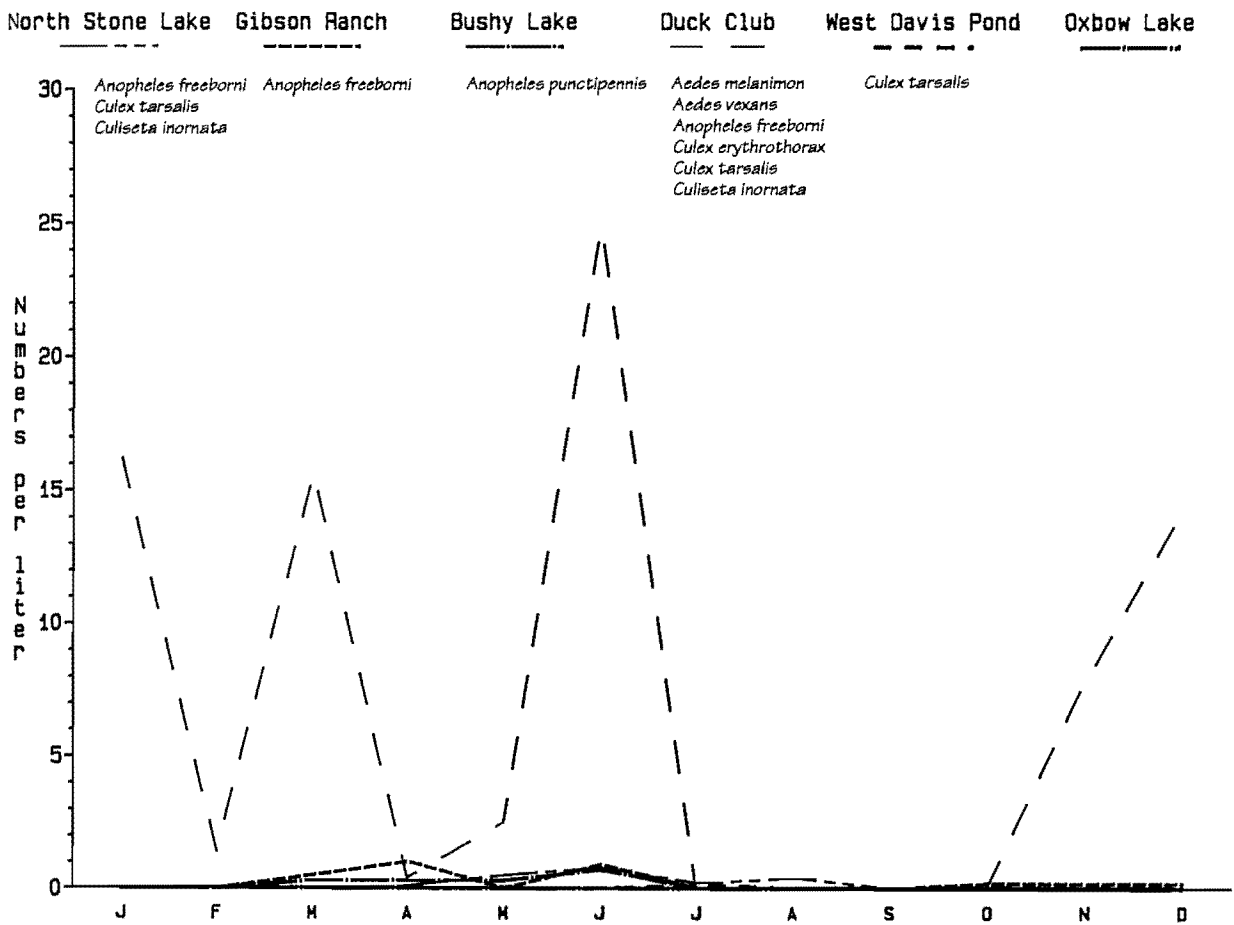


Figure 3. Culicidae density and diversity at the six wetland sites.

genera and six species collected, an average of 10.3 larvae per liter throughout the season. The other sites had significantly fewer mosquito larvae with North Stone Lake and Gibson Lake averaging only 0.2 larvae per liter (represented by three species), Bushy Lake averaging 0.1 larvae per liter and West Davis Pond less than 0.01 larvae per liter. Oxbow Lake site did not support mosquitoes during the twelve month sampling period (Fig. 3).

Mosquito populations at McCormack's Duck Club Pond consisted primarily of three species which had abundance peaks separated temporally. *Culiseta inornata* (Williston) occurred November through January, *Culex tarsalis* Coquillett occurred February through April and *Aedes melanimon* Dyar occurred May through July. Both *Cx. tarsalis* and *Ae. melanimon* produced populations early in the spring, creating what may be the initial cohort for the season. All the *Cx. tarsalis* collected from the other sites occurred after June.

The temporal distribution of mosquitoes at North Stone Lake shows small numbers of *Cs.*

*inornata* collected during April through June and small numbers of *Anopheles freeborni* Aitken collected in August. The most abundant mosquito species was *Cx. tarsalis* which occurred during June through October with a peak in August.

The temporal distribution of mosquitoes at Bushy Lake indicates that small numbers of *Anopheles punctipennis* (Say) occurred from March to June, being most abundant during June. Gibson Lake produced *An. freeborni* in the spring, April to July, and in the fall, October through early December. No mosquitoes were collected in January or February or during July through September.

McCormack's Duck Club Pond, clearly, was significantly more productive for both mosquitoes and chironomids than any of the other wetland sites sampled.

Secondary consumers are of great significance in the structure of the biological trophic system. Interacting higher on the trophic pyramid, they regulate the populations of the primary consumers. The diversity of the secondary consumer community



is indicative of the maturity and complexity of a stabilized aquatic community. Many of the members of this group are predaceous insects.

The Odonata, dragonflies and damselflies, are important predators and can be considered both secondary and tertiary consumers in the invertebrate community. Their life cycles are relatively long, usually one year, but as many as four years are necessary for some species (Merritt and Cummins 1984). They are, therefore, suited for more permanent wetland habitats but may also occur in many seasonal wetlands.

In the six observed sites, odonate density was greatest at Bushy Lake and Gibson Lake with 4.4 and 3.2 individuals per liter, respectively. In these sites, odonates were the predominant predator. In the West Davis Pond and Oxbow Lake sites, odonates were rare, averaging only 0.4 individuals per liter of sample. In these sites, a stable predator-prey relationship had yet to be established with odonates. At the North Stone Lake site odonate populations represented only part of a larger predator population, averaging only 1.1 individuals per liter. Odonates did not occur at McCormack's Duck Club Pond.

Another important group of predators are the predaceous beetles, of which the Dytiscidae are the most important. Both adult and larval dytiscids feed extensively on chironomid and culicid larvae (Smith and Enns 1969).

At the six wetland sites, major dytiscid population increases occurred in June and July, consisting mostly of adults. The greatest dytiscid numbers were found during June at North Stone Lake and McCormack's Duck Club Pond with over nine and six adults per liter of sample, respectively. At North Stone Lake the dytiscids were collected during June, July and August while at McCormack's Duck Club Pond, dytiscids occurred from November until drained in June. Ten genera of dytiscids occurred at North Stone Lake and twelve occurred at McCormack's Duck Club Pond. The other sites had scattered seasonal distribution and lower diversity.

Hemipteran predators consisted of three families; Gerridae, Notonectidae and Belostomatidae. The greatest density of these predators occurred at the sites with the greatest density of primary consumers. McCormack's Duck Club Pond and West Davis Pond had densities between 1.3 and 1.5 individuals per liter of sample, respectively, but were represented by only four and five different genera, respectively. North Stone Lake had far fewer numbers, only 0.6 individuals per liter, but had the greatest diversity

with eleven different genera. Belostomatidae, an important apex predator require some deep water in their habitat for overwintering and therefore did not occur at McCormack's Duck Club Pond.

## CONCLUSIONS

Based on the abiotic data and the community structure of the constructed wetland sites, each can be classified as either mesotrophic or eutrophic in nature. North Stone Lake is comparatively oligotrophic in nature, having lower productivity due to fewer available nutrients but having more complex trophic structures and greater diversity - representing a stable state for aquatic ecosystems. Gibson Lake and Bushy Lake are mesotrophic-like wetlands, having moderate amounts of nutrients with occasional plankton blooms. McCormack's Duck Club Pond, West Davis Pond and Oxbow Lake are human-induced eutrophic wetlands which were more productive due to abundant available nutrients and had simple trophic structures with episodic changes in population sizes.

When comparing the community structure of the six sites, we found two distinct conditions. In the mature wetland sites, such as North Stone Lake, the highest diversity of species existed but a comparatively low density of any one species occurred. North Stone Lake had a lower concentration of primary consumers, such as cladocera and chironomids, with small population eruptions, but had the highest concentration of secondary consumers, such as odonates. Among the other three newer sites, McCormack's Duck Club Pond had higher concentrations of primary consumers, with defined peaks in population size, but had lower levels of secondary consumers (e.g., odonates and belostomatids were entirely absent), indicating a shorter trophic pyramid. Eutrophism provides two major advantages to chironomids and culicids; 1) the eutrophic condition creates large changes in oxygen concentrations and frequently oxygen depletion which their competitors and predators may not tolerate and 2) eutrophic conditions create high concentrations of detritus for food (Grodhaus 1963).

It seems evident that eutrophism and a diminished invertebrate community structure are important biotic factors contributing to high primary consumer productivity. The reproductive strategy of these animals is to rapidly respond to and utilize the available resources characteristic of eutrophic

wetlands, hence the eruptive population numbers. It has been demonstrated that eutrophic conditions will lead to reduced community diversity with the selection of a few species (Meyer et al. 1982). However, eutrophism alone does not promote prodigious productivity as attested by the Bushy Lake and West Davis Pond sites. The largest difference between the McCormack's Duck Club Pond wetland and the other eutrophic wetlands was the lack of year-round, non-fluctuating water in deep basins which could support secondary and tertiary consumers.

The uniform shallow water, soft substrate and even stands of vegetation at McCormack's Duck Club Pond allow easy recirculation of nutrients, accumulation of high concentration of detritus and increased decomposition, creating abundant food for primary consumers. The seasonal drying of the site for three months each year prevents the development of complex communities and arrests the wetland at an early successional stage. This creates what is essentially a continuous littoral habitat, with high nutrient levels, which benefit a short list of invertebrates specifically adapted to these conditions.

These conditions are part of the strategy of the duck club habitat. Duck club designers and managers create and maintain shallow, seasonally flooded wetlands to produce high density primary consumers, specifically chironomids and culicids (Meyer and Swanson 1982, Euliss and Grodhaus 1987). Predators and competitors are excluded from these habitats giving dabbling ducks the opportunity for feeding lower on the trophic pyramid (Kadlec 1962). The primary dabbling duck species in California are the mallards, teals, widgeons and pintails, which feed on invertebrates, primarily chironomids, in late winter (Miller 1987). Summer drawdown is important to provide seeds of wetland plants for spring feeding ducks, and to provide soil improvement for enrichment of reflooded water in the fall for eutrophic conditions (Kadlec 1962). This strategy for waterfowl management may produce excellent habitat for dabbling ducks, however, it has also been shown to create optimal conditions for the production of mosquito species (Meyer et al. 1982).

From information gained during this study, our recommendation is to create wetlands that more closely resemble the North Stone Lake site, diverse in habitat and consequently, diverse in biota. These wetland sites include both littoral and limnetic zones which together support primary, secondary and tertiary consumers that collectively regulate the

eruption of any single population which would prevent mosquitoes from becoming dominant. Such wetlands also support a diverse vertebrate community including not only dabbling ducks, but also diving ducks, mergansers, grebes, cormorants, herons, egrets, cranes, geese and pelicans. This type of habitat should meet the goal of wetland creation with the purpose of enhancing biodiversity.

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## THE EVOLVING ROLE OF THE CALIFORNIA DEPARTMENT OF HEALTH SERVICES IN THE SURVEILLANCE AND CONTROL OF VECTOR-BORNE DISEASES

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Today I will cover four aspects of the vector surveillance and control program in the Department of Health Services. I will refresh your memories on its history, comment on its current organization and funding, touch on how the program fits with the Governor's prevention agenda, and tell you what we anticipate the future of the program to be. I have a strong personal interest in the program and, as the Deputy Director in charge of Prevention Services and earlier as Chief of the Infectious Disease Branch, have worked closely with many of the program's staff.

At your conference a year ago, Don Womeldorf elaborated upon the history of the vector surveillance and control program. Briefly, its genesis goes back to 1910, more than 80 years ago, when Dr. William B. Herms, of *Anopheles hermsi* fame, recommended creation of a mosquito control program within the Department of Public Health. As you may recall, the first local mosquito control program was begun by Dr. Herms in 1991 at Penryn, just east of Sacramento, and your enabling legislation was enacted in 1915, so the State clearly has been identified with your activities since their beginning. In 1946, the Bureau of Vector Control was formed, combining the mosquito control activities with the functions of the Sanitary Inspections Section, which was charged with plague and tularemia surveillance. The year 1946, then, saw the genesis of the State's comprehensive program in vector surveillance and control. It has always placed its emphasis on service to the public and support of local programs, and so it will remain

under my control.

You will be amused or perhaps bemused to note that from 1946 to present the program's name has changed from the Bureau of Vector Control to the Bureau of Vector Control and Solid Waste Management to the Vector Control Section to the Vector and Waste Management Section to the Vector Biology and Control Section to the Vector Biology and Control Branch to the Vector Surveillance and Control Branch and now to the Environmental Management Branch, all within the Department of Public Health or the Department of Health, or now, the Department of Health Services.

Turning now to current organization and funding, the program has been reduced to one geared to operating within specific mandates and statutory authorities. Some of you may remember that the program had once been the focal point for California's mosquito control research, and by legislative direction yielded those activities to the University of California in the mid-1960s.

In the aftermath of Proposition 13, the program sustained a major reduction in force in 1979. Most recently, in 1991, the State's bleak fiscal picture necessitated another deep cut in the program. With Don Womeldorf's retirement, the program will consist of 15 public health biologists, one administrative support position, and three clerical support positions. The organizational structure will remain a regional and District office deployment, the best configuration to meet your needs. For efficiency's sake, though, we will be consolidating into two not three regions.

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Funding is a major issue. As I am sure you have been told more than once, the difficulties faced by State programs are exacerbated if their support comes from the General Fund. The total amount of the General Fund is strictly limited and the demands upon it continue to grow. The State's vector surveillance and control program has always been supported by the General Fund, and so when General Fund cuts are imposed, the program is always vulnerable.

The only portion of the program currently supported on an ongoing basis by anything other than the General Fund is the continuing education program, and the fees assessed amount to enough for only a portion of a clerical position. By way of perspective, the program support from the General Fund approximates \$1.8 million while the continuing education fee assessment brings in only \$24,000.

Before I say more about the future and future needs of the program, let me comment that the philosophy of the Governor and his administration generally, and the Department of Health Services specifically, is geared toward prevention of disease and of environmental and other conditions which are inimical to the public well-being and enjoyment of life. Indeed, I am Deputy Director for Prevention Services, a name chosen by Dr. Coyle because it focuses attention on its mission better than did the old name of Public Health. The vector surveillance and control program, like your own programs, emphasizes prevention of vectors and the diseases they transmit. In fact, like yourselves, one difficulty faced by the program is justifying its existence when it is successful -- with no bodies in the streets, it is hard to see why a prevention program needs to exist. I am committed to assuring that the program's attention will continue to be directed toward prevention.

In discussing the future of the vector surveillance and control program, I will highlight three aspects. The first is function and scope, the second is funding, and the third is program location. Regarding function and scope, the program has been reduced to doing what it must do to comply with enabling statutes. The basic mandates are found in the California Health and Safety Code, Sections 402 and 403, Sections 2425 and 2426, as well as some related language in the Government Code, and elsewhere in the Health and Safety Code. In response to the mandates, the program focuses about 25 percent of its efforts on vector-borne disease surveillance and another 25 percent on emergency

vector control actions. Another 25 percent is invested in the training, certification, and continuing education actions. The balance goes into administering the cooperative agreement with your agencies dealing with use of public health pesticides and doing the related work such as processing pesticide use reports, consultation provided to your agencies, and public education and information.

Nearly no time is budgeted to deal with mandates regarding authorizing importation of exotic vectors and adjudicating disputes involving your agencies and other governmental entities, but those functions continue to be carried out. In summary, I do not see that there are sweeping changes in store for the program's scope and function. There will be some adjustments. For example, Africanized honey bees, sure to arrive in California this year or next, will require a focused effort, of which your agencies will be a major part.

The vector surveillance and control program has evolved into an organization designed to serve as the State's representative to a team designed around your agencies, and has been and will continue to be supportive of your actions to protect California's citizens. As you know, key members of the team at the Department's level include our biomedical laboratory scientists, our physicians and epidemiologists, who provide specialized support to the vector surveillance and control program and thereby to you.

Funding is a thorny problem. As I noted earlier, competition for General Fund dollars is intense. Even with good justification, it is exceedingly difficult to obtain General Fund support. That was vividly illustrated when the program lost its developing Lyme disease vector surveillance and prevention capability. Lyme disease causes more human cases of vector-borne disease in California than all of the rest of them put together, but that fact was not enough to maintain general Fund funding. I should add that Lyme disease epidemiologic and vector ecology research have continued under funding from the Centers for Disease Control (CDC).

Program staff have been innovative in the past year or two in seeking outside funding, and have been able to obtain support for plague surveillance and control from the U.S. Forest Service and, to a lesser extent, from the National Park Service. The small amount of outside funding, which at the moment adds up to only about \$250,000 per year, is not going to be sufficient to offset losses which may result from forced reductions at some time in the

future.

If indeed, the support you receive from the vector surveillance and control program is important to you, it may be necessary to have serious discussions with you about your providing financial support for those activities which are critical to your operations. I understand that there have been informal talks with you for some time to explore possibilities.

The last item I want to cover has to do with the future administrative location of the vector surveillance and control program. I have been looking at the several programs within Prevention Services and am

considering some reorganization. I plan to put likes with likes. In the case of the vector surveillance and control program, one option is to place it with other disease prevention and laboratory functions in the Division of Communicable Disease Control. Militating against that option is the fact that much of what the vector surveillance and control program does is regulatory and quasi-regulatory, and so it would also be appropriate to leave it with other regulatory programs in the Environmental Health Division. As promised when your officers met with Dr. Coye and myself, your views are solicited.

## THE FUTURE OF MOSQUITO RESEARCH IN CALIFORNIA: NEW PERSPECTIVES AND CHALLENGES

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My friend Don McLean presented a presidential address to the Entomological Society of America a few years ago entitled: "--Ou Sont les Neiges d'Antan". I liked the title a lot, for among other reasons because it projected an image of erudition, but also because of the message it carried. The line is from the poet Francoise Villon and is translated "Where are the snows of bygone years?". At this point you may be wondering what last year's snow has to do with the future of mosquito research, but I think there is a connection. The connection is this: Tempted as I might be to go over past accomplishments of the University of California (UC) Mosquito Research Program, and the past role the program has played in mosquito abatement in California, I will not. In the political and economic climate of California in the 1990's, past roles are relatively unimportant, and it is best for all of us to consider where we will be at the end of this century and into the next.

This is going to be a very simple talk, with few messages. I hope that this will enable you to remember easily the few points I intend to make.

### MOSQUITO RESEARCH FACULTY

For the 1987-88 academic year, which was the first full year in which I participated as Director of the Mosquito Research Program, 25 researchers submitted proposals. This year we will probably see proposals from about 16 researchers. For 1987-88, 35 proposals were submitted for funding. By the end of the 1992-93 academic year, five years later, the number had dropped to 30. This year, we expect to see about 25.

In 1987-88 there were 22 UC faculty whom I

considered participants in the Mosquito Research Program. By the end of the 1992-93 academic year, 9 of the 22 had retired, died or left the University. Another three were no longer active in the area of mosquito research. This left only ten of the original group.

