PROCEEDINGS AND PAPERS

of the

Fifty-third Annual Conference of the

California Mosquito and Vector Control Association, Inc.

January 27 thru January 30, 1985

Held at the
HILTON HOTEL
STOCKTON, CALIFORNIA

Editor — John C. Combs

Business Office
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Published February 14, 1986

Printed by
Anchor Press
Sacramento, California 95818

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SURVEILLANCE FOR ARTHROPOD-BORNE VIRAL ACTIVITY AND DISEASE IN CALIFORNIA DURING 1984

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This is the fifteenth (since 1969) in a series of annual reports made to the California Mosquito and Vector Control Association and published in the Proceedings and Papers of the Annual Conference. In last year's report we described a resurgence of activity by St. Louis encephalitis (SLE) virus, involving the Colorado River region (6 cases) and, unexpectedly, the occurrence of 3 cases of SLE in San Diego County (1 case) and Los Angeles County (2 cases) -- areas not previously considered at risk for this disease. The recognition of 2 equine cases of western equine encephalomyelitis (WEE) in Los Angeles County, one during 1983 and one which occurred in 1979 but was not documented until serologic tests were done on stored serum samples in 1983, also was unusual. The possibility that a change in the ecology of SLE and WEE viruses might have occurred or may have been overlooked in previous years, and that densely populated urban areas of southern California might be at risk of epidemic encephalitis, was of concern. Consequently, the surveillance effort during 1984 was expanded to the extent that funds were available to do so, and included a closer look at these more urban regions of the state as well as the traditionally endemic areas of the San Joaquin, Sacramento, and Imperial valleys.

Mosquito collections for virus isolation were increased, and extra sentinel chicken flocks were added to the network, so that 45 flocks, instead of the 31 flocks in 1983, were placed throughout the state, including new ones in Irvine (Orange County) and Harbor City (Los Angeles County). There was a single chicken seroconversion for WEE in Mecca (Riverside County) in late June, and one more at the same site in late July. As usual, some SLE and WEE virus isolations were made from mosquitoes along the Colorado River at Imperial and San Bernardino County sites. A total of 56 clinically suspect equine cases, reported from 17 California counties, were tested for WEE (38 tested serologically, 13 cases tested for virus in brain specimens and 5 cases tested by both serology and virus isolation attempts). No arboviruses were isolated and only one presumptive-positive WEE case was confirmed: a 7-year old horse from Fresno County with onset of illness on July 13, 1984, without any history of WEE vaccination in 1984. The complement fixation titers were 1:128 on serum samples taken on July 16 and August 10.

Except for these findings, the early and midsummer season was uneventful. However, sentinel chickens in the Irvine and Harbor City flocks in Late August unexpectedly showed seroconversions for WEE and/or SLE, and subsequently 3 isolations of SLE virus were made from *Culex tarsalis* mosquitoes collected in Harbor City September 13 and 18. Publicity about these findings and efforts to achieve better human case surveillance were followed by prompt recognition of several laboratory documented cases of SLE in the Los Angeles area. A more extensive surveillance effort followed, and by season's end it had revealed a total of 26 confirmed or probable cases of SLE, one fatal. Several other possible cases

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Table 1.-Number of mosquitoes and pools tested during 1984 by the Viral and Rickettsial Disease Laboratory Section by county and species. Source: Mosquito Virus Pool Data System, Vector Biology and Control Branch, Department of Health Services.

COUNTY	*Culex tarsalis*		*Cx pipiens* cpx		*Cx peus*		*Cx erythrothorax*		*Aedes melanimon*		OTHER SPECIES		TOTAL	
	MOSQ	POOLS	MOSQ	POOLS	MOSQ	POOLS	MOSQ	POOLS	MOSQ	POOLS	MOSQ	POOLS	MOSQ	POOLS
BUTTE	1,475	30	.	.	.	.	.	.	50	1	.	.	1,525	31
COLUSA	481	10	.	.	.	.	.	.	.	.	.	.	481	10
GLENN	250	5	.	.	.	.	.	.	.	.	.	.	250	5
IMPERL	14,989	337	.	.	.	.	.	.	.	.	113	3[a]	15,102	340
INYO	223	10	.	.	.	.	225	6	799	19	.	.	1,277	35
KERN	37,136	839	70	3	.	.	.	.	13,222	307	3	1[b]	50,431	1,150
LAKE	583	12	.	.	.	.	.	.	.	.	.	.	583	12
L A	1,446	36	1,256	32	90	4	281	7	.	.	4	2[c]	3,077	81
MARIN	1,197	25	.	.	.	.	.	.	.	.	.	.	1,197	25
MENDOC	.	.	.	.	94	2	.	.	.	.	.	.	94	2
MERCED	523	12	.	.	.	.	.	.	100	2	.	.	623	14
ORANGE	2,629	62	1,779	49	.	.	.	.	.	.	.	.	4,408	111
RIVERS	9,761	201	2,272	46	.	.	335	7	.	.	.	.	12,368	254
SACRA	9,588	199	.	.	.	.	15	1	.	.	.	.	9,603	200
SBERN	10,628	217	.	.	.	.	.	.	.	.	.	.	10,628	217
SHASTA	550	11	.	.	.	.	100	2	.	.	.	.	650	13
SOLANO	286	6	.	.	.	.	.	.	.	.	.	.	286	6
SONOMA	3,369	71	110	2	281	6	.	.	.	.	.	.	3,760	79
STANIS	166	6	46	2	.	.	.	.	13	1	.	.	225	9
SUTTER	1,955	39	.	.	.	.	.	.	.	.	.	.	1,955	39
TEHAMA	1,076	22	.	.	.	.	.	.	.	.	100	2[d]	1,176	24
TULARE	3,780	78	.	.	.	.	.	.	.	.	.	.	3,780	78
YOLO	12,028	243	.	.	.	.	.	.	118	4	.	.	12,146	247
YUBA	1,057	22	.	.	.	.	.	.	.	.	.	.	1,057	22
ARIZON	3,130	65	.	.	.	.	.	.	.	.	.	.	3,130	65
TOTAL	118,306	2,558	5,533	134	465	12	986	23	14,302	334	220	8	139,812	3,069

a. *Aedes dorsalis* b. *Culiseta inornata* c. *Culiseta incidens, C. inornata* d. *Aedes vexans*

could not be sufficiently documented to include in the official case count. The 26 cases were all apparently infected locally, not during travel to traditionally endemic areas of California or other states. Details of these cases and of the epidemiologic investigations are presented in a separate paper by Dr. R.A. Murry in these Proceedings. We were particularly helped in this search by the several county health departments in the region and by a private clinical laboratory (Microbiology Reference Laboratory) which detected the first recognized SLE cases serologically and which assisted extensively in the search for, and documentation of, further cases. Approximately 550 suspect human cases were screened serologically in this effort, including at least 352 by the Microbiology Reference Laboratory.

In total, 3,069 mosquito pools (139,812 mosquitoes) were tested during the season (Table 1). Of these pools, the majority were *Culex tarsalis*, as usual (2,558 pools, 83.3% of the total). Viruses isolated from the mosquito pools (Table 2) included 46 WEE, 25 SLE, 15 California encephalitis group, 68 Turlock, and 110 Hart Park -- 264 isolates in all. All of the WEE isolates, and all but 1 of the SLE isolates (*Aedes dorsalis*), were from *Culex tarsalis* mosquitoes. A detailed listing of individual positive mosquito pools was provided throughout the season in the 21 weekly bulletins mailed to survey participants. A final summary of these findings is available on a limited basis (R.W. Emmons).

There were 4,144 serum samples taken from chickens in the 45 sentinel flocks which were bled monthly from the end of May through October. All seroconversions occurred in flocks in southern California, except for a single WEE conversion in 1 Kern County flock (Table 3). Thus, despite the usual high *Culex tarsalis* populations throughout the state, evidence for WEE and SLE viral activity was nearly all from southern California urban and rural areas of San Bernardino, Riverside, Imperial, Los Angeles, Orange, and San Diego Counties.

The mosquito and sentinel chicken flock surveillance program was again aided by a grant from special U.C. Mosquito Control Research funds to the University of California, School of Public Health's Arbovirus Research Unit, which assigned Mary Ann Mahoney to the project.

A more intensive effort, particularly in southern California urban areas is anticipated

Table 2.-Number of viral isolates from mosquitoes tested during 1984 by the Viral and Rickettsial Disease Laboratory Section. Source: Mosquito Virus Pool Data System, Vector Biology and Control Branch, Department of Health Services.

SPECIES	COUNTY	WEE	SLE	TURLOCK	CALIF GRP	HART PARK	TOTAL
Culex tarsalis	BUTTE	.	.	2	.	1	3
	IMPERIAL	36	19	5	.	2	62
	KERN	.	.	12	.	77	89
	LAKE	.	.	3	.	.	3
	LOS ANGELES	.	3	.	.	1	4
	ORANGE	.	.	.	.	5	5
	RIVERSIDE	.	.	3	.	.	3
	SACRAMENTO	.	.	8	.	4	12
	SAN BERNARD	9	2	19	.	2	32
	SHASTA	.	.	1	.	1	2
	SONOMA	.	.	.	.	1	1
	SUTTER	.	.	3	.	.	3
	TEHAMA	.	.	1	.	1	2
	TULARE	.	.	.	.	5	5
	YOLO	.	.	4	.	9	13
	YUBA	.	.	.	.	1	1
	ARIZONA	1	.	7	.	.	8
		----	----	----	----	----	----
		46	24	68	0	110	248
Aedes melanimon	INYO	.	.	.	3	.	3
	KERN	.	.	.	12	.	12
		----	----	----	----	----	----
		0	0	0	15	0	15
Aedes dorsalis	IMPERIAL	.	1	.	.	.	1
		====	====	====	====	====	====
TOTAL		46	25	68	15	110	264

during the 1985 season. Earlier and more complete detection of viral activity and of encephalitis cases is needed, and better knowledge of the ecology of SLE and WEE viruses in these areas is essential to control and prevent potentially extensive epidemic disease.

ACKNOWLEDGMENTS.-We thank the many staff members of the Viral and Rickettsial Disease Laboratory, the Vector Biology and Control Branch, the Infectious Disease Section, and others in the California State Department of Health Services; all participating local Mosquito Abatement agencies; County Health Departments; the California Department of Food and Agriculture; private physicians and veterinarians; the Microbiology Reference Laboratory; and all others who helped in the surveillance program. This program was supported in part by special funds for mosquito research allocated annually through the Division of Agricultural and Natural Resources, University of California.

Table 3.-Serological conversions to WEE and SLE viruses in sentinel chickens, California, 1984.

Flock location	Seasonal average females per NJ per trap night*			Number (%) chickens positive							
				WEE					SLE		
	Cx. tars.	*Cx. quinq.*	*Cx. peus*	June	July	Aug	Sep	Oct	Aug	Sep	Oct
Kern, Wildife Refuge	2.7	0.0	0.0	0	0	0	0	1(6)	0	0	0
Southeast, Harbor Lake	2.7	1.4	0.4	0	0	0	0	ND**	2(13)	10(63)	NB
Orange, 20 Ranch Duck Club	1.9	0.5	0.2	0	0	1(6)	1(6)	1(6)	1(6)	1(6)	3(19)
San Bernardino, Needles	210.3	0.0	0.0	0	0	1(8)	1(8)	NB	1(8)	7(54)	NB
Coachella Valley, Mecca	56.3	3.3	0.1	1(6)	2(13)	2(13)	2(13)	NB	1(7)	5(33)	NB
Imperial, Palo Verde	10.2	1.0	0.0	0	0	2(13)	2(13)	NB	3(20)	9(60)	NB

* "Season" is disease weeks 14 through 44.

** Not bled.

All chickens were negative in May. The initial WEE seroconversion was found in the June sample; SLE positives did not occur until the August sample. 39 additional flocks (15 in the Sacramento Valley, 19 in the San Joaquin Valley and 5 in Southern California) remained negative throughout the season.

EPIDEMIOLOGIC ASPECTS OF THE 1984 ST. LOUIS ENCEPHALITIS EPIDEMIC IN SOUTHERN CALIFORNIA

Robert A. Murray[1], Laurie A. Habel[2], Katherine J. Mackey[3], Hazel G. Wallace[3], Barbara A. Peck[4], Susan Johnson Mora[5], Michele M. Ginsberg[6], and Richard W. Emmons[7]

INTRODUCTION.-In early October 1984, the Long Beach City Health Department in Los Angeles County, was notified by a private physician that a resident of that city with encephalitis had been shown by tests performed at a local private medical laboratory to have evidence of a recent infection with St. Louis encephalitis (SLE) virus. The health department subsequently learned that this laboratory had results from several other patients from southern California that also indicated recent infection with SLE.

INVESTIGATION METHODS.-Epidemiologists in county health departments throughout the area acted quickly to gather information to delineate the situation. Working with the patients' physicians and families, and often with the splendid cooperation of the private Microbiology Reference Laboratory (MRL) in Long Beach, they determined for each suspect case the pertinent medical findings, place of residence, date of illness onset and history of travel preceeding onset. Arrangements were made for the collection of blood and spinal fluid specimens from current cases and from patients who had recovered but retrospectively were considered possible SLE cases. Letters mailed to local hospitals and media publicity alerted physicians to the current situation and helped in the search for missed cases. Collected specimens were sent for preliminary testing at local public health laboratories, the MRL or other private laboratories and for confirmatory tests at the State's Viral and Rickettsial Disease Laboratory (VRDL). Tests were conducted on multiple serum specimens (whenever possible) from each suspect case using complement-fixation and indirect fluorescent antibody (IFA) methods for both SLE and western equine encephalomyeltis (WEE) virus antibodies. IFA tests for SLE and WEE specific IgM (early) antibody were performed when preliminary testing revealed the presence of SLE or WEE antibodies. A case was defined as an individual with signs or symptoms of an illness compatible with arboviral encephalitis and either a four-fold (or greater) increase in antibody titer to SLE (or WEE) antigens or the presence of IgM antibody to these agents.

RESULTS.-Between August 2 and October 21, 1984, 26 individuals had onset of clinical signs and symptoms and laboratory documentation of illnesses compatible with recent or current SLE virus infections in California. No human cases of WEE were detected in the state during 1984. Table 1 details the age, sex, location of residence, date of onset and place of probable contraction for each case in the order in which they came to the attention of public health authorities. The epidemic curve by week of illness onset and county of residence for each case are shown in Figure 1. Superimposed on this graph for reference are arrows pointing to dates when serum specimens from sentinel chicken flocks at Harbor Lake in Los Angeles County showed recent seroconversions to SLE on August 30 and September 21. Sentinel chickens in Irvine in Orange County seroconverted also sometime prior to specimen collections on October 11. Also referenced are dates when pools of *Culex tarsalis* mosquitoes collected from the Harbor Lake site on September 13 and September 18 were tested and found to contain SLE virus. Details of the findings and surveillance methods for arthropod-borne viral activity are presented in a separate paper by Dr. R. W. Emmons, et al. in these Proceedings.

All of these cases were residents of urban or suburban areas of southern California counties. Figure 2 shows the approximate residential location of each case within these counties and attack rates per million population are shown in Table 2. All but two of the cases had no history of travel away from their areas of residence. The two cases who had been in other areas of the state during the limits of the incubation period were considered unlikely to have acquired their infections in those other areas.

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Table 1.-Arboviral encephalitis cases (St. Louis encephalitis), California, 1984.

Case No.	Age	Sex	Residence City/(County)	Date of Onset	Place of Probable Contraction	Remarks
1.	52	M	Long Beach (Los Angeles Co.)	9/11/84	Los Angeles Co. area	
2.	59	M	El Monte (Los Angeles Co.)	9/19/84 (approx.)	Los Angeles Co. area	
3.	51	M	Anaheim Hills area (Orange Co.)	9/24/84	Orange Co. area	
4.	49	F	Reseda (Los Angeles Co.)	9/19/84 (approx.)	Los Angeles Co. area	
5.	28	F	Canoga Park (Los Angeles Co.)	10/1/84	Los Angeles Co. area (Possibly Mono Co.)	Camped near Devils Post Pile, 9/15/84.
6.	56	M	Westminster (Orange Co.)	10/5/84	Orange Co. area	
7.	78	F	Tustin (Orange Co.)	9/12/84	Orange Co. area	
8.	89	F	Long Beach (Los Angeles Co.)	10/5/84	Los Angeles Co. area	Husband negative for SLE in 10/29/84 spec.
9.	62	F	Long Beach (Los Angeles Co.)	10/2/84	Los Angeles Co. area	Husband negative for SLE in 10/31/84 spec.
10.	55	M	Perris/Palm Springs (Riverside Co.)	10/5/84 (approx.)	Riverside Co. area	Originally a suspect rabies case.
11.	44	M	Indio (Riverside Co.)	9/18/84	Riverside Co. area	
12.	42	F	Los Alamitos (Orange Co.)	10/10/84	Orange Co. area	
13.	54	F	Van Nuys (Los Angeles Co.)	10/1/84	Los Angeles Co. area	
14.	50	F	Los Angeles (Los Angeles Co.)	8/2/84	Los Angeles Co. area	
15.	79	F	Oceanside (San Diego Co.)	9/20/84	San Diego Co. area	
16.	19	M	Los Angeles (Los Angeles Co.)	9/21/84	Los Angeles Co. area?	Originally a suspect rabies case.
17.	32	M	Banning (Riverside Co.)	9/15/84	Riverside Co. area?	
18.	67	M	La Cañada (Los Angeles Co.)	9/28/84	Area of residence	Confined to home because of previous stroke.
19.	37	F	Long Beach (Los Angeles Co.)	10/21/84	Los Angeles Co. area	
20.	55	F	Hollywood (Los Angeles Co.)	10/5/84	Los Angeles Co. area	Lives part-time in Las Vegas, Nevada.
21.	57	M	Riverside (Riverside Co.)	9/6/84	Riverside Co. area (Possibly Shasta Co.)	In Shasta Co. until 8/23/84.
22.	19	F	Los Angeles (Los Angeles Co.)	10/4/84	Los Angeles Co. area	
23.	58	F	Pasadena (Los Angeles Co.)	10/5/84	Los Angeles Co. area	
24.	46	M	Pico Rivera (Los Angeles Co.)	9/17/84	Los Angeles Co. area	
25.	20	M	Orange (Orange Co.)	10/12/84	Orange Co. area	
26.	55	M	Palos Verdes Estates (Los Angeles Co.)	10/2/84	Los Angeles Co. area	

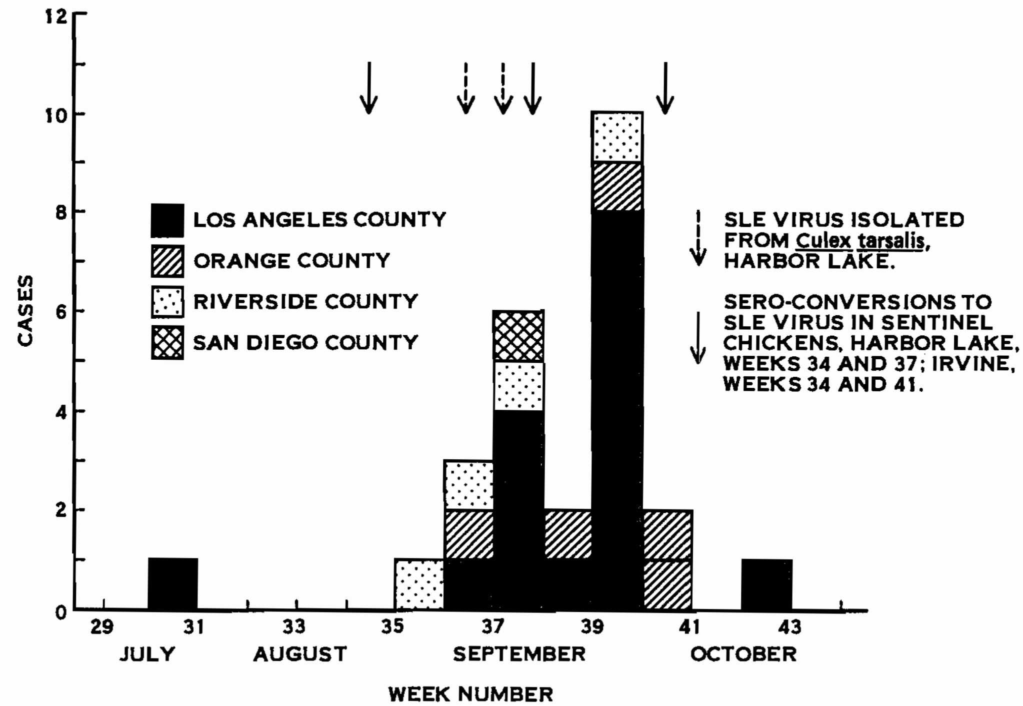

Figure 1.-St. Louis encephalitis by date of onset and county of residence, California, 1984.

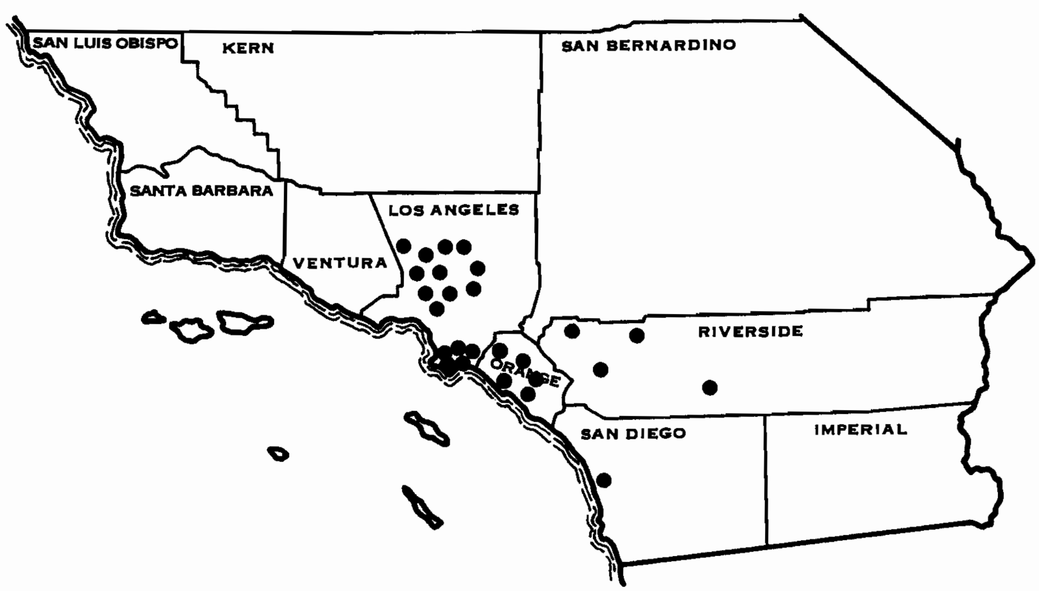

Figure 2.-St. Louis encephalitis by location of residence, California, 1984.

Table 2.-St. Louis encephalitis incidence by county of residence, California, 1984.

County of Residence	No. Cases	July 1, 1984 Population	Rate Per Million Population
Los Angeles	16	7,909,300	2.0
Orange	5	2,073,000	2.4
Riverside	4	775,200	5.2
San Diego	1	2,068,000	0.5
Total	26		

*Annual Estimates of the Population of California Counties, Report 84 E-2, State of California, Department of Finance, Population Research Unit, January, 1985.

Cases ranged from 19 to 89 years of age. The mean age was 50.5 years and the median age was 53 years. The majority of cases were males (14 males and 12 females) but this difference was not statistically significant.

One of the 26 confirmed SLE cases died on January 8, 1985 of complications resulting from her SLE infection. This victim was a 62 year old female resident of Long Beach who became ill on October 2, 1984.

Many suspect cases were also tested at the MRL, local public health laboratories and the VRDL but had insufficient or no evidence of recent or current infections with SLE virus or WEE virus. Included in this group were an 80 year old male resident of Van Nuys (Los Angeles County) who became ill on approximately September 30 and died on October 13 and a 67 year old male resident of Lomita (Los Angeles County) who became ill on October 5 and died on October 26.

DISCUSSION.-It was apparent from this epidemic that California's 1984 arboviral disease experience was a significant departure from that of past seasons and is a cause for concern that this new pattern of urban epidemic encephalitis may continue.

Figure 3 displays the number of human cases of WEE and SLE reported in California from 1950 to 1984. Extensive epidemics of WEE and SLE occurred in the state during the 1950's. This epidemic activity occurred in rural, agricultural counties in the central San Joaquin Valley area of the state.

While Los Angeles County reported several arboviral encephalitis cases in the 1950's, a review of records from that county indicated most were residents of San Joaquin Valley counties who were brought to Los Angeles for treatment. No Los Angeles County resident cases in the 1950's were found who did not have a history of immediately prior travel to endemic areas elsewhere in the state (Murray, unpublished data).

After 1959, confirmed human disease caused by WEE and SLE greatly decreased compared with the previous decade. Occurrences were largely limited to sporadic cases contracted in traditionally endemic areas of the state, namely rural areas the central California valleys, Imperial Valley and the lower Colorado River areas of Arizona and adjacent San Bernardino, Riverside and Imperial Counties.

In 1983, 6 of the 9 cases of SLE documented in California residents were associated with residence near or travel to the lower Colorado River areas of California and adjacent Arizona where extensive flooding and consequently increased mosquito activity had occurred. The other three cases in 1983, however, were residents of Long Beach and La Puente in Los Angeles County, and Vista in San Diego County. None had a history of travel away from these urban-suburban areas during the accepted incubation period for SLE. In retrospect, these three cases may have been harbingers of the epidemic activity that occurred the following season in 1984.

The 26 SLE cases documented in 1984 are the largest number of human mosquito-borne encephalitis cases recorded in the state since 1959 when 40 cases of SLE and 2 cases of WEE were reported. What makes the 1984 experience particularly unusual, though, is the distribution of locations where these illnesses were contracted. The majority of cases occurred in residents of heavily populated urban-suburban areas of three southern California counties. As shown in Figure 2, each dot represents the approximate residence location of a case. Because of the relative mobility of southern California residents in travelling to and from work or travelling for other reasons in the general area, it was not possible to pinpoint the precise location where these individuals were bitten by infected mosquitoes. Place of probable contraction in Table 1 is therefore listed as the county in which they resided. From the investigations carried out, however, it is believed that the majority of cases acquired infection in the vicinity of their residence. Thus 1984 represents an unprecedented departure from past experience with arboviral encephalitis in California in that the largest number of human cases to occur in 25 years were contracted in areas of the state never before associated with this disease.

The reasons for this new pattern of arboviral disease in California are unknown but are the subject of intensive investigation by various collaborating groups. One environmental factor temporally associated with this epidemic was an unusual weather pattern in southern California. While cases occurred during an approximately three month period, 24 of the 26 cases (92%) had onset of illness during the 6-week period from September 1 to October 13 and the median, or middle case, became ill during the week ending October 6. During this period, southern California experienced an unusually prolonged spell of very hot and humid tropical weather.

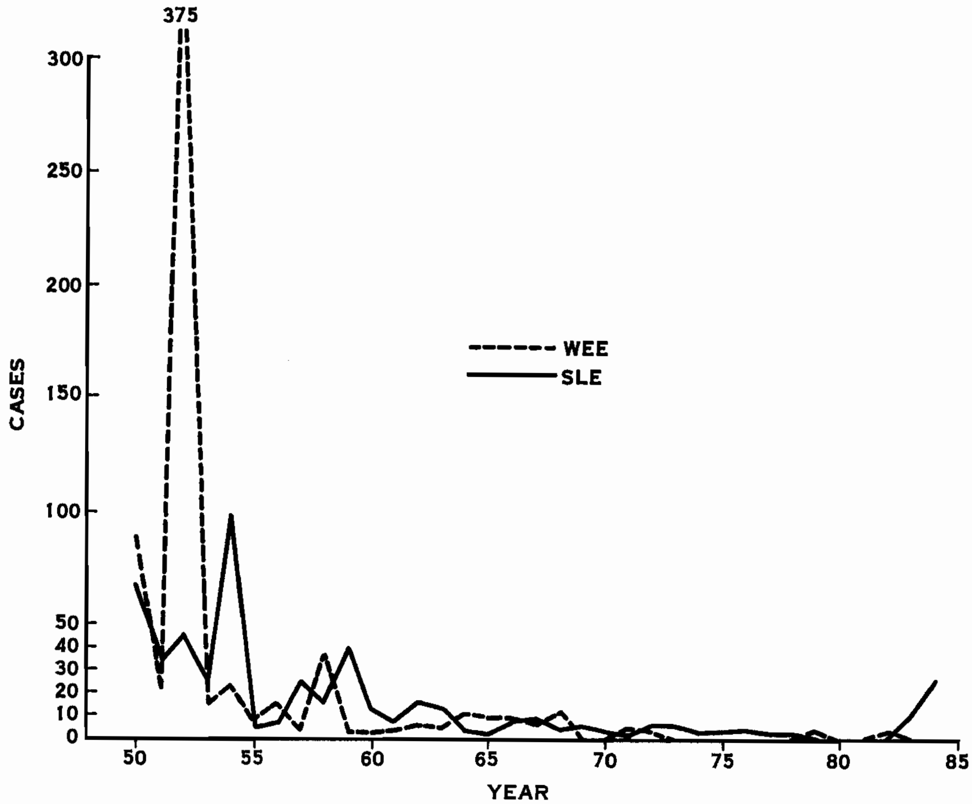

Figure 3.-Confirmed cases of western equine encephalitis (WEE) and St. Louis encephalitis (SLE), California, 1950 to 1984.

Among personal risk factors noted were several anecdotal reports by cases and their families that the individuals who contracted SLE were often in the habit of being outdoors during the evening dusk hours, periods when *Culex* mosquitoes are known to be most active. Interviews conducted by Los Angeles County Health Department epidemiologists indicated that 12 of the 16 cases in that county had a history of outdoors activity during evening hours in the two week period prior to onset of their illnesses. The one confirmed SLE infected patient who died was a woman who often worked in her yard during the evening while her husband (who was serologically negative for SLE) usually remained indoors. The significance of these findings is uncertain in the absence of data from controls who did not acquire SLE infections.

Although the crude attack rates for known cases in this epidemic are low, the actual impact of the epidemic was undoubtedly even more severe. The reported cases were all hospitalized (many for extended periods) and one died. Given the experience of other urban SLE epidemics where surveys found the ratio of infected persons to those with medically diagnosed encephalitis to be at least on the order of 200 to 1, it is likely that there were many additional cases of overt SLE illness including persons with encephalitis, meningoencephalitis or meningitis, and febrile headache, that did not come to the attention of public health officials.

In conclusion, this epidemic represents an apparent new pattern for arboviral disease in California. It shows an obvious need for ongoing surveillance and control programs to anticipate and prevent the buildup of epidemic encephalitis in the state, especially in urban areas of high population density.