Fortunately five new investigators have begun submitting proposals. However, this still leaves us with an overall reduction from 22 to 15. Three of the five new investigators have physiology or biochemistry-related programs. Only one can be considered an ecologist, and he is filling a soft-money position.

My message as it relates to UC faculty participating in the Mosquito Research Program is straight-forward and simple. The future ability of the program to address and solve problems relating to mosquito abatement and protection of people in California from mosquito-borne disease depends on our ability to attract qualified UC scientists to the program. The recent decision of Vice President Farrell to transfer two vacant faculty positions from the UC Berkeley campus to UC Davis and UC Riverside and to specify these positions as dedicated to mosquito research is a very encouraging response by the Vice President to the need for continuing faculty strength in the Program. I hope we can go forward quickly in bringing young, qualified scientists to the University.

### MOSQUITO RESEARCH FUNDING

A parallel problem is related to funds available to fund mosquito research. As I explained in the past, the only increase in "Special Mosquito Research Funds" have come from cost-of-living increases to the

amounts budgeted for salaries. In addition, there have been 5% permanent reductions in programming funding over the past three years.

### **FUTURE RESEARCH DIRECTIONS**

Is there a continuing need for research on mosquitoes in California? My answer to this is emphatically "yes"! The ever diminishing options available to mosquito abatement district managers, the complex web of governmental regulatory agencies involved in decisions affecting mosquito abatement, and the increasingly complex problems in mosquito control technology demand solutions which can be developed only after careful study and experimentation. Here are the areas I see as offering the best returns on investment of funds, people and effort:

#### **Insect Ecology.**

I have listed this area of research first because I think that our most critical problems need ecological approaches for solution. Unfortunately, it may be that it is in this area that we are most lacking in expertise. I hope that our first new faculty hire will be an aquatic ecologist. Specific areas in need of study include: insect mortality factors, wetlands ecology, mosquito life cycles, mosquito sampling, mosquito (population) genetics, mosquito identification and mosquito migration. None of these topics involve high-technology approaches, but we know too little about these subjects as they can be applied to mosquitoes in the context of modern problems.

#### **Insect Biochemistry.**

We have good faculty strength in this area, but the research is expensive, and mosquito-related subjects must compete with many other areas for attention. Subjects for study include: mosquito reproduction, mosquito host attraction, mosquito host repellency, mosquito growth and development, mosquito metabolism and mosquito (biochemical) genetics.

#### **Insect Management.**

The previous two areas involve primarily basic

approaches. There are very good opportunities for applied research as well. Some of these areas are: new classes of insecticides, environmental toxicology, IPM approaches, insecticide resistance management, application technology and non-target protection.

#### **Public Health.**

This is a badly neglected area. Our present information on the real public health importance of mosquito-borne diseases is badly deficient. If you want some reinforcement of this concept, ask Dr. Fulhorst to recount his experiences in trying to track down human disease cases in San Luis Obispo County. We need better surveillance methods, and better surveillance programs. I cannot emphasize this too much. We also need research in: host preference, disease ecology, predictive models, new and emergent diseases, emergency methods and disease assessment technology.

#### **Extension.**

This area may be outside the scope of this presentation, but it is within the scope of my duties, and it is an important area. A weak link in getting research results into the hands of users in a useful and meaningful fashion is our medical entomology extension effort. These are areas which I think need special attention: insecticide susceptibility, insecticide labeling, disease surveillance, mosquito distribution and abundance and dissemination of results of research. I think MosquitoNet has a strong role to play in the extension of research results into the field.

### **SUMMARY**

In closing, I would like to reinforce three points.

- 1) There will be a critical need for research on mosquito abatement and mosquito-borne diseases in the future.
- 2) To do this research we need a dedicated faculty trained in appropriate disciplines.
- 3) We will need adequate levels of funding.



## CHEMICAL MOSQUITO CONTROL AGENTS IN CALIFORNIA: THE PAST, THE PRESENT AND THE FUTURE

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Twenty five years ago I made my first presentation to the CMVCA. At that time I presented my plan for an applied research program that would hopefully provide some new control methods for mosquito abatement agencies (Schaefer 1968). I believe that this program was fairly successful but it is no longer applicable for the future. Numerous changes have taken place over the past quarter century; these have included large changes in the people and philosophies involved in mosquito abatement, changes in the available control agents and the initiation of legal abatement procedures. In order to provide an understanding to many of you who are relatively new to mosquito abatement, I would like to review the past, discuss the current situation, including research in progress, and make some projections about the future of chemical mosquito control agents.

### REVIEW OF THE PAST

Mosquito control in California was initiated, just after the turn of the century, with the use of petroleum hydrocarbon oils for larviciding. Other early practices emphasized the elimination of the places where mosquitoes were found to be breeding - a method which is referred to as source reduction. The most profound change occurred just after World War II when chemical control methods became available that allowed for large area treatments. Insecticides that had been discovered during the war and held as secrets became available in the post-war years. The chlorinated hydrocarbon and many of the organophosphorus compounds were broadly effective against insects and were inexpensive. Surplus World War II aircraft were also available and this allowed a

means of large-scale insecticide application. This led to the concept of mosquito elimination or eradication.

Technical advisors (including one prominent University of California scientist) encouraged California mosquito abatement managers to just go out and control mosquitoes without worry about resistance as "science would be able to provide alternative chemical agents as they became needed". The same advice was provided to the World Health Organization which initiated a massive program to eradicate malaria through the treatment of mosquito resting sites with residual insecticides. Ultimately, it became apparent that mosquitoes had the ability to adapt to repeated insecticide pressure through the selection of resistant strains. Strategies had to be changed from eradication to control.

Many of the early synthetic insecticides were based on technology developed in Germany during World War II and no patents (composition of matter) on these compounds were allowed. Thus, the costs of such materials (e.g., parathion) were reduced. Also, as these compounds generally had broad spectrum activity, their market volume was large and the cost per unit (e.g., per pound) was quite low. Thus, mosquito abatement agencies had broad spectrum, low cost materials available and such insecticides were widely used. During this period, it was easier to spray rather than to attempt to solve the mosquito problem through other means (e.g., source reduction). Often the worst mosquito problems occurred in properties having poor soil types and low productivity; land and water management on such properties were usually minimal. These situations required repetitive spraying and insecticide resistance was the result.

During the past twenty five years, several new and more selective insecticides became available

(e.g., methoprene, diflubenzuron, *Bacillus thuringiensis israelensis* toxin), but their unit costs were higher than that for the organochlorine or organophosphorus compounds that had formerly been used. It became clear that mosquito abatement agencies could not solve all problems with insecticides alone and the difficult process of legal mosquito abatement became necessary in some situations.

### PRESENT SITUATION

Many of the chemical control agents that have been used in the past are no longer available because of insecticide resistance or because the respective owner chose not to go through the expense of meeting EPA costs for renewal of registration. Some available agents do not have any established crop tolerances and cannot be applied legally on, or within drift range of, commercial crops. The costs of development of new chemical agents is steadily increasing and several of the major, past manufacturers of insecticides have exited from the market. Industrial synthesis programs do not have any new modes of action that provide a basis for making new candidate compounds. The net result is that public research laboratories, such as the University of California's Mosquito Research Laboratory, are not receiving any new candidates for testing. If a new, perfectly safe and effective chemical agent, that had only good environmental properties, were to be made available today, a minimum of approximately five years of work would be required to obtain registration. The longer it is until new materials are provided, the longer it will be until future control agents reach the user.

One course of action is to conduct mosquito control without any use of insecticides. Unfortunately, even in the most carefully integrated program, there remains a requirement for the chemical control of mosquito adults when other methods have failed to keep their numbers from reaching levels regarded as being above tolerable because of biting annoyance or because of vector potential. Extensive research on biological control agents of mosquitoes in California, and in all other research programs as well, has not shown any evidence of the possibility of being able to eliminate the need for effective chemical agents. Thus, the need for effective and safe insecticides remains as a required element in mosquito abatement.

### DIRECTIONS FOR FINDING NEW CHEMICAL CONTROL AGENTS

Reexamination of an older approach for finding new insecticides has revealed substantial potential: the evaluation of natural products for insecticidal components. In the early 1800's the observation that mosquito adults died following exposure to flowers of the pyrethrum plant (*Chrysanthemum* spp.) led to the discovery of a natural insecticidal mixture that is still in use in mosquito control today. Plants and other natural products (e.g., mosses, lichens, seaweed, etc.) are known to contain a wide variety of chemical components and they represent an enormous resource for future needs.

The pharmaceutical industry is also pursuing natural products in order to find replacement drugs to combat microorganisms that have become resistant as well as to discover treatments for diseases for which there are no effective remedies (e.g., cancers or AIDS). A complicated molecule called Taxol, isolated from the Pacific yew plant, has recently been approved for use against ovarian cancer and shows some promise against breast cancer. A very large program to develop a synthetic means of producing Taxol is progressing and is essential since the natural supply is limited. The supply of yew is currently being augmented through large-scale plantings. Artemisinin, a new anti-malaria drug, has been isolated from a plant, *Artemisia annua*, in China and is now receiving extensive testing. The Natural Cancer Institute is collecting natural products from all over the world and is testing extracts of several hundred species each week against a series of cancers and against HIV virus.

There have been several past research projects where workers were not successful in finding mosquitocidal components from plant sources; a careful review of these projects reveals poor methodology as the likely explanation.

### CURRENT RESEARCH PROGRAM

During the past two years, our laboratory has extracted and tested a wide variety of natural products. About 125 species have been tested against mosquito larvae and adults. The current procedures used to carry-out this process are outlined in Table 1.

Using these procedures, a new type of larvicide which has a  $LC_{50}$  of less than 0.01 ppm has been found in the leaves of one plant. This dosage is in

Table 1. Procedures utilized by the U.C. Mosquito Control Research Laboratory for testing extracts of natural products for mosquitocidal activity.

Step	Process or Technique
1.	Collect, identify and store samples in a freezer until time is available for extraction.
2.	Homogenize sample in a commercial food processor.
3.	Blend the sample in 95% alcohol, filter-out solids, remove the solvent in a rotating evaporator under reduced pressure and weigh out aliquots of the dried extract.
4.	Conduct bioassays against mosquito larvae and adults to determine the presence or absence of insecticidal components. Tests which show positive results are replicated and the $LC_{50}$ and $LC_{95}$ values are then determined.
5.	For natural products in which positive results are obtained, examine all computerized data bases and conduct a search of the older literature to determine whether or not the biological activity observed was previously reported.
6.	For those extracts which possess novel biological activity, isolate the active ingredient(s) using: <ol style="list-style-type: none"> <li>a. liquid/liquid partitioning</li> <li>b. column chromatography</li> <li>c. preparative gel-permeation chromatography</li> <li>d. centrifugal countercurrent-distribution chromatography</li> <li>e. preparative high-performance column chromatography</li> </ol>
7.	The purity and composition of active components is then determined using: <ol style="list-style-type: none"> <li>a. Fourier-transform infrared spectrometry</li> <li>b. visible and ultraviolet spectrometry</li> <li>c. mass spectrometry (low and high resolution) with EI, CI and FAB detection</li> <li>d. nuclear magnetic resonance (multiple techniques)</li> <li>e. x-ray crystallography</li> </ol>

the range of those of commercial organophosphorus larvicides. The compound has been isolated in pure form and most of the structure has been determined. This structure is complex and synthesis will be required to determine which portions of the molecule are responsible for the biological activity. As the compound represents a new type of chemical structure, it could provide a model for synthesis. Studies on the mode of action and safety will not be initiated until structure activity relationships have been established.

During the past year a large amount of effort was placed on the fractionation of the extracts of two weeds that showed high adulticidal activity against both *Culex quinquefasciatus* Say and *Culex tarsalis* Coquillett. The active ingredient was isolated in pure form and the chemical structure was determined; the same isomer of this compound was present in each of these plants. While the chemical structure itself was not new, its insecticidal activity had not previously been known. The chemistry is very different from any known insecticide and a new model for synthesis has thus become available. As these two plants are closely related, other species in the same taxonomic group will be collected and tested in 1993.

## OUTLOOK

Considerable changes in mosquito abatement operations have taken place in the past 25 years, especially with respect to the types and amounts of insecticides that have been used and the manufacturers that have produced these materials. This pattern will continue. The large production of relatively inexpensive, broad spectrum insecticides of the past will likely not occur again. New types of mosquito larvicides and adulticides will slowly emerge and these will have a much higher unit price than anything that is in current use. Insecticide resistance will not be as significant a problem as it has been in the past because cost will dictate minimal use and thus minimize selection pressure. The marketplace for mosquito larvicides and adulticides will be filled by fewer companies than has been true in the past and these producers will likely specialize in the global public health arena.

It is important now that research be conducted to provide the future insecticide industry with starting places on which synthesis programs can be used. With the high technology of the 1990's, this is possible.

Recently, the University of California has announced a plan for commercialization of research findings. The University is proposing the establishment of a nonprofit corporation (U.C. Technology Development Foundation) to take care of patenting and licensing. One possibility under consideration is the partial financing of this corporation from venture capitalists and stock offerings. This is a radical departure from past policies and represents the magnitude of change that we can expect in the future.

The technology now exists for us to find the chemical mosquito control agents of the future and

the mechanisms for making this technology available to the private sector are presently being put into place. I am optimistic about the future but we have to take the initiative ourselves in order to provide for our future needs.

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## WORKING FOR AMENDMENTS OF FEDERAL LAWS WHICH IMPACT MOSQUITO CONTROL

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There are three federal laws which essentially control the delivery of vector control services. These laws are:

1. The Federal Insecticide, Fungicide and Rodenticide Act, as amended (FIFRA).
2. A part of the Clean Water ACT (CWA) which is the basis for the Army Corps of Engineers' control of the dredging or filling of "wetlands".
3. The Endangered Species Act, as amended (ESA).

There are many other federal laws which may impact vector control agencies, but the three which I have listed are the ones which actively control or prevent our ability to protect the health of our public.

Let me begin by clearly saying that the statutory language, the administrative and judicial interpretations and the enforcement of all laws is political in the sense that these actions provide what the public, or at least some strong or wealthy part of the public, seems to want or need. Lobbying and political action are the driving forces used to achieve the changes which are made or prevented.

The courts are obviously political, because judges are either political appointees or they are elected. Look at the recent Supreme Court Justices' confirmation hearings if you want evidence of political involvement.

Another example is the recent decision of the Supreme Court dealing with "just compensation" for private property rights which are taken for public use. The conflict seems to be over the perceived chilling effect on the ability of Government to impose land use restrictions if the Government is required at the same time to pay for the value of what it "takes".

I submit that these three listed federal laws are being interpreted in ways which are detrimental to "our" (or now "your") ability to efficiently and safely control vector species, and to protect the public from diseases.

For the sake of organization, I want to touch on the problems which each of these federal laws cause, and then to suggest the kinds of amendments which I see as necessary. Remember that regulations are supposed to be changed to be consistent with changes in the statutes.

FIFRA has always been an agricultural law, written and reviewed by the Committees on Agriculture, yet these laws control the regulation and use of all pesticides. Public health uses of pesticides have been held to the same package of risk standards associated with agricultural doses and practices. Public health uses were acknowledged as necessary, while amendment language was considered over the years, beginning in 1971, when the 1972 overhaul of FIFRA was in process. Yet, the Federal Environmental Protection Agency (EPA) has progressively worked to restrict and eliminate uses of all pesticides.

The law requires the standard of "no unreasonable adverse effects on the environment" as the basis for the decisions which EPA is supposed to make about pesticide registration and use. The word "unreasonable" is the key. The "environment" in this standard is to include the human environment, and the definition of this phrase imposes a risk/benefit balancing requirement. However, EPA has imposed its own spin on risk/benefit balancing to emphasize risk assessment and risk reduction, with benefits such as health protection from disease vectors only getting passive, if any, consideration. We could all probably name examples of this kind of disregard for the required standards.

In fairness to EPA, I sense that this problem has

evolved because the Agency has tried to replace human value judgements with an institutional type of computerized system which has no ability to handle subjective issues like health benefits. Risks can be quantified, and safety factors determined, but benefits are too vague for an untrained person to understand and defend. The safe answer to any petition is therefore to say no, because to say yes would allow the correctness of the decision to be tested.

In congressional testimony on the need for amendments to provide for public health pesticides, a committee member asked me if I thought amendments were still necessary, because EPA had said they presently had the authority to register pesticides for public health uses. My reply was that if EPA has the authority, but apparently has not used it, that specific direction to the Agency was appropriate.

Those of you involved in vector control programs know you are in a "world of hurt" when it comes to the loss of effective pesticides. Without some drastic changes, epidemics of vector-borne diseases may have to become acceptable.

Section 404 of the CWA is referred to as the "Wetlands Section", and it is a hot issue for us as well as for people with other interests. The Corps has developed a system to consider and occasionally grant a permit for the dredging or filling of a wetland. Even mowed grass which falls into a wetland is now considered filling, and may require a permit, which shows how far this problem has progressed.

The definition of a "wetland" is still undecided, and the different Multi-Agency Delineation Manuals are still in dispute, because the decision on which one to use lacks administrative consensus. Very recently, the EPA has decided to adopt the older, more realistic 1987 edition of the manual and now, at last, the Corps and EPA at least appear to agree. The Corps was even offering classes to certify wetlands delineation experts, even though the definition of a wetland was still in dispute.

There was a strong effort last year to amend Section 404 of the CWA, but failure to find a solution was apparently permitted as a way to bash President Bush over his "no net loss of wetlands" pledge. I suggest that the term be retained, but defined to mean "no unreasonable loss of wetlands". I also suggest that a wetland could be defined as a place where two or more consecutive broods of mosquitoes can be produced.

The problems of wetland delineation and permits are not going away; they are going to get worse.

The endangered species problem is also rapidly getting out of hand. The basic problems with this law are its lack of flexibility and an unclear species definition. There is no requirement for risk/benefit balancing in the listing process; the only criteria to be considered are scarcity and "good scientific data" to support the finding of scarcity. The listing problem does not come under the National Environmental Policy Act.

The ESA currently offers a haven for abuses because the act can be used to stop changes in land use, under the banner of saving a species or subspecies or even some small segment of a subspecies of plant or animal.

Once a population is listed, all sorts of requirements are imposed on the U.S. Fish and Wildlife Service (FWS), or the National Marine Fisheries Service (NMFS). These duties include designation of critical habitat, recovery plans and a general duty to do whatever is necessary to increase the numbers of organisms so that the listed population can be de-listed. As long as an individual of a listed species is present or suspected in a mosquito breeding source, EPA and the FWS together can stop or limit control.

Listing also imposes a protection duty on all branches of the federal government, as well.

Preserving the habitat of an endangered species is now the standard being proposed. Preserving genetic diversity through habitat preservation is the logic used to justify this action. This is only one step short of advocating the preservation of all individuals of all separate populations of all organisms, which translates to "no change".

A recent Federal Register Notice proposed to list a tidewater fish as endangered. These fish live in brackish water and one suggested cause for rarity of these fish includes mosquitofish predation. If listed, this designation could stop the use of mosquitofish as a biological control agent of mosquitoes in some brackish waters.

Taken together, these three laws can prevent us from eliminating standing water by ditching and draining, from using natural controls because they may be detrimental to endangered animals, and even preventing the use of any remaining registered pesticides if rare animals or plants are present. In the midwest, the FWS has even proposed that mosquitoes and midges are food for baby ducks, and should be preserved.

I hope what I have said so far has motivated you to see the need for action. We need to make a

commitment to get reasonable changes in these federal laws.

I also hope the American Mosquito Control Association (AMCA) can get a sponsor and we can support a bill similar to Congressman Herger's H.R. 5110 of last year, which would create a Public Health Class of pesticide registration. This would encourage industry to provide products for public health use under a risk/benefit balancing scheme that would be separate from the criteria used to register pesticides for crop production. This bill recognized the low doses of pesticides we use, and the special benefit assigned to vector and disease control. It also named the Secretary of Health and Human Services for consultation of health protection needs, the same way the Secretary of Agriculture is consulted on agricultural pesticide needs.