COMPARISON OF A 1984 LOS ANGELES STRAIN OF SLE VIRUS WITH EARLIER CALIFORNIA STRAINS OF SLE VIRUS: MOUSE VIRULENCE, CHICKEN VIREMOGENIC, RNA OLIGONUCLEOTIDE AND VECTOR COMPETENCE CHARACTERISTICS[1]

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ABSTRACT

A 1984 isolate of St. Louis encephalitis (SLE) virus from the Los Angeles area did not differ significantly from 1954 and 1978 SLE viral isolates from Kern and Imperial Counties, respectively, in its peroral infectivity for *Culex* females or its ability to be transmitted perorally by infected *Culex* females. Several differences were noted, however, between these SLE viral strains in their mouse virulence, chicken viremogenic and RNase T_1 oligonucleotide fingerprint characteristics. *Culex peus* females from the Los Angeles area were the most competent vectors of indigenous and non-indigenous SLE viral strains, followed by *Culex tarsalis* and *Culex quinquefasciatus* females in that order, when they were incubated for 14 to 21 days at 25°C after ingestion of virus. The vector competence of *Cx. quinquefasciatus* and *Cx. tarsalis* females was greatly enchanced at an extrinsic incubation temperature of 32°C. Thus, given the unusually high mean daily ambient air temperatures of over 25°C during August and September 1984 in the Los Angeles area, *Cx. quinquefasciatus*, as well as *Cx. peus* and *Cx. tarsalis*, must be considered as potential vectors of SLE in the 1984 outbreak in the Greater Los Angeles area.

The transmission of St. Louis encephalitis (SLE) virus to humans in Western United States typically occurs in rural agricultural areas where *Culex tarsalis* is the principal mosquito vector and wild birds serve as hosts for viral amplification (Reeves and Hammon 1963; Monath 1980). Therefore, the human outbreak of SLE in urban Los Angeles in fall 1984 was unusual and might suggest that another mosquito vector was involved in the transmission-amplification cycle in this urban environment. One would naturally suspect mosquitoes belonging to the *Culex pipiens* Complex because they have been incriminated as the principal vectors of urban SLE in mid-western and eastern United States except in Florida where *Culex nigripalpus* is the epidemic vector. However, previous vector competence studies done in our laboratory indicated that populations of *Culex quinquefasciatus* from the San Joaquin and Coachella/Imperial Valleys were uniformly less susceptible to oral infection with indigenous strains of SLE virus than were sympatric populations of *Cx. tarsalis* (Meyer et al., 1983). Thus, if *Cx. quinquefasciatus* was an important vector of SLE in the Los Angeles outbreak, then populations in this area must be genetically more competent vectors of SLE virus than are other populations of *Cx. quinquefasciatus* evaluated in California. Alternatively, its vector competence for SLE virus may have been enhanced by the unusually high ambient temperatures that occurred in the Greater Los Angeles area in August and September 1984.

It is also possible that strains of SLE virus involved in the Los Angeles outbreak are more infectious for *Cx. quinquefasciatus* females or can be transmitted more efficiently by infected *Cx. quinquefasciatus* females than the SLE viral strains used in previous vector competence studies. Trent et al. (1981) reported that strains of SLE virus with different geographic origins can exhibit marked genetic variation by RNase T_1 oligonucleotide fingerprint analyses. Phenotypic variations observed for different strains of SLE virus include virulence for mice and monkeys (Monath et al., 1980), ability to produce viremias in House Sparrows and chickens (Bowen et al., 1980) and vector competence for *Cx. quinquefasciatus* (Mitchell et al., 1983).

Thus, the 1984 Los Angeles outbreak has raised several questions about the epidemiology of SLE in California that can not be answered satisfactorily from available data. Therefore, we initiated studies in the fall of 1984 to compare a) the biological and genetic properties of representative California strains of SLE virus and b) the vector competence of *Culex* species from the Greater Los Angeles area and Kern County for indigenous and

[1]These studies were supported in part by Research Grant AI-03028 from the National Institute of Allergy and Infectious Diseases, Contract MIPR-3504 from the U.S. Army Medical Research and Development Command and special funds for mosquito control research appropriated annually by the California State Legislature.

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non-indigenous strains of SLE virus. The results of these studies are reported here.

MATERIALS AND METHODS.-Three strains of SLE virus were selected for comparative studies: BFS 2035 from *Cx. tarsalis* collected in July 1954 at Tracy's Ranch, Kern County; IV 824 from *Cx. tarsalis* collected in July 1978 at Seeley, Imperial County; and SOUE 16-84 from *Cx. tarsalis* collected in September 1984 at Harbor Lake, Los Angeles County. Limited studies were done with 3 other SLE isolates from *Cx. tarsalis* collected in Kern County in July 1953 (BFS 1750 strain) and August 1958 (FMS 968 strain) and in Los Angeles County in September 1984 (SOUE 46-84 strain). Stock viruses were prepared as 10% homogenates of infected suckling mouse brains at intracranial passages 1 to 4. Virus was titered by plaque assay in monolayer cultures of African Green monkey kidney (Vero) cells maintained under a methyl cellulose nutrient medium (Davis and Hardy 1973).

Standard procedures were used for biological and genetic characterization of SLE viral strains. For virulence tests weanling Swiss albino mice were inoculated either intracranially (ic) or intraperitoneally (ip) with serial decimal dilutions of virus (5 mice/dilution/route of inoculation) and observed daily for 14 days for mortality. A viral strain was considered virulent if it was equally lethal for mice by ic and ip routes and avirulent if it was lethal by the ic but not the ip route (Monath et al., 1980). Viremogenic capacity was determined on 7 day old White Leghorn cockerels inoculated subcutaneously with virus and bled daily for 5 days after inoculation for viremia titer determinations by plaque assay in Vero cells and at 21 days postinoculation for antibody determinations by the indirect fluorescent antibody (IFA) test. Oligonucleotide fingerprint analysis of RNA genomes was done at the Center for Disease Control, Fort Collins, Colorado, by Dr. A. Vance Vorndam using standard procedures (Trent et al., 1981).

Culex mosquitoes for vector competence studies were collected during early November 1984 as late 4th instar larvae or pupae from various field breeding sites in Los Angeles and Kern Counties and transported in wet ice chests to the insectary in Bakersfield for emergence of adults. The Kern Colony of *Cx. quinquefasciatus* was used as a control. At 4-7 days after emergence, adult females were allowed to feed on pledgets soaked with a pool of defibrinated blood obtained from 5 chickens that had been infected subcutaneously with 1 strain of SLE virus when less than 24 hrs old and bled by jugular venipuncture 72 hrs after inoculation. Fed females were incubated at 25(±1)°C until they were evaluated individually at 14 and 21 days after feeding for ability to transmit virus by the capillary tube feeding method (Aitken 1977). Each mosquito and the contents of the corresponding capillary tube were tested for virus to determine infection and transmission rates respectively.

The effect of extrinsic incubation temperature on peroral transmission of SLE virus was determined with *Cx. quinquefasciatus* (Kern) and *Cx. tarsalis* (Yuma) females that were fed on a viremic blood preparation and then divided into 3 groups for incubation at 18°C, 25°C or 32°C. Transmission rates were determined at selected times after feeding by allowing each female to feed individually on a week-old chicken and then testing the 21-day post feeding serum samples for SLE antibody by a plaque reduction neutralization test.

RESULTS AND DISCUSSION.-Comparative studies were done to determine if a 1984 isolate (SOUE 16-84) of SLE virus from the Los Angeles area differed significantly from earlier California strains of SLE virus in its mouse virulence, chicken viremogenic, oligonucleotide fingerprint and vector competence characteristics. For comparative purposes a 1954 strain (BFS 2035) of SLE virus from Kern County was selected because it was isolated in a year when 99 human cases of SLE occurred statewide in California; and a 1978 strain (IV 824) from Imperial County was selected because it came from another geographically distinct area in Southern California and was isolated during a year in which only one human case of SLE was reported statewide.

Mouse virulence properties. Results of mouse virulence tests are presented in Figure 1. The 1984 Los Angeles County (SOUE 16-84) and 1978 Imperial County (IV 824) isolates have intermediate mouse virulence characteristics as compared to the highly virulent 1954 Kern County isolate (BFS 2035) and the relatively avirulent 1958 Kern County isolate (FMS 968). Another 1984 Los Angeles County isolate (SOUE 46-84) was also partially attenuated for mice (i.e., intermediate virulence) (Data not shown). Other studies done by Monath et al. (1980) and in our laboratory suggest that most California strains of SLE virus are moderately attenuated for mice by the ip route of infection. This includes several other strains of SLE virus isolated in Kern County during the 1950's when widespread human disease was documented. Thus, it appears that the 1984

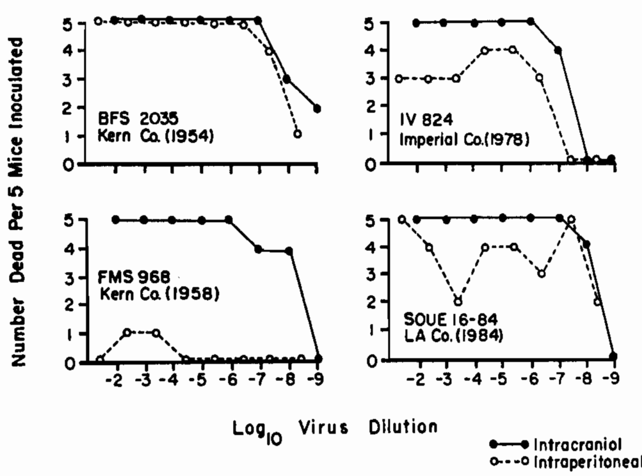

Figure 1.-Virulence of 4 California strains of SLE virus for weanling mice following intracranial and intraperitoneal inoculation.

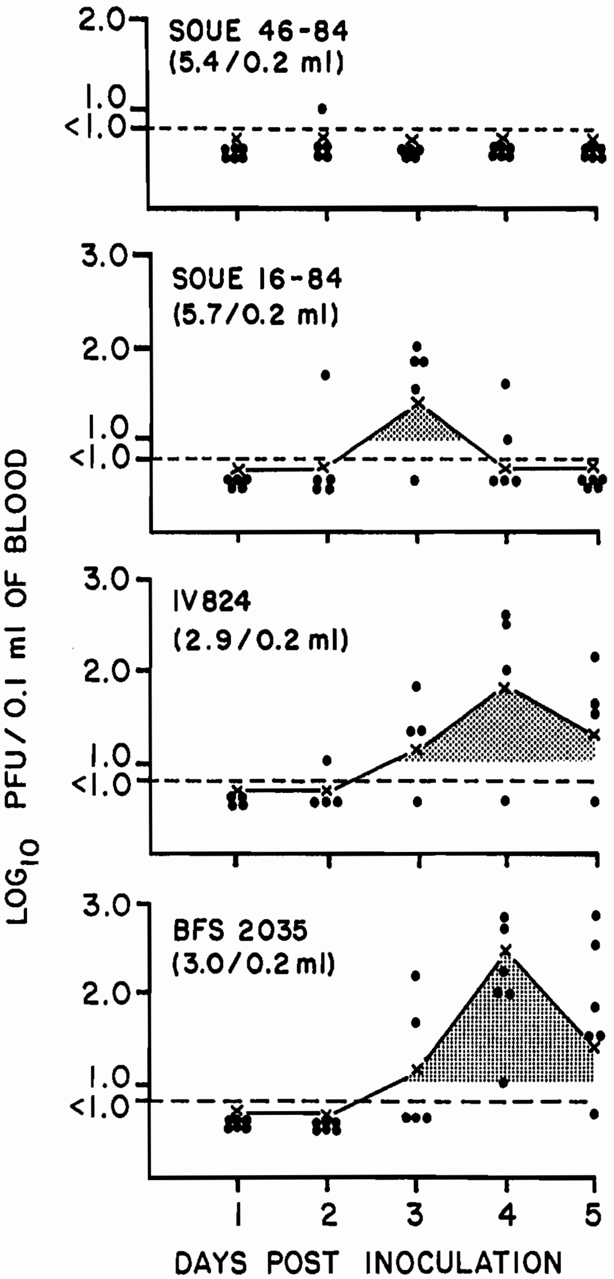

Figure 2.-Viremia profiles in 7 day old White Leghorn cockerels after subcutaneous inoculation with 4 California strains of SLE virus. Information in parenthesis is the $logarithm_{10}$ PFU of virus inoculated into each chicken. ● = viral titer in individual blood samples and x = mean viral in blood samples tested each day.

Los Angeles strains of SLE virus do not differ significantly from most California SLE isolates in their mouse virulent properties.

Chicken viremogenic properties. Viremia profiles in 7 day old chickens are depicted in Figure 2. Peak viremia titers occurred in chickens at 3 days after sc inoculation with SOUE 16-84 virus as compared to 4 days after inoculation with IV 824 and BFS 2035 viruses. Peak mean titers in chickens infected with BFS 2035 virus were about 5-fold and 10-fold higher than in chickens infected with IV 824 and SOUE 16-84 viruses respectively. However, chickens infected with SOUE 16-84 virus were inoculated with a significantly higher dose of virus and this could explain both the earlier appearance and lower titer of peak viremias (Bowen et al., 1980). Interestingly, viremia in chickens inoculated with the other Los Angeles isolate, SOUE 46-84, was detected in only 1 blood sample. All chickens became infected after inoculation with this virus since they all developed SLE antibodies. However, the viremia data with SOUE 46-84 may be misleading because we now know that this virus produces late-forming, indiscreet, minute plaques in Vero cells and thus the inoculated cell cultures may not have been incubated long enough in these tests for the plaques to fully develop. Nonetheless, plaque morphology is another phenotypic marker used to distinguish genetic differences between viral strains and SOUE 46-84 obviously differs from the other 3 SLE viral strains in this property.

Figure 3 depicts viral titers in the pooled 72 hr post infection viremic bloods obtained from chickens infected when less than 24 hrs old (i.e., wet chickens) with IV 824, SOUE 16-84 or BFS 2035 virus. It needs to be emphasized that the viral titer in a pooled blood would be similar to the blood sample in the pool with the highest titer and thus does not represent the mean titer of the 5 bloods in the pool. Nonetheless, it is evident that SOUE 16-84 and BFS 2035 viruses produced equally high viremia titers in wet chickens and

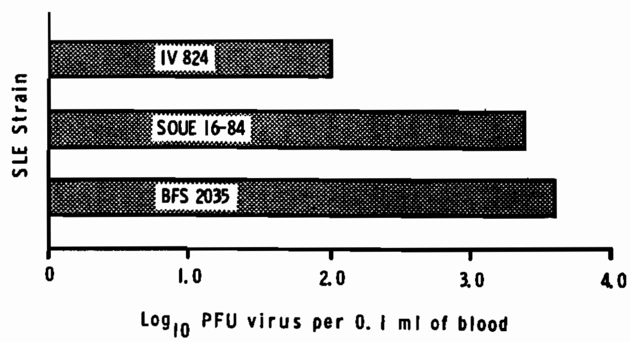

Figure 3.-Viremia titers in pooled defibrinated blood obtained 72 hrs after subcutaneous inoculation of wet chickens (i.e., < 24 hrs old) with approximately 10,000 PFU of the IV 824, SOUE 16-84 or BFS 2035 strains of SLE virus. Since titrations were done on the pooled blood, the viral titer is representative of the blood with the highest titer rather than the mean viral titer.

that these titers were higher than the highest viral titers produced by these viruses in 7 day old chickens (Figure 2). Peak titers in wet chickens infected with IV 824 virus were significantly lower than those produced by the other 2 viral strains in this age chicken. Thus, it is evident that chicken viremogenic capacity can differ from 1 SLE strain to another and that this capacity can be influenced by infecting dose of virus and the age of the chicken. Bowen et al. (1980) made similar observations when they infected nestling and adult House Sparrows with different geographical strains of SLE virus. In addition, the avian species may also influence the viremogenic capacity of an SLE viral strain. In previous studies, we found that a 1953 SLE isolate (BFS 1750) from Kern County produced high viremia titers in adult Tricolored Blackbirds, and House Finches, intermediate titers in adult Mourning Doves and low titers in adult House Sparrows. Thus, caution must be exercised when interpreting the viremogenic capacity of SLE viral strains in laboratory hosts.

Genetic relatedness of SLE viral strains. RNase T_1 oligonucleotide fingerprints were generated with RNA from the BFS 2035, IV 824 and SOUE 16-84 isolates. A fingerprint analysis revealed that there was only a 60% homology in oligonucleotides between the 1954 Kern County (BFS 2035) and 1984 Los Angeles (SOUE 16-84) isolates whereas there was a 90% homology between the Los Angeles isolate and 1978 Imperial Valley isolate (IV 834). It is not clear whether these differences represent a continuing evolution of an indigenous California SLE viral population over time or an independent evolution of isolated SLE viral populations in the different geographical areas from which these viruses originated.

Vector competence of *Culex* mosquitoes. An attempt was made to simultaneously compare the vector competence of each *Culex* species from the Greater Los Angeles area and Kern County for the SOUE 16-84, BFS 2035 and IV 824 SLE viral strains (Table 1). However, this was accomplished only with *Cx. quinquefasciatus* since a source of *Culex peus* was not available in Kern County and *Cx. tarsalis* collections from the 2 geographical areas were small and only allowed vector competence tests to be done with the indigenous viral strains.

At an extrinsic incubation temperature of 25°C, *Culex peus* derived from field collections was the most susceptible species to peroral infection with the SOUE 16-84 and BFS 2035 viral strains followed by *Cx. tarsalis* and *Cx. quinquefasciatus* females in that order (Table 1). How-

Table 1.-Results of comparative vector competence tests done with 3 *Culex* species and 3 California strains of SLE virus.

Mosquito Species and Source[1]	SLE (SOUE 16-84) Virus			SLE (BFS 2035) Virus			SLE (IV 824) Virus		
	Infection Rate	Transmission Rate 14 days	Transmission Rate 21 days	Infection Rate	Transmission Rate 14 days	Transmission Rate 21 days	Infection Rate	Transmission Rate 14 days	Transmission Rate 21 days
Culex peus									
Santa Fe Springs, Los Angeles County	77(107)[2]	11(31)[3]	47(45)	79(104)	25(36)	43(46)	45(109)	16(19)	45(29)
Culex tarsalis									
Santa Fe Springs, Los Angeles County	58(24)	14(14)	--	--	--	--	--	--	--
Hart Park, Kern County	--	--	--	40(69)	41(17)	55(11)	--	--	--
Culex quinquefasciatus									
Harbor Lake, Los Angeles County	9(75)	0(1)	0(6)	16(64)	0(4)	17(6)	3(75)	0(2)	--
Orange, Orange County	22(18)	0(2)	0(2)	--	--	--	--	--	--
Kern Colony	22(40)	0(0)	11(9)	32(40)	0(2)	45(11)	0(23)	--	--

[1]Except for Kern Colony, late 4th instar larvae or pupae were collected in the field during early November 1984 and adults emerged in the insectary.

[2]Percent females infected (number tested) after ingestion of virus from pledgets soaked with defibrinated, pooled viremic chicken blood (See Figure 3 for viral titers in viremic bloods). Females were incubated at 25(±1)°C for 14 to 21 days before being tested individually for virus.

[3]Percent of infected females that transmitted virus to capillary tubes (number tested).

ever, both viral strains were equally infectious for females of a single *Culex* species. Mosquito infection rates obtained with the IV 824 virus can not be compared directly with those obtained with the other 2 viral strains because of the lower titer of IV 824 virus in the viremic chicken blood fed to mosquitoes (Figure 3). Nonetheless, it is quite evident that *Cx. peus* females were also much more susceptible than *Cx. quinquefasciatus* females to peroral infection with this strain of SLE virus.

Peroral transmission rates determined after 14 or 21 days incubation at 25°C were similar for *Cx. peus* females infected with each of the 3 SLE viral strains and these rates were significantly higher than those obtained with field derived *Cx. quinquefasciatus* females that became infected after ingestion of these viruses. *Culex quinquefasciatus* females from the Kern Colony appeared to be more efficient transmitters of SLE virus than were field collected *Cx. quinquefasciatus* females. Although *Cx. tarsalis* females were less susceptible to peroral infection with SOUE 16-84 and BFS 2035 viruses than were *Cx. peus* females, the infected *Cx. tarsalis* females were equally or more efficient transmitters of SLE virus.

The data from the above vector competence studies corroborated other observations made in our laboratory with SLE virus and *Culex* species. *Culex peus* seems to be an excellent vector of SLE virus, a fact that has become evident only recently. Thus, this species needs to be considered as a potentially important epidemic and overwintering vector of SLE virus in those areas where it is abundant. In contrast, California strains of *Cx. quinquefasciatus* seem to be very incompetent vectors of SLE virus and therefore one might think that they were not involved in the recent SLE outbreak in Los Angeles. However, an extrinsic incubation temperature of 25°C was used in the vector competence studies. Previous laboratory studies using the BFS 1750 strain of SLE virus and the Yuma and Kern strains of *Cx. tarsalis* and *Cx. quinquefasciatus* respectively, indicated that both mosquito species were much more efficient transmitters of SLE virus when incubated at 32°C than at 25°C (Figure 4). Both species were unable to transmit virus at 18°C. Hurlbut (1973) also found that *Cx. quinquefasciatus* infected with SLE virus transmitted virus more efficiently at higher temperatures of extrinsic incubation. Ambient air temperatures in the Los Angeles area during August and September 1984 were above normal. For example, in the Long Beach area the average daily temperature exceeded 25°C on all but 5 days in the first 3 weeks of September, reaching a maximum average daily temperature of 32.5°C on September 6th. During this period infected female mosquitoes were most likely exposed to temperatures in excess of 25°C for several hrs each day. Under these conditions, *Cx. quinquefasciatus* may have been excellent vectors of SLE virus and thus should be considered as potential vectors during the 1984 Los Angeles outbreak of SLE. Certainly, more refined vector competence studies need to be done with SLE and *Culex* mosquitoes using cyclic rather than constant daily temperatures.

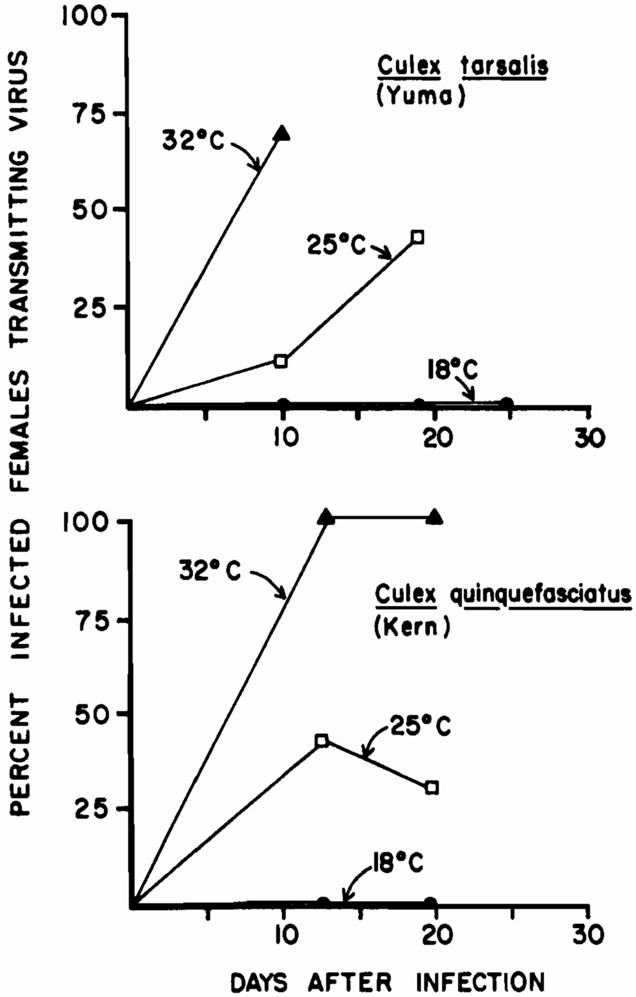

Figure 4.-Effect of extrinsic incubation temperature on the peroral transmission of SLE (BFS 1750) virus by *Culex* mosquitoes. *Culex tarsalis* and *Cx. quinquefasciatus* females ingested 10 and 1,000 PFU of virus, respectively, from pledgets soaked with defibrinated viremic chicken blood. Transmission was determined by feeding each female on an individual chicken and demonstrating specific SLE antibody in the chicken serum obtained 21 days after the mosquito fed.

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ECOLOGICAL EVIDENCE OF ST. LOUIS ENCEPHALITIS VIRUS TRANSMISSION IN SOUTHERN CALIFORNIA[1]

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Patrick Ryan,[4] Gerald Greene,[5] Noor Tietze[5]

On Friday 16 September 1983, a 70 year old retired aerospace engineer was uprooting plants in his garden in suburban Long Beach where he had been resident for many years. He began to feel badly but honored a dinner engagement at a harborside restaurant where, after an hour's wait for a table he became unusually irritable. On driving home he complained of nausea and brightness of lights (photophobia). On arrival home at 10:30 P.M. his temperature was 101.4°F.

The following day his complaints were not severe enough to call for another temperature determination. He ate only cold cereal. Lassitude appeared to be his only problem. On Sunday afternoon he noticed fibrillation of muscles in his left arm and lower extremities. Monday morning he was visibly unstable and sought advice from his family physician of 45 years. Physical support by a nurse was required to get him into the office where it was decided to terminate the medicine he had been taking for hypertension, as a possible cause of his unstable condition. On return home from this visit his temperature was 101.0°F.

He had a sleepless night but spoke rationally in conversation. However, his mobility was so abnormal that he lay on the floor. By afternoon three persons were required to lift him into a wheel chair, where he began to speak irrationally of his employment five years before. By late afternoon, with a fever of 104°F and mental aberrations he was admitted to a Long Beach hospital where he drifted through delirium into coma. A spinal fluid specimen drawn the following day showed 80 WBC with a 7% PMN and 93% lymphocyte differential.

Examination on 6 October revealed a semicomatose patient who could respond to some stimulation. Serological tests confirmed by HI and CF that he was suffering from St. Louis encephalitis. This was the index case of urban-suburban St. Louis encephalitis in Long Beach.

Extensive and intensive subsequent investigations involving Southeast and Riverside Abatement Districts failed to establish any likely exposure other than a two hour midday visit with his son at La Sierra on 8 September. It was reluctantly concluded that this was an unexplainable case whose exposure could have been at home, not far from Lakewood Park Country Club Golf Course in Long Beach. More than a year later, this patient is virtually bed ridden (Long Beach Press Telegram, 1984) although he is communicative and responsive to family and visitors.

The occurrence of the Long Beach cases in September 1984 have been documented previously (Murray, 1985). One lived within a few city blocks of the 1983 index case. History of exercise within El Dorado Park was elicited from another patient. One 1984 fatal case in Long Beach accumulated $320,000 in medical and hospital costs before she died. The economic cost of urban-suburban St. Louis encephalitis has not been generally recognized. Schwab (1968) calculated the cost of the 1966 Dallas epidemic at more than $795,500. Inflation would amplify today's costs to more than two million.

The role of wild birds in the amplification and dispersion of St. Louis encephalitis virus in urban-suburban epidemics is well documented (Work 1963, Stamm 1963). In the 1964 epidemic in Houston we isolated four strains of SLE virus from blood of captured birds in one week. (Blue Jay, mockingbird, pigeon, and goose). This was against a background of 11% SLE antibody prevalence in 2,215 birds including 70 species collected in a three month period. This spread of SLE antibody in avian hosts probably had substantial dampening effect on progression of the epidemic (Lord, Work, et al. 1973).

In the 1984 Southern California epidemic (Murray, 1985), limited sentinel chicken antibody conversions and *Culex tarsalis* isolations failed to signal widespread SLE virus transmission until a month after onset of the earliest recognized human case. Allowing for an incubation period of 10 to 14 days, exposure occurred about the middle of July, long before the celebrated excessive hot spell of September. This indicated a dispersion and amplification in the wild bird cycle as early as May, either from an overwintering focus involving the 1983 index case or reintroduction of SLE virus from somewhere else in Southern California.

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[1]For presentation at Conference of the California Mosquito and Vector Control Association, Stockton, California, 28 January 1985.

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[5]California State University, Long Beach, California.

When the geographical extent of the epidemic was finally recognized in October it became incumbent on those with available expertise to attempt to probe the avifauna of the region for evidence of what avian species were involved. This is much the same way a detective works for evidential fingerprints after a crime is committed, before the evidence is wiped away.

Having available experienced and knowledgeable persons (who co-authored this presentation) a field effort was organized to mist net and capture indigenous birds to collect blood samples for hemagglutination inhibition (HI) tests. Because the highest attack rate of more than one case per 100,000 people was recognized in the City of Long Beach, another site was selected at El Dorado Park in the eastern part of the city. A workable ecological site at the San Joaquin Marsh of the University of California Reserve System at UC Irvine, in Orange County, was identified as a location where SLE antibody conversion had occurred in the course of the epidemic (Figure 1). The County Comparative Medical and Veterinary Services took care of the area of eastern Los Angeles. Unfortunately, the limited field resources could not cover two other localities; Harbor Lakes, where serological conversions and SLE virus isolations had occurred, and the cluster of cases in central San Fernando Valley.

Patterns of mist nets were established in the San Joaquin Marsh and at El Dorado Park in eastern Long Beach as indicated in Figure 2. The passerine birds captured were bled from jugular veins. Larger resident and migrant water birds were captured or shot and some resident pigeons trapped. Four hundred and twenty-five sera have been tested to date. The diluted serum specimens were transported to UCLA for the HI testing (Work and Jozan, 1977) with results presented in Tables 1, 2, 3 and 4.

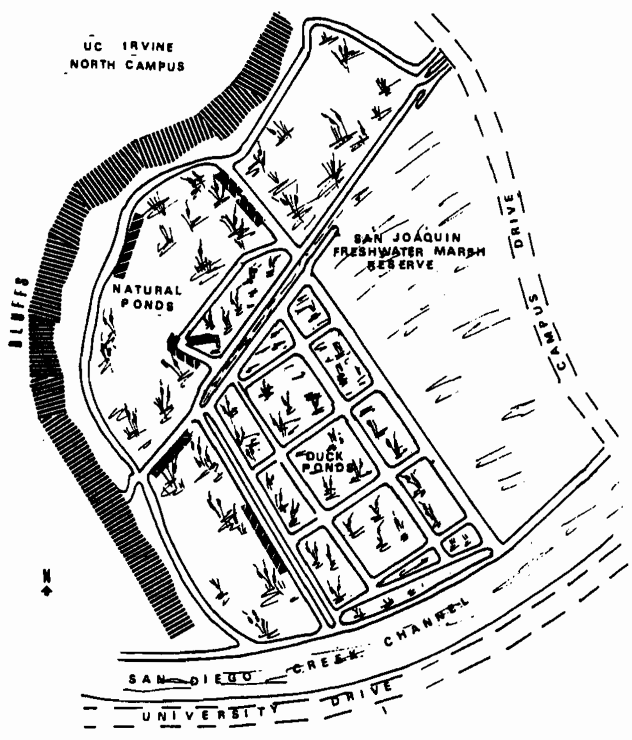

Figure 1.-Mist net locations in University of California Reserve, San Joaquin Marsh, Orange County, William Bretz, Steward.

It should be noted that with many of the smaller birds only .2ml of blood could be obtained, diluted to 1.0ml in rabbit serum diluent. The lowest titer hemagglutination inhibition obtainable was therefore no more than 1:40. Those reactions recorded as positive for all specimens were at least 1:20.

Some interpretation of the avian species sampled is in order. The lateness of the effort in view of the seasonal fall migration southward was considered important and directed the effort toward birds known to be resident, close to case occurrence. Some of the human cases actually frequented parks in Long Beach which is widely recognized as having more public parks and recreational areas per square mile, or 100,000 population, than any city of similar size in the country. Of these parks only three could be sampled. El Dorado, Lakewood and Heartwell which encompassed lakes and ponds containing Anseriformes. Mist nets were deployed only in El Dorado. Where pigeons were trapped it was known that they were of local flocks with a circumscribed range.

Of special interest were the coots because some summer breeders had migrated south to be replaced by logarithmic increases in winter populations from the north. As expected, most of the coots shot in localities of substantial SLE AB prevalence in resident avian species were essentially negative. The occasional positive probably represents either an early arrival in August or September when virus was being transmitted or are resident breeders of East Los Angeles and Long Beach which never left. These winter migrants represent an important sentinel population for detection of winter transmission. Selected winter avian populations require more careful study at another time. Significant antibody prevalence was found in resident duck, geese and pigeons but not in winter migrant coots.

The residence status of species collected at the San Joaquin Marsh University Reserve at U.C. Irvine was derived from observations by Ms. Gertrude A. Siptroth of the Sea and Sage Branch of the National Audubon Society (Siptroth, 1973). The substantial prevalence of SLE antibody in indigenous species contrasts, as anticipated, to the virtually negative results in the coots collected for us by Mr. Richard Jamison of the 20 Ranch Duck Club, which extends eastward from the U.C. Irvine University Reserve.

Two significant results emerged. There was significant incidence of SLE HI antibodies in resident wild and feral birds. The distribution of significant SLE HI antibodies was scattered, but adjacent to, localities of case occurrence or sentinel flock conversion. Of greater interest was

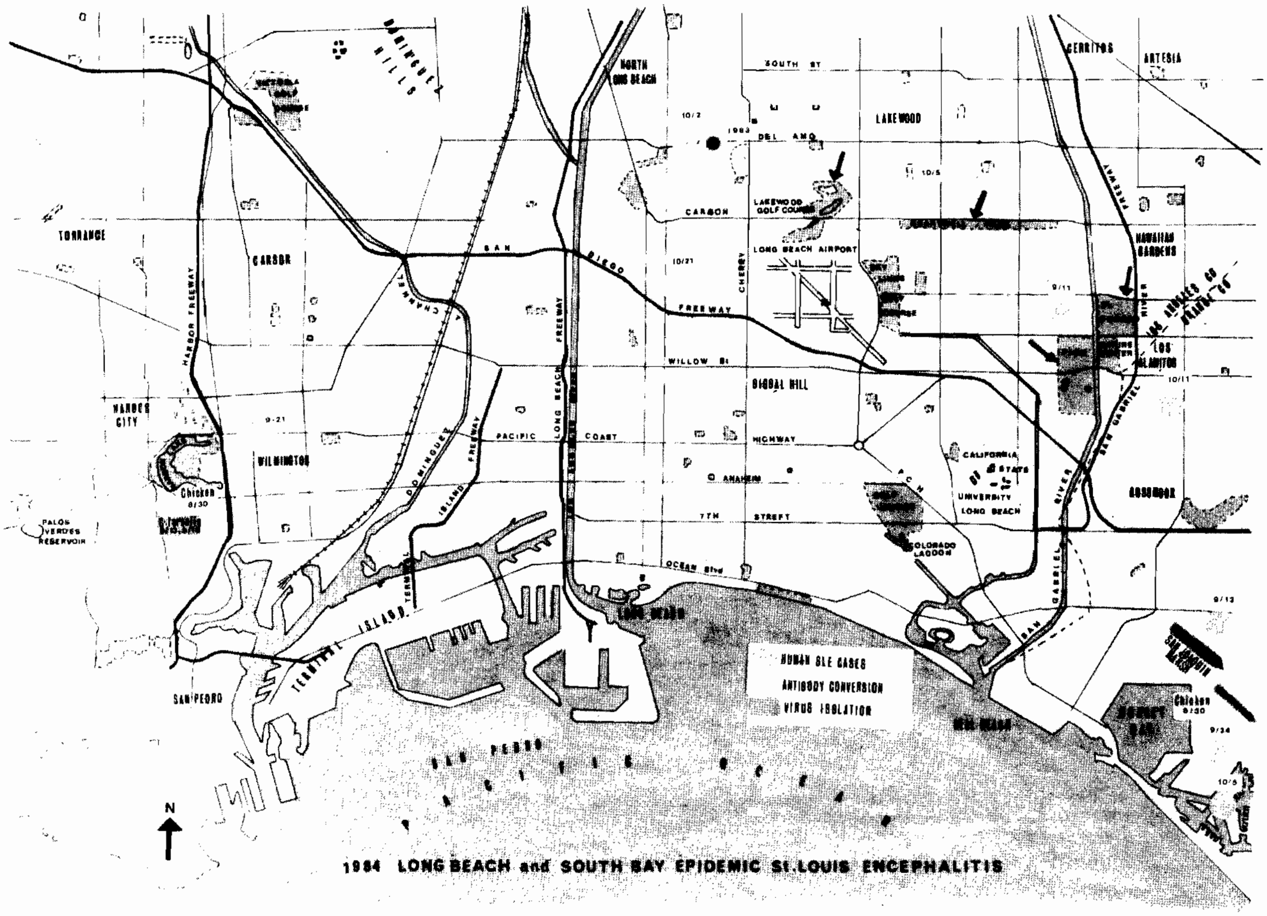

Figure 2.-Mist net patterns in the San Joaquin Marsh and at El Dorado Park in eastern Long Beach.

the association of these indigenous serological sentinels to continued light trap collections of *Culex pipiens quinquefasciatus* mosquitoes at the Long Beach and Orange County foci, as reported by Webb (1985). Recovery of *C. p. quinquefasciatus* has continued in collections in December and January reflecting favorable winter breeding conditions.

The epidemiological incrimination of *C. p. quinquefasciatus* as a potential vector to urban residents is in contrast to previously documented insignificance of this species as a vector of SLE in California. Thirteen years of research in Imperial Valley (Work, 1980) had established that SLE virus was transmitted annually by *C. tarsalis* in the Finney Lake focus and several years work on the New River had resulted in SLE virus isolations from *C. P. quinquefasciatus* which became the suspected vector of the virus to man (Magy, et al. 1976, Work et al., 1977a, 1977b, Webb, et al. 1977). Pursuit of basic mechanisms for *C. p. quinquefasciatus* transmission of SLE virus in California ended in 1980 when support for continuing these investigations was terminated.

Since 1954, when *C. p. quinquefasciatus* was identified as the vector of SLE in the Lower Rio Grande Valley (Chin, et al., 1957), this species has been found to be the vector in all urban-suburban SLE epidemics from New Jersey to the Mississippi. It is not surprising that the impact of human population pressure in the Southern California communities has produced conditions in sewage disposal and recreational area irrigation favorable to massive increases in *C. p. quinquefasciatus* breeding and activity adjacent to and among the urban and suburban dwellers of Southern California (White, 1985), similar to what happened in Houston and Dallas, Texas in 1964 and 1966 (Work, a cooperative study, 1965, Sudia, et al., 1967, Henderson, et al., 1970, Schwab, 1968).

Now that *C. P. quinquefasicatus* has once more emerged as the likely urban-suburban vector in Los Angeles and Orange Counties, questions relevant to disease control require scientific answers.

Has the threshold of *C. p. quinquefasciatus* populations supporting SLE virus transmission been reached, or surpassed?

Table 1.-Prevalence of HI antibodies to St. Louis encephalitis virus in birds collected at Irvine Marsh, Orange County, California, 1984.*

Resident			Non Resident		
Species	Number Collected	Number Positive*	Species	Number Collected	Number Positive
Song Sparrow	26	11(42.3%)	White-Crowned Sparrow	10	2(20%)
Yellowthroat Warbler	6	0	Golden-Crowned Sparrow	5	0
House Finch	2	0	Rufous-Crowned Sparrow	2	0
Black Phoebe	5	4(80%)	Audubon Warbler	4	2(50%)
Wren	1	0	Yellowtail Warbler	1	0
Ruby-Crowned Vireo	1	0	Bushtit (M/R)	2	1
Loggerhead Shrike	1	0			
Western Kingbird	2	1(50%)	TOTAL	24	5(20%)
TOTAL	53	16(32.2%)			
Resident Duck	4	1(25%)	Non Resident Coot	39	2(5.1%)

* Collections made throughout October and November, 1984.
** Positive with an HI titer of 1:20 and over.
M/R Migrant and Resident.
HI: Hemagglutination-inhibition.

Is this species involved in overwintering maintenance of SLE virus locally?

Is SLE virus now reintroduced annually by the classic bird-*Culex tarsalis*-bird cycle from somewhere like the established spring foci in Imperial Valley?

Where in Southern California might the crossover from *C. tarsalis* to *C. p. quinquefasciatus* occur?

How can the most sensitive and informative sites for surveillance by mosquito isolations and wild bird serum conversions be identified?

What resources for a sensitive pre-epidemic surveillance mechanism will be required?

How important is extension of mosquito control to presently uncovered areas: An how can it be done, including the surveillance role of the Mosquito Abatement districts?

Where are the key avian species instrumental in chains of events leading to epidemic dispersion of SLE virus?

And finally, which of the diverse ecological niches in which *C. p. quinquefasciatus* breeds and feeds are the critical foci for vector control?

These questions cannot be answered exclusively in the laboratory with domesticated mosquito populations. They will require long and intensive studies where the disease has occurred, and where it may occur again, even on a regular basis.

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Table 2.-Prevalence of HI antibodies to St. Louis encephalitis virus in birds collected at El Dorado Park, Long Beach, California, 1984.

Resident			Non Resident		
Species	Number Collected	Number Positive*	Species	Number Collected	Number Positive
Song Sparrow	4	1	Ruby-Crowned Sparrow	1	0
Yellowthroat Warbler	3	0	Golden-Crowned Sparrow	5	0
Ana's Hummingbird	1	0	White-Crowned Sparrow	5	1
Black Phoebe	2	1	Audubon Warbler	2	0
House Finch	4	0	Bushtit (M/R)	5	0
Loggerhead Shrike	1	0	Hermit Thrush	6	2
Mocking Bird	1	1	Solitary Vireo	1	0
			Lesser Goldfinch	7	1
	TOTAL 16	2(12.5%)			
				TOTAL 32	4(12.5%)

HI: Hemagglutination-inhibition.
M/R: Migrant and Resident.
* Positive with HI titer of 1:40 and over.

Table 3.-Prevalence of HI antibodies to St. Louis encephalitis virus in birds collected at Long Beach, California, 1984.

Species	El Dorado Park Golf Course	El Dorado Park Nature Center	Heartwell Park	Lakewood Country Club
Coot	NS	0/5*	NS	5/54
Mallard	0/2	5/13	0/5	NS
Muscovy Duck	0/1	NS	0/2	NS
Resident Ducks	1/4	4/15	0/1	2/2
Resident Geese	1/2	3/26	0/1	NS
TOTAL	2/9(22.2%)	12/59(20.3%)	0/9	7/56(12.5%)

*Number of birds Positive with and HI titer equal or superior to 1:20/number collected.
HI: Hemagglutination-inhibition.

Table 4.-Prevalence of HI antibodies to St. Louis encephalitis virus in birds collected in Los Angeles County, California, 1984.

Area	Ducks	Geese	Coots	Pigeons	Other Non Passerine	Passerine
Lancaster	NS	NS	NS	NS	1/1*	NS
Castaic	NS	NS	NS	NS	1/1	NS
San Fernando	NS	NS	NS	NS	1/5	NS
Granada Hills	NS	NS	NS	2/5	NS	3/10
Torrance	1/1	NS	NS	NS	NS	NS
Whittier	7/13 Adult 0/8 4 weeks	NS	NS	NS	NS	NS
Marina Del Rey	0/11	1/6	NS	1/6	NS	NS
Los Angeles	NS	NS	NS	1/1	NS	NS
Long Beach (downtown)	NS	NS	NS	6/7	NS	NS
Carson	2/16	0/2	NS	NS	0/2	NS
Downey	0/1	0/1	NS	NS	0/2	NS
Santa Fe Dam	NS	NS	9/22	NS	NS	NS

Collections made from October 1984 through January 1985.
HI: Hemagglutination-Inhibition.
*Number with Positive HI titer of 1;20 and over/number collected.

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RESPONSE OF LOCAL AGENCIES TO THE 1984 URBAN ST. LOUIS ENCEPHALITIS OUTBREAK IN ORANGE AND LOS ANGELES COUNTIES

James P. Webb, Jr.[1] and Charles M. Myers[2]

INTRODUCTION.-The 1983 human cases of St. Louis encephalitis (SLE) in Los Angeles County occurred in Long Beach and La Puente and established the second recorded endemic SLE cases from the Los Angeles basin; one case was reported in 1957 from Los Angeles County. Seven human SLE cases were documented in California in 1983, six of which were associated with living near or traveling along the lower Colorado River and one was correlated with virus transmission in northwestern San Diego County. St. Louis encephalitis and/or western equine encephalitis (WEE) virus activity is demonstrated regularly each year along the lower Colorado River, particularly in the Palo Verde Valley and the nearby Imperial Valley. These nine SLE cases represented the largest number of cases in California since 1963 when 12 cases were documented.

SENTINEL CHICKEN FLOCKS.-The 1984 encephalitis arbovirus surveillance season was relatively uneventful until early September when sera collected from sentinel chickens cooped at Harbor Lakes (Los Angeles County) and 20 Ranch Duck Club (Irvine, Orange County) yielded significant titer levels of SLE antibodies. One other sentinel chicken from the Irvine flock also seroconverted for WEE virus. Both flocks were bled on the 30th of August; the last blood collection was made during the week of July 23-31. Tests conducted on sera obtained from the Harbor Lakes flock on the 21st of September produced eight additional birds positive for SLE antibodies. Blood collected on the 11th of October from the Irvine flock produced two more seroconversions. Sentinel chicken flocks had been placed at the 20 Ranch Duck Club and Harbor Lakes sites in July of 1983, but no seroconversions for SLE or WEE antibodies were obtained during the surveillance period through September.

AGENCIES' RESPONSE. Control Operations.-The immediate response to the first notice of the 1984 SLE seroconversions in sentinel chickens by both the Southeast Mosquito Abatement District (SEMAD) and the Orange County Vector Control District (OCVCD) was to increase the mosquito control procedures at all known and suspect mosquito breeding sites. Both aerial and ground applications of insecticides were performed within a week of the confirmation of seroconversion for SLE in the sentinel chickens. Normal seasonal larviciding activities with Dursban (1%) granules (D, Figures 1, 2, 3, 4) were extended into the months of September and October (1984) in the mosquitoes' wetland production sites with the exception of the San Joaquin Marsh where Bti (5%) pellets (Bti, Figures 1, 3) were applied. Ground application of a pyrethrin fogging concentrate (3%) for adult mosquito control (P, Figures 1, 3) was also conducted in the San Joaquin Marsh, 20 Ranch Duck Club, Mason Park Marsh ("Mini-Marsh"), and adjacent wetlands in Irvine.

Mosquito Collection/Virus Surveillance.-Concurrently, both districts increased and expanded their adult mosquito collection programs. Modified CO_2/CDC light traps were employed at traditional and many new collection sites. The trapped mosquitoes were identified, pooled, and sent to the California Department of Health Services, Viral and Rickettsial Disease Laboratory at Berkeley for viral analysis. Pools of *Culex tarsalis* (encephalitis mosquito) females collected from the Harbor Lakes area on the 13th and the 18th of September tested positive for SLE virus; no viral isolates were made from the Orange County mosquito pools.

MOSQUITO ACTIVITY DATA. Surface Sites.-Examination of the New Jersey trap data obtained at the 20 Ranch Duck Club and the Harbor Lakes sentinel flocks during the 1984 mosquito season indicated that female *Culex tarsalis* numbers reached an average of 10.0 per trap night ($\bar{x}$ PTN) at the 20 Ranch Duck Club and 9.2 at Harbor Lakes during the month preceding the August 30th blood collection. Mosquito collection data from the New Jersey trap situated at the San Joaquin Marsh (approx. 0.5 miles west of the 20 Ranch Duck Club) indicated a peak of 120 ($\bar{x}$ PTN) female *Cx. tarsalis* during late July in 1984 (Figure 1); a peak of 120 ($\bar{x}$ PTN) female *Cx. tarsalis* occurred in the middle of July in 1983 (Figure 2). New Jersey trap data obtained from 14-16 sites around Orange County (exclusive of the San Joaquin Marsh) also illustrated a similar peak of *Cx. tarsalis* activity in July of 1984 (Table 1). In 1983, *Cx. tarsalis* numbers were high in May and June as well as July. A comparison of *Cx. tarsalis* activity data from 1975-1984 (Table 1) suggests that an increase in *Cx. tarsalis* numbers began in 1982 (possibly as early as 1981) and continued through 1984.

Culex quinquefasciatus (southern house mosquito) reached their peak numbers at the San Joaquin Marsh in August, September, and October in 1983 (6.5 females $\bar{x}$ PTN; Figure 4) and 1984 (3.0 females $\bar{x}$ PTN; Figure 3). Since 1974, Orange County *Cx. quinquefasciatus* populations have usually reached their peak in the autumn and early winter months (Table 2), although in 1981, June, July, and August there were high population levels as well.

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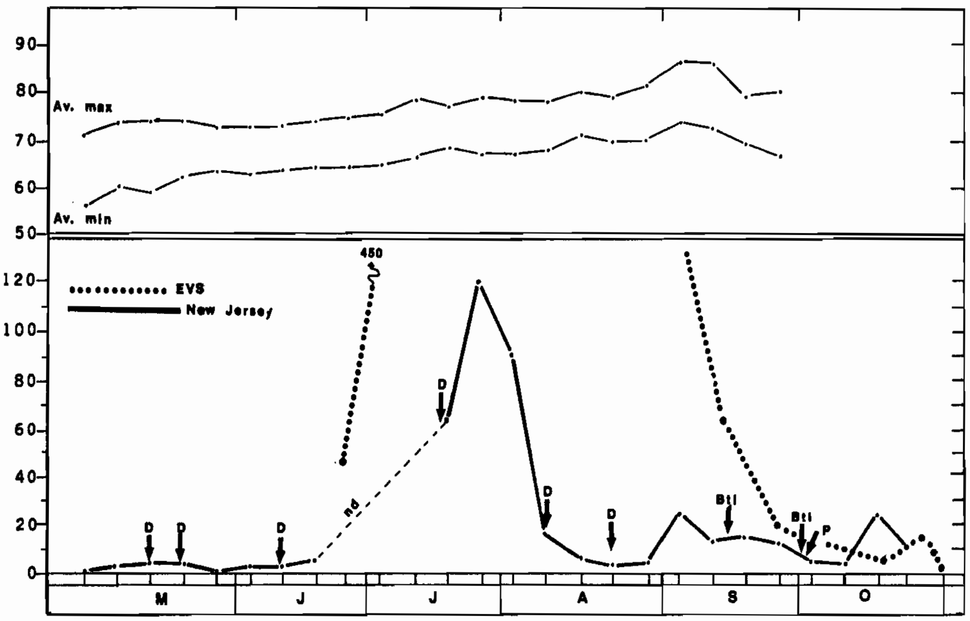

Figure 1.-The occurrence of female *Culex tarsalis* from May through October (1984) at the San Joaquin Marsh (Irvine); temperatures were recorded from Newport Beach Harbor as the average maximum and average minimum on a weekly basis.

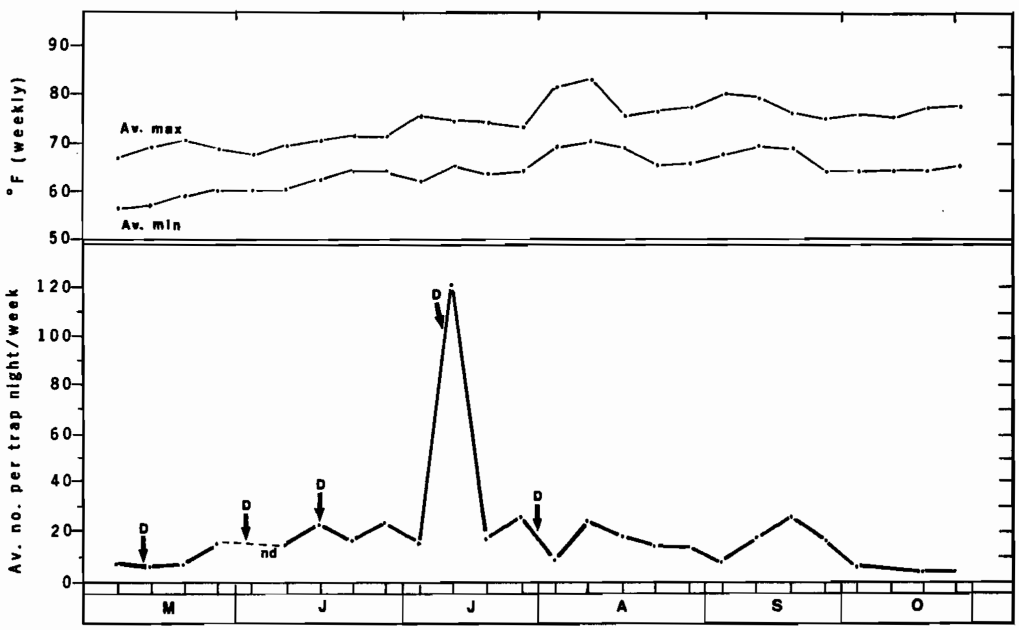

Figure 2.-The occurrence of female *Culex tarsalis* from May through October (1983) at the San Joaquin Marsh (Irvine); temperatures were recorded from Newport Beach Harbor as the average maximum and average minimum on a weekly basis.

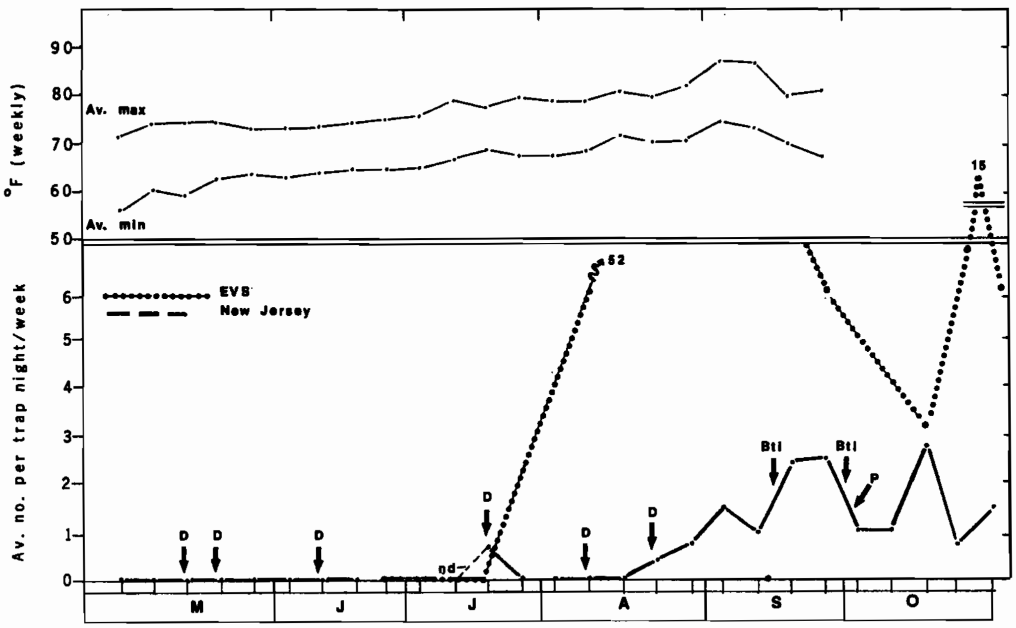

Figure 3.-The occurrence of female *Culex quinquefasciatus* from May through October (1984) at the San Joaquin Marsh (Irvine); temperatures were recorded from Newport Beach Harbor as the average maximum and average minimum on a weekly basis.

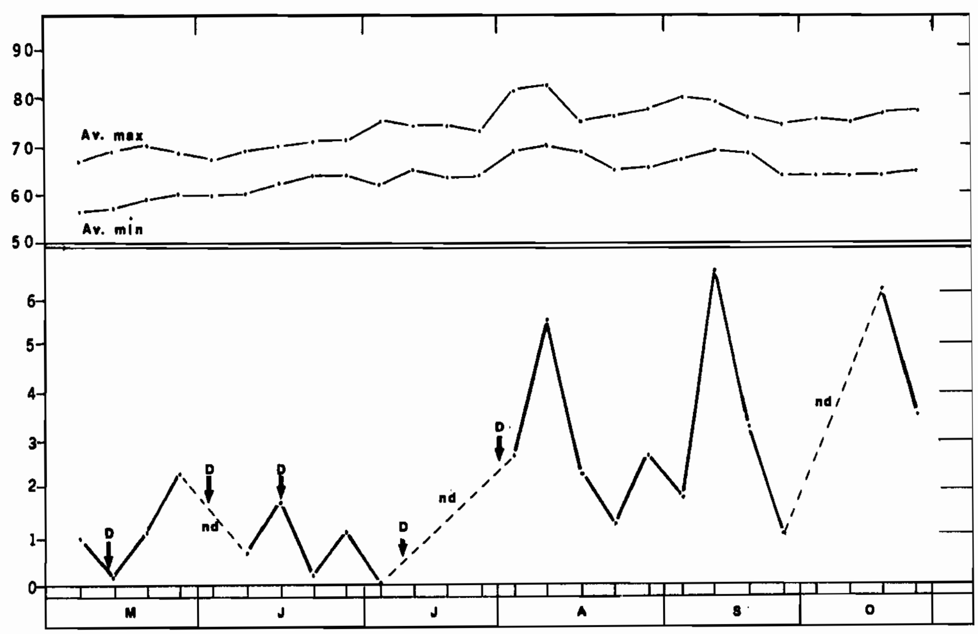

Figure 4.-The occurrence of female *Culex quinquefasciatus* from May through October (1983) at the San Joaquin Marsh (Irvine); temperatures were recorded from Newport Beach Harbor as the average maximum and average minimum on a weekly basis.

Table 1.-Average number of ♀♀ *Cx. tarsalis*. Orange County (excluding San Joaquin Marsh).

	M	J	J	A	S	O
1984	0.2	0.9	1.2	0.6	0.2	0.4
1983	0.8	1.2	0.9	0.4	0.7	0.2
1982	0.3	0.5	0.4	0.1	0.1	0.1
1981	0.2	0.9	0.1	0.2	0.2	0.0
1980	0.2	0.1	ND*	ND	0.0	0.0
1979	0.2	0.2	0.1	0.1	0.2	ND
1978	0.3	0.5	ND	ND	0.2	0.2
1977	0.0	0.2	0.3	0.2	0.4	0.1
1976	0.0	0.2	0.2	0.1	0.2	0.1
1975	0.0	0.2	0.2	0.1	0.1	0.0

*ND = No Data

Table 2.-Average number of ♀ *Cx. quinquefasciatus* per trap night. Orange County (excluding San Joaquin Marsh).

	A	M	J	J	A	S	O	N
1984	0.02	0.02	0.04	0.04	0.24	0.41	0.27	0.20
1983	0.12	0.14	0.09	0.06	0.22	0.39	0.29	0.15
1982	0.01	0.04	0.06	0.07	0.04	0.04	0.05	0.25
1981	0.01	0.06	0.21	0.41	0.69	0.16	0.07	0.02
1980	0.01	0.01	0.01	0.02	0.01	0.00	0.04	0.04
1979	0.05	0.03	0.02	0.02	0.20	0.12	0.02	0.01
1978	0.07	0.04	0.08	0.05	0.03	0.07	0.20	0.05
1977	0.02	0.02	0.04	0.08	0.03	0.20	0.06	0.03
1976	0.01	0.02	0.03	0.03	0.14	0.09	0.15	0.11
1975	0.02	0.01	0.03	0.03	0.02	0.02	0.01	0.09

Table 3.-Average number of ♀♀ *Cx. quinquefasciatus* per trap night - underground drainage.

1983 (1984)	SANTA ANA (ORANGE COUNTY)	PICO RIVERA (LOS ANGELES COUNTY)
J	11 (5)	2 (1)
F	4 (122)	0 (4)
M	6 (92)	2 (30)
A	6 (541)	1 (8)
M	73 (111)	2 (30)
J	199 (438)	165 (184)
J	97 (358)	161 (136)
A	23 (505)	28 (75)
S	131 (346)	148 (76)
O	26 (70)	14 (46)
N	28 (4)	12 (9)
D	10 (1)	3 (20)

Underground Sites.-Underground storm drains in Orange and Los Angeles counties yielded *Cx. quinquefasciatus* specimens throughout the year in 1983 and in 1984 (Table 3; M. S. Dhillon, unpub. data). The 1984 records indicate relatively high numbers of southern house mosquitoes from February through October, particularly in the Santa Ana undergound site in Orange County.

Wild Bird Blood Arbovirus Surveillance.-In October and November of 1984, Drs. Telford H. Work and Martine Jozan from the School of Public Health, University of California, Los Angeles were invited to assist in the investigation of SLE and WEE virus transmission in Orange County. Drs. Work and Jozan quickly set up a bird collecting and blood sampling program at the San Joaquin Marsh and associated adjacent wetlands. Using Hemagglutination Inhibition techniques to determine the presence of antibody titers to SLE virus in the collected bird sera, they were able to show significant levels of SLE antibodies in resident song sparrows (11/26 = 42%), black phoebes (4/5 = 80%), ducks (1/4 = 25%), and western kingbirds (1/2 = 50%). A similar program was initiated by the UCLA research group at the El Dorado Park in Long Beach (Los Angeles County) in cooperation with Dr. Hazel Wallace (Long Beach City Public Health Department).

PHYSICAL CONDITIONS. Temperature.-The influence of relatively high temperatures (80°F) has been suggested by a number of researchers to be significant in the transmissibility of arbovirus by mosquitoes during the late summer and early fall months. Meteorologists have documented a worldwide warming trend during the past decade and the years of 1983 and 1984, in particular, have had average temperatures significantly above normal (Figure 5) in the Los Angeles Basin through the months of July, August, and September.

Precipitation.-Rainfall patterns (Figures 6, 7) from the April through October (1980-1984) demonstrated near normal to below normal amounts of precipitation. In 1983, however, April experienced 2-4 inches above normal; 1.5-2 inches above normal fell in the early autumn. Overall, 1983 was an exceptionally wet year, averaging from 15-19 inches above normal in the Los Angeles Basin. In contrast, 1984 was a very dry year (4-6 inches below normal).