Wetland legislation will be back this year. Congressman Edwards from San Jose had already introduced the Environmental Coalition's Bill. We need to join forces to continue to support a bill such as the one introduced by Congressman Hayes in the last Congress. We need to have a benefit factor built into the decision making equation on wetland modification, and even to get mosquito breeding recognized as a health concern worthy of correction.

The Endangered Species Act has to be changed to allow the needs of human animals to be considered as part of the decision making process on endangered or threatened status. The concept of what is a "species" must also be addressed.

Of the three laws, prospect for changes in the ESA is the best. This law has so many problems associated with it that public opinion is driving hard

for change. There are already three large coalitions made up of people and organizations representing agriculture, industry, housing, water and government. The extent of the changes to benefit public health interests will depend on our commitment and support. In the world of politics, nothing is for nothing.

The environmental activists have their agenda for amendments, as do many other interest groups, but vector control people still seem to lack any strong consensus. At the present time, the AMCA Board requires specific authorization of any statement I might make on wetlands or endangered species, on their behalf, until it is approved by that Board.

An action plan is necessary if the nationwide public health needs are to be adequately addressed. As an organization, and as individuals, we all need to make commitments to winning the battle, including finding ways to finance getting our message out to lawmakers.

Most of you know my hope for a strong AMCA program, working for legislative changes in Washington. Every district should be an active part of this effort.

The AMCA Board must agree to delegate authority to whoever it chooses to represent it, the same way a District Manager is responsible to a Board of Trustees, without having to clear every action or statement beforehand with the Board.

Our collective experience on political issues shows clearly that we either commit our resources to winning, or we will collectively wither and die.

With that observation, I leave you to consider the fate of your district or business, and what sort of commitment you will keep.

## ETHICS VERSUS ENVIRONMENTALISM IN VECTOR CONTROL

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When I was asked to discuss "ethics" in vector control I was sent a suggested title with the knowledge that I was free to change it to suit my needs. However after seeing the one listed in your program, I felt it suited my needs to leave it as written. For me, the thinking expressed in the title exemplifies the greatest obstacle to resolving the conflicts that exist between public health and resource professionals in the areas of wetlands. We seem to feel that as advocates for public health we wear the white hats and that environmentalists, through their efforts in behalf of wetlands establishment/protection, are a threat to our standard of living and are thus "unethical".

This kind of thinking on our part is arrogant just as it is when our colleagues in the resource agencies state the case for wetlands/wildlife concerns as the nations number one priority. It is high time that we, both disciplines, understand and accept the importance and relevance of each other's field. One is as important as the other and any joint planning efforts should set high goals for wildlife as well as public health and safety. We should go to these

cooperative planning sessions having considered ways to help the others involved achieve their stated goals and objectives.

Hundreds of years ago when health professionals tried to tell people that their illnesses were caused by "invisible" organisms, they were viewed as extremists. Only when their efforts at establishing better sanitation led to minimizing illness did they become trusted members of the community. We must look to the accomplishments of our colleagues in the resource agencies and understand that their goals of enhancing or establishing "viable" wetlands is in the best interest of humans as well as wildlife. They too must accept that our goal of minimizing mosquitoes and other vectors in their projects will only serve to make the finished project better for wildlife as well as humans.

A stated position of mutual respect and cooperation must be the new biological ethos that drives the future efforts of biologists regardless of which agency they represent. Ultimately we all answer to the present and future inhabitants and for them we must do our best.



## FORMATION AND FUNDING OF A SPECIAL DISTRICT IN THE POST-PROPOSITION 13 ERA

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Government provides a number of services such as health, education and safety in return for which the individual gives up some freedom in the process of being governed. Throughout our history, governments have served three major purposes: maintaining order, providing public goods and promoting equality. Established governments then use their power to tax citizens in raising revenue to spend on public goods such as police, fire, health and recreation.

Government is broken down and localized thereby allowing people at the local level to participate in the political process. The impact of their policy allows the community the protection and services that are important to them. At the local level, differences exist with respect to cities and counties. Counties serve as the administrative arm of the State which imposes mandates on them to provide social services to the needy. In contrast, cities have the right to enact and enforce legislation, giving them greater freedom of action to operate in providing services. Diverse elements that exist within a community may not be met by a city or county due to population size, economic resources or other characteristics. For this matter, smaller public units are formed to respond and serve the local condition. This unit of government is known as a special district.

A special district as defined by the Controller of the State of California is a "legally constituted government entity which is neither a city nor county, established for the purpose of carrying on specific activities within ... defined boundaries".

Special districts are generally established by residents of an area to provide a public service not provided by city or county government and are classified according to the function they perform. These functions can be as diverse as providing water, waste disposal, libraries or mosquito abatement.

The first California special district was the San Joaquin Valley Irrigation District, formed in 1887 to provide irrigation water for those within a defined area. Currently, within California there are 58 counties, 434 cities and over 5,000 special districts (exclusive of school districts). Although some special districts are located within or include cities, most are usually located in unincorporated areas. Often, suburban residents choose to form a special district to obtain municipal type services rather than annex into existing cities or incorporate a new city because they either think city government will bring higher taxes or they are concerned with the nature of controls such as zoning or loss of community identity.

Some special districts are authorized by law to perform a single function (e.g., providing water, street lighting or abating mosquitoes), while other districts provide multipurpose activities. The majority of special districts in California are single purpose districts with only 10% (about 500) being multipurpose districts. Multipurpose districts (e.g., community or county service areas) often offer a range of services from sewage and water to safety and recreation.

Special districts are further classified as either enterprise or non-enterprise, depending on their revenue source. Enterprise special districts (e.g., water and waste disposal districts) are financed through user fees which cover the cost of services provided. Non-enterprise special districts (e.g., mosquito abatement and fire protection service districts) are supported primarily through property taxes. Approximately 46% of California special districts serve in a non-enterprise capacity. With the passage of Proposition 13 in 1978, the number of non-enterprise special districts has decreased while the number of enterprise special districts continues to expand at the same growth rate as prior to 1978.

Special districts are additionally classified as either independent or dependent with regard to the type of governing body under which they operate. A dependent district functions under a city council or a county board of supervisors, while an independent special district has an independent board of directors appointed by the county board of supervisors or incorporated cities within the boundaries of the district. Fifty-eight percent of California's special districts are self-governed, independent districts.

It takes authorization by the State of California to form a special district. The California state legislature has authorized the formation and operation of 55 general types of special districts. The funding, taxing, type of activities and governing body is detailed for each type of district. For example, by 1967 there were 190 acts for as many different kinds of districts and these did not even include the school districts.

In 1963 Local Agency Formation Commissions (LAFCO's) were established in each county by state statutes. These commissions review the merits of proposals for new districts including incorporation and annexation. Several years later, additional authority was given to these commissions to encourage reorganization and dissolution of existing districts.

Special districts rely mainly on three local revenue sources: property taxes, special assessments and/or user/service fees. The degree of dependence on one or several funding source(s) will vary according to the kind of district and the type of public service provided. Enterprise special districts such as water and sewage disposal districts are supported almost exclusively by user or service fees, while non-enterprise special districts such as mosquito abatement and fire protection districts rely heavily on property taxes to fund the service(s) they provide.

The Butte County Mosquito Abatement District (BCMAD), a non-enterprise independent special district, provides the single function of the suppression of nuisance and vector mosquitoes for the residents of Butte County. The District's mosquito control program is based upon the concepts of Integrated Pest Management (IPM). In IPM, naturally occurring or introduced predators assist in reducing immature mosquito populations. Chemicals, such as oils, are applied to the water surface to control larval mosquitoes and botanical-based chemicals are used to control adult mosquitoes if mosquito populations are not maintained below certain tolerance threshold

levels by these predators. In addition, source reduction is utilized to assist and encourage property owners to adapt alternatives such as physical management improvements with regard to farming practices. At times, legal abatement procedures are initiated to compel property owners to comply to the District's recommendations to eliminate or alleviate mosquito problems caused by improper irrigation methods.

In 1948 a group of citizens petitioned the Butte County Board of Supervisors to form a special district that could provide mosquito control throughout all of Butte County. Although the Oroville Mosquito Abatement District was formed in 1915 and the Durham Mosquito District in 1917, these two districts did not meet the demands needed for mosquito control for the remaining communities and rural areas of Butte County. Since Butte County government did not have the expertise in the area of functions, finances and administration for mosquito control, the Butte County Mosquito Abatement District was formed in 1948 to meet the demand of providing this public service to the citizens of Butte County. Presently the BCMAD includes all of Butte County except the areas of Oroville and Durham which remain separate and served by their own districts.

Butte County Mosquito Abatement District, as an independent district, is governed by an appointed Board of Trustees which has the ability to determine the price of services they provide and tax the residents accordingly. The ten-member Board of Trustees has five members appointed, one each, by the governing bodies of the cities of Chico, Oroville, Paradise, Gridley and Biggs. Five additional members are from the county at large and are appointed by the Butte County Board of Supervisors. Recently an eleventh member was appointed to the Board with the annexation of the community of Hamilton City into the District. Trustee members are appointed for a two year term. After this two year term, they can serve, if reappointed, a four year term. The eligibility requirement for the governing body member is residence within the territory of the district and having a similar political philosophy as the appointing authority.

The appointed Board of Trustees conduct their fiscal and policy activities without substantial review by local government. Within the parameters of the state legislature, which places limits on their finances, and supervision by the State Department of Health, the Board of Trustees can determine their budget and financing objectives so that all policy decisions will

be carried out. The board is, therefore, autonomous when it comes to establishing District policy and budget matters.

Trustees are selected for the two year term and cannot be removed by the appointing authority unless upon substantiation of serious charges. Since the formation of the District in 1948, however, no trustee has been dismissed due to improper conduct. In general, this tenure can only be terminated by resignation, poor health, death or change in the local political power. Often, the president of the board is elevated to presidency by vote of the board membership. Usually the presiding term is one year or until the board composition changes through subsequent appointments. The continuance of the same person as president of the board over an extended period is not unusual. The District Board convenes on the second Wednesday of each month with a monthly stipend of thirty-five dollars paid to each trustee for regular meeting attendance. The meeting of the District Board is usually attended by the District Manager and Assistant Manager. Although these meetings are open to the public, they are not often attended by either media or citizens, except when controversial issues occur.

One important feature of the BCMAD common to many other special districts is area flexibility. The district includes both rural and urban territories of Butte County without regard to the boundaries of other agencies that cover all or part of the same land, with the exception of the areas that are included in the Oroville and Durham Mosquito Abatement Districts. The District has additional flexibility through legal authorization to annex territory not originally part of the district, as occurred in 1987 when the community of Hamilton City was annexed into the district, making it a multi-county district.

By and large, the District serves in a non-enterprise capacity where the services are financed through general property taxes to guarantee that all who benefit also pay. It is a service-oriented agency rather than regulatory, providing public service to individual property owners. The District can legally issue bonds or implement service charges to finance district activities. The issuance of bonds is not a major method of district financing since little, if any, district revenue is acquired through borrowing (indebtedness). Secondly, a bond proposal must have a two-thirds vote of approval from District residents which is difficult to obtain in the post Proposition 13 era.

Alternately, the BCMAD, through the California

Health and Safety Code, can obtain revenue through charges for services rendered through the establishment of service areas and/or benefit assessment fees. A service area is a defined area established by the district through resolution to provide additional mosquito control. Upon approval by the District Board, a service charge can be instituted and a fee levied against any or all parcels of land within the district or specific service areas to pay for the cost of vector surveillance and control in that area.

In 1989 BCMAD established a service area consisting of all land inside the outer boundaries of County Service Area 114, including the city of Chico. The basis for the service area was to provide source maintenance for the prevention of mosquito breeding. Agencies previously responsible for maintenance of the flood control system in these areas have not been able to provide channel maintenance to prevent mosquito breeding in the bodies of water within this system.

A benefit assessment fee can be imposed by the District to carry out vector surveillance and control projects for any part of the district. This can benefit the entire district or it can benefit a single zone. If the projects proposed by the District Board is for one or more zones, financing is obtained on a per household or per parcel charge within the benefit assessment area. The District established such a benefit assessment fee within all the land in the Hamilton City Service District boundary known as the community of Hamilton City.

In 1987, the District initiated zones of benefit to finance continued vector surveillance and control methods by the purchase of capital equipment. Zone 1 encompassed the entire area already within the district except for an area the boundaries of which are the same as those of the Central Chico Redevelopment Project Area. Zone 2 included the boundaries as those of the Central Chico Redevelopment Project Area. An assessment of one dollar (\$1.00) per parcel was levied for all the parcels in Zones 1 and 2 for fiscal year 1987-88 for the purpose of augmenting programs operating under the same objectives as the present vector surveillance and control programs. This assessment sunsetted in 1988 since the revenue collected provided the necessary funds for the capital purchases of these items for the continuance of the District's mosquito control program. The benefit assessment provided critical funding for the District which lost revenue when the City of Chico returned only seventy percent of the

tax increment to the District while placing the remaining thirty percent in the city coffers.

Under the Jarvis-Gann tax-limiting initiative known as Proposition 13, property assessment values were fixed at 1% of the market value at 1975 levels and could increase by no more than 2% per year until a property is sold - at which time the property assessment would be increased to reflect the market value of the property. Changes to the maximum allowed increase in assessed value of 2% per year are only allowed for new construction, additions to existing property or change of ownership. The maximum allowed 2% growth rate is below Butte County's historical growth experienced in property values, restricting the growth in the District's tax base below that needed to keep pace with moderate increases in expenditures due to inflation.

The substantial reduction in local property taxes hurt not only local government, but special districts such as mosquito districts throughout all of California. An additional problem with Proposition 13 dealt with the fact that counties, cities, school districts and other special districts had their own tax rate.

Prior to Proposition 13, BCMAD was able to levy up to fifteen cents (\$0.15) on each one hundred dollars of taxable secured and unsecured property in Butte County. If the rate produced exceeded \$0.15 but was less than \$0.40, the Butte County Board of Supervisors were required to approve the rate to determine the necessity of their expenditures and any amount over \$0.40 required voter approval.

At the passage of Proposition 13 no existing law specified how the property tax revenues under the one per cent cap would be apportioned among the governmental jurisdictions. The legislative interpretation of Proposition 13, Assembly Bill 8 (AB8), established the division of tax revenues among the various governmental agencies based upon their percentage of the tax rate over the prior three fiscal years starting from 1977. This established the District's present apportionment rate factor of \$0.01445. This is in contrast to the rate of \$0.11 set by the District Trustees and/or Board of Supervisors that BCMAD used prior to Proposition 13. This change in tax rates on revenue collected by the District has had far reaching impacts.

Statewide, the revenue special districts received from property taxes, declined from \$954 million in 1977-78 to \$532 million in 1978-79. The passage of Assembly Bill 8 (AB8) was only a short term solution to local finance problem and aimed at

increasing the property tax base for special districts and local government.

To distribute this shifted money to special districts, Assembly Bill 8 (AB8) established the Special District Augmentation Fund (SDAF) whereby instead of the revenue going directly to each special district it went to the Board of Supervisors of each county who had complete discretion in the allocation of these funds. Multi-county districts such as Sutter-Yuba MAD or Sacramento-Yolo MAD received their money directly since their jurisdictional boundaries include all or part of an adjacent county and they were in place prior to the passage of Proposition 13.

Yearly, the Butte County Auditor must compute the size of the augmentation fund so that the Butte County Board of Supervisors can distribute these monies among the various governmental agencies operating within the county. Using a ratio based on the Districts bail-out in 1977-78 and the growth in its assessed value each year, the Butte County Auditor reduces the property tax allocation to the BCMAD and places this amount in the SDAF. The amount placed in this fund is the District's contribution to the total amount. By September 30th of each year, the Butte County Auditor notifies the Butte County Board of Supervisors of the monies in the fund and within thirty days the Board of Supervisors allocates the augmentation monies after holding a public hearing. The Butte County Board of Supervisors base their allocation on the priority of service, need and/or the District's contribution to the augmentation fund. The basis of this allocation changed as Butte County's general revenue funds were being utilized for state-mandated programs. Over the intervening years, the Butte County Board of Supervisors' attention to program discretion has been directed to funding fire protection and library districts.

To illustrate this, let's review the situation of the Butte County Mosquito Abatement District (Table 1). In 1978-79 the Butte County Board of Supervisors allocated \$20,084.54 to the District from the Senate Bill 154 (SB154) bailout of special districts; the District's contribution to the SDAF was over \$50,000 (a loss of \$30,000). In 1979-80 the Butte County Auditor computed BCMAD's contribution to the SDAF as \$50,602.48. The Butte County Board of Supervisors responded by allocating \$64,246.25 from the SDAF to the BCMAD (a gain of \$9,000).

Butte County Mosquito Abatement District's contributions to the SDAF grew from 1979-80 until it stabilized at \$76,120.70 in 1983-84 and remained the same until 1986-87. The District's allocations

from the fund were not steady however, as the Butte County Board of Supervisors shifted various amounts each year in response to its changing budget needs. BCMAD participated in the SDAF during a seven year period and in five of those seven years the District was allocated less than what they actually contributed into the fund.

Table 1. BCMAD's contributions to and allocations from the SDAF over the seven year period, 1979-87.

Fiscal Year	BCMAD Contributions (\$)	Butte County Allocations (\$)	Gain (Loss) (\$)
1979-80	50,602.48	20,084.54	(30,000)
1980-81	55,771.43	64,246.25	9,000
1982-83	63,330.88	57,090.00	(6,000)
1983-84	76,120.70	60,386.72	(16,000)
1984-85	76,120.70	85,116.17	9,000
1985-86	76,120.70	71,201.00	(5,000)
1986-87	76,120.70	65,939.48	(11,000)

For this reason the District opted not to continue their participation in the augmentation fund. Instead they became a multi-county district in 1987 with the annexation of the Glenn County community of Hamilton City into their territory. By doing this, the District did not have to participate in the SDAF. Mosquito abatement districts listed as bi-county in the state tax code prior to 1978 did not have to participate in the SDAF, instead all revenues go directly to them rather than through their respective County Boards of Supervisors.

The post-Proposition 13 era for the BCMAD has seen a reduction in personnel and resulting reduction of public service. Providing service for mosquito control has been oriented to urban areas leaving some of the rural areas with less service. Since the passage of Proposition 13 in 1978, the District has lost an estimated 50% of its revenue base, including inflationary increase. No additional personnel have been hired leaving the District with fifteen full-time staff.

Butte County, on the other hand, has experienced growth with the construction of residential and commercial areas. For example the city of Chico has had a growth rate of 35% within the last decade. This has amounted to a rate of growth of four to six percent whereas the county of Butte has experienced between a two to three percent growth rate within this same period. The District has

attempted to serve this new growth with no additional personnel with the exception of hiring seasonal personnel for a five to six month period each year.

The fiscally conservative District Board of Trustees has also been reluctant to authorize a tax rate increase. Several reasons can be concluded for adopting this position. If the District Board wished to authorize a special tax to enhance mosquito control programs, a two-thirds majority of voters would be required to approve the tax. The opinion of the Board was that the majority of constituents would not support this special tax. Secondly, the philosophy of the Board of Trustees was that the voters in 1978 made an overwhelming declaration that every Californian has the basic right not to be taxed out of their home. Since their constituents voted for Proposition 13, they felt compelled to live within its spirit.

The revenue sources available to BCMAD are generally quite narrow. The District derives 84-95% of its revenue from property taxes; the remainder is obtained from benefit assessment fees and other miscellaneous nontax sources. In 1988 approximately 16% of the District's revenue was from interest and benefit assessment service charges. The extremely heavy reliance of the BCMAD on this highly restricted revenue base contrasts strongly when compared to the diversification permitted by city and county government.

The District has enjoyed considerable fiscal and administrative autonomy from local government. However, Proposition 13 and now Community Redevelopment law has reduced these characteristics. Redevelopment provides a mechanism for the implementing public agency to eliminate slums and blight by encouraging infrastructure improvements (e.g., curbs, sidewalks, etc.), stabilizing and increasing the tax base, improving housing and increasing employment opportunities. Redevelopment provides the tool that creates a localized source of revenue for these projects within a defined area. When an agency forms a redevelopment area, the assessed value of the designated area is determined. This is known as the base value or base tax. The difference between the new tax and the base tax is the tax increment. Under the redevelopment process the redevelopment agency can negotiate a portion of the tax increment thereby creating this localized tax revenue or passing through 100% of this tax increment to the affected taxing agency (i.e., special district).

The City of Chico created three redevelopment

project areas beginning in 1980 with the Southeast Chico project area containing 1,850 acres, the Chico Municipal Airport Project Area in 1983 with 1,160 acres, and the Central Chico Project Area in 1985 with 385 acres. Tax increment pass-through agreements for each of Chico's redevelopment project areas was negotiated with BCMAD. The District will receive 70% of the tax increment in each of the three defined redevelopment areas with 30% going to the City of Chico's redevelopment agency. The 30% loss of the District's property tax revenue limits its ability to provide service to these areas equal to what is received by the public throughout District.

Since the passage of Proposition 13, local governments have been forced to find alternative ways of competing for available revenues with escalating costs of services. Redevelopment is an alternative localized revenue source that has become popular to municipalities to stimulate the tax base, create jobs and to make the necessary public improvements. Conversely, the eroding away of property tax revenue by redevelopment has hampered the District's function of providing mosquito control for the residents in Butte County.

## HOW CAN SPECIAL DISTRICTS SURVIVE REGIONAL GOVERNMENT?

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Regionalization, in simple terms, may be described as seeking to establish a balance between the activities of man and the environment in a prescribed bioregion through holistic planning. Problems such as air or water pollution, disposal of solid waste, or depletion of natural resources would be remediated within the region without damage to the environment. A tall order!

To answer the question posed by the title, I might be somewhat introspective to start. In our present socio-political climate, there are two compelling forces in my work life: The need for our mosquito control efforts to be effective in spite of growing concern about the environment and the need to accomplish the job in spite of an enormous fiscal crisis.

At the Conference on Economics in December, I heard a number of speakers present information to then President-Elect Clinton and Vice President-Elect Gore that indicated a growing vision that it is essential to strike a balance between man and the environment. Lilia Clement, one of the speakers, captured the vision well when she implored:

"The future of America is people, machines and the environment working together without doing each other harm."

The second force that is acting on me is the state budget crisis. This is a particularly difficult problem since the state has taken the approach that they will simply shift the financial burden to local government rather than make structural or systemic changes to fix the problem. It leads me to three questions:

1. Why are they not fixing the budget problems?

2. Why are they diverting our attention to regionalization?
3. Are the two issues connected in some deep way?

### EXPLORING OUR CRISES BY WAY OF QUESTIONS

In the form of questions, I would like to take a fast journey through our world of non-regionalized urban sprawl. The questions are not to be answered here but are to be seen as a way to explore the problem.

Why in our high-technology world do we have to work harder and harder, usually both family members, to just maintain our standard of living?

Why do our children have to work even harder than we to educate themselves; and, yet, they will probably not reach our standard of living?

Why does greater and greater effort against illegal drugs seem to have no effect?

Why, in spite of decades of social aid, has poverty persisted and grown in our nation?

Why, in a society with economic, educational and individual freedoms, do we fill the prisons to overflowing with our citizens?

How is it that the great freeways in Los Angeles create traffic flows averaging 35 miles per hour.

Why is greater and greater effort in schools producing less competent employees?

Why do so many workers hate their job?

Why do so many students hate school?

Why is the worst day fishing better than the best day working?

Why do most of the workers in our society feel under-employed?

Why do we have to watch 5½ hours of TV a day?

Do we really need mint flavored dental floss?

Do we really need virtual reality skiing?

Why doesn't your doctor listen to you?

Why doesn't your check-out clerk at the supermarket listen to you?

Why doesn't your son, daughter, wife or husband listen to you?

Why don't your employees or students listen to you?

Have you listened to your own internal dialogue lately?

Do those questions tend to make you think something might be wrong? The first few questions pointed out problems that will not go away; anomalies of our system. The other questions explore a kind of fragmentation and sense of meaninglessness in our lives.

Take for instance the problem of poverty. We might listen to an economist and he might say, "Stoke the economic fires. Create demand. Jobs will follow." And what if they don't? We might listen to a sociologist and he might say, "Help the unemployed and poor". But perhaps the approach would build in disincentives to work and thereby create helplessness and paralysis. We seem to attack the problems with a limited view.

I catch myself trying to be blind to poverty on the streets. Lawrence Kasdan in the movie "Grand Canyon" has Actress Mary McDonnell speak about

this issue in her role as a housewife in Los Angeles:

"The world doesn't make any sense to me any more. There are babies lying around in the streets. There are people living in boxes. There are people ready to shoot you if you look at them. And we are getting use to it. The world is nuts. It makes me wonder about all the choices we make."

**A question from the audience: How did we get in this mess?**

I believe Fritjof Capra, author of the "Turning Point" which became the basis for the movie "Mind Walk", can help us answer that. He says we are in a crisis of perception. The unique perceptions of a community of humans are acquired in parallel with language. Jared Diamond found an aboriginal tribe in New Guinea who were able to identify a phenomenal number of birds in their environment. You can bet it was linked with their survival. Our cognitive processes have been fashioned over the hundreds of thousands of years as hunter-gathers to acquire skilled perceptions for survival in a particular environment. The perceptions of western man, according to Capra, have been fashioned by the Cartesian/Newtonian view. These powerful thinkers of the 17th Century created a lens through which the western world still views their world. It creates the metaphor of the machine to represent nature and the universe. It is a reductionistic, analytic method that seeks to isolate to understand. It has been extremely powerful; resulting in industrialization and tremendous scientific and technical achievements. But, it also creates fragmented thought and fragmented actions, both in things and in our relationships.

## PARADIGM WARS

Let me give you an example of the fragmentation that the Cartesian view creates. Figure 1 shows how the disciplines of medical entomology and wildlife biology have separated under the influence of our Cartesian view. The result is that we are over-specialized. We break up a holistic system (a wetland) and divide the responsibility of mosquito control and wildlife management to scientific communities with narrow perspectives. The result is "paradigm wars" that reduce the likelihood of creative, holistic solutions to wetland problems.



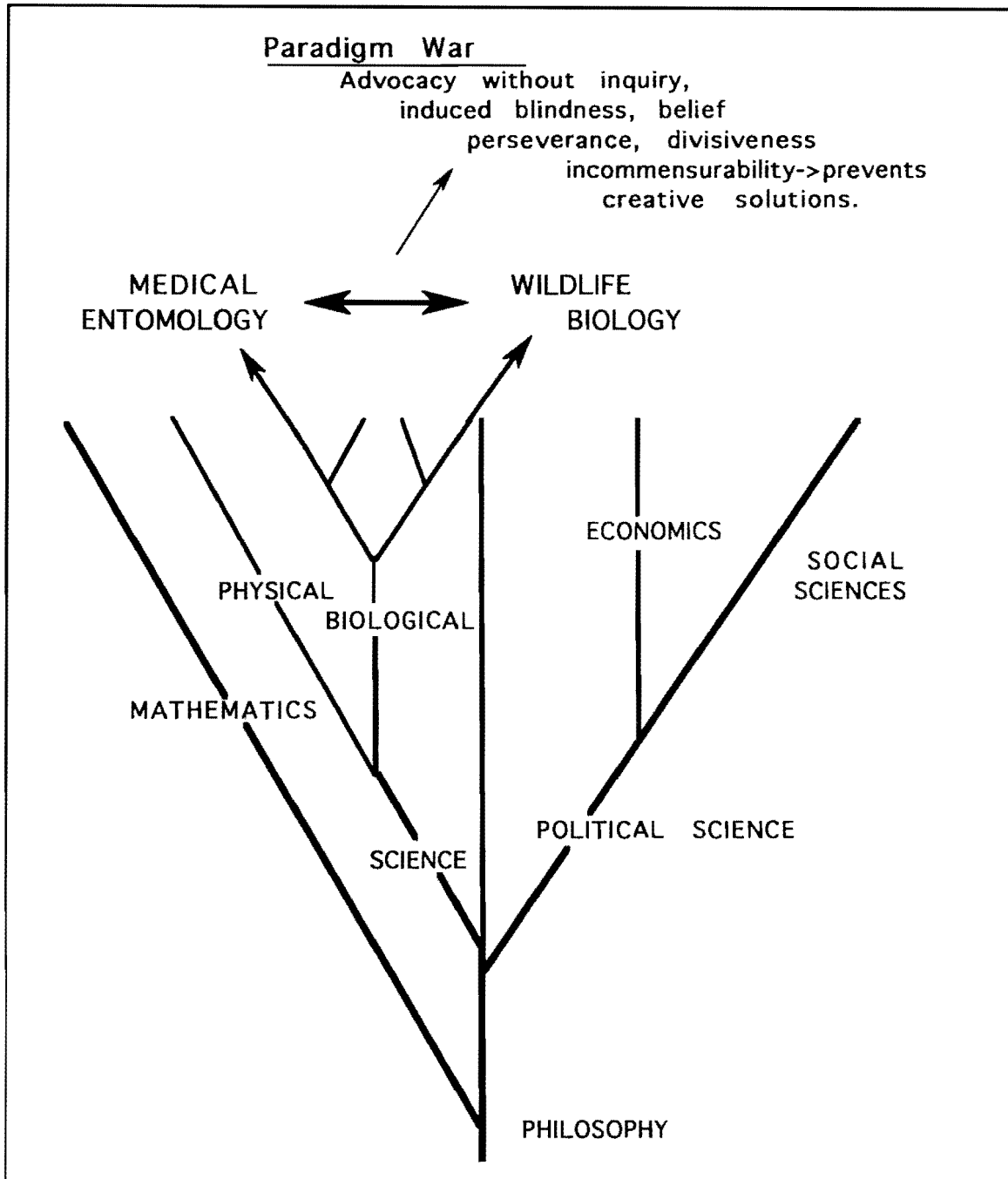


Figure 1. The branching of disciplines illustrating the roots of Paradigm Wars (branching is illustrative only).

These kind of inefficiencies are played out in our socio-economic system a thousand times and create a tremendous burden of cost to the citizens.

**Another question from the audience: How do we behave in this fragmented system that reinforces the problem?**

There are probably many ways. I can only take a narrow slice, but one serious problem is the way we foster fragmentation through our interpersonal communications. I want to show you a depiction of how we might have acted prior to the time of Descartes and Newton. This is a video of the movie "Dances With Wolves". This scene is where

members of the Indian tribe are in dialogue. Note that all of the males in the tribe were able to surface their ideas to be heard by all before a serious decision was made. They were strategizing and learning together through dialogue. The individuals were connected.

Today, in our fragmented world, it is much different. This scene is from a Lawrence Kasdan's movie called "Grand Canyon". It depicts a person who takes unilateral control of a situation and maintains control through his behavior strategies. This behavior is described by Chris Argyris (1985) as Model I behavior. It is operated in the service of gaining control. In our hierarchical society it is rampant. The action strategies are to seek to be in unilateral control; win, don't lose; and avoid creating negative feelings.

This kind of behavior limits individual and organizational learning as well as creating an array of individual and organizational defenses that are designed to combat it. As a result, the feelings of individuals being disconnected are reinforced in our organizations and society.

**Another question from the audience: How can we change it?**

Behavioral change that is common in a socio-economic system is difficult to change. It may require a two-pronged attack. Kasdan helps us understand change. His movies "The Big Chill" and "Grand Canyon" both provide wonderful insights into change. The "Big Chill" is a reunion of college friends who find they have mostly bought-in to the system. A sell-out they would not have believed of themselves when they were in college. In "Grand Canyon" a producer of violent movies is shot in the leg; transforms in the morning dawn into a new person to make responsible films, returns to the system and a few weeks later he is making violent films again. In real life we have Jerry Rubin, free speech radical, who upon entering our socio-economic system becomes the consummate "yuppie" and a new, soft Mike Ditka after his heart attack, who within months back in the NFL system reverts to the garrulous Mike Ditka we all love to hate.

A system structure reinforces some behaviors and punishes others. It pulls you toward a way of thinking and acting. The first principle of individual change may be:

**YOU CANNOT BE A SNOWFLAKE IN  
A BOWL OF RICE KRISPIES**

Therefore, to create change, Kasdan may be saying we need to change the structure of the system. We can call this top-down change or outside-in change.

We also may need to take individual action to change the way we think. Everything we see around us in this room, except our flesh and blood, is the product of thinking; and, of course, it feeds back on us to reinforce our thinking. The way we think really makes a difference. If we are capable of another way of learning and thinking, we could take a more holistic view. We need to learn across the disciplines (Fig. 2). This is called integrative or transparadigm learning. I know of at least one of us, Chuck Taylor at UCLA, who is a transparadigm learner. This kind of approach could be called bottom-up or inside-out change because it starts with the individual rather than the system. As we learn across the disciplines, I believe we will not only be reconnecting our world, we will be reconnecting ourselves to the world and to each other.

**A somewhat frustrated question from the audience: What does this have to do with regionalization?**

The movement of the state legislature toward regionalization gives us both an opportunity to make a change in the structure of government, a system structure, and a very rare opportunity to reinforce our thinking in a holistic way. This is both top-down and bottom-up change. I see regionalization as a very rare opportunity for real change that could move us toward the vision expressed by Lilia Clemente. It is also, at least as it was proposed last year, aimed directly at a good number of the anomalies of our Cartesian view. In regionalization, we have a chance to make a real difference in the world.

**Another question from the audience: Tell me what is meant by regionalization today?**

A few years ago the Inner Agency Natural Areas Coordinating Committee divided the state into nine bioregions. The committee found that the main acceptable criteria to draw the regional boundaries were watershed and commute distance.

The regions, according to the staff of the commission, are not set in stone. This work may

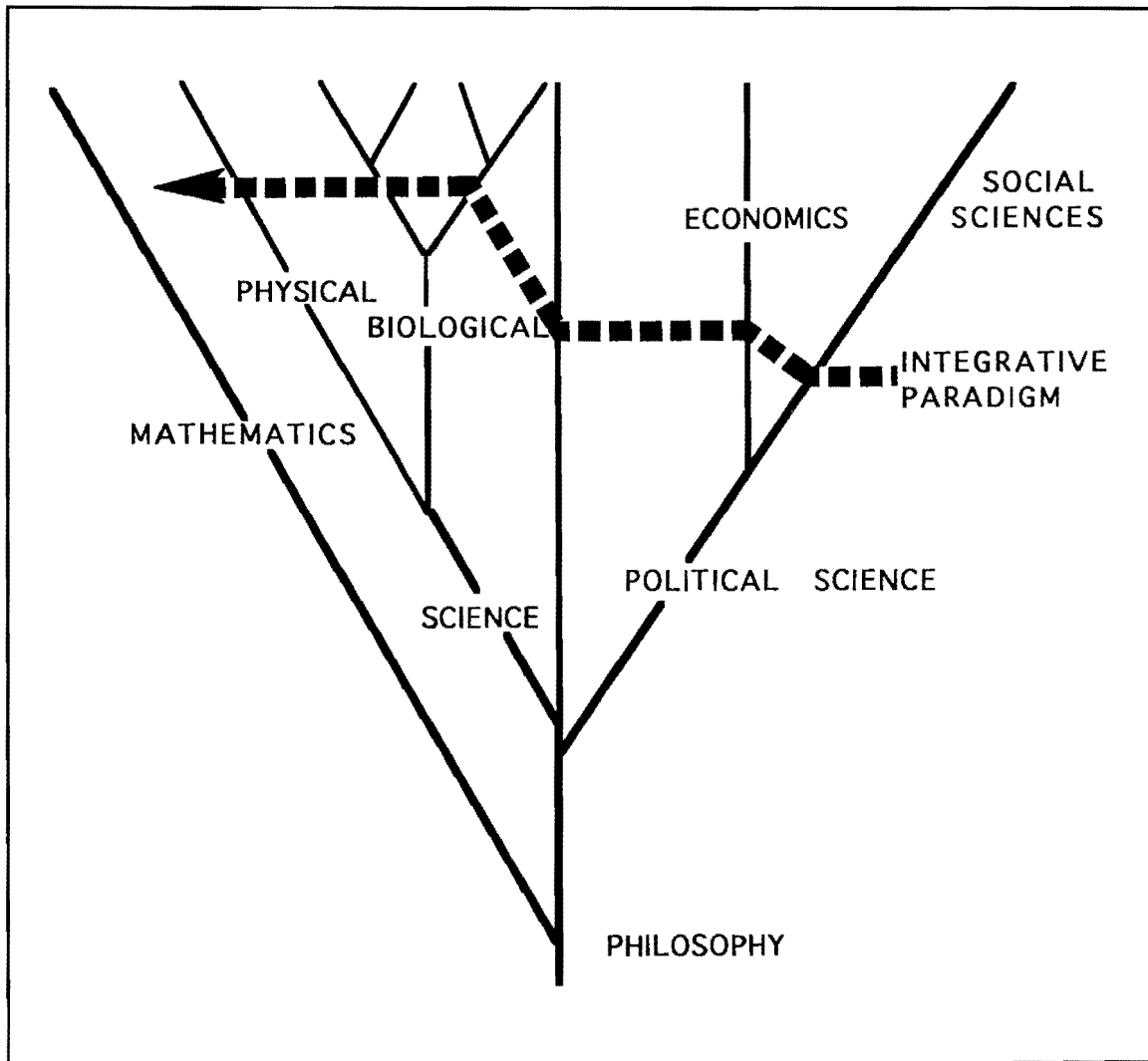


Figure 2. Learning path of integrative paradigm emphasizing connections and interrelationships. Learning path of traditional reductionistic paradigm emphasizes isolation of phenomena.

provide the starting point for much of the thinking of legislators that wish to provide legal mechanisms for regionalization.

Becky Morgan, State Senator in San Mateo, was to introduce another bill this year to regionalize the Bay Area. It is not directed at wholesale consolidations of special districts as was the regionalization movement of the 1970's reported by Marv Kramer (1971, 1973). It would consolidate air quality, transportation, and housing. Housing Association of Bay Area Governments, South Bay Air Quality Management District, and the Metropolitan Transportation Commission would be the only forced consolidations. The state would simply require environmental standards and leave it up to local

government to find a way. It should streamline legislation to allow local agencies to consolidate if it is appropriate.

Ms. Morgan also indicated that legislators Presley, Brown, and Farr are also expected to introduce another bill this year that would include the Los Angeles Basin. She feels their approach would not be much different.

Last year's report of the Bay Vision 2020 Commission can help us understand why regionalization is currently being considered in the San Francisco Bay Area. It specifies the following as some of their objectives for regionalization:

1. To preserve the special qualities of the

Bay Area that are being lost to unmanaged growth.

2. To better coordinate government at a regional level.
3. To recognize that the Bay Area is a region with respect to the environment, the economy and government.
4. To minimize the impact of increased population growth.
5. To encourage high-density housing and more open space.
6. To manage housing to reduce commute travel.

The likelihood that these bills will pass is high. The Governor, according to Senator Morgan's staff, is only lukewarm because of the fiscal crisis.

### CONCLUSIONS

I believe Mosquito control agencies should seriously look at consolidation with other mosquito or vector control agencies if it will provide enough of the following benefits:

1. Economies of scale.
2. Financial stability associated with being "regional".
3. More effective voice in the planning and regulatory arena.
4. Control over cross-border mosquito problems.

I do not believe merging with larger multi-purpose government will work. It can destroy the flexibility and rapid response that is so necessary in

vector control. We have data that abounds to prove this if anyone is skeptical.