Discussion.-Although studied (Olson 1977), no well-defined explanation has yet been advanced that can clearly correlate the coincidence of ambient temperatures above 80°F occurring for several consecutive days and the appearance of human SLE cases usually during the months of July, August, September, and October. Two feasible hypotheses have emerged in attempts to explain the high mean daily temperature/SLE cases phenomenon. One suggests that warmer temperatures induces more people to leave windows and doors ajar or to spend more time outside in the evening, thus, increasing the chances of exposure to more mosquitoes. The availability of air conditioning may also be an important factor in this regard. The other indicates that vector competency increases relatively in *Cx. tarsalis, Culex peus* (foul water mosquito), and *Cx. quinque-*

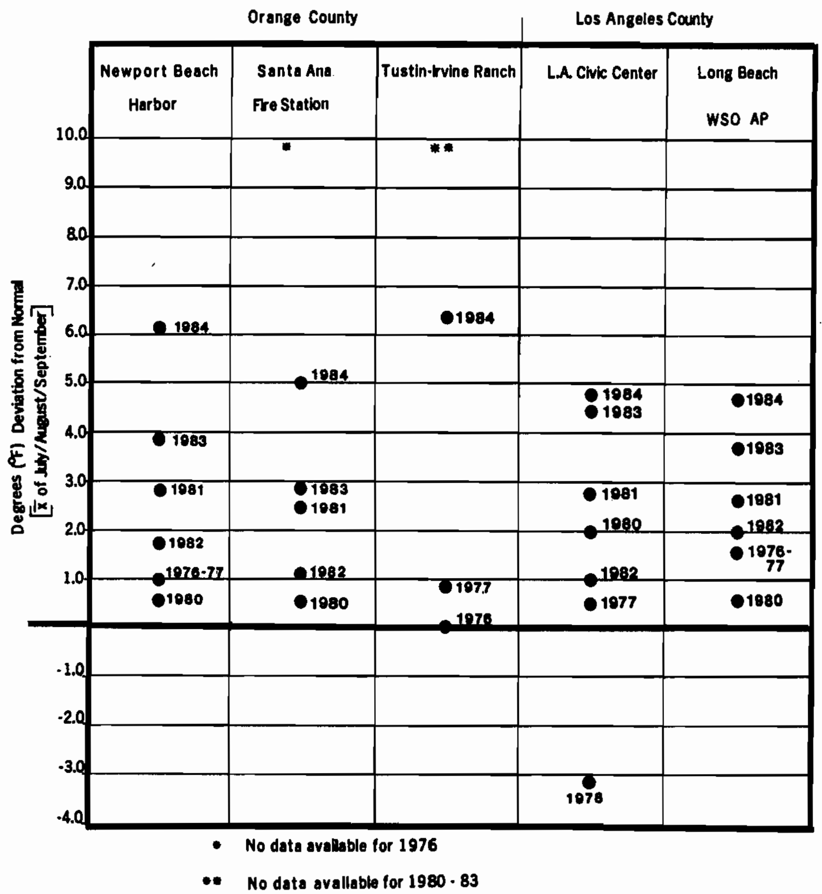

Figure 5.-The average degrees (°F) deviation from normal for the months of July, August, and September from three localities in Orange County and two localities in Los Angeles County for the years 1976-1977 and 1980-1984.

fasciatus when higher ambient temperatures are maintained, thus, increasing chances of virus transmission.

Previous studies (Keith 1981) have indicated that the combination of early season rains followed by a period of drought conditions has been implicated in previous SLE epidemics. This situation favors the production of large numbers of pools of stagnant water that provide optimal conditions for the development of *Culex* species. In the San Joaquin Marsh (Orange County) meteorological and other environmental factors have seemingly favored the production of *Culex* species mosquitoes that do well in stagnating waters. Beginning in 1981, a number of events furthered the successful production of *Cx. tarsalis* and *Cx. quinquefasciatus*. Alteration of the marsh by human activities (herbiciding, burning, and cutting) has accelerated the production of the tule stands. Cattails (*Typha latifolia*) and sedges (*Scirpus* sp.) have returned each year in greater biomass as a result of the thinning out attempts and because the ponds are filling in with sediment. Where once there were large open surface areas of pond water with fringes of tules along the shoreline, there are now practically no open water surfaces at all. Winter rains, pump water, and water piped from the nearby water treatment plant maintain the water levels in the marsh until May and June when the usual drought conditions begin. The ponds are nearly dried by late August and the water quality changes from a relatively clean water (*Cx. erythrothorax* and *Anopheles freeborni* habitats) to aquatic conditions that support *Cx. tarsalis* and *Cx. quinquefasciatus* larvae in large numbers. Table 4 illustrates the change in the relative numbers of *Cx. tarsalis* and *Cx. erythrothorax* (cf., the years 1975-1980 and 1981-1984). Mosquito production is further enchanced because the thick stands of tules also protect the mosquito larvae and pupae from the mosquito eating fish.

These conditions have been somewhat paralleled at Harbor Lakes (Los Angeles County), especially during the years 1983 and 1984, when extensive redevelopment operations were begun. Both Harbor Lakes and the San Joaquin Marsh lose water only by evaporation, thus, increasing

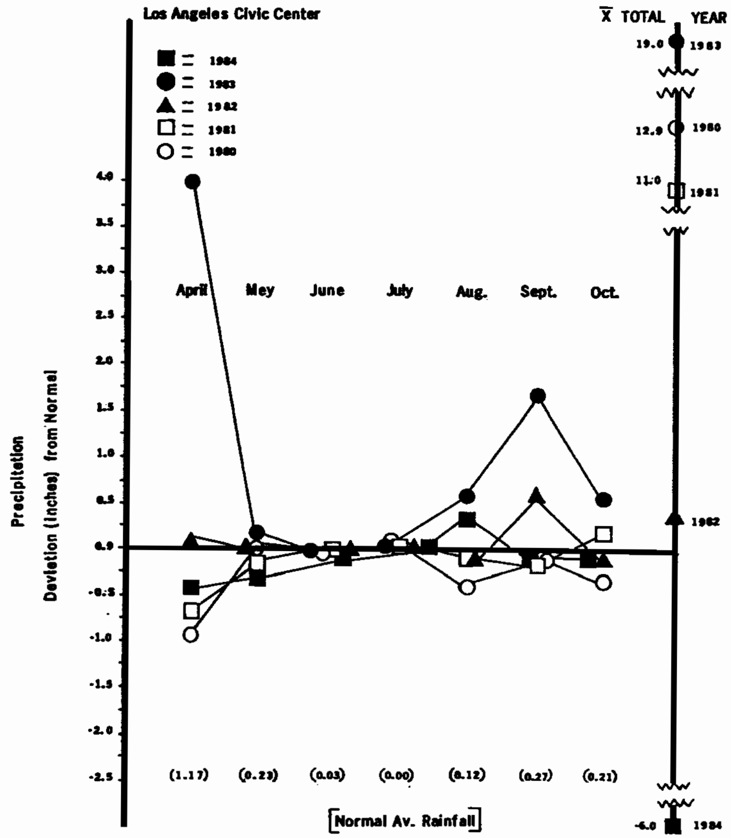

Figure 6.-The deviation from normal of rainfall (inches) at the Los Angeles Civic Center (Los Angeles County) during the months of April through October, 1980-84; average total rainfall for the years 1980-1984. (NOAA Records.).

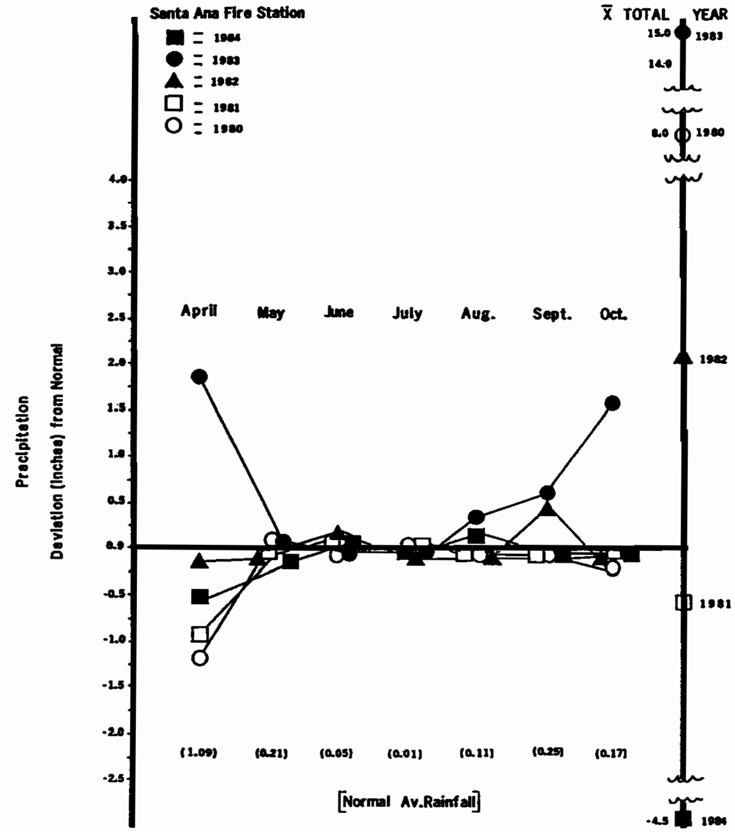

Figure 7.-The deviation from normal of rainfall (inches) at the Santa Ana Fire Station (Orange County) during the months of April through October, 1980-1984; average total rainfall for the years 1980-1984. (NOAA Records.).

the change of water quality from relatively clean water to more polluted conditions as the drought season proceeds through the later summer and early autumn months.

Investigations of SLE epidemics in both urban and rural environments nationwide have provided insights into the evaluation of the SLE transmission cycle that occurred in the Los Angeles Basin in 1984. Southern California is, however, ecologically very different from other SLE epidemic sites. Field studies must, therefore, be established that peruse the broad host/vector and environmental relationships in the SLE transmission cycle. It is recommended that extensive field investigations be conducted in both the wetland habitats and the urban habitats including the underground drainage systems in the latter. Determination of the reservoir hosts (birds and mammals) as well as the significant vector(s) (*Cx. tarsalis, Cx. quinquefasciatus,* and *Cx. peus*) is also important for early warning of virus transmission and the development of effective vector control operations.

ACKNOWLEDGMENTS.-We extend our appreciation to Dr. Jack E. Hazelrigg and Dr. Frank W. Pelsue, Southeast Mosquito Abatement District, Southgate and Dr. Major S. Dhillon, Department of Entomology, University of California, Riverside, for use of unpublished data. In addition, we thank Gilbert L. Challet, Manager, Orange County Vector Control District (OCVCD) for assistance and suggestions, and Mr. Stephen G. Bennett (OCVCD) for graphics production.

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Table 4.-San Joaquin Marsh (Irvine) New Jersey light trap data. ♀ *Cx. tarsalis*/ ♀ *Cx. erythrothorax*.

	MAY	JUNE	JULY	AUGUST	SEPT.	OCT.
1984	29/21 (1.4)	70/125 (0.6)	1310/72 (18.2)	836/18 (46.4)	414/10 (41.4)	318/1 (318.0)
1983	273/1335 (0.2)	552/1803 (0.3)	1263/543 (2.3)	559/155 (3.6)	490/43 (11.4)	121/56 (2.2)
1982	ND (-)	ND (-)	ND (-)	ND (-)	ND (-)	ND (-)
1981	436/39 (11.2)	62/0 (62.0+)	307/26 (11.8)	98/0 (98.0+)	ND (-)	12/3 (4.0)
1980	238/38 (6.2)	307/201 (1.5)	121/172[A] (0.7)	ND (-)	39/112 (0.3)	38/131 (0.3)
1979	100/16 (6.3)	83/36 (2.3)	32/213 (0.2)	105/244 (0.4)	163/165 (1.0)	96/133[A] (0.7)
1978	171/95 (1.8)	151/269 (0.6)	76/177[A] (0.4)	22/83[B] (0.3)	56/142 (0.4)	103/218[A] (0.5)
1977	53/21 (2.5)	129/354 (0.4)	44/422 (0.1)	50/185 (0.3)	45/171 (0.3)	219/127 (1.7)
1976	30/4 (7.5)	100/98 (1.0)	94/308 (0.3)	61/68 (0.4)	96/304 (0.3)	106/143 (0.7)
1975	38/2 (19.0)	23/22 (1.0)	110/224 (0.5)	132/129 (1.0)	72/107 (0.7)	56/26 (2.2)

ND = No data, trap malfunction.

[A] 1 week's data missing.

[B] 2 weeks only.

PERSPECTIVES AND PREDICTIONS FOLLOWING THE ST. LOUIS ENCEPHALITIS OUTBREAK IN SOUTHERN CALIFORNIA

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I was asked to give you my perspective and predictive views on the epidemic of St. Louis encephalitis (SLE) that occurred in southern California in 1984. That is not a simple task and much of what I say will reinforce views expressed by the other speakers on the preceeding panel.

Perspectives. The presentations of the panel left no question of the general surprise that 25 cases of SLE were diagnosed in the greater metropolitan area of Los Angeles, Orange, San Diego and Riverside Counties in 1984.

As background, it was known that SLE and western equine encephalomyelitis (WEE) viruses had been highly active in the southern portions of Imperial, Riverside and San Bernardino Counties and adjacent areas of Arizona in recent summers, and cases had occurred in that area in 1983. It would have surprised no one if there had been a flurry of SLE or WEE cases in those areas in 1984.

In contrast, the history of encephalitis in the greater metropolitan area was quite different as only one SLE case had been confirmed in the 37-year period 1945-1982 and there had been only a few WEE cases in the area in humans or equines in those years. During the same 38-year period, hundreds of cases of the two diseases were diagnosed in the San Joaquin and Sacramento Valleys. Three cases of SLE had been reported from the southern urban area in 1983 but the rarity of such cases in the area had raised questions if they were exposed elsewhere or possibly were misdiagnosed. The mosquito abatement districts in the region had not reported any unusual increase in suspected vectors in 1983 or 1984 and no infection with WEE or SLE viruses had been detected in the several sentinel chicken flocks in the area in 1983. All available evidence indicated that SLE and WEE viruses were not well established in the metropolitan area.

In spite of the above evidence, in August and early September 1984 sentinel chickens at Irvine and Harbor Lake became infected with SLE virus, mosquito pools yielded SLE virus and these occurrences coincided with three weeks of unusually hot weather. However, populations of *Culex tarsalis, Culex quinquefasciatus* or *Culex peus* had not increased dramatically to the numbers usually associated with effective spread of infection to man. A single sentinel chicken had converted to WEE in August in the urban area. However, this single WEE conversion did not indicate widespread activity. The expected pattern in California and other far western states was for WEE virus activity to precede the appearance of SLE virus.

At this time, and in spite of poor reporting, SLE cases began to turn up and occurred in unexpected numbers over a large area of the metropolitan region. When onset dates were determined, the onset of some cases had preceded viral detection by the surveillance system in birds or mosquitoes.

Twenty-five cases are now known to have occurred in a population of over 12,000,000 people. This is a low attack rate as compared with urban outbreaks in the midwest or historically in the central valley of California. This number and more cases occurred in some years of the 1940's and 1950's in much smaller populations in the valley area. We never will know the total number of cases in 1984 but even if it was only 25, we can be relatively certain that an additional 10,000 or more persons probably had inapparent or mild infections and did not come to medical attention.

Questions raised by this experience. As I indicated in a prior presentation this morning, the heart of research is the reexamination of accepted concepts on the basis of new facts. The principal new fact that emerged was that SLE virus was present over an extensive metropolitan area in 1984 and had infected people in sufficient numbers to cause 25 cases. This was a new experience in this region of California. We must determine what happened in 1984 if we are to prevent the occurrence of such events in the future in our state.

Some questions that this experience has raised are:

(1) How was the virus introduced and was it a new strain with unusual infectivity and pathogenic traits? So far there is no evidence that this was the case.

(2) How did it spread over such a large area so quietly and apparently rapidly?

(3) Were the residual marshes, reclaimed water areas and recreational waters in the region key sites for introduction and establishment of infection? It is possible that our earlier experiences in rural areas merely led us to suspect this was the case because of the ecology of such sites as they obviously support abundant bird and mosquito populations.

(4) Which of the three species of *Culex* common in the area, and that we know

are potential or proven vectors of SLE virus, was the primary vector? Was an interaction of known efficient primary vectors such as *Cx. peus* and *Cx. tarsalis* and a secondary vector *Cx. quinquefasciatus* necessary for this event to occur? This is the subject of ongoing research.

(5) Were previous threshold levels of vector populations that were thought to be necessary for epidemic spread of SLE virus invalid in this situation? If so, was it because a population of 12,000,000 people made man a competitive host for mosquito bites as compared with birds? Did the hot weather cause people to increase their outdoor exposure to mosquito bites?

(6) What is the blood feeding range or frequency of feeding of *Cx. tarsalis, Cx. peus,* and *Cx. quinquefasciatus* on different hosts in a metropolitan area? We know from carefully done studies in rural and smaller urban-suburban areas that these 3 species feed mostly on birds but include a range of domestic and wild mammals and man as blood sources. Indeed, we have come to accept that this feeding pattern is essential if these species are to maintain infection cycles in birds and spread infection to other hosts. We simply do not have similar data from the southern California metropolitan area.

(7) Was it possible that SLE virus had become established as a transovarial infection that was spread from female mosquitoes to their progeny? Had the virus spread throughout an extensive area over several years as a covert infection causing no problems until the unusually hot period in the summer changed the characteristics of the virus so it multiplied rapidly in vectors and was transmitted to people? We know SLE virus can be transovarially transmitted at a low rate in mosquitoes.

The panel presentations demonstrated the importance of my questions and some are already being studied. It is clear that we have the methods available to study these questions in this unique environmental setting. I believe the 1984 epidemic was different in many ways from that of previously studied SLE epidemics that occurred in metropolitan areas of the midwest and eastern United States or rural areas of California.

Predications for the future. I was asked to predict what will happen next year and further into the future. It is tempting to respond, "If I had God as a consultant, my task would be easy." You know that I do not have that privilege.

It would be less than candid it if I did not remind you again that we expected cases in the San Bernardino, Imperial, Coachella Valley area in 1984. Both WEE and SLE viruses were active in this area in 1984 and a few cases of SLE were detected. The cases occurred in residents of the desert area of Riverside County. We did not expect cases where the majority occurred in the Los Angeles Basin area.

My first prediction for 1985 is, if we carry out intensive and costly studies looking to answer the questions I raised, we can almost be certain that the virus will disappear for a few years. That has been my experience and that of many others over the past 40 years when studies were carried out following an epidemic. History tells us that major metropolitan outbreaks of SLE have rarely continued for a second year. In most instances, the disease has decreased or disappeared in the second year and has not recurred for a considerable period in the same urban areas. Sometimes, it has seemed that the cheapest control program is to try to do follow-up studies to research the problems associated with a prior epidemic.

My second prediction requires that I know in advance: the temperatures in the Los Angeles Basin next summer, the extent and effectiveness of the mosquito control programs and the success of the virus in surviving over the winter in the same area where it prevailed in 1984. I can not know this information in advance and have to await developments. All I know is that there still will be millions of susceptible persons in the area and the virus became widespread in 1984 in spite of control efforts. If the exact conditions that prevailed in 1984 were to recur it would be reasonable to expect cases to occur again in 1985.

I urge that research be carried out on the problems uncovered and intend to support such activity. I obviously encourage development of an intensified surveillance program including better case finding and verification. I recommend the intensive control of larval populations of all 3 suspect vectors species in 1985 to minimize viral activity. There is an excellent basic organization, staff, and "know how" for vector control in the area that should be able to handle the problem.

AIR CONDITIONING AND TELEVISION AS PROTECTIVE FACTORS IN ARBOVIRAL ENCEPHALITIS RISK[1]

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ABSTRACT

Exposure to arboviral disease vectors may be altered by changes in human behavior. Human behavioral factors that may influence exposure to arboviral disease vectors include protection from vectors by remaining indoors during vector feeding periods. In California, the advent of air conditioned housing and television has coincided with a general decrease in arboviral encephalitis. Air conditioning and television encourage persons to remain indoors during summer evenings which is the primary time when infected *Culex tarsalis* transmit Western equine encephalomyelitis and St. Louis encephalitis viruses. The attack rates for these 2 diseases in 33 counties in the central valley of California, 1945-1982, were compared with the extent of household air conditioning and television. Encephalitis rates had a significant negative association with television ownership in these counties. A telephone survey conducted in Kern County revealed that air conditioner and television utilization times corresponded closely to the feeding times of *Cx. tarsalis*, and respondents indicated a preference to remain indoors during this time because of these appliances. It is concluded that changed behavioral patterns may protect from vector-borne disease and be complementary to vector control programs.

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[1]This research was funded in part by Research Grant AI-3028D from the National Institute of Allergy and Infectious Diseases, Biomedical Research Support Grant 5-S07-RR-05441 from the National Institutes of Health, and by special funds for mosquito research allocated annually through the Division of Agriculture and Natural Resources, University of California.

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GEOGRAPHIC DISTRIBUTION AND INCIDENCE OF CANINE HEARTWORM IN A SIX COUNTY AREA OF NORTHERN CALIFORNIA

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INTRODUCTION.-In 1984, the Sacramento County/Yolo County Mosquito Abatement District, in cooperation with the Sacramento Valley Veterinary Medical Association, conducted a canine heartworm prevalence study. The purpose of this study was to determine the current incidence and geographic distribution of dog heartworm in the two District Valley counties (Sacramento and Yolo) and four adjacent foothill counties (Placer, El Dorado, Amador and Calaveras).

A recent veterinary hospital survey in Northern California encompassing Alameda, Contra Costa, Napa, Solano, Sonoma, Stanislaus, Merced, San Mateo and Marin Counties (Acevedo and Theis 1980) reported canine heartworm prevalence rates ranging from an estimated 4.0% to 40.0%. Unfortunately, Sacramento Valley and Mother Lode veterinarians were not included in the survey. Another study of the region's heartworm activity (Weinmann and Garcia 1980) determined that *Dirofilaria immitis* Leidy, the dog heartworm parasite, was present in the native coyote population of El Dorado County, but did not examine the canine population.

Little has been done to assess the current status of heartworm in canines residing in the region, so a survey was undertaken to determine this.

METHODS.-On the 22nd of March, a letter and survey forms were mailed to 102 veterinarian hospitals in Sacramento, Yolo, Placer, El Dorado, Amador and Calaveras Counties asking them to participate in the canine heartworm prevalence study. Local veterinarians were asked to submit monthly survey forms from April until December 1984. For each month the veterinarians indicated the number of dogs tested; the number of dogs positive for *Dipetalonema*; the number of dogs positive for *Dirofilaria*; the number of cats positive for *Dirofilaria*; and the type of test used to detect infected dogs and cats (Figure 1). If a dog was positive, the breed, the owner's address and whether the dog was local or transient were indicated.

RESULTS AND DISCUSSION.-Of the 102 veterinarian hospitals contacted, 34 responded to the survey. A 33.3% response to a canine heartworm survey is comparable to the percentages reported by other investigators; 21% to 28% (Noyes 1980) and 35.9% (Schlotthauer et al., 1979). Responses to the survey ranged from 25% of the veterinarians in Amador County to 100% in Calaveras County (Table 1).

Response to the survey varied geographically. Foothill veterinarians in Placer, El Dorado, Amador and Calaveras Counties had a greater percentage response to the survey than valley veterinarians in Yolo and Sacramento Counties, 41.6% compared to 28.8%. Foothill veterinarians' interest in the survey may have been prompted by their awareness of the heartworm activity in the foothill counties. However, most of the veterinarian hospitals participating in the survey (19 or 56%) were from the two valley counties. Fifteen hospitals, or 44% of the total responding, were from the four foothill counties.

During the nine month study, 5,592 dogs were examined for heartworm infection; 2,152 in Placer, 2,042 in Sacramento, 855 in El Dorado, 334 in Yolo, 153 in Amador, and 56 in Calaveras County (Table 2). Foothill veterinarians tested 58% (3,216) of the dogs while valley veterinarians tested 42% (2,376) of the dogs.

Table 1.-Number of veterinarian hospitals that responded to 1984 heartworm survey.

County	Number of Veterinarian Hospitals Surveyed	Number Responded To Survey	Percentage
Sacramento	59	17	28.8
Placer	16	8	50.0
El Dorado	15	5	33.3
Yolo	7	2	28.6
Amador	4	1	25.0
Calaveras	1	1	100.0
TOTAL	102	34	33.3

Table 2.-Six county summary of incidence of canine heartworm.

County	Number Dogs Tested	Number Dogs Positive	Percent Dogs Positive
El Dorado	855	107	12.5
Amador	153	18	11.8
Calaveras	56	6	10.7
Placer	2,152	122	5.7
Yolo	334	11	3.3
Sacramento	2,042	66	3.2
TOTAL	5,592	330	5.9

Of the 5,592 dogs tested 330 were found infected with heartworm parasites. Most of the cases (122) were reported from Placer County, with El Dorado reporting 107; Sacramento 66; Amador 18; Yolo 11; and Calaveras 6. The four foothill counties accounted for 253 or 76% of the cases, while the two valley counties reported 77 or 23% of the cases.

In the survey area most of the canines positive for heartworm were local dogs. Of the 330 positive dogs, 18 or 5% were transient. Sacramento County had the highest number of positive transient dogs (12), El Dorado was next with two; Amador, Calaveras, Placer and Yolo Counties each reported one. Considering the number of local infected dogs it would appear that heartworm is endemic in the canines of counties surveyed.

No felines were reported infected with heartworm during the study. In a survey of this type a lack of cats reported infected with heartworm is not surprising, since reported heartworm infections of cats in other surveys has been rare. A six year survey of Illinois veterinarians (Noyes 1978) indicated that 0-5 cats per year were diagnosed for *D. immitis*.

Also, no dogs were reported positive for *Dipetalonema reconditum* Grassi. This is a little unusual since reported *Dip. reconditum* prevalence rates in other surveys (Otto 1972) ranged from 2% to 55%. A lack of dogs reported infected may be a result of the testing techniques. Ninety-three percent of the dogs tested were by the Difil filtration technique, 6% by a modified Knott's test and less than 1% by either radiography, antibody or necropsy. No blood samples were tested by the direct smear method.

The overall percentage infection rate for the study was 5.9%. El Dorado County had the highest infection rate, 12.5%; while Sacramento County had the lowest infection rate, 3.2%. In all foothill counties with the exception of Placer County the infection rates were three to four times greater than the valley rates (Figure 2). The Placer County prevalence rate of 5.7% was comparable to the valley rates of 3.2% and 3.3%. One reason cited for the lower infection rate in Placer County

SACRAMENTO/YOLO MOSQUITO ABATEMENT DISTRICT

AND

SACRAMENTO VALLEY VETERINARY MEDICAL ASSOCIATION

HEARTWORM PREVALENCE STUDY

HOSPITAL NAME AND ADDRESS: ____________________

PHONE: ____________

Month, year: ____________

Number of dogs tested: ____________ Type of test: ____________

Number of dogs positive for Dipetalonema: ____________

Number of dogs positive for Dirofilaria: ____________

Number of cats positive for Dirofilaria: ____________

POSITIVE CASES:

Breed	Street Address	City	Zip Code	Local Dog (yes/no)

Figure 1.-Hospital Survey Form.

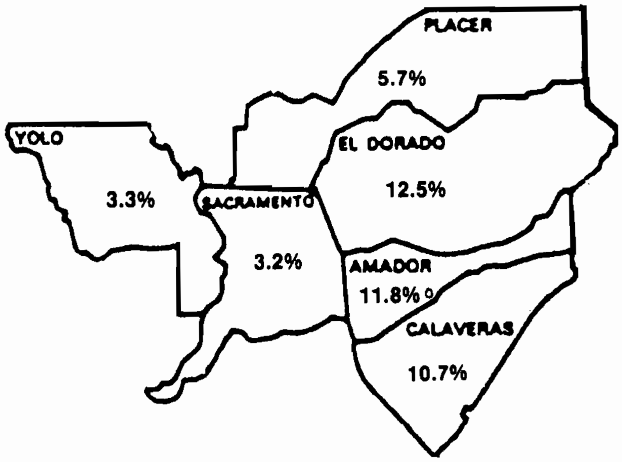

Figure 2.-County Prevalance Rates.

(Richard Pollack, DMV, person communication, Granite Bay Veterinary Clinic) is the large number of dogs on preventive medication, and extensive testing program and public education in that county.

Survey results indicated that the geographic distribution of canine heartworm in the six county area varied with the location of population centers, elevations and local vegetation types.

In El Dorado County with a 12.5% prevalence rate, most of the heartworm activity occurred around four of the eighteen reported communities. Placerville (38), Aukum (18), Shingle Springs (16), and Garden Valley (15) accounted for 82% of the cases. Heartworm activity was also reported from Georgetown (4), Cool (4), Greenwood (3), Pilot Hill (3), Camino (3), Grizzly Flats (2), Diamond Springs (2), Pollock Pines (2), Lotus (2), Mosquito Camp (1), Rescue (1), Somerset (1), Coloma (1), and Pleasant Valley (1) (Figure 3). The Aukum area cases illustrate the focal nature of the disease. Of the 18 reported cases, 17 occurred at one kennel.

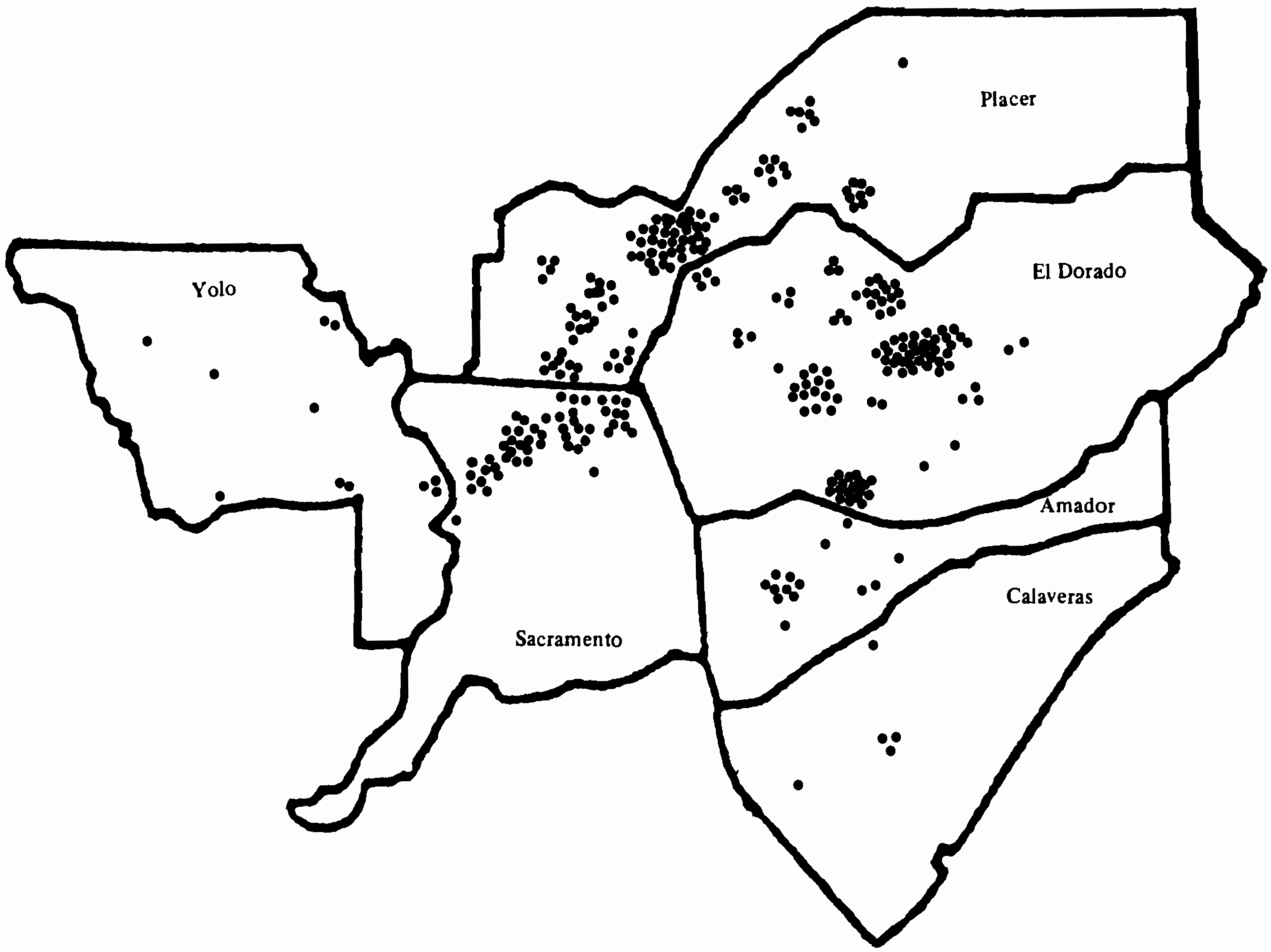

Figure 3.-Approximate Geographic Distribution of Canine Heartworm Cases.