Beyond just our agencies, however, I support regionalization vigorously. I believe it is a step in the direction of holistic thinking that can help us resolve the many problems that simply won't go away: the anomalies of the Cartesian view. It can be a force to begin to re-connect the fragments in our world. The forces of regionalization give us an opportunity to make deep changes. Changes in both the way we think and in the system that reinforces our thinking.

I also tend to believe that there is a connection between regionalization and the budget crisis. I believe that non-coordinated regional action has depleted fisheries, depleted timber, degraded the natural environment, created urban sprawl, and condemned people to long, costly commutes. In our business of mosquito control, it contributes to inefficiencies by fragmenting government in artificial ways and fostering "paradigm wars". This and other costs not listed has surely been a significant drag on the economy of the state, contributing to the fiscal crisis. Regionalization and the state budget crisis may well be connected in a deep way. We may not be able to escape the fiscal crisis unless we correct the problems of fragmented government.

President Clement asked me to discuss the question: Can we survive Regionalization? My answer is in the form of another question: Can we survive without it?

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## MOSQUITO CONTROL IN THE PEOPLE'S REPUBLIC OF CHINA<sup>1</sup>

William K. Reisen<sup>2</sup>

Department of Biomedical and Environmental Health Sciences  
School of Public Health  
University of California  
Berkeley, California 94720

The film described the People's Republic of China program to eliminate mosquitoes, one of the four pests targeted for eradication by the Citizen's Committee of the Patriotic Health Campaign. Towns that markedly reduced mosquito problems and eliminated mosquito-borne disease through source reduction and urban development projects were awarded special certification as mosquito-free towns.

Urban control programs emphasized source reduction through community participation and sanitation projects. Major source reduction projects included draining marshes to develop recreational lakes, covering drainage channels, and cleaning and cementing river banks. For peridomestic problems, mosquito inspectors visited residences and provided education on methods of eliminating standing water in domestic and peridomestic water storage vessels and automobile tires to eliminate *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse). This approach was been used extensively on Hainan Island which experienced outbreaks of Dengue Type 3 in 1979-1982 and Type 2 in 1985-1988.

Improved drainage and the use of water traps in in-flow pipes prevented underground breeding in storm drains by *Culex quinquefasciatus* Say, previously an important urban vector of filariasis. Fish also are used to eliminate mosquito larvae in both domestic water storage jars and ornamental ponds. Areas unsuited to the above methods were larvicided with formulations of *Bacillus thuringiensis* var. *israelensis* or *Bacillus sphaericus* (both produced in

China) or were treated with oil. When necessary, tree rows and thickets were sprayed with ULV adulticides such as Sumithion dispersed with backpack sprayers.

In rural towns with adjacent rice fields, a mosquito-free zone was established by larval and adult control. Mosquito production from rice fields was reduced by intermittent irrigation which dried the fields and killed the immature stages before emergence. In permanent fields too wet for rapid drying, local fishes were used for mosquito control and later as food.

In rural or hilly regions where mosquito control was not practical or had not been successful, government programs encouraged personal protection by sleeping under bed nets impregnated with permethrin or deltamethrin. Community education combined with careful disease surveillance and rapid treatment have been successful in these areas in reducing the prevalence of previously endemic mosquito-borne diseases such as malaria transmitted principally by *Anopheles dirus* Peyton and Harrison, *Anopheles minimus* Theobald and *Anopheles sinensis* Wiedemann, and filariasis transmitted by *An. sinensis* and *Cx. quinquefasciatus*.

### Acknowledgments.

The technical support of D. Fish, N.Y. Medical College, and T. Davis, Colorado Department of Health, in transferring this film to VHS format was appreciated.

<sup>1</sup> This presentation consisted of a videotape produced by the Office of the Patriotic Health Campaign and provided courtesy of Lu Bao-Lin, Professor of Medical Entomology, Institute of Microbiology and Epidemiology, P.O. Box 130, Beijing 100850, People's Republic of China.

<sup>2</sup> Visit to Beijing in July 1992 organized by the Citizen Ambassador Program, D.D. Eisenhower Building, Spokane, Washington 99202 and hosted by the Chinese Association for Science and Technology, Beijing, People's Republic of China.

## SANTA CLARA COUNTY VECTOR CONTROL DISTRICT'S CUSTOMIZED COMPUTER DATABASE SYSTEM

Russ Parman, Don Warner<sup>1</sup> and Stan Husted

Santa Clara County Vector Control District  
976 Lenzen Avenue  
San Jose, California 95126

The Santa Clara County Vector Control District (SCCVCD) serves a population of approximately 1.5 million over an area of 1,312 square miles. Operations are divided into inspecting and treating 561 inventoried sources for mosquitoes and rats, and responding to over 4,000 service requests from the public each year.

The previous database consisted of a system which stored service request information including requestor data and type of request. Requests were routed to the correct field technician by linking work districts to census tract information. Since there was no information linking street addresses to census tracts, data-entry personnel were required to manually look up and enter a census tract before the proper work district and technician could be assigned. There was no provision for entering time usage data or information on inventoried breeding sources.

Requirements for the new system included alleviation of these inadequacies, as well as monthly and quarterly automated management reports on services rendered and records of inventoried sources linked to census tracts. Also, the program needed to be expandable and adaptable to future changes.

A consultant (Don Warner, Data Bases Unlimited) was hired to create a relational database system which would retain the function of the old program and add the required enhancements. The system was based on Dbase IV ver. 1.5 (Borland International, Inc.) database management software. For service requests, address information was automatically linked to city and census tract data;

upon entering an address, the correct census tract was retrieved from a 25,000-record file and corresponding work district and technician were assigned to the request. Each request was printed automatically upon completion and routed to the technician. Additional reports were made available which summarized the types of request for each city, work district or technician. Time usage data were recorded for each employee on a weekly basis, and were used to prepare quarterly and monthly reports from the manager to each of the 15 participating cities within the district. All of the data entry and reporting functions were accessed via customized pull-down menus. The system also included various automated file maintenance functions.

The time required for the quarterly management report was reduced from approximately four days to ten minutes. Census tract determination time during service request processing was reduced by 80-90%.

The Dbase IV program provides a great deal of flexibility for future requirements, which will include the recording of all inventoried-source inspections and treatments, and integration of pesticide use data with pesticide inventory. Ad-hoc queries of data are easily designed and implemented through the Dbase software, resulting in an increase in the amount of workload information available to manager and supervisors.

For districts which do not have a great deal of computer expertise on hand, custom programming may present a viable solution for meeting the ever increasing demand for information processing.

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<sup>1</sup> Data Bases Unlimited, 1824 P Street, Sacramento, California 95814.

## EXECUTIVE DIRECTOR'S ANNUAL REPORT FOR 1992

Donald A. Eliason

Executive Director  
California Mosquito and Vector Control Association  
197 Otto Circle  
Sacramento, California 95822

The big issue faced by CMVCA during 1992 was the prolonged fight in the legislature over the state budget and its impact on special districts. The Association and its members spent considerable time and energy on this matter over the summer months as the battle went on. Ultimately, the shift of property taxes from local governments resulted in loss of revenues of approximately 10% in most districts, although a few lost considerably more and a few (those not receiving property taxes and those serving multi-county areas) lost none. Combined losses suffered by CMVCA corporate members was approximately 7% of their combined revenues.

While the loss of revenues was painful for districts that lost them, the Association was strengthened by the challenge. We learned a lot about the legislature and about our own potential to have an influence on certain aspects of the budget process. Contacts made with legislators or their staff by our members, many for the first time, were important in the final outcome. Our efforts, combined with those of other special districts, were influential in convincing the legislature that the "superpot" was not acceptable and that there must be a cap on the amount of the shift of property tax revenues from any district. Each member who took part in the effort to educate their legislators are to be congratulated. The losses could have been much worse than they were without their efforts along with the superb work of our lobbyist, Ralph Heim.

A decision was made to become proactive with regard to investigating administrative and technical options in dealing with the Africanized Honey Bee (AHB). The Board approved developing and holding an AHB workshop/training session at UC Davis. The session was well attended and the Davis Entomology staff did an outstanding job in covering the subjects included in an outline developed by B. Fred Beams.

The Management Workshop held in Ontario

featured a one day session on "Systemic Management" presented by Dr. William Reckmeyer and a half-day session presented by Betty Yee and Peter Detwiler on the state budget crisis and its implications for funding problems facing the districts in 1993.

Use of the memorandum of understanding between the CMVCA Research Foundation and individual districts, developed last year to provide support of encephalitis virus surveillance, has proven to be a successful way of providing stable funding for support of the cooperative effort. It enables the VRDL to anticipate their work load and ensure an adequate laboratory staff is on hand when needed. Without CMVCA support, the VRDL would have been forced to discontinue testing of surveillance samples. Loss of our surveillance program would have been disastrous, disabling our ability to prevent human cases by intensifying control efforts when virus activity is detected in sentinel chickens or mosquitoes. Surveillance data is also vital for use in support of the districts' work in discussions with legislators, regulatory agencies and the public.

CMVCA ended 1992 in good financial condition with \$296,474 in reserves, an amount equal to 98% of the 1993 annual budget. These reserves will be important when facing the impact of the state's serious economic problems on district funding.

Our annual conference ending the CMVCA 1992 administrative year was held in January, 1993 at the Red Lion Inn in Bakersfield. Of the 332 registrants and 31 companions attending, 191 attended the annual banquet and 58 attended the Trustee Luncheon.

A new corporate member, San Gabriel Valley MAD, was welcomed into the Association. We welcome the new district and its first manager, P. Sue Zuhlke, into our Association.

## C.M.V.C.A. YEAR-END COMMITTEE REPORTS: 1992

Charles H. Dill

CMVCA Vice President  
Marin-Sonoma Mosquito Abatement District  
556 North McDowell Boulevard  
Petaluma, California 94954

The following reports reflect the activities of the standing committees, sub-committees and ad hoc

committees of the CMVCA for the year 1992 as submitted by the chairperson(s) of each committee.

### BIOLOGICAL CONTROL SUB-COMMITTEE

Craig Downs (Chair)

Robert L. Coykendall, Jean G. Kovaltchouk, Vicki L. Kramer, Steve Mulligan, Richard T. Ramsey, Gary Reynolds, Werner P. Schon, Stan A. Wright and Joseph J. Cech, Jr. (Consultant)

In 1992 the Biological Control Sub-Committee produced a revision of the chapter on biological control of mosquitoes for the vector control training manual. Along with the chapter, a list of new references was included. Another charge of the committee was to search out and disseminate information regarding the development of new biological control agents, mainly *Lagenidium gigant-*

*eum* and *Bacillus sphaericus*. *Lagenidium* is moving through the registration process but no one has stepped forward to produce the fungus. *Bacillus sphaericus* is being used in Europe and there exists an interest by at least one company to pursue registration in the United States, time will tell if anything materializes.

### CHEMICAL CONTROL SUB-COMMITTEE

David A. Brown (Chair)

Michael W. Auburn, Jerry M. Davis, Carol Evkhanian, Mickey Gutknecht, Allen Inman, Ronald D. Keith, Leo F. Kohl, Joseph R. Krygier, Ed Lucchesi, Scott E. Monsen, Steve Mulligan, Frank W. Pelsue, Robert Quiring, Kenneth R. Townzen, Jim Wanderscheid, David B. Whitesell, Mir S. Mulla (Consultant), Charles H. Schaefer (Consultant) and Burnell Yarick (Consultant)

During 1992, the Chemical Control Sub-Committee was alerted to the possible loss of pyrethrin and piperonyl butoxide products due to the reregistration process all products are having to undergo as a result of the 1988 changes in the

Federal Fungicide, Rodenticide and Insecticide Act (FIFRA). Many chemical companies are choosing to drop registrations rather than perform the tests necessary to retain certain uses because of the extremely high costs involved in this process.



Unfortunately, many of the uses being dropped will limit the ability of public health agencies to adequately control mosquitoes and other vectors. The IR-4 program has been contacted to support those pesticide uses that may be lost due to this reregistration process.

The Sub-Committee strongly supported federal legislation that was introduced last year in support of minor use pesticides, especially as they relate to public health. While the legislative session ended before the proposed bill could be voted on, it is understood that the bill will be introduced again and the Sub-Committee intends to continue the strong support legislation of this type deserves.

The Sub-Committee continued working with CAL-EPA in preventing the loss of pesticides due to

SB-950 when the loss of those pesticides would have a detrimental impact on public health.

MosquitoNet was utilized by the Sub-Committee, allowing interested users to receive current information on public health pesticides and related issues. This forum will continue to be used by the Sub-Committee, and it is hoped that the system will eventually incorporate data from the Department of Pesticide Registration as to current materials labeled for the control of insects important to mosquito and vector control agencies.

And finally, we will continue to support and encourage the development of safe pesticides, both chemical and biological, for use in public health operations.

## COMPUTER COMMITTEE

Bruce F. Eldridge (Chair)

David A. Brown, Craig Downs, Jack E. Hazelrigg, Ronald D. Keith, Barbara A. Kozusko,  
Marilyn M. Milby, Fred C. Roberts, Malcolm A. Thompson, Patrick Turney  
and Charles Taylor (Consultant)

The Computer Committee was involved in the development of the statewide computerized mosquito information system, MosquitoNet. By the end of 1992, MosquitoNet had 86 users, broken down as follows: California MAD's, 38; University of California, 17; California Department of Health Services, 5; other state users, 16; out-of-state users, 10. The number of subject areas (forums) has grown to ten. Areas covered are: Chemical Pesticides, Laws, Vector-Borne Diseases, Wetlands, Management, General Information, Research, Calendar, IGR's and Endangered Species.

During 1992, the basic system used by MosquitoNet, The Major BBS by Gallacticom, Inc. was upgraded from version 5.0 to 6.03. This improved the system considerably by allowing full

screen editing, searching for key words, immediate access to menus and other enhancements. The system was also upgraded to allow four simultaneous callers (from the previous two).

Committee members spent much of the year informally training new users. Two demonstrations were presented. One was at the quarterly Board of Directors meeting in Bakersfield, the other at the 1992 annual conference.

The Sysop of MosquitoNet is John Gimnig. He is a graduate student in the Department of Entomology at UC Davis, and is supported at 1/4-time salary by CMVCA. The other expense to CMVCA is the lease of four telephone lines. The total costs to CMVCA in 1992 were approximately \$10,000.

## CONTINUING EDUCATION COMMITTEE

B. Fred Beams (Chair)

Vic Baracosa, Glenn E. Bissell, William L. Boynton, David, G. Farley, Stan R. Husted,  
Leo F. Kohl, Ed Lucchesi, Minoo B. Madon, Lal S. Mian, Joyce Bradley (Consultant)  
and Bruce F. Eldridge (Consultant)

The committee met four times in 1992, and discussed or took action on the following:

1. Reviewed 103 continuing education programs submitted to the committee for approval. Of these, 100 were approved for credit.
2. The computer programs have been cleaned up by Susan Magy and will be much easier to use at the regional level.
3. Planned and implemented a two-day Africanized Honey Bee Management Training program held at UC Davis in October. This course can be used as a model for future training programs.
4. Dr. Joyce Bradley was replaced by Dr. John Poorbaugh as training and certification officer for the California Department of Health Services, Environmental Management Branch. Dr. Poorbaugh, in turn, retired in December, 1992 and was replaced by James R. Clover.
5. Recommended to the Board of Directors that the name of the committee be changed to the "Certification and Training Committee". The Board approved the change at the November 1992 quarterly meeting.
6. David G. Farley assumed Chairmanship of the committee at the January 1993 annual meeting.
7. The regional training coordinators for 1993 are:  
Sacramento Valley - Michael R. Kimball  
Coastal - Robert F. Schoeppner  
No. San Joaquin Valley - Ed F. Lucchesi  
So. San Joaquin Valley - Charles W. Smith  
So. California - B. Fred Beams

## DISEASE CONTROL SUB-COMMITTEE

William K. Reisen (Chair)

James R. Caton, Arthur E. Colwell, Jerry M. Davis, Patricia A. Gillies, Ronald D. Keith,  
Robert L. Kennedy, Lal S. Mian, Melvin L. Oldham, Allan R. Pfunter, Harvey I. Scudder,  
Robert A. Murray (Consultant), Ronald R. Roberto (Consultant), William C. Reeves (Consultant)  
and Charles R. Smith (Consultant)

Specific charges of the Disease Control Sub-Committee for 1992 were: 1. Complete guidelines for interagency response to plague in California, 2. Evaluate the potential consequences for local vector control agencies of cutbacks in the Department of Health Services, 3. Evaluate the outcome of research on the potential use of the comb-stick/filter paper method for obtaining serum samples from sentinel chickens and make recommendations and 4. Develop proposals for a statewide approach to Lyme disease.

Considerable progress was made towards the

completion of our charges during 1992, due largely to the efforts of the Sub-Committee with support from the Executive Director, several branches of the California Department of Health Services and the School of Public Health at UC Berkeley. Progress on each charge is summarized below:

1. Interagency guidelines for plague control were completed and approved by the Board in early 1992. Plague and malaria guidelines are currently being circulated among collaborating

agencies for their approval and/or recommendations.

2. The CMVCA has addressed cutbacks by the Department of Health Services in surveillance by assuming a greater fiscal and organizational responsibility for the Encephalitis Virus Surveillance (EVS) Program. Member and nonmember CMVCA agencies currently are billed for services based on the number of mosquito pools and chicken sera submitted for testing each year. Following Sub-Committee recommendations, 1992 EVS activities emphasized the testing of sentinel chicken sera (137 flocks tested bi-weekly in 1992 as compared to 85 tested every four weeks in 1991). The number of mosquito pools tested consequently decreased from 4,589 in 1991 to 2,329 in 1992. This shift in emphasis provided increased sensitivity at comparable cost. In 1993, the organization of chicken purchases and bleeding supplies will most likely be assumed by the Disease Control Sub-Committee for the 1994 season.
3. Collaborative research between the Viral and Rickettsial Disease Laboratory and the School

of Public Health at UC Berkeley, sponsored, in part, by the CMVCA Research Foundation, developed and evaluated a new method for bleeding sentinel chickens. Antibodies to both WEE and SLE viruses were detected readily by EIA in several drops of blood collected onto filter paper from a small lancet prick of the chicken's comb. The specificity and sensitivity of this blood spot method agreed well with sera collected concurrently by venopuncture in both laboratory and field evaluations. This new method will be used to bleed sentinel chickens during the 1993 surveillance season and should provide saving in both supply and labor costs.

4. A draft of a publication entitled "Interagency Guidelines for Lyme Disease Surveillance" was completed, circulated among the Sub-Committee and is undergoing final revision. The Lyme disease guidelines should be completed during mid-1993.

In addition, a revision of the EVS guidelines was completed and circulated among the Sub-Committee. A final revision should be completed and approved during early 1993 so as to be in place during the summer of 1993.

## EMERGING PROBLEMS SUB-COMMITTEE

John R. Stroh (Chair)

James R. Caton, Charles H. Dill, David C. Martinez, Irvin R. Schauer, Harry A. Scott, Charles R. Smith, Charles W. Smith, Bruce F. Eldridge (Consultant), Robert S. Lane (Consultant), William K. Reisen (Consultant), Robert A. Murray (Consultant) and Jeffrey A. Myer (Consultant)

In 1992, the Sub-Committee focused its attention on two subjects; the issues of used tire storage regulations and Africanized Honey Bee migration into California.

The California Integrated Waste Management Board adopted emergency regulations on June 26, 1991 for permitting waste tire facilities (WTF) which store or stockpile more than 500 waste tires at a specific location. The emergency regulations set forth procedures and requirements necessary to obtain WTF permits and establish technical standards for storage and disposal of waste tires at WTF's and solid

waste facilities. The emergency regulations became effective on February 10, 1992.

The Sub-Committee provided vector control criteria for inclusion into the regulations. Section 17353 of the regulations define vector control measures that must be included in a permit application for a WTF. A complete description of the program can be found in Title 14, Division 7, Chapter 3, Article 4.1 of the Natural Resources Code.