Three vegetation types, foothill woodlands, pine/fir woodlands and cultivated pastures predominated in areas where positive heartworm cases occurred. In El Dorado County, the elevation for positive heartworm areas varied from 680 feet at Lotus up to 3,980 feet at Pollock Pines.

In Amador County 11.8% of the dogs tested had heartworm. The majority of heartworm activity was found around the town of Sutter Creek (10). Infections were also reported from six other areas, Pine Grove (2), Pioneer (2), Fiddletown (1), River Pines (1), Volcano (1), and Jackson (1).

The predominate vegetation types in the areas where positive cases occurred were pine/fir woodlands and foothill woodlands. The only exception to this was Sutter Creek, where grassland vegetation predominated. The elevation in the areas of positive heartworm cases ranged from 1,198 feet at Sutter Creek up to 2,951 feet at Pioneer.

The third highest prevalence rate was reported by Calaveras County veterinarians. The area around San Andreas (3) accounted for most of the activity in Calaveras County. Three other communities, Jenny Lind (1), Mokelumne Hill (1) and Arnold (1) also reported heartworm cases.

Foothill woodlands was the predominate vegetation type in heartworm areas. The highest reported elevation for a heartworm infection occurred at Arnold (4,000 feet). Cases ranged from an elevation of 243-4,000 feet.

Placer County veterinarians found an infection rate of 5.7%. A large focus of activity occurred around the city of Auburn. Fifty-nine cases, representing 48% of the county total, were distributed around Auburn. Three other smaller foci occurred in Foresthill (10), Rocklin (9), and Roseville (8). Twelve communities were heartworm positive including Loomis (7), Applegate (7), Colfax (6), Granite Bay (6), Lincoln (4), Meadow Vista (4), Penryn (1), and Alta (1).

The predominate vegetation types in the positive heartworm area are pine/fir woodlands and cultivated pastures. However, 48% of the positive heartworm activity occurred around the foothill woodland area of Auburn. The elevation in areas where positive heartworm cases occurred varied from 161 feet at Roseville to 3,590 feet at Alta.

The second lowest prevalence rate was reported in Yolo County where 3.3% of the dogs tested had heartworm. Although there were only 11 positive cases, these cases were widely distributed. Positive infections were reported from the communities of West Sacramento (3), Davis (2), Knights Landing (2), Winters (1), Capay (1), Madison (1) and Brooks (1).

In Yolo County, most of the reported cases were near riparian woodland habitats. The elevation in areas where positive heartworm cases occurred varied from 18 feet in West Sacramento to 300 feet in Brooks.

In Sacramento County, all the cases were reported from the Northern half of the county; roughly the area between Highways 80 and 50 from downtown Sacramento to the city of Folsom. Heartworm activity was concentrated in three areas; one in Carmichael, the others near Folsom and the downtown section. Heartworm activity in Sacramento County may be expanding. One veterinarian located in the Pocket area reported his first case in eight years of practice. Cases were reported from the communities of Sacramento (40), Orangevale (8), Carmichael (6), Citrus Heights (5), Folsom (3), Fair Oaks (3) and Rio Linda (1).

The elevation of positive heartworm areas ranged from 25 feet in downtown Sacramento to 300 feet in Orangevale.

CONCLUSIONS.-Fifty-six communities in the survey area were positive for heartworm infections. In all counties surveyed with the exception of Sacramento, the geographic distribution of reported cases was widespread. Cases were reported ranging in elevation of 18 feet in the valley up to 4,000 feet in the foothill counties. Cases occurred in five vegetation types in the study area; foothill woodlands, pine/fir woodlands, cultivated pastures, grassland and riparian habitats.

Survey results indicated that testing, number of positive cases, and prevalence rates were greater in the four foothill counties than the two valley counties.

ACKNOWLEDGMENTS.-We would like to thank Richard Pollack, DMV, for his assistance in composing and initiating the survey.

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THE HISTORY OF MOSQUITO CONTROL IN CALIFORNIA

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In October 1984, the Cooperative Extension of the University called together a Working Group on Mosquito Control and Research. The purpose of the 2-day workshop was to identify voids in the University's research program and to develop priorities for future programs. Many people in this audience participated in that meeting. Dr. Fontaine asked me to make an introductory presentation on "Historical Perspectives of Mosquito Research in California".

While I protested being given that task, I was assured it would be no problem as I could do it "off the top of my head". As you can see, anything "off the top of my head" at this stage of life would have very little substance. I finally had no alternative but to accept that the chore was the price to be paid for surviving over 40 years in the University, devoting my career largely to mosquito research (with the state providing necessary money) and knowing almost all the people involved in the history of mosquito control research in California. I dug into my memory bank and reread many documents and reports, including reviews of the history of the CMVCA and University research programs. This resulted in some notes which I spoke from. The program Chairman for today's meeting, Mr. Hazeleur, who did not participate in the workshops, then had the bright idea I should give the same talk today. I apologize for his judgement and to those persons who heard the earlier presentation.

I am not going to talk "off the top of my head", as the subject deserves better than that. I will describe starting points and turning points in the 80 year history of the program and I hope give you some perspective views. The review will emphasize that the success of the research depended on the involvement of many individuals and agencies who identified problems and frequently joined in a mutual effort to resolve the problems. The research has demanded the continuous reexamination of accepted knowledge in light of new facts and the practical application of revised approaches.

1904-1930: the first 26 years of mosquito research in California.-Mosquito research in California started 80 years ago with the recognition that salt marsh mosquitoes in the San Francisco Bay region were interfering with development of the area and the health of the residents. In 1904, Professor C. W. Woodworth of the Department of Entomology, University of California, Berkeley, was asked to review the problem and to conduct a control campaign in the Burlingame area of San Mateo County. In 1905, the Burlingame Improvement Club allocated $2,000 to implement a program based on recommendations of Dr. H. J. Quayle, U. C. Berkeley, who had been assigned to the problem. San Rafael soon followed the lead. The Burlingame program emphasized water management and the San Rafael program emphasized oiling. Both were successful. Based on this success, the University of California Agricultural Experiment Station issued the first bulletin on mosquito control.

In 1909, the State Department of Health and residents of several regions of the Sacramento Valley recognized the importance of malaria as an endemic disease. Dr. W. B. Herms, a new member of the University faculty, was deputized as an Assistant Health Officer, given a budget of $700 from the Penryn Fruit Company and sent to Placer County to determine if control was feasible. By 1910, Oroville and Bakersfield began malaria control programs, which were formalized in 1915 when the state legislature passed the Mosquito Abatement Act. By 1921, malaria transmission as an endemic mosquito-borne disease had ceased. However, we still concern ourselves today with the potential for malaria reintroduction. From 1910 to 1912, S. B. Freeborn, W. B. Herms, H. F. Gray, and associates carried out a mosquito survey of California and malaria surveys of central California.

In 1919, the state legislature allocated $10,000 to the University for research on mosquito control in the Sacramento Valley. In 1920, the California Mosquito Control Association was formed, and in 1921 the first effort at biological control was based on the introduction of *Gambusia* near Redding.

As a final development in this first period of slightly more than 20 years, Freeborn published the first edition of The Mosquitoes of California in 1926, listing 36 species, summarizing their habits and distributing and providing keys for their identification. I still periodically reexamine my copy of this publication with interest and respect.

The important characteristic of this first 26 year period was that a very small group of scientists in the University worked closely with the State Department of Health, concerned local communities, and the state legislature to:

1. Define the principal mosquito problems that faced the state. They were malaria control and pest mosquito control as they affected urban and agricultural development.

2. Develop the first knowledge of the mosquito species involved and their biology in California.

3. Recommend a combination of the best

available physical, biological and chemical control methods to control the problem.

4. Establish legislation to implement control based on research findings.

We would do well in any period of our history to emulate those pioneers; their groundwork was well done. Accomplishments involved not just research by the University but the collaborative effort and interest of all persons concerned with mosquitoes and the diseases they carry.

1930-1950" the second two decades of mosquito research.-The next 20 year period, 1930-1950, saw many new faces in mosquito research, and major developments occurred.

The first conference on mosquito control was held in Berkeley in 1930 and included University, State Department of Health, and mosquito control personnel.

In that same year, Dr. Karl F. Meyer and his associates from the University recovered the virus of western equine encephalomyelitis from the brain of a horse in Merced County. Their report established the cause of a disease that in 1930 affected some 6,000 horses and mules with a 50% case fatality rate in the San Joaquin Valley. The description of cases provided epidemiological evidence that the virus was carried by mosquitoes or other biting insects and that it also infected people. In 1933, St. Louis encephalitis was found in Missouri and was soon proved to occur in California as a disease of humans.

These findings opened a Pandora's Box of problems requiring research, and a research unit was developed at the Hooper Foundation at the University of California, San Francisco, to study them. The problem was not insignificant as the State Department of Health and Agriculture reported that there had been 1,383 cases of encephalitis in people and 3,370 cases in horses from 1936-1945. Subsequent research in the early 1940s revealed that the principal mosquito vector of both viruses in the Central Valley was *Culex tarsalis* and that the basic cycle involved birds. The research set the stage for establishment of a program in the State Department of Health and local mosquito control districts for surveillance and control of encephalitis in California.

In 1940 and 1944, Herms and Gray published the 2 editions of their book Mosquito Control. These classics clearly show that the mosquito control problems in California had already gone through a significant change. It is not an exaggeration to say that man had altered the ecology of the state with reference to primary mosquito sources. Extensive new sources of mosquito production had developed that required reexamination of the problems.

In the earlier decades, springtime flooding of the Central Valley had maintained marshes, sloughs, and high level water tables. By the 1940s, rivers were being dammed and diked, major new irrigation systems were supporting increased agricultural development and urban developments and populations were expanding rapidly. Although marshes had been drained or stabilized and rivers diked, the rural regions had new types of mosquito producing areas related to irrigation. At the same time, septic tanks, cesspools, and privies were being relaced with sewers, waste water treatment plants, dairy drains, and industrial waste water ponds. The major mosquito problems had become man-made rather than natural in most of the heavily populated areas of the state.

For the first time, a new class of insecticides was mentioned, first DDT and then the related hydrocarbons. These materials were a product of research during World War II. Their possible value, limitations, and methods of application in California demanded a research effort.

World War II also led to a resurgence of concern about malaria. The Federal Malaria Control in War Areas Program included a unit in California, housed in the State Department of Health, beginning in 1942. At the end of World War II, the scene was set for major development in both research and control. The state legislature and the governor were in a receptive mood to provide support. In June 1944, the Senate passed a resolution entitled "Relating to the Investigation of the Malaria Hazard in California." In January 1945, the state legislature received a report representing the range of concerns of the State Department of Health, the University and mosquito control agencies. The report was titled "A Report on Investigation of the Disease Bearing Mosquito Hazard in California." The report urged a shift from wartime concerns, almost solely with malaria, to a broadened program of disease and pest mosquito control for the state.

At that time, the University had a very small research staff. It was almost limited to the Hooper Foundation program, of which I was a member, and to Drs. R. M. Bohart and S. F. Bailey who had just returned from the Armed Forces. The control resources of the State consisted of 29 Mosquito Abatement Districts (MADs) covering only 4,645 square miles, a population of slightly over 1 million people and a budget from local taxation of $369,000. The report predicted that the cost of an affective mosquito-borne disease control program for the state might eventually reach $10 million. How I wish that prediction was true today, as it would make the taxpayers happy. The response of the legislature in 1945 was to allocate $400,000 to the State Department of Health for subvention to MADs and $200,000 to demonstrate the role of *Culex tarsalis* as a vector of encephalitis.

The above actions provided a real shot in the arm for research and for control programs based on the findings. The Bureau of Vector Control was organized in 1947 and subventions required that the MADs maintain records of mosquito abundance and control efforts, add entomologists to their staffs, and establish standards for the qualifications of managers and operators.

The outstanding characteristics of this second 20 year period were:

1. Recognition that the encephalitides, a pair of new mosquito-borne diseases,

should and could be managed through mosquito control.

2. Realization that new approaches were needed to control mosquitoes associated with the changed ecology of the state and to use the new insecticides.

3. A dramatic extension of the areas and populations under control.

1950-1970.-The 1950-1970 period was a time of maximum growth and diversification of mosquito research in California. It also was a time when a combination of public health, biological, and administrative developments arose that challenged our abilities to cope with unanticipated problems. Let me illustrate this with 5 examples.

1. In 1952, we experienced the largest epidemic of encephalitis in the history of the state: 375 WEE and 45 SLE cases, a total of 420 cases. We also had the largest epidemic of malaria in modern times, 35 cases at Lake Vera.

2. In both pest and vector species there was a widespread development of resistance to DDT and other hydrocarbon insecticides, which extended to the organophosphorus compounds. Research revealed the mechanisms by which mosquitoes inactivated these compounds and their genetic basis. However, we still needed to perfect practical ways to bypass this problem.

3. The Fresno Field Station of the Vector Control Section of the State Department of Health was established in 1953 in response to the need for a centralized and integrated research effort on mosquito biology and control in California. This program had broad objectives which included support of the limited University research programs.

4. In February 1965, a report was prepared on "Proposed Expanded Research and Extension Programs for the Control of Mosquitoes Affecting the Health and Well Being of Man and Animals." This report represented the best joint effort of University, State Department of Health, and mosquito control personnel.

5. On June 21, 1965, the state legislature, unexpectedly to most of us, proclaimed that as of July 1 of that year, all responsibility for research on mosquitoes and the budget for the Fresno Field Station was transferred to the University.

I prefer not to discuss the rightness or wrongness of that decision. Possibly the most important thing was that the impossible was accomplished through the combined efforts of personnel of the University and the State Department of Health. In less than 2 weeks a budget of $141,000 and 12 professional staff members at Fresno and 6 on subassignment to the University at Bakersfield and Davis were transferred to UC and program continuity was assured. The effectiveness and contributions of Dr. Ralph Barr as Director of the Fresno Field Station cannot be overstated. The Fresno Field Station had made major contributions to mosquito research. At that time, other programs of the State Health Department took the responsibility for carrying forward the programs on diagnosis and surveillance of diseases spread by mosquitoes.

Partially promoted by the reallocation of State Health Department funds, but also by budgetary support within the University system, major research programs rapidly developed in the mid-1950s and 1960s on the Riverside, Los Angeles, Davis and Berkeley campuses. Indeed, by 1970, no other University and few major government agencies had professional staffs and research facilities equal to those of the University, fully supported by the Vector Control and other programs of the State Department of Health and mosquito control agencies that covered most of the densely populated areas of the state. The value of the continuous representation in support of the mosquito research program in Sacramento by the California Mosquito and Vector Control Association can never by overstated.

It became very clear during this period that an accelerated research program was needed that would encompass neglected areas. Included were alternative approaches for management of mosquito populations other than by insecticides. Priority subjects for study were biological agents, physical alternation of agricultural environments, improved management of excess water or wastewater from metropolitan areas and resolution of issues raised by persons concerned with environmental quality.

1970-present.-I will spend a minimum amount of time on the period since 1970. It is the current period and you know the most about it.

In 1975, a special appropriation of $300,000 was allocated to the University by the legislature for research on mosquitoes and mosquito-borne diseases. Again this program was vigorously supported in Sacramento by all concerned parties: the CMVCA, State Department of Health, and the University. Subsequently, coordinators were recruited: Drs. Carl Mitchell, Edmond Loomis and Russell Fontaine. Awarding and policy committees were appointed that had broad representation. Since 1973, annual reports have been issued that have world-wide distribution. In 1978, the third edition of The Mosquitoes of California was published by R. M. Bohart and R. K. Washino, and covered 47 species in the state as compared with 36 species in 1926.

This program stimulated new research and an assurance of the continuity of established research efforts. Resources available in California today for mosquito research are:

(1) state funds of $557,800 annually for

committee allocation to University researchers based on their research proposals,

(2) departmental budgets of $684,300 for faculty and staff involved in research on mosquitoes.

(3) awards to research workers who, through their own initiative, have almost doubled the above budgets by receiving grants and contracts from federal, private and other sources that total $1,162,800, and

(4) $59,000 each year in local funds provided by mosquito control agencies in direct support of the University research effort.

We may not have money to support all the research that could or should be funded, but we have come a long way from $10,000 in 1919 or $600,000 in 1945 to a total of almost $2,500,000 in 1984-85.

The University now has a large professional staff dedicated to research on mosquitoes. If one compares the projects currently being carried out and the program for this meeting as compared with the same information from 1975, it is obvious that the research effort is constantly changing its focus in an effort to face today's problems. That is how it should be.

Equally important, the State Department of Health Services has a well organized surveillance program on vector-borne diseases and vector and pest populations. This effort is closely integrated with those of local vector control and health agencies and state personnel are in constant consultation with those agencies and the University.

Finally, California's major resource related to mosquitoes is the corporate membership of the CMVCA which in 1984 represented some 60 agencies with an annual budget from local taxation of $24 million covering an area of 31,000 square miles and 15 million people. Many of the districts are carrying out applied research.

Few areas in the world have a total mosquito research resource comparable to California. It is staffed with competent professionals and has a broad base of agencies supporting the research and applying promising new research findings. To conclude, the research on mosquitoes and mosquito-borne diseases in California represents a collaborative effort of all of the above agencies and each has a serious responsibility to carry out its portion of that effort with dedication, initiative and ingenuity as they have since 1904.

A HISTORICAL REVIEW OF MOSQUITO PREVENTION IN CALIFORNIA. PART I (1904 - 1946)

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INTRODUCTION.-One of my particular interests in mosquito control has been in the area of mosquito prevention. As a member of the CMVCA historical archives committee, I wanted to make a contribution to the history of this vitally important aspect of mosquito control in California.

The process of mosquito prevention consists of two main parts. The first part would be the initial actions taken to prevent environmental conditions responsible for propagating mosquitoes. These actions would be classified as primary prevention, a term now used in other areas of public health. The second part would be the physical modification of an existing mosquito source to eliminate or reduce mosquito populations. This second part would be classified secondary prevention, commonly referred to as "Source Reduction": A term adopted by the California Mosquito Control Association's Water Resources Committee at the 1951 Annual Conference. This committee became the Source Reduction Committee in 1956.

The major activities involved in primary and secondary mosquito prevention include engineering design, construction and maintenance, public education, interagency cooperation and code enforcement of mosquito prevention standards. They also include direct operational intervention either by the control agency or property owner to modify the environmental conditions causing a habitat to produce mosquitoes. Historically, these environmental modifications have taken the form of filling low areas, construction of drainage and water circulation channels, installation of water control structures, clearing channels of vegetation and debris to enhance natural flow, changes in irrigation practices, and proper field design.

THE EARLY YEARS (1904 - 1930).-The first organized mosquito prevention program took place in the salt marshes of San Rafael, Marin County, in 1904. Information from letters and newspaper articles of that day indicate that the salt marsh mosquitoes were so pestiferous that persons waiting at the San Rafael train station wore head nets. Smudge pots were also operated at the train depot. Because of the severe mosquito problem, real estate could only be sold during the winter time. The San Rafael Improvement Club sent a letter to Professor C. W. Woodworth, Entomologist, University of California, Berkeley campus, requesting assistance in solving the mosquito problem. In response to the request, the Professor sent one of his graduate students, Mr. H. J. Quayle, to survey the problem. Quayle recommended two basic control measures. One was to drain the tidal ponds by constructing a series of small hand-dug drain ditches that would connect up to the main tidal channels. The other approach was to spray a mixture of crude oil and kerosene on impounded waters to kill the mosquito larvae and pupae. The Improvement Club conducted a series of community fund raising activities to obtain funds to hire persons to do the control work. At the recommendation of Professor Woodworth, a Reverend E. H. Ashman was hired to direct the control operation. This early entomological survey by Woodworth and Quayle indicated that two species were the prime offenders. *Culex squamiger*, now called *Aedes squamiger*, occurred early in the spring and *Culex curii*, later renamed *Aedes dorsalis* was found to be more prevalent in summer months.

A year later, in 1905, the Burlingame Improvement Club followed San Rafael's example and requested help from Professor Woodworth to conduct a mosquito survey and develop control recommendations. One of the lessons learned from the earlier San Rafael experience was to emphasize ditching as a more permanent solution to mosquito control and less effort on "oiling" which had to be repeated after every monthly high tide.

The amount of money actually expended in San Rafael for the control work during 1905 was about $2,000. The larger portion of this was expended for labor in ditching and diking. A key observation cited in one of Professor Woodworth's reports stated "It was observed both at San Rafael and at Burlingame that in no case where the tides had free access to a pool where eggs were laid were mosquitoes produced, and an attempt was therefore made to connect every pool by means of a ditch with the channels up which the tide came".

With the achievement of successful control of salt marsh mosquitoes demonstrated in selected areas of San Rafael and Burlingame, members of the Entomological Department at the University of California, Berkeley, directed their attention to a special study of two regions of the state; the overflow lands along the Sacramento river, and the foothill counties of the Sierra Nevadas. In both cases the most prevalent mosquitoes were the *Anopheles*, and throughout the two regions malaria was very common.

In December 1909 a letter from the Penryn Fruit Company of Placer County was sent to the University of California's Agricultural Experiment Station, requesting help in fighting the malaria mosquito. In response to the request the Department of Entomology in Berkeley sent Professor William B. Herms to meet with community leaders of Penryn, and the other nearby towns of Loomis, Newcastle, and Auburn. Herms evaluated the ma-

Figure 1.-The work at Penryn begins - left to right. Benjamin Bairos, Herbert Leak, Lawrence Woodworth, W. B. Herms.

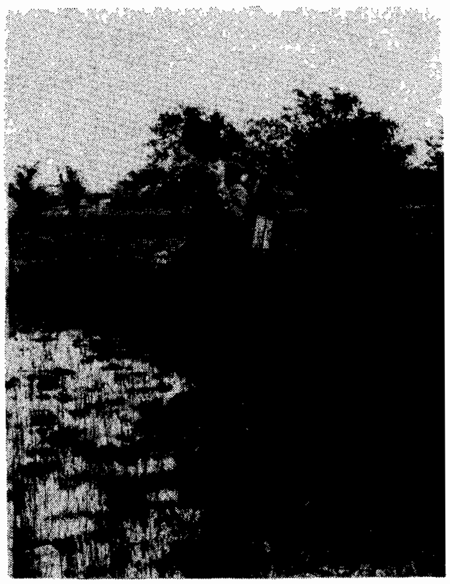

Figure 2.-Oiling a mosquito source-Oroville, 1916.

laria mosquito problems, conducted a public education campaign, and developed voluntary local funding. Field agents Harold F. Gray and Mr. Cornell assisted Professor Herms in these activities. During the same year (1910) the City of Oroville requested help in developing a program similar to Placer County. The funds raised for the control program at Oroville were from a community sponsored tag day. The ladies' club of Oroville sold mosquito tags on street corners in the business section of town. The mosquito control work at Penryn was the first organized anti-malaria control program to be carried out in the nation. The major source of malaria mosquitoes in Butte and Placer county areas during this early control program were overflow and seepage areas from irrigation ditches, dredger pools and other placer mining tailings, and water holding containers.

A large portion of these *Anopheles* mosquito sources in the Sacramento Valley were associated with residual water areas from newly formed irrigation service areas adjacent to the Sacramento River. In the Los Molinos and Anderson service areas many of the irrigation canals and supply laterals were hastily constructed, unlined, and subject to excessive seepage. This is paradoxical because real estate interests were pushing hard to develop irrigation districts in order to enchance the sale of farm land, and attract settlement in northern California, referred to in the regional news media as "Superior California". These newly developed irrigation projects, however, provided ideal conditions for malaria mosquitoes to be produced in enormous numbers, causing the human malaria cases to reach epidemic levels. As a result of this malaria threat, the settlers wanted their money refunded on their newly purchased farm lands. Strong appeals for help were sent to the University of California from these newly formed irrigation districts to help in controlling the malaria problem. For the next six years, Professor Herms and Harold Gray expanded their anti-malaria activities into Shasta, Butte, Glenn, and Merced counties, and the City of Bakersfield. On August 15, 1917, Mr. Harold F. Gray was appointed State District Health Officer by the State Board of Health, and assigned to continue his anti-malaria control work in the northern district of the State. This decade of 1910 to 1920 was the beginning of a distinguished career as public health engineer, health officer for the City of Palo Alto, mosquito control and, later, expert as a University lecturer, manager of the Alameda Mosquito Abatement District, president of the California Mosquito Control Association in 1933, and American Mosquito Control Association president in 1949. He was co-author with Dr. Herms of the text "Mosquito Control: Practical Method for Abatement of Disease Vectors and Pests", which served as a basic text for mosquito control internationally. Throughout his career, Mr. Gray provided leadership. He was a firm advocate of mosquito prevention through environmental modification. The early concepts of mosquito control that guided both Dr. Herms and Harold Gray in the formative years in California were derived through the teaching and guidance of Colonel J. A. LePrince of the U.S. Public Health Service based upon his experience during the anti-malaria campaigns in Panama.

In Harold Gray's 1918 Annual Report to the State Board of Health he described oiling for control of larvae and pupae as a very useful measure for the control of mosquito breeding but emphasized that it should be used simply as a measure supplemental to more permanent measures such as draining and filling. "If reliance is placed on oiling alone, the expense is continual throughout the breeding season and from year to year. Filling and draining are more costly during the first year or two but the expense thereafter reduces greatly, and in the course of not more than five years the initial cost of draining is more than exceeded by the constant cost of oiling". Draining is still the most useful means of achieving mosquito control where large areas of shallow seepage results from defects associated with newly constructed irrigation ditches and control structures. Considerable breeding areas in the northern Sacramento Valley were due to blockage of natural drainways by railroads, county roads and irrigation canals and laterals.

During 1912 and 1913, Dr. Herms and Harold Gray alternated as members of a traveling staff of University experts on the Agricultural and Horticultural Demonstration train that traveled from San Diego to the Oregon border, stopping at communities along the route to disseminate information on agriculture. One-half of a railroad baggage car was set up as a display for rural sanitation. They presented talks on mosquito and fly control, stressed the need for conserving irrigation water, and for area-wide drainage systems as the major approach to achieve mosquito control.

In Professor Herm's book, "Malaria: Cause and Control", published in 1913, a chapter is devoted to education as a vital link in the science of applied hygiene in controlling malaria and preventing mosquito breeding. "If once the people of a town or village catch the vision of better things, and are taught how to realize these things, the problem is largely solved, for there will surely be some who will put the matter to the test". The first step in educating the community in those early anti-malaria campaigns was to present lectures at public meeting halls, well illustrated by means of charts, lantern slides, and live specimens. The next phase was to follow up with brief newspaper articles and store window displays of living insects and poster narratives in the downtown business section.

One of the most important educational factors cited by Dr. Herms was with the school children. "The school children are vested in the classroom and the story of the mosquito wriggler is told, how the mosquito carries disease and how to prevent it. Classroom demonstrations with the living wrigglers are made and field trips taken to nearby swamp lands to see where the mosquito lives. Interesting essays are then written by the children and the best may be published in the local papers. The lessons learned at this time will be applied at once, and generation of citizens is reared with some knowledge of practical hygiene".

A major event that occurred during the early years was the passage of the Mosquito Abatement Act of 1915 by the State Legislature. This provided a mechanism for tax monies and set forth the powers and organizational structure of special districts to control mosquitoes on an area-wide basis, independent of city and county boundary lines. However, it was not until 1927 that the law was revised to give the Board of Trustees of a mosquito abatement district the power to compel the owner of any land to bear the expense of mosquito control work that was made necessary by the landowners operation or changes in land use.

Prior to the enactment of the 1915 Mosquito Abatement District Act, the first definitive county legislation in California to exterminate mosquito larvae was enacted in 1912 by the County of Tehama, Ordinance No. 46. Ordinance No. 46 ordained that no person, firm or corporation was allowed to cause stagnant water to exist within 2,000 feet of any occupied dwelling house, and upon finding mosquito larvae the person would be liable to a fine, arrest and imprisonment and if not drained or treated in a manner satisfactory to the health officer, the said nuisance shall be abated by the health officer and the cost recaptured by a lien upon the property.

Professor Herms outlined his recommendation concerning the use of irrigation water in Appendix II of his 1913 text on Malaria Control. It was directed to irrigation districts. His recommendations are as follows: 1) No irrigation water shall be so used that there will be any overflow onto adjoining property, or so that water in a stagnant condition collects and remains in any part of the water users property for more than 24 hours after the time the irrigation has been stopped nor more than six days from the commencement of irrigating. 2) Have educational session for their farmer clients each March. 3) Shall maintain a proper drain and prevent seepage around low spots in borrow areas next to ditches. 4) Provide circulars to landowners and prospective buyers giving concise directions to prevent breeding of mosquitoes.

The first local community to take advantage of the 1915 Mosquito Abatement Law was East Marin County. On November 3, 1915, a petition for the formation was presented to the Board of Supervisors and on November 16, a resolution to form a Mosquito Abatement District was passed by the Board. In 1916 the Three Cities Mosquito Abatement District of San Mateo County formed and became operational that same year. Noble Stover became Superintendent of Three Cities District and in 1916 also assumed the same position for the Marin County District. Mr. Stover was the most influential leader from 1915 to 1930 in the technology related to salt marsh mosquito control in the San Francisco Bay area. He was an engineering graduate from the University of California at Berkeley, specializing in irrigation and drainage. He also took courses in entomology and as a student under Professor Quayle worked during the summers on the initial drainage efforts at Burlingame before 1910. In 1913 he was employed by a volunteer San Mateo County organization for the purpose of salt marsh mosquito control. He was largely responsible for passage of the Mosquito Abatement District Act of 1915. He organized and became the superintendent of the Contra Costa Mosquito Abatement District in 1926. Mr. Stover's achievements in mosquito control and salt marsh reclamation were considerable. At one time he had at least seventy miles of marsh-land in the vicinity of San Francisco Bay under his control, and literally thousands of acres of land that had been major mosquito sources were reclaimed. His operational reports indicate the tremendous effort and ability in critical negotiations he carried out with large corporate landowners to convince them to modify their marsh land holdings to eliminate extensive mosquito-breeding areas. One of Noble Stover's assistants during early mosquito control campaigns in San Mateo County was Lawrence Woodworth; Professor C. W. Woodworth's son, who subsequently, in 1917, became the first manager of the newly formed Dr. Morris Mosquito Abatement District in Kern County.

One of the most comprehensive technical papers on control of salt marsh mosquitoes was published in July 1927 in the American Journal of Public Health, authored by Edward Stuart, California State Board of Health and Noble Stover, dealing with tide-water circulation ditches, installation and operation of tide gates, pumping and how to deal with the cracked ground problem in reclaimed tidal lands. In 1930, Mr. Stover was elected as first president of the Conference of Mosquito Abatement Officials, the forerunner of the present California Mosquito and Vector Control Association.

One of the significant contributions to mosquito control in the early years was the statewide malaria-mosquito survey conducted by Dr. William B. Herms and Dr. Stanley B. Freeborn, which commensed in 1916, and was financed by the State Board of Health. In 1917, as part of this statewide survey, World War I military cantonment areas were inspected for malaria and pest mosquito sources in San Diego, Imperial Beach, Camp Fremont (Santa Clara County), Monterey, and Sacramento. Survey results were sent to Colonel Lynch, Chief Sanitary Officer at the Presidio of San Francisco. This important work culminated in the University publication, "Mosquitoes of California, 1926," by Dr. Freeborn.