Mr. Kenneth Townzen, Regional Chief of the California Department of Health Services, Environmental Management Branch, should be

recognized for his perseverance in ensuring the requirement to vector control in waste management programs.

The planned entry of Africanized Honey Bees (AHB) into California was also investigated by the Sub-Committee. Because California's mosquito abatement and vector control districts were identified in the AHB Action Plan for the State of California, it was felt that the Sub-Committee should establish a position on CMVCA's involvement with future surveillance and control activities. The CMVCA Continuing Education Committee, chaired by B. Fred Beams, spearheaded a plan to educate and train

mosquito and vector control technicians in emergency response to the AHB. A workshop sponsored by San Diego County and Imperial County addressed specific action plans for the anticipated arrival of the AHB from Mexico and Arizona. CMVCA Executive Director, Don Eliason, participated in a study of AHB programs in Texas and brought back information to be used by California mosquito and vector control agencies. The Association is also participating on a committee of state and local agencies that will deal with AHB in the future. AHB will be a specific charge for Sub-Committee action in 1993.

### ENTOMOLOGY SUB-COMMITTEE

Stephen L. Durso (Chair)

Frank Ennik, Jack E. Hazelrigg, Ronald D. Keith, Leo Kohl,  
Edward Magoon, Richard P. Meyer, James P. Webb, Glenn M. Yoshimura  
Lucia T. Hui (Consultant) and Michael Stimmann (Consultant)

The Sub-Committee devoted much of 1992 to the completion of the mosquito identification manual for the mosquitoes of California. At the end of the year, the manual, entitled *Identification of the Mosquitoes of California*, had been completed by the authors, R.P. Meyer and S.L. Durso, and was ready for printing early in 1993. This manual presents completely illustrated identification keys, distributional tables and biological notes for the 52 currently recognized species in the state of California.

The manual also offers a comprehensive glossary and morphological diagrams for easier understanding of terms used in the keys. In addition, work is well underway on a series of four companion guides to the common mosquitoes of each of the four major geographic regions of the state.

The Sub-Committee also completed the revision of four chapters in the vector control technician training manual and forwarded them to the Publications Committee.

### HISTORICAL ARCHIVES COMMITTEE

Ernest E. Lusk (Chair)

John C. Combs, Peter B. Ghormley, Earl W. Mortenson, Felix H. Ocko, Melvin L. Oldham,  
Irvin R. Schauer, Harvey I. Scudder, Robert F. Schoepner and David E. Reed (Consultant)

The committee met at each of the four quarterly CMVCA Board of Directors meetings during 1992.

The concept of using barcoding and a computer program to catalogue and retrieve archival material regretfully had to be shelved for the present, largely due to time and financial considerations.

Cataloguing of the photo and slide collection is

nearly complete thanks to the efforts of Earl Mortenson.

John Combs dedicated staff and computer time to complete a computerized record of all the archival material in the collection in Visalia. A printout of this record is available to interested individuals.

## MANAGEMENT COMMITTEE

Elizabeth A. Cline (Chair)

Mitchell J. Bernstein, Dennis D. Boronda, David A. Brown, James A. Camy,  
Lue Casey, Gilbert L. Challet, Major S. Dhillon, Craig Downs,  
Carol Evkanhian, David G. Farley, Herbert Marsh, Ronald L. McBride,  
Kevin Pinion, Theresa Stratton, John R. Stroh, James Wanderscheid,  
David B. Whitesell, Charles M. Myers (Consultant)

The Management Committee faithfully fulfilled its charges again this year. Through the hard work and cooperation of the committee members, the following goals were accomplished:

### Management Seminar

A Management Seminar was held for managers, trustees and agency personnel. The focus of this seminar was on management and future planning. We also tried to deal with the State budget problems now and in the future. The seminar was not as well attended as we would have liked, however, those in attendance gave it high praise for relevance and effectiveness. We would like to continue these seminars annually and we expect that attendance will improve as we get past some of our budgeting problems caused by the indecision of our State legislators.

### Resource Directory.

The Committee made revisions to the Resource Directory which will be distributed in February. The distribution process will be slightly different this year.

We will be distributing the directory to the Committee members first and then they will distribute them to their regions at regional meetings along with a short orientation session to familiarize the members with the directory. We will be stressing intra-agency distribution also so that more people within each agency have access to this resource. In addition, we will be working toward the goal of getting the Resource Directory onto MosquitoNet so that member agencies can access it through their computer modems. We also hope to add new and exciting information to the directory each year.

### CMVCA Training Manual.

Committee members worked on revisions for several chapters in the CMVCA Training Manual. Those revisions have been turned over to the Publications Committee and Ernie Lusk, editor of the revised Training Manual.

Overall, it was a good year and this committee is looking forward to new challenges and continuing service next year.

## PUBLICATIONS COMMITTEE

Peter B. Ghormley (Chair)

John C. Combs, Stephen L. Durso, Ernest E. Lusk, Wesley R. Nowell,  
Glenn Yoshimura, Bruce F. Eldridge (Consultant) and Minoo B. Madon (Consultant)

The Publications Committee worked diligently during 1992 to eliminate the backlog of Publications awaiting printing or in preparation.

The Committee and *Proceedings* Editor, Stephen L. Durso, were able to bring that publication up-to-date by publishing two volumes (1991 and 1992 volumes) in a single year. The Committee has been

fine tuning the *Proceedings* over the last several years and has made significant progress in upgrading the contents, presentation and preparation of the yearly volumes. It should be much easier in the future to publish the *Proceedings* within the same year as the annual conference from which it is based.

Vector Control Technician Training Manual

Editor, Ernest E. Lusk, collected all revised chapters for that manual from the committee chairpersons assigned the revisions and collated them into an early draft from which future editing can be done.

The major emphasis of the 1993 Publications

Committee will be the completion of the training manual, the initiation of work to update the other training manuals, the publication of the statewide and regional mosquito identification guides and the timely publication of the *Proceedings*.

## WAYS AND MEANS COMMITTEE

Allan R. Pfunter (Chair)

Dennis D. Beebe, Lue Casey, Gilbert L. Challet, Elizabeth A. Cline, Charles P. Hansen, William Hazelaar, Allen Inman, Don J. Layson, Herbert J. Marsh, Grant W. McCombs, Kevin Pinion, Theresa Stratton and Donald A. Eliason (Consultant)

In 1992, the Committee responded to the following specific charges: 1. Analyze standing committee standard charges, 2. Continue assessment of structure and function of committees, 3. Review the CMVCA written policies, 4. Review funding mechanisms for EVS programs and 5. Assist in the election of an Associate Member to the Board of Directors.

Charges one and two were combined with number three as all are items requiring written policies. A policy format utilizing numbered series (100, 200, etc.) was developed. This approach allows changes to be inserted without a total policy revision and/or reprinting. Policies for Personnel, Committee Structure and Standing Charges, and Duties of Board Officers were drafted and submitted to the Board of Directors for review. Additional policies will be developed in 1993.

In response to charge four, the Committee asked for, and was granted, a motion by the Board of Directors to instruct the Executive Director to determine an administrative fee to be applied to each EVS sample requested by a non-member agency.

The election procedure present in the Bylaws was followed and an Associate Member Representative was elected.

In addition to the aforementioned tasks, the Committee addressed the items noted below:

1. Evaluated the current member classes to determine if redefinition was in order. After considerable discussion and input from the CMVCA Regions, the Committee agreed to

maintain the present system but to re-evaluate it in the future.

2. Regarding the membership fee structure, the Committee requested that a records search be made to determine if an "old" policy exists defining what materials are to be included with the Associate Member package. The current mail-out costs about \$60 per member annually, while the membership fee is \$30.
3. Some members requested that the current days of the week designated for the annual conference be reviewed as the schedule requires weekend travel. After discussion by the Regions, the Committee agreed that the current schedule be maintained.
4. The Committee approved a request by the Trustee Corporate Board to institute a "Trustee Outstanding Service Award". The request was subsequently approved by the membership.
5. In November, a request was made to expand the information regarding the role of trustees as outlined in the Trustee Manual. After review, the Committee referred the request to committee member Grant McCombs. Member McCombs and Chairman Pfunter's recommendation for a minor addition to the Trustee Manual has been forwarded to the 1993 Chairman, Charles Hansen.

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

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

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

Jeffrey W. Beehler was the recipient of the 1993 award at the 61st Annual Conference held in Bakersfield. The other finalists were Charles F. Fulhorst, John E. Gimnig and J. Wakoli Wekesa. The four finalists' papers are printed on pages 121-137.

### **Previous William C. Reeves New Investigator Award Winners:**

1993 - Jeffrey W. Beehler  
1992 - Darold P. Batzer  
1991 - David R. Mercer  
1990 - Gary N. Fritz  
1989 - Truls Jensen  
1988 - Vicki L. Kramer

## 1993 WINNER

### WILLIAM C. REEVES NEW INVESTIGATOR AWARD

1993 Proc. Calif. Mosq. Vector Control Assoc.

61: 121-124

#### THE EFFECT OF ORGANIC ENRICHMENT AND FLOODING DURATION ON THE OVIPOSITION BEHAVIOR OF *CULEX* MOSQUITOES

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##### ABSTRACT

Differential oviposition, based on the nutrient content of the water and the duration of habitat inundation with water, was shown to account for the differential distribution of *Culex* mosquitoes in temporarily flooded habitats in two experiments. Water samples taken from duck club ponds which were flooded for less than one week and for six weeks were more attractive to ovipositing *Cx. quinquefasciatus* than were distilled water controls in laboratory tests. However, water samples taken from ponds flooded for less than one week were more attractive to ovipositing *Cx. quinquefasciatus* than samples taken from ponds flooded for six weeks. The degree of nutrient supplementation and the duration of flooding was shown to affect oviposition by *Cx. quinquefasciatus*, *Cx. stigmatosoma* and *Cx. tarsalis* in studies conducted in experimental ponds. Ponds which received the highest levels of supplementation and were the most recently flooded, received the highest number of *Culex* egg rafts.

Mosquito larvae are not randomly distributed in the aquatic environment, rather they have a clumped distribution both spatially and temporally. Spatially, larvae are found in specific niches of the total larval habitat available. This spatial clumping is related to the proximity of habitat edges (Walton et al. 1990), emergent vegetation (Walton and Mulla 1990) and the presence of predators. Another aspect of this distributional clumping is related to the duration of flooding in a given habitat. Immediately after inundation, production of larval mosquitoes increases to a maximum during a period of 2-3 weeks post-flooding, but then the populations decline over time to substantially lower levels. Mulla (1990) hypothesized that this decline could be due to four factors: 1) an increase in predator pressure, 2) a decrease in attractiveness of the habitat to ovipositing

mosquitoes, 3) a combination of 1 and 2, or 4) the depletion of the food supply.

Walton and Mulla (1991) demonstrated that colonization by macroinvertebrates which are predaceous on mosquito larvae, occurs 2-3 weeks after habitat flooding. However, there are no studies which relate temporal and spatial mosquito larval abundance to a dynamic pattern of differential oviposition in temporary water habitats. Our objectives were to determine the degree of oviposition based on nutrient load and flooding duration in temporary flooded habitats. To investigate the intensity and distributional patterns of oviposition, studies were conducted in duck club ponds in the Prado Basin and in experimental ponds breeding natural populations of *Culex* mosquitoes.



### PRADO BASIN STUDIES

Throughout southern California, duck club ponds comprise expansive artificially-created wetlands for waterfowl. These ponds have been shown to be important breeding sites for *Culex* mosquitoes (Walton and Mulla 1991, Durso and Burguin 1988).

In the Prado Basin (Riverside County), the Schuekle Duck Club ponds are flooded each fall to provide habitat for wildlife and recreational opportunities for hunters. Water samples were taken from Prado Basin duck club ponds which were flooded for different durations and compared for attractancy to ovipositing *Culex quinquefasciatus* Say in the laboratory. Water samples from ponds which had been flooded for six weeks were compared to distilled water controls and water samples from ponds which had been flooded for less than one week were also compared to distilled water controls. Field water samples were assayed in small waxed paper cups (5 cm high x 7.5 cm diameter) for attractancy against each other or distilled water controls in replicated small cages (23 x 23 x 23 cm) in a photoperiod-controlled laboratory insectary (14L:10D including a 2-hour evening twilight period). Twenty laboratory-reared, blood-fed *Cx. quinquefasciatus* were placed in each of six cages overnight with two randomly placed cups containing water samples to be tested. The next morning, the deposited egg rafts in each test cup were counted and the number of egg rafts transformed to square roots ( $\sqrt{x}$ ), an appropriate transformation when the variance is proportional to the mean (Snedecor and Cochran 1980). Paired t-tests were used to compare treatments.

Water samples taken from ponds flooded for both six weeks and less than one week were significantly more attractive ( $P < 0.025$ ) to ovipositing *Culex quinquefasciatus* than were the distilled water controls (Fig. 1). The most interesting results were from comparisons of water samples from ponds which had been flooded for less than one week and those which had been flooded for six weeks. Both of these groups were attractive to ovipositing mosquitoes when compared to distilled water controls, but when compared to each other, water from the most recently flooded ponds was significantly more attractive ( $P < 0.025$ ). The data thus provide strong evidence that at least some of the temporal larval distribution patterns in duck club ponds and other managed marsh ecosystems are due to differential attractancy to ovipositing mosquitoes.

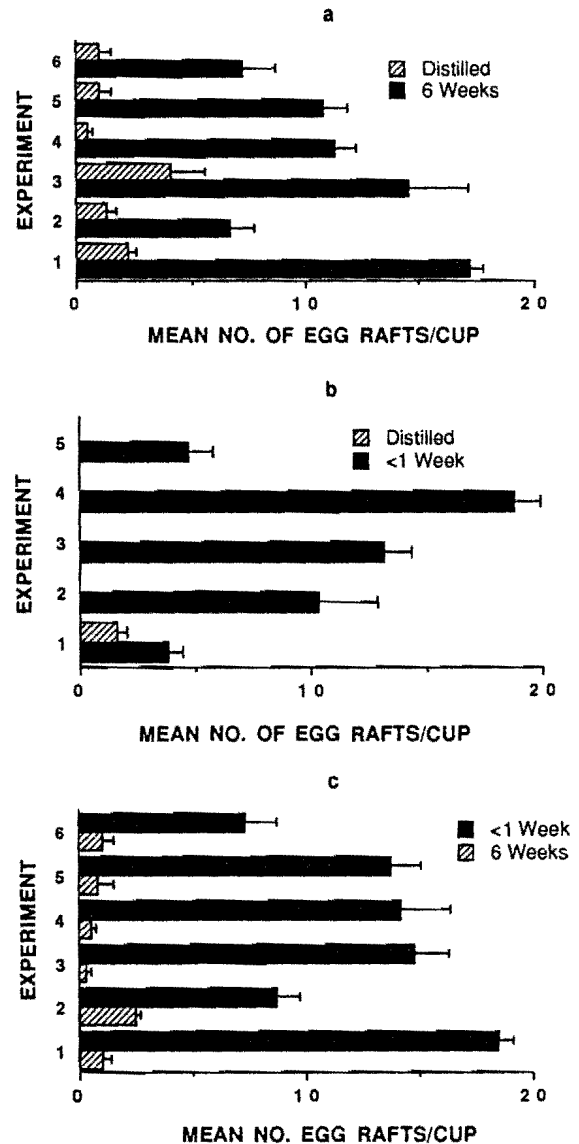


Figure 1. The ovipositional response of gravid *Culex quinquefasciatus* to a) water taken from duck club ponds flooded six weeks versus distilled water controls, b) water taken from duck club ponds flooded less than one week versus distilled water controls, and c) water taken from duck club ponds flooded less than one week versus water taken from duck club ponds flooded six weeks.

### EXPERIMENTAL POND STUDIES

The second group of experiments were conducted at the Aquatic and Vector Control Research Facilities at Oasis in the Coachella Valley and in Riverside. Experimental ponds (30 m<sup>2</sup> in Oasis and 27 m<sup>2</sup> in Riverside) were flooded to a

mean depth of 35-38 cm to directly measure the changes in oviposition levels of *Culex* mosquitoes over time and to determine the effect of different levels of nutrient supplementation on oviposition behavior. Before flooding, the ponds were supplemented with 0, 1 or 4 kg of rabbit chow (Hare Raiser Pellets, Poultryman's Cooperative Association, Atascadero, California). Oviposition levels were compared by counting *Culex* egg rafts along a 11 meter x 4 cm transect which was located along the north and west edge of each pond. Egg rafts are generally deposited along the edges of these ponds. Subsamples of rafts were returned to the laboratory and reared individually to the fourth instar larval stage where they were identified. Egg raft counts were transformed to square roots and analyzed using multiple regression methods (Box et al. 1978) to compare the effects of nutrient supplementation and temporal factors.

Significant differences in *Culex* oviposition based on flooding duration, as well as the effect of differential nutrient supplementation levels, were shown in ponds at both Oasis and Riverside (Fig. 2). Ponds which received the highest levels of nutrient supplementation had significantly more ( $P < 0.025$ ) *Culex* egg rafts than did other ponds. Ponds receiving the 1 kg supplement and the controls were not significantly different in attractancy.

Subsamples of the egg rafts from the Oasis ponds were reared individually in the laboratory to the fourth instar larval stage and identified as *Culex tarsalis* Coquillett and rafts collected at the Riverside ponds were a mix of *Cx. quinquefasciatus* and *Culex stigmatosoma* Dyar. As expected, based on the experiments in the Prado Basin duck club ponds, there was a strong temporal component to the attractancy of the ponds to ovipositing mosquitoes as related to the flooding time. Ponds received more egg rafts initially and the number of rafts declined with time in all treatments ( $P < 0.01$ ).

In summary, oviposition attractancy in a temporary water habitat is a dynamic process. Soon after flooding, ponds become attractive to ovipositing *Culex* mosquitoes and that attractancy subsequently declines. This attractancy may be related to the production of bioactive compounds which are either metabolic by-products of rapidly increasing bacterial populations or breakdown products of the organic substrate itself. Millar et al. (1992) identified from fermenting grass infusions 3-methylindole, a tryptophan derivative, which is highly attractive to ovipositing *Cx. quinquefasciatus*. In these studies,

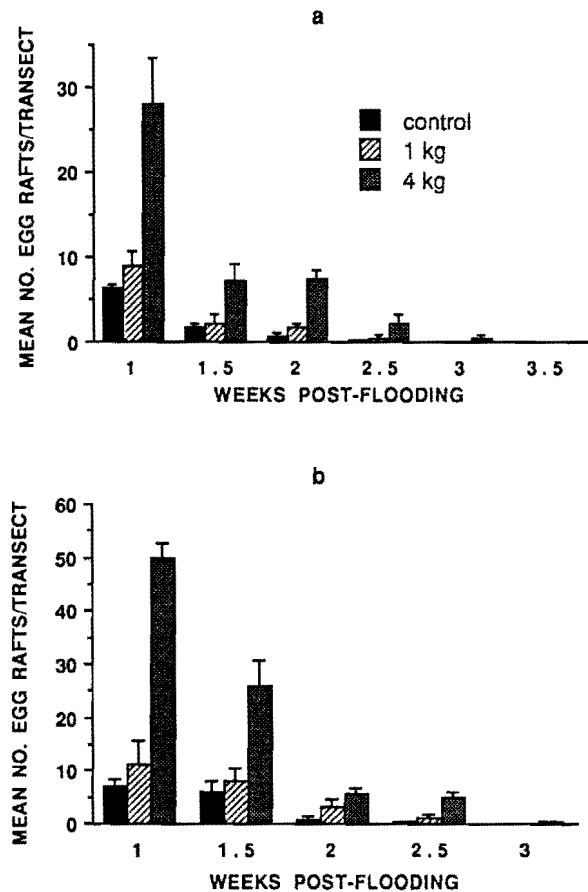


Figure 2. The mean number of *Culex* egg rafts deposited over time post-flooding along a transect in ponds which receive different levels of organic supplementation at a) Oasis and b) Riverside.

the level of nutritional supplementation was clearly shown to be a factor in the selection of an oviposition site. Any model of the population dynamics of mosquitoes must include the changing attractancy to ovipositing mosquitoes and nutrient content of the larval habitat, as well as the effects of predation.

#### ACKNOWLEDGEMENTS

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## THE GEOGRAPHIC DISTRIBUTION OF A CALIFORNIA ENCEPHALITIS-LIKE VIRUS (BUNYAVIRIDAE) IN ASSOCIATION WITH *Aedes squamiger*

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California (CAL) serogroup viruses are antigenically closely related viruses belonging to the genus *Bunyavirus* (Bunyaviridae). These viruses appear to be maintained in nature by vertical (transovarial) transmission in mosquitoes in conjunction with horizontal transmission cycles that involve mosquitoes as vectors and mammalian species as amplifying hosts (LeDuc 1979, Turell 1988). Jamestown Canyon (JC) virus and California encephalitis (CE) virus are the CAL serogroup viruses known to occur in California (Reeves et al. 1983). Several CAL serogroup viruses, including CE and JC viruses, are recognized human pathogens (Grimstad 1988).

*Aedes squamiger* (Coquillett) is a pestiferous salt marsh mosquito in many coastal areas of California. Its present-day distribution consists of geographically isolated populations located as far north as Sonoma County and as far south as Baja California in Mexico (Bohart and Washino 1978). In 1989, a CAL serogroup virus was isolated from adult *Ae. squamiger* reared from larvae collected from a salt marsh at Morro Bay, San Luis Obispo County, California (Eldridge et al. 1991). In cross-neutralization tests, this virus was closely related to the prototype strain of CE virus (BFS-283) and, therefore, has been referred to as a California encephalitis-like (CE-like) virus.

Eldridge (1990) considered the hypothesis that CAL serogroup viruses coevolved with *Aedes* (*Ochlerotatus*) mosquitoes. On the basis of morphology, *Ae. squamiger* is a member of the

subgenus *Ochlerotatus*. If the association between the CE-like virus and *Aedes squamiger* is the result of a coevolutionary process, then geographically distinct populations of *Ae. squamiger* should be infected with the same CE-like virus or remarkably similar viruses.

The objective of the present study was to test the hypothesis that CAL serogroup viruses coevolved with *Aedes* (*Ochlerotatus*) mosquitoes.

### MATERIAL AND METHODS

#### Selection of Collection Sites.

*Aedes squamiger* larvae and pupae were collected from 18 salt marshes located throughout the geographic range of this mosquito species (Fig. 1). Emphasis was placed on sampling populations of *Ae. squamiger* in close proximity to metropolitan areas in both northern and southern California.

#### Collection and Processing of Mosquitoes.

Larvae and pupae were collected during the late winter and early spring of 1991 and 1992 from intertidal pools by dipping with a pint-sized container. Mosquitoes collected in this manner were transported alive in water-filled containers to the Entomology Laboratory at the University of California, Davis, where they were mass-reared to the adult stage. Newly emerged adults were identified and segregated by species and gender into pools of up to 50 individuals and stored at -80°C. The frozen

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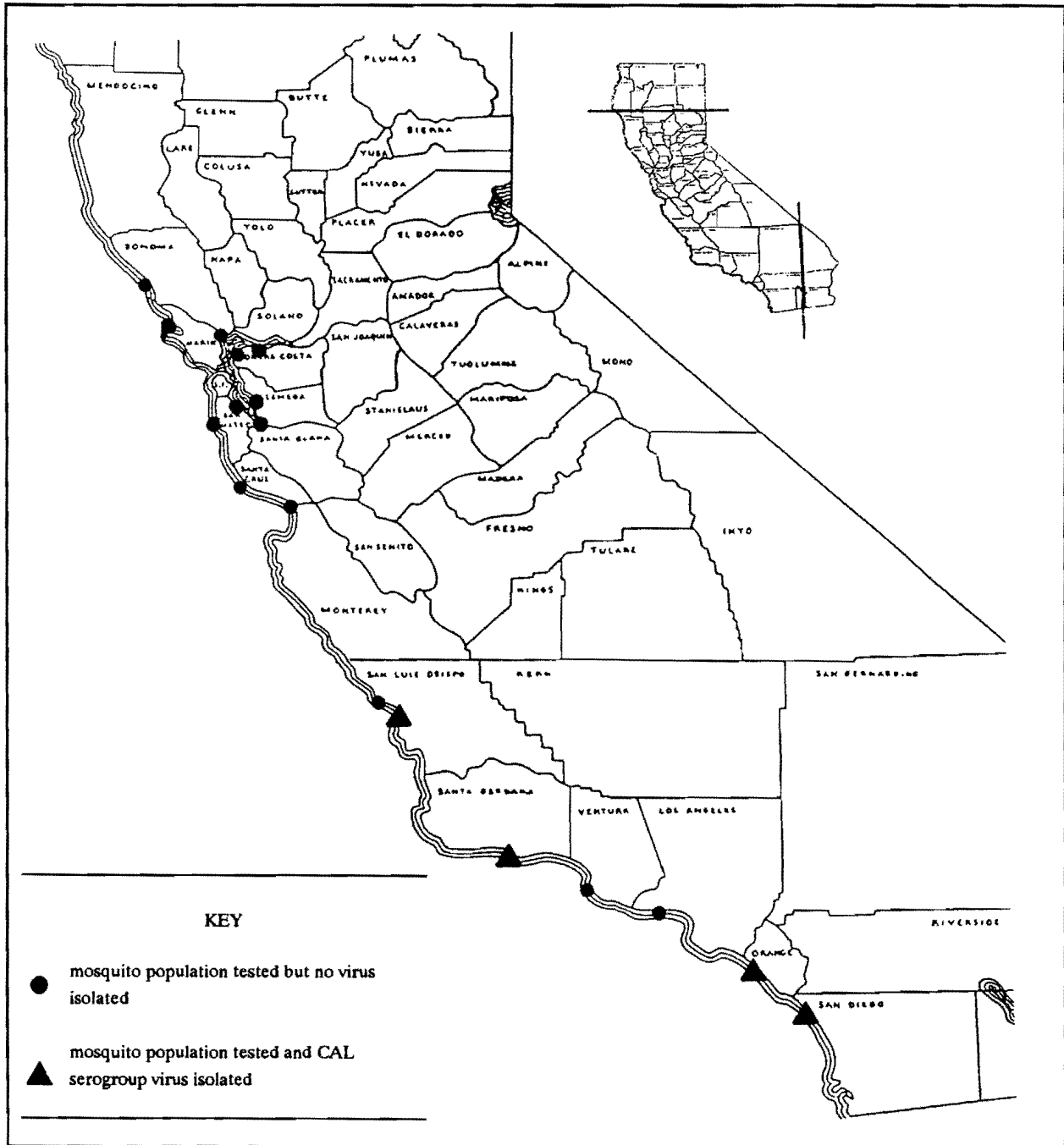


Figure 1. Locations of *Ae. squamiger* populations tested for vertical transmission of a CAL serogroup virus.

mosquito pools were subsequently transported on dry ice to the Virus Laboratory at the University of California, Berkeley, for virus assay.

**Virus Isolation and Identification.**

Mosquito pools were assayed for virus in Vero cell cultures (Campbell et al. 1991). Stock viruses were prepared as supernatant fluids from infected cell cultures and frozen at -80°C.

Virus isolates were identified by an in situ

enzyme immunoassay (EIA) (Faran et al. 1986) adapted for use in Vero cell monolayers in 96-well plates. This assay can be used to accurately determine if a virus is (or is not) a member of the CAL serogroup but cannot be used to accurately determine if a CAL serogroup virus is a strain of either the CE serotype or the JC serotype. Reference viruses used in the EIA included 2 strains of the CE serotype from California (prototype strain BFS-283 and strain DAV-457) and the Jerry Slough variant of the JC serotype (strain BFS-4474 from California). Hyperimmune mouse ascitic fluid (HIMAF) prepared against each of the reference viruses also was used in the EIA. All of the field isolates were tested against BFS-283 HIMAF and BFS-4474 HIMAF. Only some of the field isolates were tested against DAV-457 HIMAF.

#### Statistical Methods.

For the purpose of analysis it has been assumed that each positive mosquito pool contained a single infected mosquito and the mosquitoes collected from each field population and tested for virus formed a representative sample of the field population. A point estimate of the proportion of each population infected

with virus was calculated by dividing the number of positive pools by the total number of mosquitoes tested for virus. In addition, a 95% confidence interval (C.I.) for the proportion of each population infected with virus was calculated using an exact method.

## RESULTS

Larvae and/or pupae were collected from 18 geographically distinct populations of *Ae. squamiger* in 14 counties of California (Fig 1). A total of 42,142 adults was reared from these larvae and/or pupae and then tested for virus in 957 pools. Twenty-six viruses were isolated from these 42,142 mosquitoes (Table 1). Ten isolates were from mosquitoes collected in San Luis Obispo County (population #13), 1 isolate each was from mosquitoes collected in Santa Barbara County (population #14) and Orange County (population #17), and 14 isolates were from mosquitoes collected in San Diego County (population #18). All of these isolates have been identified with the EIA as CAL serogroup viruses.

No virus was isolated from mosquitoes collected

Table 1. Point estimate and 95% confidence interval for the number of infected mosquitoes (per 10,000 population) in each population of *Aedes squamiger* tested for transovarial transmission of a California serogroup virus.

Population	County	Mosquitoes tested	Virus isolates	Point estimate	Confidence interval
1	Sonoma	38	0	0	0 - 925
2	Sonoma	449	0	0	0 - 82
3	Marin	2,718	0	0	0 - 14
4	San Mateo	6,352	0	0	0 - 6
5	San Mateo	963	0	0	0 - 38
6	Contra Costa	4,731	0	0	0 - 8
7	Contra Costa	2,153	0	0	0 - 17
8	Alameda	3,881	0	0	0 - 10
9	Santa Clara	249	0	0	0 - 147
10	Santa Cruz	64	0	0	0 - 560
11	Monterey	1,447	0	0	0 - 26
12	San Luis Obispo	112	0	0	0 - 324
13	San Luis Obispo	6,488	10	15	6 - 25
14	Santa Barbara	1,963	1	5	1 - 29
15	Ventura	3,443	0	0	0 - 11
16	Los Angeles	1,487	0	0	0 - 25
17	Orange	729	1	14	1 - 76
18	San Diego	4,875	14	29	14 - 44
Totals		42,142	26		

north of San Luis Obispo County (mid-point of the geographic range of *Ae. squamiger*) even though a total of 23,157 mosquitoes (55% of the mosquitoes tested), representing 11 populations, was collected in this region and tested for virus.

A point estimate and 95% C.I. for the proportion of each population infected with virus are presented in Table 1.

## DISCUSSION

Viruses belonging to the CAL serogroup are primarily maintained in nature by subpopulations of vectors capable of highly efficient vertical (transovarial) transmission (Turell and LeDuc 1983). These subpopulations probably constitute a very small proportion of each vector population.

In the present study, vertical transmission of a CAL serogroup virus was demonstrated in 4 of 18 isolated populations of *Ae. squamiger*. Importantly, these four populations are each separated by a distance of at least 70 miles. Based on data collected in this study, the proportion of a population that is vertically infected with a CAL serogroup virus is on the order of 10/10,000. Failure to detect virus infection in 12 of the mosquito populations studied, therefore, can be attributed to chance (i.e., the number of mosquitoes was too small to be reasonably certain that the population was not infected with a CAL serogroup virus). For each of these populations, the upper limit of the 95% C.I. for the proportion of the population infected with virus was greater than 10/10,000 (Table 1). In contrast, failure to detect virus infection in two of the populations was probably due to the absence of infection. No virus was isolated from populations #4 and #6 even though a very large number of mosquitoes was tested from each of these populations. The upper limit of the 95% C.I. for the proportion of populations #4 and #6 infected with virus was 6/10,000 and 8/10,000, respectively. These findings indicate that the geographic distribution of CAL serogroup viruses in association with *Ae. squamiger* is focal yet widespread.

Cross-neutralization tests to determine the relationship between the 26 virus isolates are in progress. If the antigenic relatedness among the isolates is high compared to their antigenic relatedness to other CAL serogroup viruses (e.g., CE, JC, LaCrosse and snowshoe hare) then it is likely that the four mosquito populations are infected with the

same virus (i.e., a CE-like virus). Such a finding would indicate a long-standing relationship between the CAL serogroup virus and *Aedes squamiger* since the geographic separation between these mosquito populations is probably sufficient to prevent present-day exchange of virus by various means (including wind-carriage of infected mosquitoes and migration of viremic vertebrate animals). This finding would also support the hypothesis that CAL serogroup viruses coevolved with *Aedes (Ochlerotatus)* mosquitoes.

At present, the human health and veterinary significance of the CE-like virus in its association with *Ae. squamiger* have not been determined. However, laboratory studies of vector competence suggest that *Ae. squamiger* is able to transmit the virus during the process of blood-feeding in nature (Kramer et al. 1992). Furthermore, preliminary results of serological surveys conducted at Morro Bay indicate that humans and animals (both wild and domestic) have been infected with a CAL serogroup virus in the past (Fulhorst, unpublished data). If the CE-like virus in its association with *Ae. squamiger* is discovered to be a human health problem then sampling of *Ae. squamiger* populations in close proximity to large metropolitan areas, especially in the northern geographic range of this species, should be done. Future studies should include viral tests on more *Ae. squamiger* populations and a large number of mosquitoes from each population sampled. Eldridge et al. (1992) have suggested that a minimum of 10,000 mosquitoes from each population be tested for virus.

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## GENETIC ANALYSIS OF THE POPULATION SUBSTRUCTURE OF *Aedes hexodontus* IN THE WESTERN UNITED STATES

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### ABSTRACT

Polyacrylamide gel electrophoresis was used to examine isozyme variation at eleven loci in eight populations of *Aedes hexodontus* and one population of *Aedes tahoensis*. Data were analyzed using the BIOSYS-1 computer program. UPGMA and Distance Wagner dendrograms were constructed using Nei's distance and Roger's similarity indices. *Aedes hexodontus* shows some differentiation and three subpopulations could be seen within California. Distance Wagner phylogram supports the hypothesis that *Ae. hexodontus* entered California during the Pleistocene Epoch and was stranded on the mountains as the glaciers receded. Restricted gene flow resulted in the southern populations becoming more differentiated than the northern populations. Oregon and Washington populations do not fit this hypothesis; the dendrograms indicate that they are more closely related to populations in the southern Sierra Nevada of California. However, PAUP analysis shows these populations to be more closely related to the northern California populations. This apparent discrepancy may be due to the smaller sample sizes from Oregon and Washington.

*Aedes (Ochlerotatus) hexodontus* Dyar is a Holarctic mosquito which is widely distributed in North America. Its geographic range encompasses the Canadian tundra and extends into the mountainous regions of the western United States. *Aedes hexodontus* can be a severe pest in recreational areas (Carpenter 1962) and has been noted as one of the most vicious biters in the Arctic tundra (Wood et al. 1979). In addition, several viruses have been isolated from this mosquito, including Jamestown Canyon virus (Campbell et al. 1991) and snowshoe hare virus (MacLean et al. 1977, Wagner et al. 1975). Both of these viruses have been associated with clinical cases of human disease. The basic biology of this mosquito is not well understood as it is difficult to colonize in the laboratory and to identify accurately. Consequently, its importance in disease transmission is also not well understood.

Electrophoresis provides an excellent tool to gain a basic understanding of this mosquito. Electrophoresis can be used to 1) aid in identification, 2) determine geographic distribution, 3) determine

population substructure, 4) determine the amount of gene flow between subpopulations, 5) help explain variation in vector competence and 6) aid in reconstruction of phylogenies. Recent studies have advocated using electrophoretic keys in identification especially in mosquitoes which are difficult to identify (Munstermann 1988, Brust and Munstermann 1992). Several studies have examined the population substructure of *Aedes aegypti* (L.) and *Aedes triseriatus* (Say) to explain variation in vector competence (Powell and Tabachnick 1980, Wallis et al. 1983, Munstermann 1985). *Aedes hexodontus* provides an excellent opportunity to study population substructure as subpopulations may be geographically isolated. This mosquito is restricted to high elevations in the mountainous regions of the western United States and several authors have reported geographic variation in its morphology (Knight 1951, Wood 1977), suggesting that there are several genetically distinct subpopulations of *Ae. hexodontus* in North America. This paper presents the results of a preliminary study of the population substructure of

*Ae. hexodontus* in California, Oregon, and Washington.

## MATERIAL AND METHODS

### Collection and Rearing.

Mosquitoes were collected as larvae and pupae by several individuals between 1989 and 1992 from several sites in the Sierra Nevada and Trinity mountains of California and from the Cascade mountain range of Northern California, Oregon and Washington (Fig. 1). Field collected larvae were reared to adulthood in the laboratory at the University of California, Davis. The larvae were kept in plastic pans (30 x 20 x 8 cm) and fed a ground, sifted mixture consisting of Tetramin® fish food, guinea pig

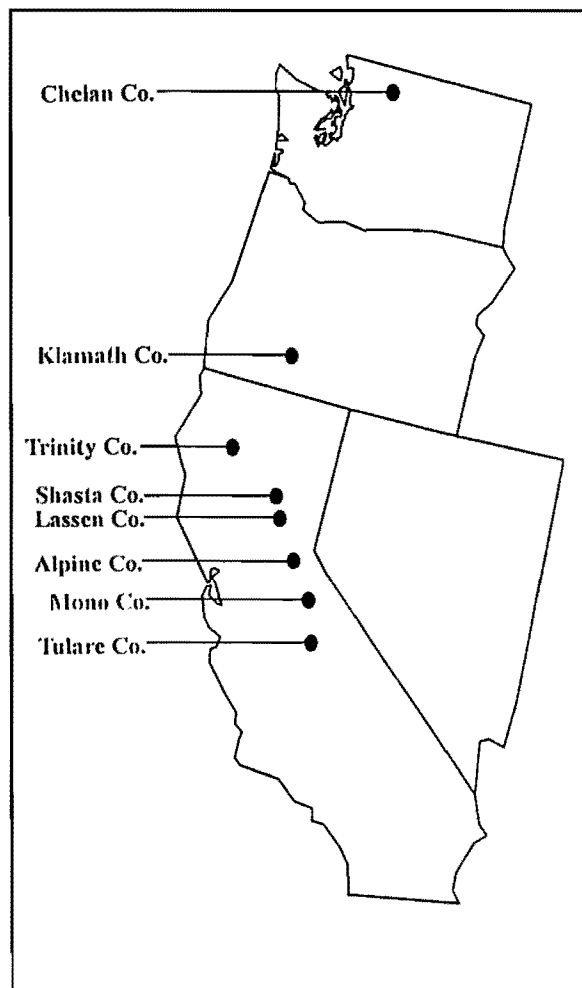


Figure 1. *Aedes hexodontus* populations examined in this study.

food, yeast powder and liver extract. Upon pupation, mosquitoes were moved to four liter adult cages. One to two days after emergence, the adults were identified and placed in Bio-Freeze® Vials (Costar Corporation, Cambridge, MA). These were immediately transferred to an ultralow temperature freezer maintained at  $-80^{\circ}\text{C}$  until the specimens were used for electrophoresis.

### Electrophoresis.

Mosquito proteins were separated using polyacrylamide electrophoresis with a 5% slab gel matrix (Munstermann 1980, Bloem 1991). Electrophoresis was carried out on Hoeffer® units (Hoeffer Scientific Instruments, San Francisco, CA) with four slab gels per unit. The gel slabs were 18 x 8 x 0.75 cm and the buffer system was Tris-citrate (pH 7.1). Mosquitoes were homogenized in 40  $\mu\text{l}$  of double-distilled water with 1% Triton-X to enhance the release of soluble proteins and 0.5% bromophenol blue as a marker front. One to two  $\mu\text{l}$  of the homogenate was added to the wells of the slab gels and the specimens were run at 300 volts for approximately two hours. The homogenates were refrozen after each run so that up to twelve enzyme systems could be assayed for each mosquito.