During the decade of the 20's, much of the concern of the State Board of Health and the University was directed to the struggling mosquito abatement districts in the Sacramento Valley. The 1915 Mosquito Abatement Act was a direct benefit to the San Francisco Bay Area districts, however, for the small districts in the northern California area, which were primarily organized as anti-malaria districts, their assessed valuation was often so low that even the maximum 10% tax rate provided in the Act provided less than $1,000 per year to handle the major mosquito sources that surrounded these valley communities.

Surveys by Louva G. Lenert and Edward T. Ross of the State Board of Health of nine northern California communities were conducted during 1922 - 1923 to evaluate the malaria mosquito problem, the level of funding and the operational capability of the local mosquito abatement districts. Survey results indicated that initial drainage and improvement of drainage channels could not be provided from funds at hand and also permit the necessary maintenance work during the remainder of the season. In all of the communities visited the lack of knowledge of the latest known control methods was very apparent. Each mosquito abatement district acted entirely upon its own initiative and without advice as to the most effective and economical methods of application.

The study recommended that the State Board of Health should have a mosquito specialist well versed in the latest scientific and economic methods to provide consultation to the local programs. The State Board of Health should also provide financial assistance to those districts with an endemic foci of malaria. The report recommended that $60,000 of State funds be provided for the first year's work. In April 1922, the State Board of Health secured a shipment of the mosquitofish *Gambusia affinis* from Texas and established a hatchery in the lily pond at Sutter's Fort in Sacramento. In the spring of 1923, 600 mosquitofish were sent by the State Board of Health from the Sacramento hatchery to the Oroville Mosquito Abatement District for mosquito control in the dredger ponds.

THE DEPRESSION YEARS (1930 - 1940).-At the first conference of the Mosquito Abatement District Officials in California held in Berkeley, December 16, 1930, a panel discussion was held on mosquito problems associated with construction of the State highway system. The panel stressed the advisability of getting in touch with the highway engineers early in the planning of a road project. A great deal of difficulty was cited in getting changes made in a project after the final plans had been approved.

There was a interesting discussion at this conference on the use of the airplane in mosquito abatement work. Harold Gray conducted experiments with the Curtiss-Wright Flying Service in the Alameda Mosquito Abatement District and concluded the airplane was especially valuable for observation and mapping work. It could possibly be used to apply a pesticide provided an effective material against mosquito larvae could be found and that suitable equipment could be developed to apply liquids for larval control.

The impact of the Depression caused a significant reduction in the tax revenues for the mosquito abatement districts. Reductions as great as 50% were common starting in 1931 and 1932. Professor Charles G. Hyde, member of the Alameda County Mosquito Abatement District Board of Trustees and Dean of College of Civil Engineering, University of California, Berkeley, in his address to the 1932 Conference of Mosquito Abatement Officials stated that budget reductions would force districts to cut down on much of the permanent mosquito prevention work which should be done. He added that oiling goes on forever and there is just as much the second and third years as there was the first. "Temporary measures result in permanent taxation".

The cesspool as an important breeding source of mosquitoes was a major subject of discussion at the 1932 Conference. Campaigns to change to a properly covered septic tank or a municipal sewage system and the need to cooperate with the local health department were recommendations being promoted by the Conference participants. The icebox drain was described as one of the more difficult mosquito sources in urban areas. Apparently it was common practice to simply bore a hole through the floor and let the water run under the building. This often was the case with small restaurants.

The nation's unemployment problems during the depression years fostered a number of federal and state-funded work relief programs which provided employment to a vast army of unemployed persons. In 1933 Dr. Herms was named state director of the federally funded pest mosquito control program under the Civil Works Administration. In a letter from Harry Hopkins, the CWA Director, California authorized $415,000 to be expended in two months for mosquito control. The project authorized the hiring of 1,650 workers, 34 supervisors and $15,000 for shovels, wheelbarrows, and rubber boots. No project would last more than 45 days. Dr. Herms' first directive announced that this would be a drainage project not an oiling operation. "We will do ditching and plenty of it. The object is to put men to work." The State Emergency Relief Act (SERA) and the Work Project Administration (WPA) employment assistance programs provided additional funds along with a generous supply of concrete pipe for mosquito drainage projects. At the 1935 Annual Conference of Mosquito Abatement Officials in California, a panel discussion was held to discuss the value of the emergency relief programs in mosquito prevention activities. The participants on the panel represented a cross-section of the state's mosquito control programs, and all had very positive reports on the value of this

Figure 3.-Ditching the marsh, 1935.

extra manpower. Without this governmental assistance (CWA, SERA, WPA) most of these mosquito source reduction projects would not have been possible.

During the decade of the 30's the cost of larvaciding with oil by backpack sprayer was about $5.00 per acre. Consequently, the use of hand labor, the Fresno scaper, and the dragline were more effective and economical for reducing mosquito sources than application of 25 to 30 gallons of oil per acre by hand application.

The Depression years caused a deterioration in living conditions. Residential window screens fell in poor repair and, at the same time, maintenance efforts on irrigation ditches were not carried out, producing seepage and improved conditions for anopheline breeding in the Central Valley region. These deteriorated environmental conditions caused a significant increase in numbers of cases of locally acquired malaria distributed from San Joaquin County north to Shasta County. At the request of the State Department of Public Health, Dr. Herms and Dr. L. L. Williams of the United States Public Health Service made a tour in 1936 of the Sacramento Valley. Dr. Herms reported that "We were much impressed with large numbers of foreign automobiles on the road and in camp for transients, and many of those out of state automobiles bearing license plates were from states notoriously malarious. The transients camped under bridges, along rivers and sloughs, in direct contact with breeding places for Anophelines. Dr. Herms visited a state migratory labor camp known as Camp

Waybur #7, located in the Sutter Basin. The camp doctor had diagnosed seven cases of malaria and there were another fifteen persons that appeared ill and suspect. There were 85 men living in the camp about a half mile from a rice field. The camp was ordered closed and the migrants were moved to Camp Applegate near Auburn, Placer County. While at Camp Waybur #7 Dr. Herms collected 250 *Anopheles maculipennis (freeborni)* in a 20 minute period by aid of an aspirator under one of the mess hall tables at mid-day. Many of these state labor camps would provide food and shelter in exchange for six hours of labor per day.

Harold Gray, Manager-Engineer of the Alameda Mosquito Abatement District, gave a talk on the engineering aspects of mosquito control at the 1936 State Mosquito Control Conference and was instrumental in setting the stage for mosquito control on the Central Valley Water Project. He recommended to the State Board of Health that the State should monitor the project for mosquito prevention using the model used in the TVA projects. This initial recommendation helped pave the way for establishment of a mosquito prevention monitoring program of the Central Valley Project conducted by the Bureau of Vector Control, State Department of Health, starting in 1949 with the assignment of two engineers, William J. Buchanan and William C. Warner from the USPHS and later establishing a position of Water Project Consultant in the Bureau of Vector Control staffed by Marvin Kramer.

THE WAR YEARS(1941 - 1946).-Richard F. Peters, newly appointed Mosquito Control Officer for the State Department of Public Health, set the stage for involvement of mosquito control in the war effort in his talk "Mosquito Control in the Vicinity of Military Zones" at the Annual Conference of Mosquito Control Officials in 1941. The State Department of Public Health was named sponsor of a State-wide National Defense WPA Mosquito Control Project. The primary objective was to control mosquito sources for a distance of five miles from military bases and National Defense areas. The U.S. Public Health Services provided engineers to survey and supervise mosquito control units. During 1941, 31 National Defense areas were listed for inclusion in the mosquito control effort. These installations varied from a PT boat base at Morro Bay in the salt marshes to bomber bases in Merced and Fresno.

Further discussions held at the 1941 annual California Mosquito Control Association Conference turned attention from mosquito prevention through water conservation, drainage and code enforcement to emergency measures for larviciding under war-time conditions. Emphasis was placed on developing power spray equipment to get the job done in less time with fewer man-hours. Also at that time field trials were conducted with pyrethrum mixed with oil-soluble emulsifying agents that allowed for spraying pasture areas which could not have been sprayed with the heavy application of oil. The lack of available manpower during war-time provided extra incentives for more efficient larviciding methods.

Disaster aid was a major topic during the early war years. Mosquito abatement districts developed plans to have power sprayers, trucks, pumps, etc., available for National Defense to fight fires caused by incendiary bombs and to respond to emergency mosquito and fly control after bombing attacks on urban areas.

At the 1944 CMCA Conference, considerable attention was given concerning the danger to civilian populations on the Pacific Coast from mosquito-transmitted diseases in returning military personnel--malaria, dengue, and filiarisis, were diseases mentioned as possible threats to California. The need to protect the State population against possible epidemics of imported vector-borne disease gave rise to a special University of California study committee that prepared a "Report on the Disease-Bearing Hazard of Mosquitoes in California". This report resulted in State Assembly Bill No. 28 (1946 Special Legislative Session) that provided subvention funds for local control programs, and for conducting studies and demonstrations on disease surveillance and control technology as determined by the Department of Public Health's Advisory Committee of scientific experts and local mosquito control representatives. Standards governing State aid to local mosquito control agencies were adopted by the State Board of Health. They set forth as one of the basic program principles implementation of measures aimed at the progressive reduction of known mosquito breeding sources.

The preoccupation with the new insecticide DDT in the late 40's appeared to be a salvation to the control agencies at that time. Here was a very effective material that could control both larvae and adults at .10 to .25 lbs to the acre, mixed with water and an emulsifying agent at about 1/3 the cost of former larvacides, and offered a new level of mosquito control never before dreamed of. This panacea for mosquito problems was short-lived. In the early fifties mosquitoes developed resistance to DDT to the degree that other chemicals were sought and again Harold Gray had his opportunity to make a strong appeal for returning to the concepts of mosquito prevention through environmental management as the primary method of mosquito control.

In retrospect, many of the problems facing mosquito control in California during the 1980's would be a repeat of what occurred in the late 1940's and early 1950's, i.e., the shift away from mosquito prevention through environmental management toward increased reliance on short-term larval and adult control agents. If Harold Gray were with us at the present he would again refer to his hallmark speech of 1950 "Which Way Now" presented at the 18th CMCA Conference and his words would have as much relevance today as they did then.

RECENT AMENDMENTS TO THE MOSQUITO ABATEMENT DISTRICT AND PEST ABATEMENT DISTRICT ACTS

Don J. Womeldorf[1], Allen R. Hubbard[2], and William E. Hazeltine[3]

Mosquito Abatement Districts (MADs) are authorized under Sections 2200 et seq., Health and Safety Code; Pest Abatement Districts (PADs) receive their authority from Sections 2800 et seq. of the Code. There have been numerous changes from the first enactment of the MAD act in 1915 and the PAD Act in 1939. Most amendments have been minor and designed to meet specific needs as they have developed. However, the passage of Proposition 13 in 1978 drastically affected District funding, and AB 1396 (Chapter 277, Statutes of 1980) required the Department of Health Services (DHS) to review all statutes relating to public health. These events resulted in major legislative changes embodied in SB 628 (Chapter 1055, Statutes of 1983) and SB 2162 (Chapter 911, Statues of 1984). Other 1984 changes included AB 2811 (Chapter 1085, Statutes of 1984), which dealt with fly nuisance abatement, and AB 3417 (Chapter 884, Statutes of 1984), which made minor technical corrections.

Both SB 628 of 1983 and SB 2162 of 1984 were authored by Senator Dan McCorquodale, representing Santa Clara and Stanislaus Counties. Others who were importantly involved in the two bills were Linda Milam, District Representative for Senator McCorquodale; Peter M. Detwiler, Senior Consultant to the Senate Local Government Committee; and Robert J. Beckus and Ralph A. Heim, California Advocates, Inc.

SB 628 was known as the Emergency Mosquito Abatement Funding Act of 1983. It rescinded the provisions of 1980 legislation which had appropriated one-time emergency matching funds for a single year, and added new authority for MADs, PADs and counties to fix a standby charge (a specific type of assessment against property but not based upon its value) for emergency mosquito abatement trust funds.

SB 2162 was an extensive recodification of the MAD and PAD Acts. Under the mandate of AB 1396, the DHS conducted an in-depth review of the public health statutes through the use of working groups with representation from agency associations and private organizations. The MAD and PAD Acts were reviewed by groups which included many individuals from the California Mosquito and Vector Control Association (CMVCA). The total time invested in the review of the District Acts approached 2000 manhours. When the DHS decided against including any special district statutes in its proposed recodification of the public health laws, the CMVCA decided to seek separate legislation, which became SB 2162.

Many changes made by the 1983 and 1984 bills were nonsubstantive. They only simplified or clarified the language, and are not discussed here. The substantive key changes in the MAD and PAD Acts are annotated below. The section numbers refer to the Health and Safety Code as of January 1, 1985. In general, the amendments affect governing boards, nuisance abatement, and financing.

<u>Governing Boards</u>

1. MADs.

   A. Trustee appointments by counties and cities are optional except for a required minimum number of trustees (Sec. 2240).

      Formerly, a city in or partly in an MAD was required to appoint a member of the Board of Trustees. A county was likewise required to appoint a member representing unincorporated areas. The amendment makes such appointment discretionary on the part of the city or county, but requires a county to appoint a minimum board of five members if that number is not otherwise reached.

   B. Trustees may be reappointed for terms of two or four years (Sec. 2245).

      Formerly, a trustee's term of office was for two years. The amendment specifies that a first term is not to exceed two years, but allows subsequent consecutive reappointments of the same person to be for either two or four years. The appointing body makes the choice on length of term.

   C. Trustees may receive a maximum of $50 in lieu of actual expenses (Sec. 2248).

      The increase from the previous limit of $35 is the first since 1969.

2. PADs.

   A. There are no changes in the appointment or terms of trustees. All PAD trustees serve at the pleasure of the Board of Supervisors.

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[1]Technical Consultant, CMVCA Legislative Committee.

[2]Chairman, CMVCA Legislative Committee.

[3]Member and Past Chairman, CMVCA Legislative Committee.

B. The allowance in lieu of actual expenses is $50 (Sec. 2851).

The increase from $25 is the first since 1959.

Nuisance Abatement

1. MADs.

Definitions of nuisance have been moved up to a section including several other definitions.

A. Water which produces mosquitoes is an abatable nuisance [(Sec. 2200(d), 2200 (e)(2)].

Formerly, the law allowed abating, as nuisances, specified mosquito breeding places. The amendment identifies water as property and makes the person or agency owning or controlling the water responsible for abating a public nuisance if the water is a breeding place.

B. Fly abatement is constrained [Sec. 2200 (e)(1)].

Formerly, the simple finding of fly larvae or pupae was prima facie evidence that the site was a breeding place. The statute, as amended by AB 2811 at the request of the California Farm Bureau, now goes on to provide several safeguards for agricultural operations which are suspected of causing public nuisances because of fly breeding.

C. Rodent presence or evidence is a nuisance [Sec. 2200(e)(3)].

This is new language. It will make it easier to abate as nuisances rodents or places where rodents shelter or feed.

D. The Director of the DHS is to arbitrate disputes between an MAD and a state or local agency, which has been ordered to abate a nuisance, on ways to prevent recurrence of a nuisance (Sec. 2283.5).

Formerly, the arbitration authority was limited to disputes an MAD might have with a state agency. The amended statute includes local agencies and is consistent with the PAD Act.

E. The Director of the DHS is to arbitrate disputes between government agencies on the need and means for abating or preventing nuisances (Sec. 2294).

This is new language. It extends the arbitration powers to general situations where agencies disagree on methods and materials to be used to abate or prevent a public nuisance.

2. PADs.

A. Water which produces mosquitoes is an abatable nuisance [Sec. 2800.5(b)].

This is new language. While it allows abating water in which mosquitoes are breeding, it does not extend (as does the MAD Act) to defining water as property.

B. The Director of the DHS is to arbitrate disputes between government agencies on the need and means for abating or preventing nuisances (Sec. 2804).

This is new language. It extends to general situations the arbitration powers dealing with the specific situations in Sec. 2863.

District Financing

Most changes in District financing provisions were needed as a result of 1978's Proposition 13, which limited ad valorem taxes (those based upon the value of property) to 1% of the fair market value. Since MADs and PADs formerly received almost all of their funding from property taxes, they were especially hard hit. Proposition 13 allowed imposition of "special taxes," not based upon property values, by a 2/3 vote of the electorate. Proposition 13 was silent on the matter of benefit assessments, which may be imposed on parcels (not the value) of property. A follow-up to Proposition 13 of 1978 was Proposition 36 of 1984. It would have limited the use of special taxes, benefit assessments, and other sources of funding not already limited by Proposition 13. Proposition 36 was rejected by the voters, and so the funding options in the MAD and PAD acts remain viable.

1. MADs.

A. An MAD may levy by resolution or ordinance a service charge or benefit assessment against parcels of land [Sec. 2270(l)].

This is new language. It allows an MAD to assess specified non-ad valorem tax-revenues.

B. An MAD may establish zones of benefit and may adjust taxes or assessments on land, improvements and personal property according to the benefits derived (Sec. 2291-2291.4).

This is language formerly limited to algae control but now broadened to vector surveillance and control.

C. An MAD is to receive its share of the 1% property tax (Sec. 2302).

This language replaces now-obsolete taxing authority. It recognizes limitations under Proposition 13 and establishes an MAD's right to its fair share of the ad valorem tax.

D. An MAD may impose a special tax upon approval by 2/3 of the voters at an election (Sec. 2303-2309).

Formerly, an MAD had the power to set a tax rate of up to $0.15/$100 assessed valuation, while the Board of Supervisors could increase the rate to $0.40/$100. A greater increase required approval by a majority of the voters at an election. The new language recognizes limitations and procedures established by Proposition 13 and subsequent legislation; any special tax is above and beyond the District's share of the 1% property tax.

E. An MAD, by ordinance, may fix a stand-by charge to establish an emergency mosquito abatement trust fund (Sec. 2315-2319).

This new language allows an MAD to fix a benefit assessment to set aside a limited amount of money for emergencies. The principal may be released only upon a finding by the Director of the DHS that a public health threat exists.

2. PADs.

PADs have differed historically from MADs in that there have been two optional methods of providing funding. One was ad valorem tax like MADs, the other a tax based upon area. A PAD was required to specify at the time of its formation which basis it would use.

A. Zones of benefit are authorized [Sec. 2822.5, 2871.5(b)].

Language has been added to existing sections dealing with taxation to allow for zones of benefit.

B. A PAD may levy a service charge or benefit assessment [Sec. 2855(i), 2871.9].

Language has been added to provide the authority to PADs by cross-reference to the MAD Act.

C. A PAD is to receive its share of the 1% property tax (Sec. 2871).

This language replaces now-obsolete taxing authority. It recognizes limitations under Proposition 13 and establishes a PAD's right to its fair share of the ad valorem tax.

D. A PAD may tax on the basis of area only, or in combination with some other basis (Sec. 2871.5).

This amendment allows a PAD to use both ad valorem and area taxes.

E. A PAD may impose a special tax upon approval by 2/3 of the voters at an election (Sec. 2871.8).

This new language recognizes limitations and procedures established by Proposition 13 and subsequent legislation.

F. A PAD may use procedures authorized for an MAD to establish an emergency mosquito abatement trust fund (Sec. 2877-2878).

This new language grants the authority to a PAD by cross-reference to the MAD Act.

COMMITTEE ACTIVITIES OF THE CALIFORNIA MOSQUITO AND VECTOR CONTROL ASSOCIATION, INC.

January 1984 - January 1985

James R. Caton[1]
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COOPERATIVE PLANNING.-Charles H. Dill, Chairman. The Committee met several times where they were brought up-to-date on the progress Earl Mortenson of VS&CB and the Chairman were making in the continuing discussion with California Fish & Game and U.S. Fish and Wildlife. A meeting on January 22, 1985 has been scheduled with state officials to finalize the state's version of the agreement.

ENTOMOLOGY AND BIOLOGICAL CONTROL.-Charles P. Hansen, Chairman. During 1985 several interesting articles appeared in Bio-Briefs under the leadership of Bob L. Coykendall. Members are encouraged to submit articles, particularly of an operational nature, to Bob for publication in future newsletters.

A questionnaire on mosquito light trap uniformity was sent out to all vector control agencies. A summary of the results should be available in early 1985. The Committee's objective is to see greater standardization and reliability in Districts' reporting methods.

A series of Bio-Notes, patterned after the Mosquito Notes, was the primary goal during 1985. These one-sheet handouts are designed for general public usage, to be used primarily by mosquito control technicians in their daily operational program. The Committee will complete the final version on the midge, Bti, mosquitofish and mermithid nematode and present to the CMVCA Publications Committee in early 1985.

ENVIRONMENT.-Fred C. Roberts, Chairman. The Committee met three times during the year 1984. The major accomplishment during the year was when the Committee set up a presentation by the California Department of Fish and Game to explain the use of the Natural Diversity Data Base that is available to all Districts. The Districts, by utilizing this data base, can obtain vital information about rare and endangered species in their Districts.

EQUIPMENT.-Claude L. Watson, Chairman. The Committee was reluctant in reporting this past year for the lack of new equipment being introduced into the mosquito control program.

Norman B. Akesson with U. C. Davis has advised that studies are being further made on cold fogger and aircraft nozzles for ultra low volume spraying that may be of interest when finished.

The question was put forth to the Committee of putting on an Equipment Show, but for the lack of new equipment, the Committee felt it would not be worth while at this time. We are in hopes that in the near future such a show can be put together, as was in past years for the interest of all employees of the Mosquito Abatement Districts through-out the State. Not only do the employees enjoy these shows but much is learned from seeing other District's equipment and the methods that are used in mosquito control.

LEGISLATIVE.-Allen R. Hubbard, Chairman. The following is a list of the accomplishments of the Committee for 1984.

1. Worked very hard in writing, reviewing and testifying toward the passage of SB 2162, an extensive recodification of the MAD and PAD Acts.

2. Reviewed and worked closely with Norman Waters, D-Plymouth, and The California Farm Bureau on how AB 2811 would affect fly control efforts. AB 2811 was intended to provide safeguards for agricultural operations which are suspected of causing public nuisances.

3. In April, 1984, the Committee recommended to the CMVCA Board of Directors that it go on record in opposition to Proposition 36, the Jarvis Initiative.

4. On August 15, 1984, the Committee and the CMVCA went on record in support of AB 2635. It was important that the legislature assert its intention that pesticides be a statewide concern and that it should be administered in that manner.

5. The Committee recommended support for SB 2197, the Exotic Vector Importation bill.

6. The Committee reviewed AB 2013 which requires the inventory of all underground facilities for hazardous materials and AB 1362, which provides for a regulatory program administered by county or city agencies to enforce construction and monitoring standards.

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[1]Vice President, California Mosquito and Vector Control Association, Inc.

RESEARCH.-Jack E. Hazelrigg, Ph.D., Chairman. The Committee accomplished its major duties in FY 84-85 by reviewing the more than 30 major research and student mini-grant proposals that focus on various aspects of California mosquito control--both applied and basic. These proposals, submitted by researchers from the University of California (Depts. of Entomology, School of Public Health, Experimental Research Stations, etc.), were critiqued in March '84 during a 2-day ERC meeting held at the University of California, Davis, with Russell E. Fontaine, Sc.D. (Cooperative Extension, Mosquito Control Research Coordinator).

The ERC's review-concerns principally address proposal content that either directly meets the relevant and practical needs of MADs directly, or, indirectly does or promises to. A proposal is rated chiefly on this basis and also scientific merit. Duration of the researchers proposed work, his adherence to schedule, publication of information, and past performance(s) are also considered.

The rated proposals this year, as in the past, were submitted to the Mosquito Research Technical Committee to use in their subsequent review and evaluation of the same research proposals before funding is awarded.

WAYS AND MEANS.-Charles Beesley, Ph.D., Chairman. The Committee compiled and distributed to the Board of Directors a Board Manual. The manual as such covers five main sections for Board referral: Duties, Committees, Procedures, Conference and Minutes with some subsections. The Committee is aware that this manual is still an unfinished product, primarily because of the scope of the project and the continual list of questions that come up while working on it. Nevertheless, it recommended acceptance of the manual by the Board and adoption for use in 1985. At the end of the year, questions can be forwarded to the Ways/Means Committee to be looked into, therefore polishing the manual as time goes by. This is meant to be a working document and will need revising continually, thus the reason for using it as is.

AD HOC CENTRAL OFFICE.-Stephen M. Silveira, Chairman. The Committee continued its implementation of the Committee's March, 1983, recommendations to the CMVCA Board of Directors. Our goal, then and now, is an efficient and cost effective Central Office.

The Board of Directors approved the purchase of an IBM computer system with dual disk drive, monitor, Printwheel printer and sheet feeder. The software programs, "Chartpack" and "Textpact 6" were selected for accounting and wordprocessing purposes. The total cost for this package was $12,756.88. The system was delivered to the Central Office on June 27, 1984.

Training was provided for the Central Office secretary by IBM. A part-time clerk was hired to assist with "setting up" the computer. On November 30, the Board approved hiring a temporary computer operator to expedite the completion of the "Proceedings". The Committee's recommendation that all part-time and temporary personnel be terminated on January 17, 1985 was approved by the Board of Directors.

The Committee will be re-evaluating the Central Office staff needs. Some aspects to be considered are the reason(s) for extra help and the type of personnel needed -- "permanent" part-time help, temporary extra help for special projects or full-time, or clerk, secretary, computer operator, etc. Hopefully, this study will enable the Committee to develop a procedure to insure timely completion of projects.

Executive Director Marvin C. Kramer resigned April 24, 1984 and Committee member John C. Combs accepted the assignment of interim director.

AD HOC CMVCA/JPA ADMINISTRATION REVIEW.-Gilbert L. Challet, Chairman. The charge of the Committee was to determine the feasibility of a joint CMVCA and JPA office. This we hope would save money for both agencies. The Committee met with a number of people over the year including attorneys, risk management specialists, and association executives to determine the feasibility of combining the two offices with one administrator in charge. At this time, we are preparing a "Request for Proposal" to solicit bids for administration of this joint office. The time table for this project will extend through January, 1986, according to an implementation schedule developed by the Committee.

CALIFORNIA CONFERENCE OF DIRECTORS OF ENVIRONMENTAL HEALTH PRESENTATION

Richard L. Roberts, President-Elect
135 Palmyra Street, Auburn, California 95603

Once again, it is my privilege to meet with you and extend the warmest greetings and best wishes from the CCDEH and the CAEHA. Both President Martin Winston from Placer County and Bill Norman, Director of Environmental Health from Merced County who is the Chairman of our Vector Committee were unable to attend. Both asked me to express their regrets, their regards and they extend to you their sincere wish for a successful conference here in Stockton.

You may recall that last year I discussed with you the five areas where your Association and ours have an opportunity to work together for mutual benefit. I will summerize these areas.

- Certainly at the local level, where we both have public health vector control responsibilities. There are many examples where MAD's, PAD's and VCD's are well coordinated with the local health agency and mutually support each other. Once again, I encourage you to keep in frequent contact with your local health officer and Director of Environmental Health.

- The reason I am here today is because of a long-standing agreement to exchange Association speakers at our annual meetings. I'm told that Bill Hazeleur will attend our conference in September, to be held at the Granlibakken Resort in the North Tahoe area.

- The California Conference of Local Health Officers - Environmental Health Committee where a member of your association is represented. Bill Hazeltine serves you well on the Committee.

- We have had a couple of mutual projects working out of a task force group. The most recent is the development and the continued refining of the 1983/84 "Guidelines, Checklists, and Standards for Vector Prevention in Proposed Developments". The task force is now encouraging that these guidelines and standards be shared with the local planning agency. I am told that Marin and Sonoma MAD's have already had workshops and comments were favorable. San Bernardino County will soon adopt them as a part of their development code. This project has been a major effort and the people involved from our respective associations and the SDOHS/VBCB staff involved are to be commended.

- Finally, the SDOHS Vector Control Advisory Committee where both of our Associations are represented. I will defer an update on the Committee's activities to either Dr. Reeves, Chairman of the Committee or Don Womeldorf.

This year our organization will emphasize two areas of Vector concern through our Vector Committee chaired by Bill Norman. --First, the increasing trend of homesite development into rural area endemic to plague and increased exposure to other disease vectors where homesites may come into closer contact with mosquitoes, flies, ticks and kissing bugs. Tell me about it! I am the District Manager for the new and successfully operating West Valley Vector Control District located in the southwest corner of San Bernardino County and serving the urban areas of Chino and South Ontario and Montclair and the vast agricultural preserve in the Chino Valley. Within the district boundaries are 15-20 poultry ranches, 280 + dairies, calf raising operations and horse estate sub-divisions. In addition to some 200 dairy waste water ponds, we have dairies that literally abut apartment house and condominium developments. Further, our County is committed to develop the Chino Hills, where 30,000 new homes will be developed adjacent to the Diamond Bar Development where plague has been recently found. Our experience there might be useful to others and could lead to further recommendations to be included in the development Guideline document I mentioned earlier. I'd like to introduce part of our staff. Lyle Stotelmyre, Supervisor of the County-wide Vector Control Program and resident entomologist and Allen Pfuntner, Field Operations Manager for the WVVCD. If you ask them questions - be prepared - they will give you complete answers. Our District Board of Trustees will be arriving to attend this meeting soon and I hope you will have the opportunity to meet them. Bill Sitton, President, Lisa Van Grueningen, Vice-President, Bill Walker, Secretary, Nancy Sitso and Wayne Stringer.

Second, the need to continue to overcome inadequate funding of local vector control services. At least two counties with different funding approaches have been successful. In San Bernardino County, our Board of Supervisors, after a 60% favorable advisory vote, formed the WVVCD. Even with the Jarvis' Prop. 36 hanging over their heads, the WVVCD Board of Trustees, with courage and a calculated risk, set their benefit assessment rates, contracted with the County, received a loan from the County, and were in business two to three months after the Board of Supervisors formed the District. A record, I'm sure! At this time, on behalf of the District Board of Trustees, I express our appreciation to you for your assistance in assuring that SB 2162 was adopted as an urgency measure which allowed the District to begin collecting assessments with the November Tax Roll.

In Alameda County, with a 70.8% support vote, a Board of Supervisors governed County Service Area was formed to provide funds to the Environmental Health Department to perform all vector services except mosquitoes which will continue with Fred Roberts' program. The emphasis will be rat control.

Aside from vector issues, our conference is very interested in how the Governor proposes to reorganize this administration in the area of toxics management. As you probably recognize, we at the local level must deal with various State Departments in the area of toxics and hazardous waste; State Department of Health Services/Toxic Substance Control Division, State Department of Food and Agriculture, Water Resources Control Board, Waste Management Board and Air Resources Board to name a few. These State agencies have a certain amount of autonomy with different goals and objectives which lead to conflicting ways of delivery of services, duplicative and overlapping turf battles and a general frustration on the part of local government and industry. We support a reorganization and the Governor as of three weeks ago will release his proposal within the next 100 days. In the meantime, Assemblymen Willie Brown and Frizelle have proposed legislation on possible reorganization.

We appreciate this time on your program. Your program is broad in scope and has technical depth and should appeal to everyone. I look forward to seeing you again next year.

PERIPHERAL DITCHING FOR SOURCE REDUCTION IN A WILDLIFE AREA

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ABSTRACT

Surface water draining rates of wetland cells with and without peripheral ditching were compared, in the Mendota wildlife area. Water drained from ditched cells at a significantly greater rate, 2.8x faster, than from cells not ditched (P < 0.01). However, the duration of standing water in all cells, ditched as well as not ditched, was sufficient to allow the development of *Aedes melanimon* Dyar immatures to the adult stage.