The mosquitoes were assayed for eleven loci using the standard histochemical staining techniques (Steiner and Joslyn 1979, Shaw and Prasad 1970). The enzyme systems assayed are listed below with abbreviations and Enzyme Commission numbers (Commission on Biochemical Nomenclature, 1984):

Aconitate Hydratase	(ACO-1, 4.2.1.3)
Glycerol Phosphate Dehydrogenase	( $\alpha$ -GPDH, 1.1.1.18)
Hexokinase	(HK-1, HK-2, 2.7.1.1)
Hydroxyacid Dehydrogenase	(HAD, 1.1.1.30)
Isocitrate Dehydrogenase	(IDH-2, 1.1.1.42)
Malic Dehydrogenase	(MDH-1, 1.1.1.37)
Malic Enzyme	(ME, 1.1.1.40)
Mannose-6-Phosphate Isomerase	(MPI, 5.3.1.8)
Peptidase	(PEP, 3.4.11)
6-Phosphogluconate Dehydrogenase	(6-PGDH, 1.1.1.43)

### Data Analysis.

Results were analyzed using the BIOSYS-1 computer program (Swofford and Selander 1981). Nei's genetic distance (Nei 1978) and Roger's similarity were calculated and dendrograms were constructed using the unweighted pair group method with arithmetic averages (UPGMA) (Sneath and Sokal 1973) and Distance Wagner procedures (Rogers 1972) using *Aedes tahoensis* as an outgroup. An additional dendrogram was constructed with the Phylogenetic

Analysis Using Parsimony (PAUP) computer program (Swofford 1991).

## RESULTS

*Aedes hexodontus* was highly polymorphic at three loci (ACO-1, IDH-2 and PEP) while rare polymorphisms (<10%) were present at four loci (HAD, MDH-1, ME and 6-PGDH) (Table 1). Peptidase (PEP) was the most polymorphic with seven different alleles over all the populations observed. The two other highly polymorphic loci were mainly two allele systems. Aconitate Dehydrogenase (ACO-1) was fixed at the fast allele in the Tulare County population but was nearly fixed at the slow allele in the Trinity County population. The populations from geographic areas located between these two as well as the Klamath County, Oregon and Chelan County, Washington populations had intermediate frequencies of each allele. The Trinity County population was also nearly fixed for the fast allele at the Isocitrate Dehydrogenase (IDH-2) locus. The percent polymorphic loci was highest in the Lassen County and Klamath County populations (45.5%) and lowest in the Trinity County population (9.1%). Mean heterozygosity ranged from 3.5% in Tulare County to 10.1% in Klamath County.

The UPGMA dendrogram (Fig. 2) shows three separate subpopulations within the *Ae. hexodontus* populations of California. One population from south central California consists of Mono County and Tulare County populations. Individuals from Alpine County, Lassen County and Shasta County (all in northern California) comprise another subpopulation. The Trinity County population also forms its own separate subpopulation. The Klamath County, Oregon population and the Chelan County, Washington population are grouped with the south central California population. The Distance Wagner dendrogram (Fig. 3) shows a stair-step pattern with the south central California populations being the most highly diverged and the northern California populations being the least diverged. Again, The Klamath County and the Chelan County populations are grouped near the south central California populations.

The PAUP analysis (Fig. 4) grouped the *Ae. hexodontus* populations into three subpopulations. One subpopulation consisted of Tulare County, Mono County and Alpine County Mosquitoes from Lassen County, Shasta County and Trinity County were

grouped into a second subpopulation with the third subpopulation comprised of the Chelan County and the Klamath County populations. However, these groupings were not as pronounced as those seen in the UPGMA clustering analysis.

## DISCUSSION

The subpopulations identified by the UPGMA dendrogram roughly correspond to the mountain ranges from which the mosquitoes were collected (Hinds 1952). Mono County and Tulare County encompass the southern part of the Sierra Nevada. The more northern populations are from the boundary region where the Sierra Nevada and the Cascade Range meet. The Alpine County population is unusual in that it is much farther south than either the Shasta County or Lassen County populations. It is not clear what forms the boundary between the northern and south central subpopulations in California. The Trinity County population appears to be isolated from all other populations. This collection came from the Trinity Alps in the Klamath Mountains which are separate from both the Sierra Nevada and the Cascade Range. The populations from Klamath County, Oregon and Chelan County, Washington are unusual in that they are grouped with the south central California populations. As these are from within the Cascade Range, they would be expected to be grouped with the northern California populations. This result may be due to the small sample size from these populations and the small number of loci observed in this study.

The Distance Wagner dendrogram shows a stair-step pattern which is evidence of restricted gene flow between all the populations observed in this study. This dendrogram suggests that *Ae. hexodontus* entered California during the glaciation of the Pleistocene Epoch and was subsequently stranded at high elevations in the Sierra Nevada as the glaciers receded. The restricted gene flow resulted in the stair-step pattern observed in the Distance-Wagner as the south central populations began to differentiate first and the more northern populations last. Thus, *Ae. hexodontus* appears to have spread in a different manner than *Ae. triseriatus*. Munstermann (1985) found no evidence of clinal variation in this mosquito in the eastern United States according to a UPGMA clustering analysis. However, the UPGMA clustering analysis of *Ae. hexodontus* populations do not suggest any evidence of clinal variation either. It may be that

Table 1. Allele frequencies at seven loci in eight populations of *Ae. hexodontus*.

Locus	Population (County)							
	Tulare	Mono	Alpine	Lassen	Shasta	Trinity	Klamath	Chelan
<b>IDH-2</b>								
(N)	18	12	24	24	11	12	9	8
A	.000	.000	.000	.021	.000	.000	.000	.000
B	.139	.125	.167	.146	.091	.042	.222	.188
C	.833	.875	.833	.833	.909	.958	.778	.813
D	.028	.000	.000	.000	.000	.000	.000	.000
<b>ME</b>								
(N)	18	9	24	24	11	12	9	8
A	.000	.000	.000	.083	.000	.000	.000	.000
B	.000	.056	.000	.000	.000	.000	.000	.000
C	1.000	.944	1.000	1.000	1.000	1.000	1.000	1.000
<b>HADH</b>								
(N)	12	6	19	24	11	12	9	8
A	.000	.000	.000	.000	.045	.000	.000	.000
B	1.000	1.000	1.000	1.000	.955	1.000	.889	1.000
C	.000	.000	.000	.000	.000	.000	.111	.000
<b>PGD-1</b>								
(N)	12	5	19	24	11	12	9	8
A	1.000	1.000	1.000	1.000	1.000	1.000	.944	1.000
B	.000	.000	.000	.000	.000	.000	.056	.000
<b>ACO-1</b>								
(N)	18	11	24	24	11	12	9	8
A	.000	.136	.500	.667	.591	.958	.000	.063
B	1.000	.864	.500	.333	.364	.042	.833	.938
C	.000	.000	.000	.000	.045	.000	.167	.000
<b>PEP</b>								
(N)	12	6	18	22	11	11	9	8
A	.000	.000	.000	.023	.045	.000	.000	.000
B	.000	.000	.083	.068	.000	.045	.000	.063
C	.917	.750	.833	.841	.909	.545	.889	.813
D	.000	.000	.000	.000	.000	.000	.000	.063
E	.042	.000	.000	.023	.045	.409	.111	.063
F	.042	.250	.056	.045	.000	.000	.000	.000
G	.000	.000	.028	.000	.000	.000	.000	.000

clinal variations are best detected using the Distance Wagner procedure. As with the UPGMA dendrogram, the Klamath County, Oregon and Chelan County, Washington populations are grouped near the south central California populations. Again, this may be an artifact of small sample size and the low number of loci studied.

The PAUP analysis corresponds very well to the geographic distribution of the *Ae. hexodontus*

populations observed in this study. This analysis grouped the Alpine County population with Tulare and Mono Counties. However, this subpopulation does not represent a strong grouping. Chelan County and Klamath County are also grouped together although this too is not a strong grouping. The northern California subpopulation consisting of Lassen, Shasta, and Trinity Counties show a somewhat stronger grouping. The PAUP dendrogram

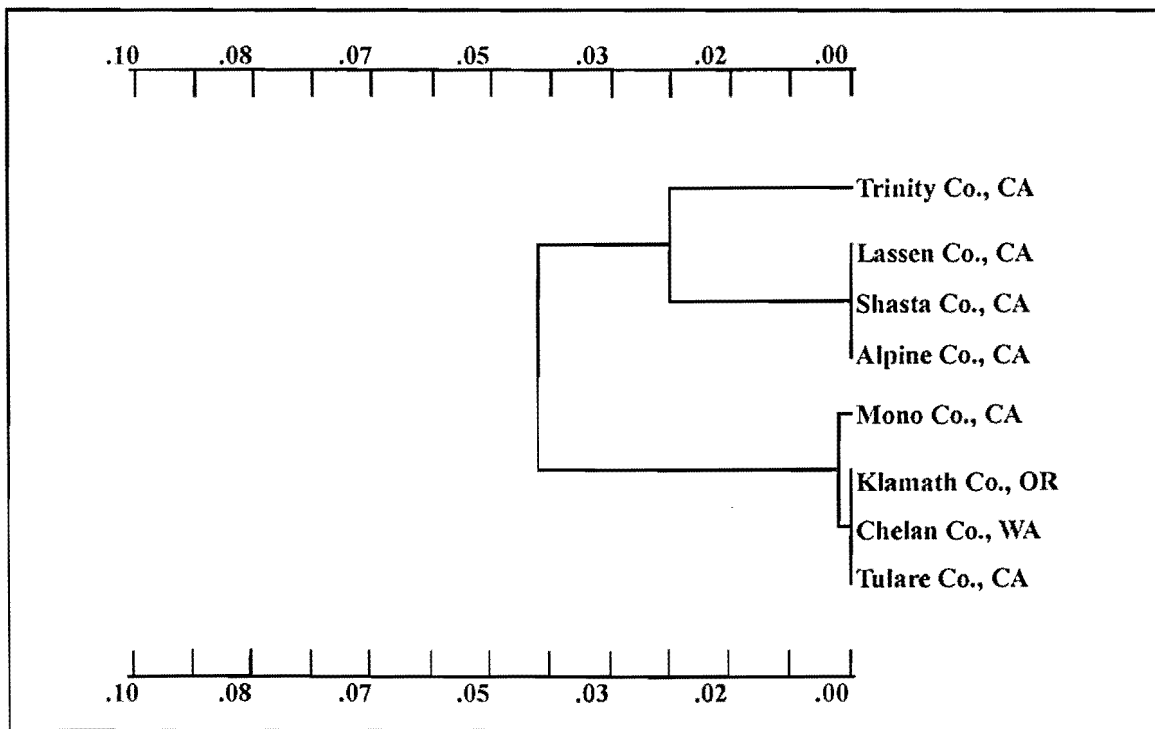


Figure 2. UPGMA phenogram of *Ae. hexodontus* populations produced using Nei's unbiased genetic distance.

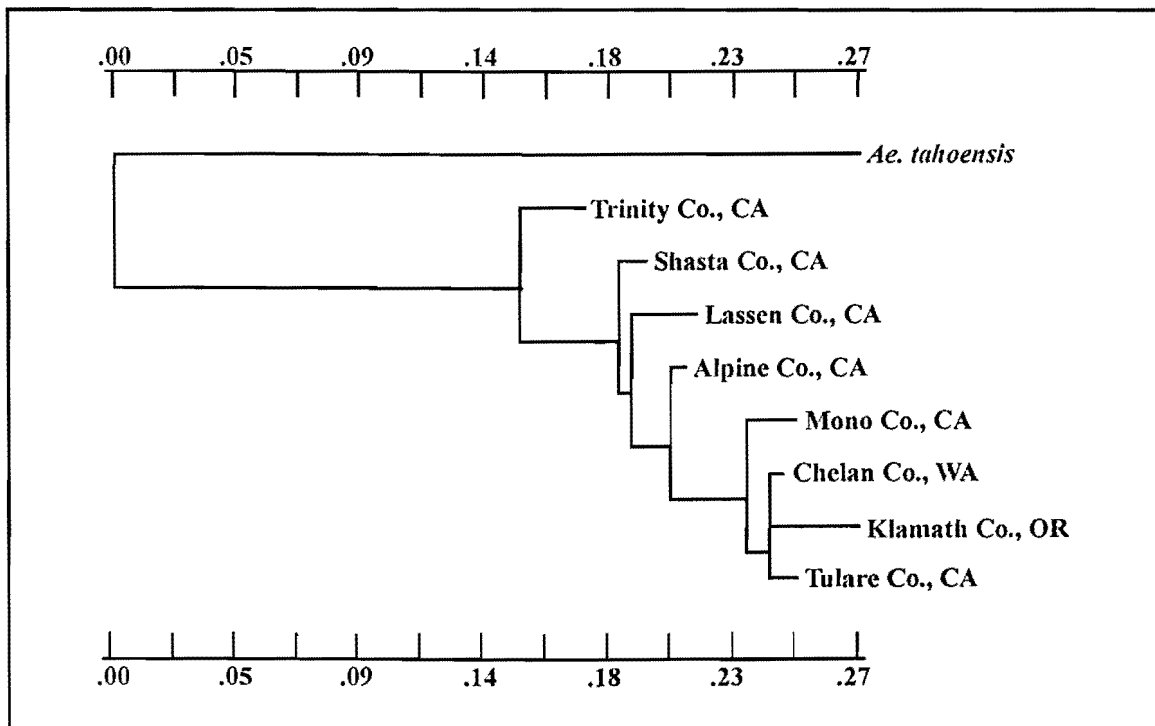


Figure 3. Dendrogram of *Ae. hexodontus* populations produced using Distance Wagner procedure and Rogers genetic distance coefficient.

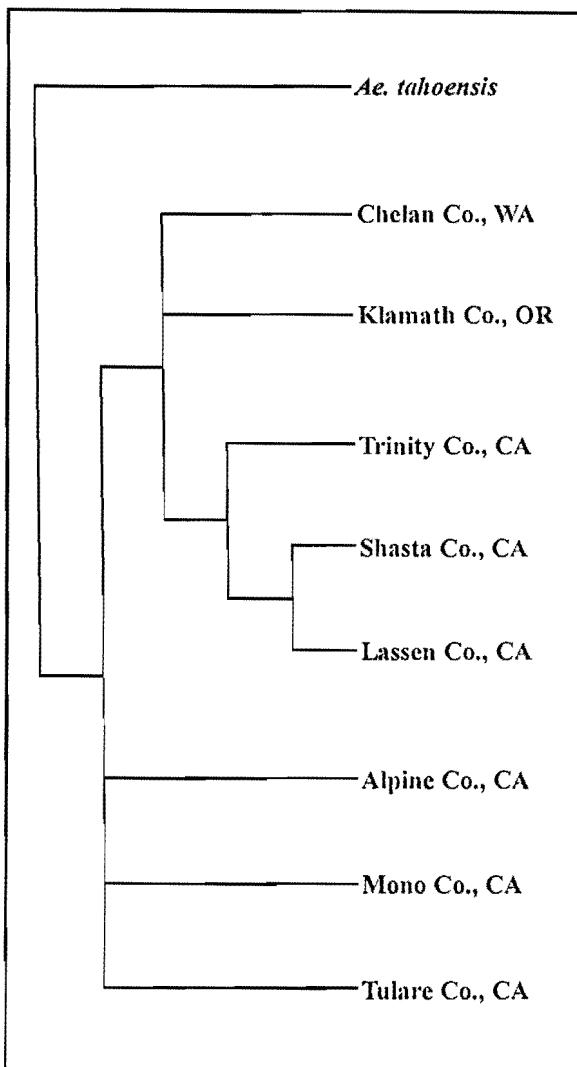


Figure 4. PAUP analysis of *Ae. hexodontus* populations using a strict consensus of 4 trees with *Ae. tahoensis* as an outgroup.

and the Distance Wagner dendrogram are similar in that both suggest that the Lassen County and Shasta County populations are more highly diverged than the Trinity County population. However, the Distance Wagner dendrogram suggests that the Trinity County population is the least divergent of all populations examined in the present study. As noted by Dr. Arthur Shapiro (personal communication), a PAUP analysis may not be the best method with which to analyze data from subpopulations of the same species. Because this analysis uses the presence or absence of an allele as a character state, a rare allele may have a strong effect on the shape of the tree.

The population substructure of *Ae. hexodontus* warrants further investigation. *Aedes hexodontus* is a widespread mosquito and restricted gene flow may have led to the formation of several subpopulations or even cryptic species as seen in the *Aedes communis* (DeGeer) complex (Brust and Munstermann 1992). In addition, *Ae. hexodontus* shows considerable geographic variation in morphology (Knight 1951, Wood 1977). Investigation of Rocky Mountain and Canadian populations will reveal more on the population substructure and historical patterns of migration of *Ae. hexodontus*. In addition, analysis of population substructure will provide a basis for determining variations in vector competence in *Ae. hexodontus*.

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## THE POPULATION STRUCTURE OF MOSQUITOES IN VARIOUS HABITATS ASSOCIATED WITH THE RICE CULTURE AGRO-ECOSYSTEM OF NORTHERN CALIFORNIA

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### ABSTRACT

The unlimited mosquito breeding habitats in rice agro-ecosystems and the increasing costs of vector control warrant new tactics for suppression of mosquito populations. It is particularly important to identify, with certain precision, where in the agro-ecosystem one should direct control measures. This agro-ecosystem provides various habitats for mosquitoes - rice fields, pastures, riparian and mixed habitats. The difference between such habitat types may affect the structure of adult mosquito populations.

Accordingly, the objectives of this study were to determine the distribution of adult populations of *Anopheles freeborni* Aitken and *Culex tarsalis* Coquillett in four habitat types during the summers of 1991 and 1992. In particular, we determined whether proportions of blood-fed females varied with abundance and whether mosquito abundance, blood feeding rates and sex ratios are associated with riparian, pasture, rice or mixed habitats within the rice culture agro-ecosystem of northern California.

The study involved weekly sampling of adult mosquito populations from walk-in red boxes during the summers of 1991 and 1992 in Sutter County, California. Sampling was done at three sites in each of the four habitat types. The definitions of habitat were based on field observations and confirmed using a satellite image of the study area taken on June 7, 1991, when all rice fields were fully flooded. The

land use patterns surrounding our trap sites was consistent throughout the study period. Habitat effect on mosquito abundance, blood-feeding rates and sex ratios were analyzed by a 2-way analysis of variance (ANOVA) and Tukey's (honestly significant difference) tests.

Significantly, high numbers of adult *An. freeborni* occurred in riparian and mixed habitats than in rice and pasture habitat (Tukey's test,  $p < 0.05$ ). Such a pattern was not evident for *Cx. tarsalis*. Riparian and pasture habitats had significantly higher proportions of blood-fed *An. freeborni* females than did rice and mixed habitat types, however, the proportions of blood-fed *Cx. tarsalis* females did not vary significantly between habitat types. The proportions of blood-fed *An. freeborni* ( $r^2 = 0.43$ ,  $p < 0.05$ ) and *Cx. tarsalis* ( $r^2 = 0.37$ ,  $p < 0.05$ ) females in riparian habitats decreased with increasing abundance. There was no correlation of blood-feeding rates and abundance of *An. freeborni* and *Cx. tarsalis* females for the other habitat types. The sex ratio of *An. freeborni* in pasture habitat was significantly female-biased ( $X^2 = 11.98$ ,  $df = 4$ ,  $p < 0.01$ ), unlike the other habitat types where it did not vary significantly from unity (1:1).

We conclude that riparian and mixed habitats contain highest densities of adult mosquitoes. Therefore, surveillance and control efforts should be focused on such habitats.



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