INTRODUCTION.-The Mendota wildlife area, 3800 ha (9390 ac) administered by the California Department of Fish and Game, is arranged into distinct wetland cells; each is managed to produce one of several vegetative types. Predominate types are barnyardgrass *Echinochloa crusgalli*, swamp timothy *Heleochloa schoenoides*, alkali bulrush *Scirpus maritimus* and a combination of these and spikerush *Eleocharis spp*. Cells are flood irrigated and manipulation of irrigation is important in the selection of dominant vegetation. Proper drainage also is necessary to inhibit such undesirable plant species as cattail *Typha domingensis*.

To decrease the time needed to drain excess water after irrigation, the management at Mendota has initiated a program of constructing peripheral, surface ditches within cells. Since ditching has proven a successful mosquito source reduction method in various salt marshes (Provost 1977), we endeavored to determine the impact of peripheral ditching on standing water time and mosquito production at Mendota.

MATERIALS AND METHODS.-Six wetland cells were selected for study. A continuous peripheral ditch adjacent to the cell borders in each of three barnyardgrass cells, #45-6 [30 ha (74 ac)], #13-2 [21 ha (52 ac)] and #4-5 [19 ha (47 ac)], was constructed by wildlife area personnel with a custom-made ditch plow drawn behind a Versatile 800 tractor. The ditch (Figure 1) was ca 35 cm deep and 50 cm across and the thrown soil formed a levee on the border side of the ditch. Two barnyardgrass cells, #45-3 [12 ha (30 ac)] and #1-2 [13 ha (32 ac)], and an alkali bulrush cell, #27-1 [32 ha (79 ac)] were left not ditched for comparison as a control group.

Within each cell, 6 sampling stations were located at low elevation sites to determine the rate of disappearance of water. Water depth was recorded at each site until surface-water drainage was complete. To correct for elevation differences between sites the final depth was subtracted from previous measurements for each site. These values were combined in a linear regression of water depth by time for each cell. The resultant slope (cm/day) was corrected for evaporation using daily class A pan evaporation data for the specific time interval [USDA Murrieta Farms weather station, within 4 km (2,5 mi)].

In each cell the mosquito population was sampled at the beginning of the draining process. Number and stage of immatures was recorded for each of 50 dips/cell.

RESULTS AND DISCUSSION.-The rate at which surface water disappeared from a wetland cell was greater in those cells that had peripheral ditching. Derived regression slopes (adjusted for evaporation loss) are shown in Table 1 along with 0.95 confidence limits. Regressions were highly significant (P < 0.01) for all slopes as determined by correlation coefficients. The average water removal rate for ditched cells was -4.67 cm/day; a value 2.8x greater than the average for those cells not ditched (-1.66 cm/day).

Statistical comparison of regression slopes showed highly significant separation between ditched and control groups. Slopes within the control group did not differ, nor did those within the ditched group (P < 0.01). Water removal was significantly increased in those cells which were ditched.

Although calculations accounted for water loss through evaporation, loss due to percolation could not be determined. Soil found in the study areas was tentatively classified by the USDA, Soil Conservation Service as follows: cell #'s 45-3, 27-1, 45-6, and 4-5, Tachi clay; #1-2, Lillis clay; and #13-2, Westcamp clay loam and Lillis clay. All of these soil classes are characterized by very slow water permeability. Percolation in such soils is minimal and standing water is primarily lost through evaporation and surface water draining. Therefore, our calculated slopes correspond primarily to the surface water drainage rate for each cell.

Water stood for sufficient time in all cells to produce adult *Aedes melanimon* Dyar (Table 2). The shortest interval between the onset of flood irrigation and completion of drainage was 13 days (#45-6). In all cases there was enough time for adult development between the sampling date and

Table 1.-Surface water draining rates (linear regression statistics) of Mendota wetland cells with and without peripheral ditching. Values adjusted for evaporation loss from class A pan data.

Cell #	Slope (cm/day)	0.95 Confidence Limit Lower	Upper	Correlation Coefficient
		(Not ditched)		
45-3	-1.360	-1.524	-1.196	-.9823
27-1	-1.840	-2.511	-1.168	-.8517
1-2	-1.781	-2.138	-1.424	-.9375
		(Ditched)		
45-6	-6.071	-6.672	-5.469	-.9813
13-2	-4.495	-5.637	-3.354	-.8333
4-5	-3.438	-4.115	-2.761	-.9148

Table 2.-Estimation from dip samples of *Aedes melanimon* populations in Mendota wetland cells and time intervals showing duration of standing water.

Cell #	No. Immatures/dip Mean	S.D.	Stage(s)	Time Interval (days)[1/] A-B	B-C	S-C	Water Depth[2/] (cm)
			(Not ditched cells)				
45-3[3/]	0.08	0.44	IV, P	9	10+	10+	23+
27-1	0.30	0.70	III, IV	6	11	6	16
1-2	0.27	0.44	IV	5	9	8	19
			(Ditched cells)				
45-6	0.10	0.30	IV	6	7	6	42
13-2	0.22	0.64	II, IV, P	6	11	11	61
4-5	0	0	-	4	11	9	40

1/ Time interval from (A-B) initiation of flooding to completion of flooding; (B-C) completion of flooding to completion of draining; and (S-C) sampling date to completion of draining.

2/ Water depth subject to draining, difference between first and last records.

3/ Sampling was terminated before draining was complete.

Figure 1.-A Mendota wetland cell with a newly ditched periphery. Cattail growth (left foreground) is enhanced by poor drainage.

complete drainage. The minimum duration was 6 days in cell #'s 27-1, and 45-6. Control measures, aerial larviciding, were applied to all cells except #4-5, which did not produce a larval hatch, and #45-3.

While peripheral ditching did not reduce standing water duration below that necessary for adult development, the depth to which the cells were flooded prolonged the draining process. Ditched cells were flooded to much greater depths than were the control cells. The reason for this disparity, other than possible physical characteristics, is not known. Had the control cells, with their lower flooding depths, drained at rates similar to ditched cells, resultant draining times would be reduced by an average of 6.5 days.

In an earlier study the infiltration or percolation of pasture water in eastern Fresno County was determined with regard to the need for implementation of vertical drainage (Mulligan et al. 1979). An infiltration rate below an arbitrary value of 6.85 cm water loss per day in the absence of surface drainage indicated that increased percolation, via vertical drainage, was needed. Surface water drainage rates of ditched fields were below this value, which indicated that other factors must also be manipulated to reduce standing water time sufficiently to effect mosquito control. A prime factor is the prolonged time needed to flood prior to draining (Table 2). Further, the cell with the greatest drainage rate (#45-6) had been leveled prior to ditching. Land leveling might increase the efficiency of ditching. A combination of water management procedures is needed, ditching alone is not sufficient to control *A. melanimon* at the Mendota wildlife area.

ACKNOWLEDGMENTS.-The cooperation of personnel of the Mendota wildlife area (California Department of Fish and Game) and the Fresno Westside Mosquito Abatement District is gratefully acknowledged. This research was supported by the University of California Mosquito Research Program.

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STUDIES OF THE DEVELOPMENT OF AN INTEGRATED MOSQUITO CONTROL STRATEGY FOR THE FALL RIVER MILLS AREA[1]

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ABSTRACT

Mosquito problems in the communities and outlying areas of Fall River Mills and McArthur in Shasta County are being studied to determine a comprehensive approach for their management. Light traps and larval monitoring stations were operated from early April through mid-September and set up adjacent to residential areas, irrigated pastures, and wild rice farms. *Culex tarsalis* and *Anopheles freeborni* were the two predominant species coming to light traps and accounted for 45% and 20% of the total mosquitoes collected, respectively. Larval surveys indicated that wild rice fields were a major source for both *Cx. tarsalis,* and *An. freeborni*. The major species found in irrigated pastures were *Ae. melanimon, Cx. tarsalis,* and *Ae. vexans,* while seepage and runoff from rice fields produced largely *Cx. tarsalis*. Fish populations released into fields increased dramatically through the summer and this correlated well with a decrease in *Cx. tarsalis* larvae.

INTRODUCTION.-Mosquito problems in the communities and outlying areas of Fall River Mills and McArthur in Shasta County are being studied to determine a comprehensive approach for their management. In this high mountain valley, elevation 1000 meters, irrigated pastures have typically been a major breeding source for several species of mosquitoes including *Aedes melanimon, Ae. nigromaculis, Ae. vexans* and *Ae. dorsalis*. In 1982 the cultivation of wild rice was initiated with 80 acres and this subsequently expanded rather quickly to include over 1000 acres in 1984. Because wild rice is grown in shallow water for several months during the spring and summer, it was anticipated that certain mosquito species would exploit this particular habitat. In light of this, a major emphasis of this study was to assess the contribution of wild rice cultivation to the mosquito fauna of the area as well as to investigate feasible mitigating measures.

The commercial production of varieties of wild rice, *Zizania palustris*, had its modest beginning in California in 1977 with the harvest of about 300 acres. Since that time acreage has expanded continuously, increasing to about 2400 acres in 1982 followed by approximately 6000 acres in 1984. The Sutter-Yuba basin currently contains about two-thirds of this acreage while Shasta, Lassen, Mendocino and Lake Counties account for the remainder (Winchell and Dahl 1984).

As mentioned above, prior to the introduction of wild rice, the primary mosquito problems in the Fall River area consisted of floodwater *Aedes spp.* produced with the irrigation of pastures. As some of the wild rice acreage has displaced some of these irrigated pastures it is expected that *Aedes* production may have declined in the area, at least to some extent.

MATERIALS AND METHODS.-In order to assess the production and movement of mosquitoes, light traps and larval monitoring stations were operated from early April through mid-September. Figure 1 shows the light trap locations in the valley. Trap 1 was set up in a golf course approximately 3 miles from the nearest rice field while traps 2 and 5 were situated within 50 yds of wild rice. Trap 5 also had irrigated pastures relatively nearby. Traps 3 and 4 were placed about 1/4 mile from both rice and irrigated pastures, the latter trap located near a recreational lodge adjacent to lava rock formations.

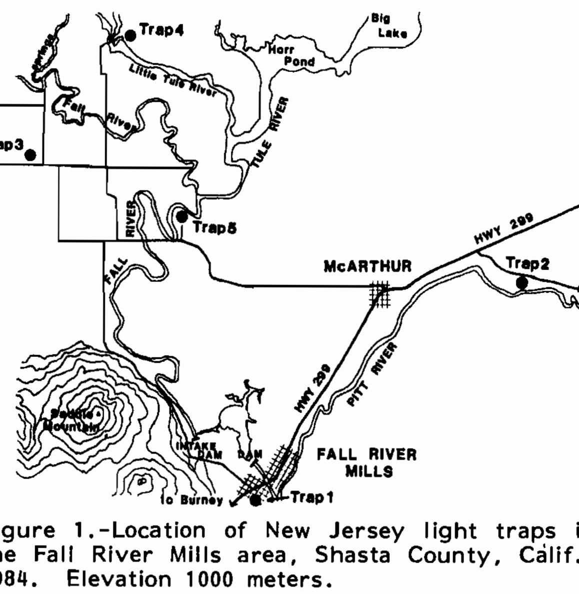

Figure 1.-Location of New Jersey light traps in the Fall River Mills area, Shasta County, Calif., 1984. Elevation 1000 meters.

[1]This research was supported in part by Special Funds for Mosquito Research in California.

In other studies two wild rice farms were selected to determine whether the mosquitofish *Gambusia affinis* could be used effectively in this habitat to reduce *Anopheles* and *Culex* populations. One farm was located near Pittville and the other farm was in the Glenburn area. Heavy wire mesh and fine window screening were placed around weirs to prevent the fish from moving between the test and control fields. Two monitoring systems, a dipping regime and minnow traps, were set up in each field to assess the fish and larval mosquito populations throughout the rice growing season. A maximum of 15 fields were selected for use at the Pittville farm while 6 fields were selected at Glenburn. Seventy-five dips were taken at one-meter intervals along a transect at the low end of each field. Fields ranged from 1 to 3 acres at the Pittville farms to 5 to 20 acres at the Glenburn farms. Dipping was conducted at biweekly intervals for a total of 5 times from June 7th through August 3rd. Two to three 1/8 inch mesh minnow traps were set per field on 3 occasions from the last week in June to just before the fields were drained in mid-August. The site at Glenburn was dropped from our studies in mid-July when the fields were inadvertently drained due to a ruptured irrigation pipe.

RESULTS AND DISCUSSION.-*Culex tarsalis* and *Anopheles freeborni* were the two predominant species coming to light traps and accounted for about 44% and 20% of the total mosquitoes collected, respectively. Other species collected were *Ae. melanimon* (18%), *Culiseta inornata* (10%), *Ae. vexans* (3%), and *Ae. dorsalis* (< 1%; Table 1).

Adult mosquitoes were initially detected in May and remained in low numbers through the third week of June. Between the last week of June and the first week of July a sharp rise in activity was noted among the principal species. Populations peaked about mid-July and declined thereafter with most mosquito activity at very low levels by mid-September (Figs. 2 & 3).

The attraction of *An. freeborni* to light traps was particularly sharp and relatively high in areas adjacent to rice fields (Fig. 2). Over 75% of all *Anopheles* were recovered over a 5-week period starting the first week in July, with a single peak of activity noted in mid-July. This pattern of *An. freeborni* activity presents an interesting contrast with that recorded in the central valley. Light trap data from Gray Lodge Wildlife Refuge in Butte County have shown peak *An. freeborni* populations between late August and mid-September - some 5 weeks later than peak populations seen at Fall River Mills. Apparently the more northerly latitude and the elevational differences of over 1000 meters are the primary reasons for the relatively short and sharp activity period of *An. freeborni* in the Fall

Table 1.-Summary of the mosquitoes collected in each light trap in the Fall River Mills area, Shasta County, Calif., 1984[1].

Trap	Total	*An. free.*	*Cx. tars.*	*Ae. melan.*	*Ae. nigro.*	*Ae. vex.*	*Ae. dors.*	*Cs. inorn.*
1	681	153	344	75	24	5	5	75
%[3]		22.5	50.5	11.0	3.5	0.7	0.7	11.0
2	3,996	1,784	1,813	15	80	1	9	294
%		44.4	45.4	0.4	2.0	0	0.2	7.4
3	8,776	807	3,238	2,740	131	229	56	1,575
%		9.2	36.9	31.2	1.5	2.6	0.6	18.0
4	4,125	1,396	906	1,178	370	78	29	168
%		33.8	22.0	28.5	9.0	1.9	0.7	4.1
5	10,915	1,614	6,340	1,194	214	640	69	844
%		14.8	58.1	10.9	2.0	5.9	0.6	7.7
TOTAL		5,754	12,641	5,202	819	953	168	2,956
%		20.2	44.4	18.2	2.9	3.3	0.6	10.4

[1] Traps set April 23 - September 15 and collected at weekly intervals.

[2] males and females combined.

[3] % total of each species/trap.

River area.

Both male and female *An. freeborni* occurred in light traps near rice fields at the same time in mid - late June, which indicates that these fields were colonized about 2 - 3 weeks earlier by females which had moved out of their overwintering sites, taken a blood meal, and laid their eggs to produce the first generation of the year. An overwintering site was recognized near the location of trap 4 as indicated by the recovery of female *An. freeborni* about 6 weeks earlier than their recovery from traps immediately adjacent to wild rice. The lava rock formations in this area have deep cracks and crevices that could be used as an overwintering site by mosquitoes.

Table 1 summarizes the actual numbers of mosquito species collected at each of the light traps. *Cx. tarsalis* was collected in relatively large numbers in light traps near wild rice fields. Population levels of this species remained high throughout the summer, dropping precipitously in mid-September (Fig. 2).

Light traps stationed relatively close to irrigated pastures showed the highest numbers of floodwater *Aedes spp.*, as would be expected (Table 1, Fig. 3). Traps 1 and 4 , situated in more residential areas, showed variable results. Trap 1, situated farthest from wild rice fields and irrigated pastures, had the fewest numbers of mosquitoes, whereas trap 3, adjacent to wild rice fields and irrigated pastures, had high numbers of both *Cx. tarsalis* and *Ae. melanimon* but only moderate numbers of *An. freeborni* (Table 2, Figs. 2 & 3). Trap 4 located next to the Lava Creek Lodge showed moderate but prolonged activity of *An. freeborni, Cx. tarsalis* and *Ae. melanimon*. *Ae. vexans*, a particularly severe biter, was only recovered from two traps, both fairly close to irrigated pastures. Larval surveys correlated well with light trap data and indicated that wild rice fields were an important source for both *Cx. tarsalis* and *An. freeborni*. *Cx. tarsalis* appeared in large numbers in these fields by early June and *An. freeborni* larvae were recovered starting in late June (Fig. 2). The major species found in irrigated pastures were *Ae. melanimon, Cx. tarsalis*, and *Ae. vexans*, while seepage and runoff from rice fields produced largely *Cx. tarsalis*.

Results of the minnow traps indicated that the mosquitofish had dispersed throughout the test and control fields, apparently due to fluctu-

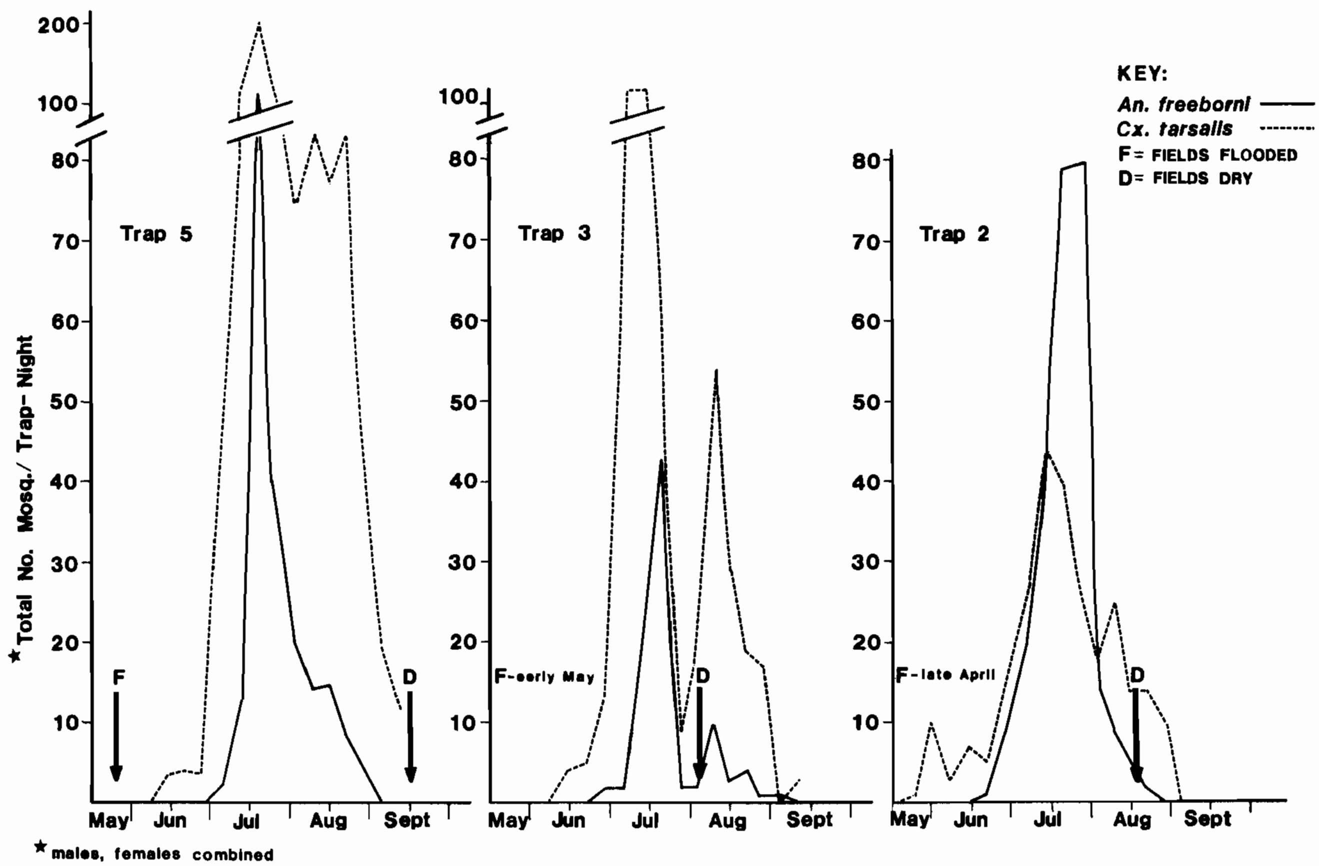

Figure 2.-*An. freeborni* and *Cx. tarsalis* populations collected in light traps adjacent to wild rice fields, Fall River Mills, Shasta County, Calif., 1984. Elevation 1000 meters.

ating water levels breaching the fairly low levees. The fish populations increased dramatically through the summer (Fig. 4) and this correlated well with a decrease in *Cx. tarsalis* larvae (Fig. 5). *An. freeborni* larvae, which were in relatively low numbers throughout the season, increased to about 0.2 larvae/dip in mid-July but decreased to less than 0.1 larvae/dip by the first week in August. The decline in mosquito larvae, particularly *Cx. tarsalis*, in all fields showing a corresponding increase in mosquitofish, is suggestive of control. However, the presence of fish in the control fields precludes further analysis.

Large populations of predatory insects were collected in both the minnow traps and dipping regime (Table 2). Coleopterans, odonates and notonectids were the most common insects found and their relative abundance in the minnow traps and in the dippings are recorded in Figures 4 and 5.

Studies for the forthcoming year are planned to confirm and quantify in more detail the production of mosquitoes in the various habitats, the potential for the use of mosquitofish in an integrated control program and the role of the large populations of naturally occurring predatory insects in wild rice fields.

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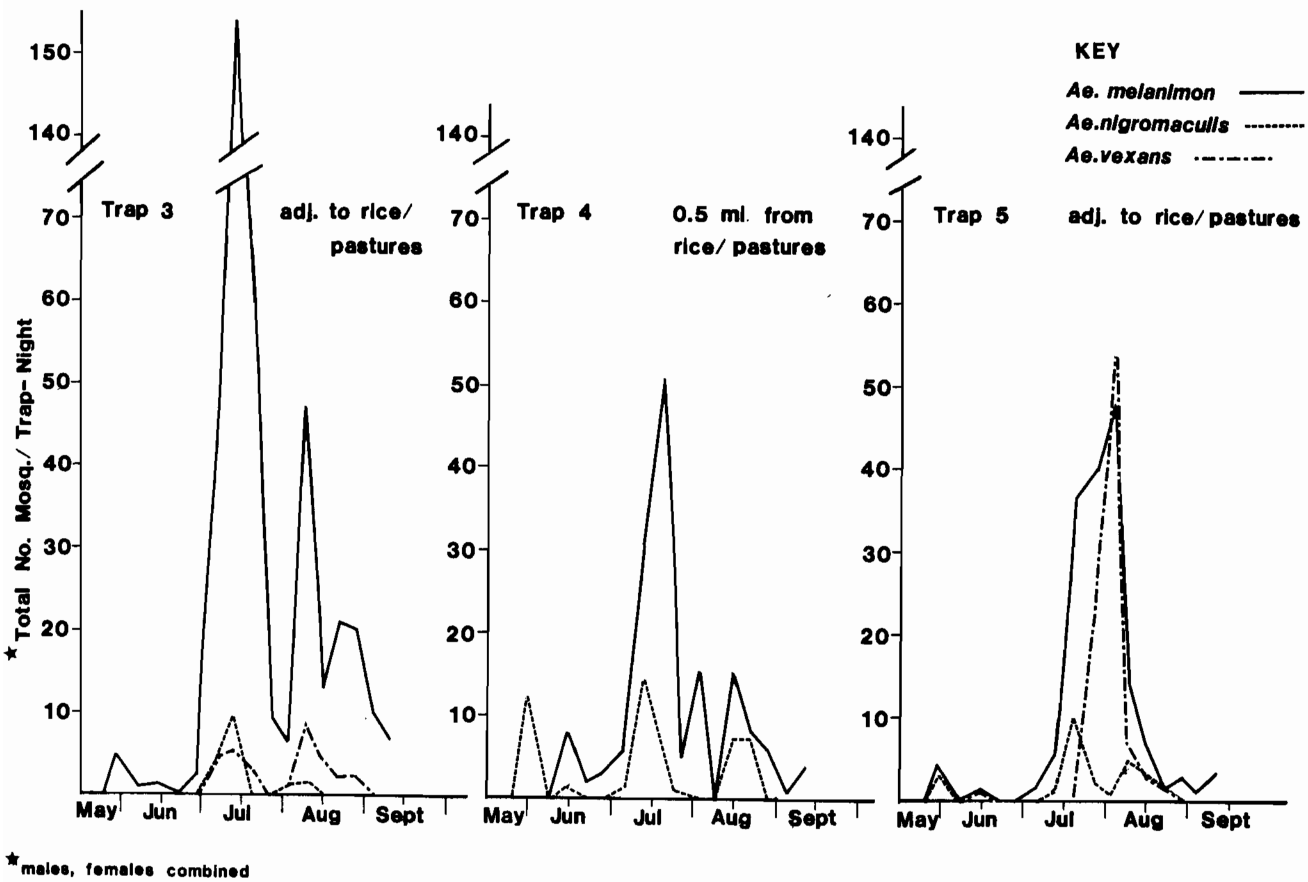

Figure 3.-*Aedes*, spp. populations collected in light traps near irrigated pastures, Fall River Mills, Shasta County, Calif., 1984. Elevation 1000 meters.

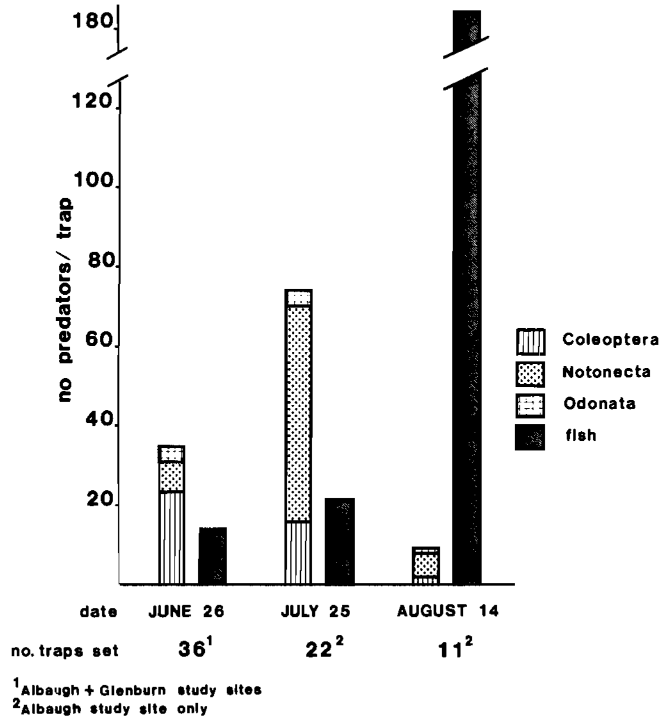

Figure 4.-Results of minnow traps set in wild rice fields in Shasta County, Calif., 1984. Elevation 1000 meters.

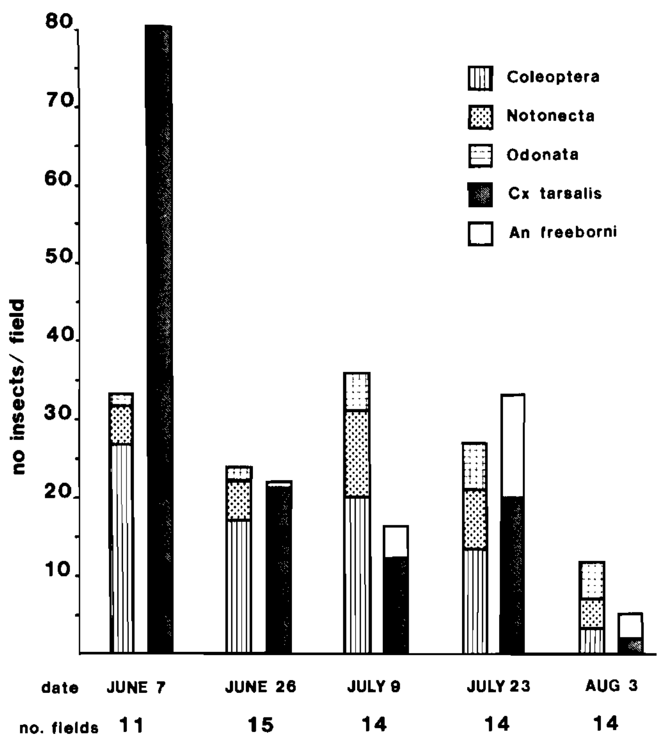

Figure 5.-Aquatic insects collected in dippings in wild rice fields in Shasta County, Calif., 1984. Elevation 1000 meters. (75 dips/field)

Table 2.-Fish and Aquatic Insects Collected[1] in Wild Rice Fields, Shasta County, Calif. 1984. Elevation 1000 meters.

Order (suborder) Family	Species	Common Name
Fish		
Poeciliidae	**Gambusia affinis*	mosquitofish
Centrarchidae	*Lepomis*, sp.	sunfish
	Lepomis cyanellus	green sunfish
	Pomoxis annularis	white crappie
	Pomoxis nigromaculatus	black crappie
	Micropterus dolomieui	smallmouth bass
Coleoptera		
Hydrophilidae	**Tropisternus lateralis*	water scavenger
	T. obscurus	beetles
	Hydrophilus triangularis	"
	Berosus, sp.	"
	Enochrus, sp.	"
Dytiscidae	*Dytiscus marginicollis*	predaceous water
	Graphoderus occidentalis	beetles
	**Agabus*, sp.	"
	Acilius, sp.	"
	Hydroporus, sp.	"
Amphizoidae	*Amphizoa insolens*	
Histeridae		
Haliplidae		
Hemiptera		
Notonectidae	*Notonecta kirbyi*	backswimmers
	**N. undulata*	"
	**N. unifasciata*	"
	N. shooteri	"
Belostomatidae	*Belostoma*, sp.	giant water bugs
Hebridae		velvet water bugs
Corixidae		water boatmen
Saldidae		shore bugs
Gerridae		water striders
Odonata (Anisoptera)		dragonflies
Libellulidae	**Pantala hymenaea*	
Aeshnidae	**Anax junius*	
Odonata (Zygoptera)		damselflies
Lestidae		
Ephemeroptera		mayflies
Baetidae	*Caellibaetis*, sp.	
Diptera		
Culicidae	*Anopheles freeborni*	mosquitoes
	Culex tarsalis	

[1] Collected by standard dipper and 1/8-inch mesh minnow traps.

* Most common species collected.

THE NAVY DVECC'S ROLE IN CALIFORNIA VECTOR CONTROL

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The United States Navy's first substantial commitment to vector control began during World War II, when it was realized that vector-borne diseases such as louse-borne typhus, malaria, yellow fever, and dengue were often determining factors in the outcome of combat. For example, in some Pacific theater actions malaria casualties were four times higher than conventional battle casualties. To reduce Navy and Marine Corps morbidity due to such vector-borne diseases, the Navy Medical Department began a network of preventive medicine units. In 1958, the Navy Disease Vector Ecology and Control Center (DVECC) Alameda, California, was commissioned as part of that growing preventive medicine program.

The purpose of this paper is to familiarize the California vector control community with DVECC's role in vector control operations and how these operations impact upon California's programs.

Most of the Naval installations in California have complete pest management plans, within which Mosquito Abatement District (MAD) contracts for mosquito control are an integral part. For many years MADs have provided expert advice and control measures to these activities. DVECC Alameda also provides technical guidance to these California installations, as well as those Navy installations throughout the western United States and the Pacific. DVECC is tasked with four basic programs:

1) Operations -- provide situation-specific entomological advice, review shore station pest management plans, and conduct technical assistance visits.

2) Applied Research -- test new products that may increase the efficiency of arthropod control programs in the military.

3) Medical Entomology Information -- serve as an information bank on arthropod-vectored diseases.

4) Training -- provide instruction on shipboard, shore station, and contingency arthropod control as well as certification training for all aspects of military pest management.

Ultimately, these four programs support our commitment to contingency response. We must be ready to send a fully prepared vector control team anywhere under any conditions at short notice.

Non-military contingency operations include disease prevention in disaster or refugee relief situations, and disease suppression in outbreaks. For example, as members of Operation New Life in 1975, we sent vector control teams to Guam and Camp Pendleton to assist Vietnamese refugees and to guard against the importation of dengue. Refugee programs at Camp Pendleton were also monitored by MAD and State Health personnel.

Our most recent military operation occurred in Lebanon. Climatically and geographically Lebanon is much like California. But the conflict-related breakdown of sanitation and public health services have greatly increased rodent and filth fly problems. DVECC sent a medical entomologist and two preventive medicine technicians with two tons of vector control equipment and pesticides to Beruit in 1983 to provide vector control services to the Marine Corps.

Historically, DVECC has assisted the shore installations as well as the fleet by performing pest management surveys and providing technical assists in concert with adjacent MADs. Additionally, our constant preparation for contingency operations forces us to undertake activities that may be of further value to the California vector control community. For example:

1) We constantly evaluate new equipment and pesticides in conjunction with the Armed Forces Pest Management Board. Through bimonthly Mosquito Abatement Council meetings with the Coastal Region MADs, Public Health, and other regional military installations, we can compare our findings with those of other agencies.

2) We routinely gather and disseminate information on the prevalence and control of disease vectors throughout the United States and overseas. Like all public health workers we strive to remain current on the latest in disease incidence, vector survey and control methods, and resistance problems. We use the Defense Pest Management Information Analysis Center to assist us in compiling vector-borne disease updates for critical regions of the world. Literature references on new techniques and analysis of vector control experiences are also reviewed.

3) Our training department instructs over 350 military and civilian vector control personnel each year. All courses are designed to surpass EPA and State of California standards for Pesticide Applicator certification in public health and other categories. We strive to produce personnel that are more than technicians; they are technologists who are responsible for survey, identification, control, and evaluation. Certified personnel

are expected to be able to independently confront any emergency situation with confidence.

In each of these programs, we have been strongly supported for years by local MADs and State Health personnel. By way of reciprocation we have sponsored equipment workshops with the civilian community, and we are currently assisting with the Continuing Education Program for several MADs. The Navy and the civilian public health community have both profited from such cooperation. We hope to continue in that spirit, advancing the knowledge of vector control for all concerned.

MOSQUITOFISH-GREEN SUNFISH INTERACTIONS IN EXPERIMENTAL RICE FIELDS: EFFECTS ON MOSQUITO ABUNDANCE

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ABSTRACT

An experiment was conducted to determine the effect of green sunfish (*Lepomis cyanellus*) on the population size and habitat utilization of mosquitofish (*Gambusia affinis*) and subsequent effects on mosquito abundance. The addition of *L. cyanellus* resulted in decreased abundance and altered habitat utilization of mosquitofish. Despite reduced mosquitofish populations, the addition of green sunfish resulted in reduced *Anopheles freeborni* populations.

Eight large (500 m^2) rice plots at the UC Davis Rice Research Facility were randomly assigned to 2 treatments: 1) *G. affinis* adults (sex ratio 1:1) alone and 2) *G. affinis* adults plus *L. cyanellus* adults (8-10cm TL). One hundred fish of each species were stocked in the appropriate plots. Aquatic fauna were monitored throughout the course of the season by minnow traps and by dipping.

By the end of the season, green sunfish reduced mosquitofish density by approximately 50%. *L. cyanellus* were collected in much greater numbers in minnow traps at the bottom of the borrow pits than at the top of the borrow pits or within the rice stands. This appeared to influence the spatial distribution of *G. affinis*. Mosquitofish utilized all 3 of these habitats in the absence of green sunfish, but in its presence, use of the bottom of the borrow pit declined significantly.

Culex tarsalis populations were too low to examine for treatment effects. Despite reduced *G. affinis* populations, *A. freeborni* populations were significantly lower in the combined fish species treatment than in the treatment containing only *G. affinis*. Our other experiments (see Blaustein and Washino. 1983. Proc. Calif. Mosq. and Vector Control Assoc. 51:42; Blaustein and Karban. 1984. Proc. Calif. Mosq. and Vector Control Assoc. 52:120; Blaustein and Karban. 1985. Proc. Calif. Mosq. and Vector Control Assoc. 53: in review) have demonstrated that *L. cyanellus* by itself does not reduce *A. freeborni* populations. Therefore, the enhanced control caused by the addition of *L. cyanellus* to *G. affinis* in the rice plots is most likely due to indirect interactions. The shift in habitat utilization of *G. affinis* in the presence of *L. cyanellus* may cause the mosquitofish to forage more where mosquitoes are present. *L. cyanellus* may also reduce alternative prey abundance resulting in greater predation intensity of *G. affinis* on mosquitoes.

EFFECTS OF MOSQUITOFISH, GREEN SUNFISH, VEGETATION AND THEIR INTERACTIONS ON MOSQUITO ABUNDANCE IN RICE FIELD ENCLOSURES

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ABSTRACT

Studies evaluating the effects of mosquitofish *Gambusia affinis*, green sunfish *Lepomis cyanellus*, vegetation type (rice vs. southern naiad) and their interactions on mosquito abundance were continued (see Blaustein and Washino. 1983. Proc. Calif. Mosq. and Vector Control Assoc. 51:42; Blaustein and Karban. 1984 a,b. Proc, Calif. Mosq. and Vector Control Assoc. 52:120; Blaustein and Karban. 1985. Proc. Calif. Mosq. and Vector Control Assoc. 53: in review) in 1984. Our results indicate that the two vegetation types do not differ with respect to the amount of refuge provided to mosquitoes against fish or to mosquitofish against larger conspecifics and green sunfish. Our results also indicate that under conditions where *G. affinis* populations are severely depressed by green sunfish, mosquito control is negatively affected.

The experiment was conducted at the UC Davis Rice Facility in sheet aluminum rings ($0.75m^2$) forced into the rice substrate. Fifty six rings were randomly assigned to 7 treatments. Four treatments contained rice: 1) no fish, 2) *G. affinis*, 3) *L. cyanellus*, and 4) both fish species. Three additional treatments contained only southern naiad: 1) no fish, 2) *G. affinis*, and 3) both fish species. *G. affinis* rings received 1 gravid female at the beginning of the season and a second gravid female two weeks before termination of the experiment. *L. cyanellus* rings received 2 fish (7.5-8.5cm TL) at the beginning of the season followed by a third fish two weeks prior to the end of the experiment. Fauna were monitored throughout the course of the season by minnow traps and by dipping. At the end of the experiment, fish were collected by sweeping. Data for mosquitoes, flatworms (*Mesostoma* sp.) and fish were analyzed by analysis of variance (repeated measures design). Means were compared using Duncan's multiple range test ($p = 0.05$).

Green sunfish drastically suppressed mosquitofish abundance in both habitats. The two habitats also supported similar numbers of mosquitofish. Significant differences in flatworm abundance between the two habitats were not demonstrated. Flatworms were depressed in the sunfish treatments and were depressed even more in the mosquitofish treatments. Treatment effects were similar for *Anopheles freeborni* and *Culex tarsalis*. There was no habitat effect for either species of mosquito. Mosquitofish suppressed mosquito populations equally well in both habitats. Mosquito populations were not suppressed below control levels in any of the sunfish or sunfish-mosquitofish treatments. Thus, large green sunfish may reduce the efficiency of mosquitofish to control mosquitoes in either rice or southern naiad habitats.

OPERATIONS WITH COMMERCIALLY AVAILABLE *BACILLUS THURINGIENSIS* SEROTYPE H-14 FORMULATIONS IN KERN COUNTY, CALIFORNIA

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ABSTRACT

Dip samples taken at 11 aerial application sites treated with *Bacillus thuringiensis* serotype H-14 (Bti) showed that the presently available commercial formulations were effective mosquito larvicides when used according to labeled instructions. Among nontarget insects, percent reductions of chironomid larvae were higher than those of all other taxa, but were lower and more variable than those of fourth instar mosquito larvae. Cost savings of 73% per treated source were achieved in ground operations using Bti in place of Golden Bear 1356 larvicide oil.

INTRODUCTION.-The Kern Mosquito Abatement District encompasses approximately 1,600 square miles of Kern County in the southern San Joaquin Valley, California. Approximately 80 percent of all control operations are directed against the larvae of four mosquito species: *Culex tarsalis* Coq., *Cx. quinquefasciatus* Say, *Aedes melanimon* Dyar and *Ae. nigromaculis* (Ludlow). *Cx. tarsalis* is of concern because of its role as the primary vector of western equine and St. Louis encephalomyelitis viruses.

For more than two decades, insecticide resistance has been a major factor influencing the choice of larvicides (Womeldorf et al. 1972), and in recent years has encompassed all available organophosphorus (OP) compounds and extended to the synthetic prethroids as well (Yoshimura et al. 1983). While the carbamate propoxur has remained an effective adulticide, larval control has emphasised the use of larvicide oil. Since 1974, Golden Bear (GB) 1356 larvicide oil (Witco Chemical Company) has been the major component of our operations because of its effectiveness against OP resistant larvae and pupae, relative safety, and utility as a diluent for other compounds.

The major drawback to using GB has been its expensiveness: at 1984 prices GB cost 17 times more per acre than parathion at the standard aerial application rates of 3 gal. and 0.1 lbs AI/acre, respectively. For this reason commercial formulations of *Bacillus thuringiensis* serotype H-14 (Bti) were investigated as an alternative to GB. Bti has proven to be an effective larvicide against a wide variety of mosquito species under both laboratory (Garcia and Des Rochers 1979, Ignoffo et al. 1981) and field conditions (Eldridge and Callicrate 1982, Goettel et al. 1982, Stark and Meisch 1983). Its safety toward most nontarget organisms has been well documented (Miura et al. 1980, Garcia et al. 1981) and it exhibits no cross resistance to OP insecticides (Sun et al. 1980). During 1984, eight commerical Bti products were registered in California, represented by wettable power (WP), liquid concentrate (LC), granular and slow-release formulations. The present paper evaluates Bti as an operational tool and compares its cost with that of GB under different phases of district operations. The effect of Bti on nontarget aquatic insects also in included, with particular reference to chironomid larvae because of their importance as food for overwintering waterfowl in wildlife areas (Euliss 1984).

METHODS AND MATERIALS.-Evaluation sites were selected from flight request sheets filed daily by field operators. Preference was given to those sites having low pupal numbers and low to moderate organic pollution. Sites ranged in size from 5 to 360 acres, with varying degrees of water quality and vegetative cover. Most sites were situated in agricultural areas with standing water which supported populations of *Cx. tarsalis* or *Cx. quinquefasciatus* larvae.

Prespray samples were taken between 0630 and 0800 hrs, just prior to aerial applications; postspray samples followed within 17-48 hours. At each location, from 1 to 3 samples consisting of 20-50 dips each were taken using a 400 ml dipper. Each dip was poured into a concentrator (Driggars et al. 1978) with a mesh size of 0.3 or 0.4 mm. Samples were fixed in the field with 95% ETOH.

Organisms were counted under a dissecting scope and identified according to Usinger (1956). Abundant taxa were subsampled in 5 or 10 randomly chosen 1 cm^2 sections of a 53.5 cm^2 counting dish and their numbers estimated based on total dish area. Usually no less than two dishes were required to contain a sample.

Pre and postspray sample means were compared using Wilcoxon's two sample test (Sokal and Rohlf 1969). Percent reduction data were subjected to arcsine transformation and compared by t-test. Proportions of nontarget taxa in pre and postspray samples were compared using chisquare against a null-hypothesis of equal distribution of numbers in pre and postspray samples.

Cost evaluations were based on 1984 prices. Amounts of Bti used in ground operations were estimated from average tank-mix rates of liquid concentrate formulations and total volume output of spray equipment. Amounts used in aerial treatments were estimated based on average application rates for the season.

Aerial applications were made using an Ayres Thrush Commander. Spray volume varied from 3 to 8 gal./acre depending upon vegetative cover and acreage. The commercial products used were: Teknar (Sandoz, Inc.), Bactimos flowable concentrate, corncob granules, and wettable powder (Biochem Products), Vectobac Aqueous Suspension and Vectobac-G corncob granules (Abbott Laboratories). LC and WP formulations were pre-mixed in an 800 gallon mechanical agitation tank before aircraft loading. All products were applied according to labeled rates and instructions.

RESULTS.-During 1984, 36 prespray samples were taken, 18 of which were suitable for postspray evaluation. These represented 13 flight requests at 11 different locations, for totals of 573 and 591 dips in pre and postspray samples, respectively. A summary of the results is presented in Table 1.

Average numbers of mosquito immatures collected are presented in Figure 1. Except for pupae, mean numbers/dip of all stages were significantly greater in prespray than in postspray samples ($U = 272.5$, $p<.001, n_1, n_2 = 18, 18$). Overall percent reductions were 81, 89, 84 and 71% for instars one through four, respectively. Total percent reductions for all stages (including pupae) ranged from 22 to 100 percent, and as expected showed a significant negative correlation with the proportion of pupae in prespray samples (Spearman's rank correlation, $r_s = -.633$, $.01>p>.001$, 16 df).

Average numbers of nontarget insects are shown in Figure 2. Wilcoxon's tests revealed no

Table 1.-Description and results of 13 Bti aerial applications.

Flight#	Product	Rate/Ac.	Stages	Mean No./dip(1-p) Pre(n)	Post(n)	%Red.	Situation
640	Bact. WP	10 oz.	1-p	1.6(30)	.03(30)	98	Rice; short variety, moderate cover.
"	"	"	"	8.3(30)	3.0(30)	64	Rice; short variety, heavy weeds.
"	"	"	"	4.9(30)	.57(30)	88	Rice; short variety, moderate cover.
6151	"	6 oz.	1-4	.27(48)	0 (48)	100	Barley; light cover.
5904	"	8 oz.	1-p	1.8(34)	1.4(34)	22	Sugarbeets; plants decaying.
1699	"	12 oz.	1-4	.60(50)	.02(48)	97	Flooded grass; moderate cover.
5506	"	8 oz.	1-p	12.9(45)	1.4(46)	89	Alfalfa; light cover.
6124	Teknar	1.5 pts.	1-4	.83(30)	0 (30)	100	Rice; light cover, oil refinery water.
"	"	"	"	2.2(30)	.33(30)	85	Rice; light cover, oil refinery water.
941	"	2 pts.	1-p	2.0(20)	1.0(20)	50	Flood water; heavy cover.
"	"	"	"	.70(20)	.05(20)	93	Flood water; light cover.
6119	Bact.Gran.	7.5 lbs.	1-3	1.3(30)	.30(30)	77	Rice; light cover, oil refinery water.
"	"	"	"	.80(27)	.41(27)	49	Rice; light cover, oil refinery water.
1883	"	8 lbs.	1-p	17.0(30)	4.9(28)	71	Cotton; light cover.
2015	"	5 lbs.	1-p	.48(25)	.24(25)	50	Tall grass; heavy cover.
1881	Vect.Gran.	10 lbs.	1-p	2.3(45)	.29(45)	87	Cotton; light cover.
2528	"	"	"	4.9(28)	.27(30)	94	Cotton; moderate cover.
4641	"	"	"	6.2(21)	.05(40)	99	Cotton; heavy cover.

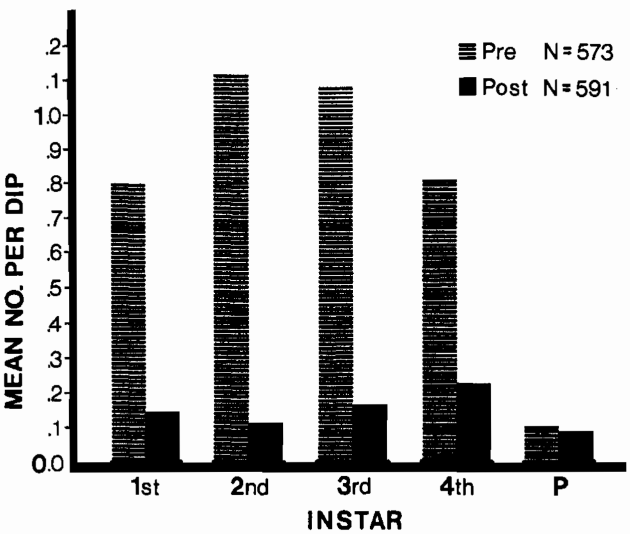

Figure 1.-Mean numbers of mosquito larvae and pupae collected per dip in 18 pre and 18 postspray samples from 11 locations treated with Bti H-14 during 1984.

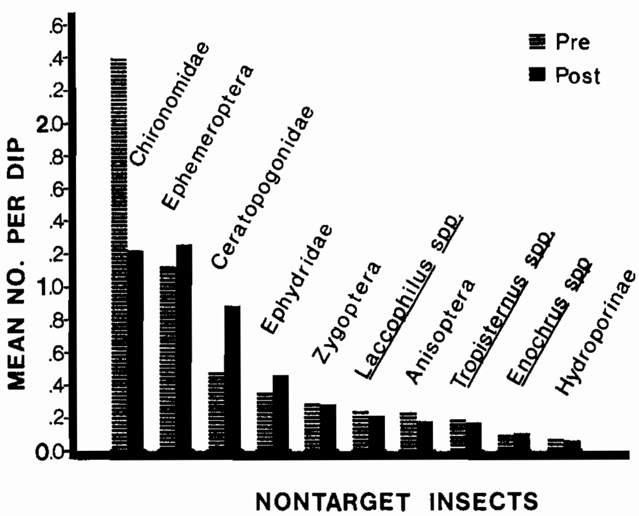

Figure 2.-Mean numbers of nontarget insects collected per dip in 18 pre and 18 postspray samples from 11 locations treated with Bti H-14 during 1984. Sample sizes are as shown in Figure 1.

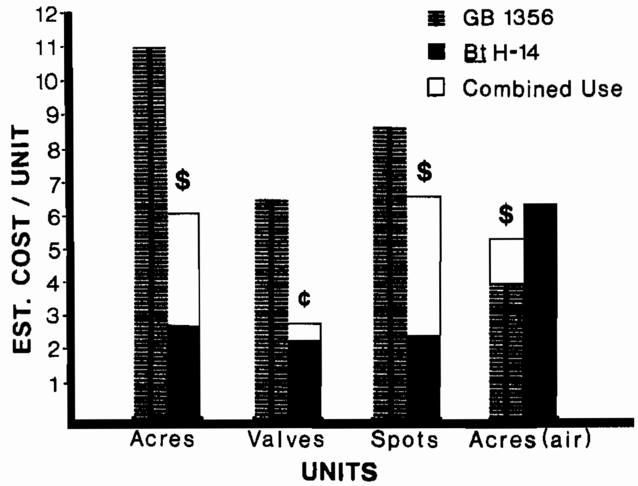

Figure 3.-Estimated material costs of GB 1356, Bti H-14, and combined use for ground (Acres, Valves and Spots) and aerial larviciding operations during 1984.

significant differences in pre and postspray abundance for any of the taxa. However, arcsine transformed percent reductions averaged significantly higher for chironomid larvae than for all other nontarget insects (t = 2.095, .02<p<.05, 31 df) and chi-square revealed a highly significant reduction from prespray numbers (chi-square = 218.29, p<.001, 1 df). Combined proportions of mayflies, ephydrids and ceratopogonids in postspray samples increased significantly over prespray samples (chi-square = 96.51, p<.001, 1 df). Chironomid percent reductions were significantly lower than those for fourth instar mosquito larvae (t = 2.25, .02<p<.05, 27 df), and were much more variable among samples (Coefficient of variation: chironomid = 87%; mosquito = 27%).

Cost comparison: Urban. 1,575 urban locations were treated in 1984 using GB or Bti. The combined cost of both control agents was $4.23 per location, 13% lower than the 1983 cost of GB. No comparisons were obtained for curbing operations (storm drains and catch basins) because of early-season control failures which occurred while using Bti LC at tank-mix rates of 2 pints/10 gallons.

Rural: 9,975 acres, 115,166 irrigation valves and 1,630 small spots (<one acre) were treated in 1984. Use of Bti LC at 1.5 to 2 pints/acre (e.g., tank-mixes of 6-8 fl. oz. LC/2-gal. handcan or 1.5-2pints/10 gal. in vehicle mounted sprayers) resulted in an overall cost reduction of 73% per source compared to GB. Average treatment costs for these sources are presented in Figure 3. Combined operational use of GB or Bti resulted in cost reductions of 43, 58 and 22 percent for acres, valves and spots, respectively, or an overall savings of 42% over the exclusive use of GB.

Aerial applications: Of the 23,975 acres treated for larvae in 1984, approximately 30% were treated with the higher rates of Bti products at an average cost of $6.29 per acre. Total material cost averaged $4.71 per acre in 1984, an increase of 32% over 1983 costs. Roughly half of this increase resulted from using Bti products at high labeled rates in place of GB; the remainder was due to a substantial decrease in the use of inexpensive OP compounds.

DISCUSSION.-The present results conformed well to previous studies. Mosquito mortalities averaged greater than 80% for instars 1 through 3, with reduced mortality in fourth instar and pupae. The slightly reduced mortality of instar 1 was attributed to recruitment from egg-hatch, since some postspray samples were taken two days after treatment.

Mortality was not recorded for nontarget insects except chironomid larvae. Previous research had demonstrated generally lower susceptibilities for this group than for mosquitoes (Ali et al. 1981), with responses varying among habitats and species (Miura et al. 1980, Garcia et al. 1980, 1981, Mulligan and Schaefer 1981). These studies have indicated that chironomid larval populations quickly rebounded after Bti applications, so it would seem unlikely that late season (October) treatments would significantly reduce the winter food supplies for overwintering waterfowl in wildlife areas. Further research is needed to elucidate the late-season population trends of important chironomid species.

Predatious insects generally are recognized as valuable in the regulation of larval mosquito populations (Schaefer et al. 1981), but at present offer only a supplement to more conventional means of control. During the present study, analysis of 35 prespray samples (data not presented) revealed a weak but significantly positive correlation between predator and mosquito abundance among samples (r_s = .353, p<.05, 33 df), suggesting that certain habitats or sub-habitats were attractive to both groups. Conversely, samples from one rice field showed a highly significant negative correlation between numbers of late instar damselfly naiads (*Enallagma* sp.) and mosquito larvae (r_s = -.964, p<.001, 5 df). Bti allows maximum exploitation of the aquatic insect community while providing the degree of immediate control expected from chemical applications.

The operational safety and efficiency of the commercial products cannot be overstated. Our operators and foremen greatly preferred the use of liquid Bti formulations over GB because they were much cleaner to work with. In practice, the concentrates were dispensed daily into wide-mouth 500 ml nalgene bottles and distributed to operators. For aerial applications the WP formulation was preferred because of its ease of handling and loading. Approximately 59 acres could be treated at the maximum labeled rate (12 oz/acre) using one 44 lb. bag; the same acreage would require almost three 5-gallon containers of liquid concentrate (at 2 pints/acre) weighing a total of approximately 118 lbs. Our limited experience with the granular products indicated good to excellent control in heavily vegetated habitats, but their cost was considered exorbitant for routine control operations. We achieved some excellent results with LC rates as low as 1 pint/acre and WP rates as low as 6 oz./acre, but overall results were more variable than with the higher rates. More judicious selection of application sites should lead to greater success at the lower rates and reduce the cost below that of GB.

The substantial cost savings achieved using the commercial Bti products in ground applications has established them as a permanent part of our operations. Future work will include investigation of the control failures in curbing operations.

ACKNOWLEGMENTS.-I wish to thank Dr. W. K. Reisen for his review of the manuscript, and the staff of the Kern Mosquito Abatement District for their assistance during this study.

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INSECTICIDE SUSCEPTIBILITY OF MOSQUITOES IN CALIFORNIA: STATUS OF ORGANOPHOSPHORUS RESISTANCE IN LARVAL *CULEX TARSALIS* THROUGH 1984, WITH NOTES ON RESTORATION OF SURVEILLANCE PROGRAM

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INTRODUCTION.-The Resistance Surveillance Program has as its mission the provision of certain services to local mosquito control agencies. They include: 1) conducting a statewide mosquito insecticide resistance surveillance program (currently emphasizing species which vector encephalitis and malaria), 2) demonstrating the relationship between laboratory analyses and field efficacy of insecticides used by control agencies, 3) providing analyses and interpretation of test data, and 4) providing training on the techniques of laboratory and field determination of insecticide resistance.

Due to budgetary cuts, resistance surveillance was dropped by the Vector Biology and Control Branch in 1979. The function has been restored, at least in part with one person assigned to provide service statewide.

The purpose of this report is to update the record with resistance levels determined during 1984 and request the continued support and assistance of control agencies to accomplish program objectives.

METHODS AND MATERIALS.-Bioassays were performed according to the methodology described by Gillies and Womeldorf (1968). Data were evaluated by graphic and probit analysis to obtain estimates of LC_{50}s and LC_{90}s. Tests were considered invalid if there were fewer than three data points, lack of mortality above or below 50%, greater than 10% mixed species or pupation, and statistically significant heterogeneity of the data points.

RESULTS.-Table 1 lists the highest laboratory demonstrated levels of organophosphorus (OP) resistance per agency, and resistance status of larval *Culex tarsalis* populations recorded through 1978 (Zboray and Gutierrez, 1979). The highest levels obtained during surveillance activities in 1984 are listed separately for comparison. Higher levels were reported for five of the agencies listed. Status was determined by the criteria

Table 1.-Highest laboratory demonstrated levels of organophosphorus resistance (in ppm), and resistance status[1] of *Culex tarsalis* larval populations recorded through 1978[2] compared to those recorded in 1984.

AGENCY	MALATHION			PARATHION			FENTHION			CHLORPYRIFOS		
	Year	LC_{50}/LC_{90}	Status	Year	LC_{50}/LC_{90}	Status	Year	LC_{50}/LC_{90}	Status	Year	LC_{50}/LC_{90}	Status
Burney Basin	1984	0.025/0.069	S	1984	0.0025/0.0082	S	1984	0.0038/0.0092	S	1984	0.0014/0.0046	S
	1974	0.015/0.034	S	----	-------------	-	1974	0.0026/0.0042	S	----	-------------	-
Shasta[3]	1984	0.049/0.24	I	1984	0.0021/0.003	S	1984	0.0096/0.032	R	1984	0.0013/0.004	S
	1967	0.087/1.0	I	1977	0.0024/0.0036	S	1977	0.013/0.032	R	1977	0.0028/0.014	R
Tehama Co.	1984	0.058/0.53	I	1984	0.0042/0.0096	I	1984	0.0036/0.012	I	1984	------------	-
	1968	0.053/0.52	I	1964	0.0031/0.0058	S	1971	0.0056/0.011	R	1972	0.00053/0.0008	S
Butte Co.[3]	1984	----------	-	1984	0.0007/0.007	S	1984	------------	-	1984	0.00026/0.0016	S
	1966	0.10/0.75	R	1972	0.0045/0.011	I	1971	0.0079/0.022	R	1968	0.00028/0.00042	S
Kings	1984	----------	-	1984	0.012/0.043	R	1984	0.014/0.037	R	1984	---------------	-
	1972	0.75/6.4	R	1972	0.054/0.16	R	1972	0.04/0.10	R	1972	0.027/0.077	R
Northwest	1984	0.27/0.50	R	1984	0.044/0.16	R	1984	0.084/0.21	R	1984	0.11/0.25	R
	1971	0.016/0.029	S	1971	0.0016/0.0025	S	1971	0.0026/0.0038	S	1971	0.00024/0.00054	S
Coachella	1984	0.011/0.028	R	1984	-------------	-	1984	-------------	-	1984	0.0052/0.015	R
Valley	1972	0.073/0.14	S	1970	0.016/0.042	R	1972	0.0086/0.039	R	1971	0.026/0.11	R
San	1984	0.44/1.9	R	1984	0.056/0.11	R	1984	-------------	-	1984	--------------	-
Bernardino Co.[4]	1983	0.01/0.047	S	----	----------	-	----	-------------	-	1983	0.0093/0.039	R
Riverside	1984	----------	-	1984	0.018/0.058	R	1984	-------------	-	1984	--------------	-
County[4]	1972	0.097/0.58	I	1972	0.0054/0.014	R	1972	0.0058/0.017	R	----	--------------	-

1. S = Susceptible; I = Intermediate; R = Resistant.
2. Zboray et al. 1979.
3. Local mosquito control agency data, 1984.
4. Unpublished data, 1972 and 1983.

Table 2.-Correlation of resistance status to the expected effect upon mosquito control operations, and criteria for status determination in order of progression in the development of resistance[1] based on the failure threshold[2] dose (T.D.).

Ratio = LC_{90}/LC_{50}.

Susceptible - Anticipate good control.

(1)	(2)
LC_{50}<T.D.	LC_{50} = T.D.
LC_{90}≤2xT.D.	LC_{90} = 2xT.D.
Ratio ≶ or = 2.00	Ratio = 2.00

Intermediate - Anticipate erratic control if field conditions are less than optimal.

(3)
LC_{50}<T.D.
LC_{90}>2xT.D,
Ratio>2.00

Resistant - Anticipate control failures.

(4)	(5)
LC_{50} = T.D.	LC_{50}>T.D.
LC_{90}>2xT.D.	
Ratio>2.00	

1. Gillies et al. 1968.
2. Brown et al. 1963; Womeldorf et al. 1966.

provided in Table 2.

Table 1 does not necessarily identify the most resistant population that may occur in an agency. However, considering the higher levels, this is a possibility. It also does not illustrate the change in the level of a population from one year to another. On the contrary, the values listed in the table reflect the highest levels collected from randomly selected populations that happened to be at the proper larval stage when sampling occurred at the agency.

The data in Table 1 are limited to those agencies at which *Cx. tarsalis* samples were tested. Levels reported through 1978 for those agencies not listed can be obtained by referring to Zboray and Gutierrez (1979).

Table 2 provides the correlation of resistance status to the expected effect upon control operations in the field. It also provides the criteria necessary to determine resistance status. These standards are based upon the failure threshold concept (Brown et al. 1963; Womeldorf et al. 1966) which uses the LC_{50} as a guide to predicting the response of a particular mosquito population to control operations.

DISCUSSION.-During 1984 an attempt was made to test as many populations as possible statewide. Limited time was available, though, since testing did not begin until mid-July. The biggest problem was locating *Cx. tarsalis* populations in large enough larval numbers for testing. They often occur dispersed, in low densities, or mixed with other species. The San Joaquin Valley was particularly troublesome in all these aspects.

Although testing disease vector populations is the present priority of our program, many bioassays were performed during 1984 on populations of other mosquito species. To do so was a common request of the agencies that were visited. It is important to note that providing resistance information on those pest populations that an agency is concerned about will be of increasing priority. Currently it is important to gather as much information as necessary on the status of disease vector populations statewide to determine the problems that might be incurred with the use of OP insecticides to avert the spread of a disease outbreak. Testing will continue until enough data are collected to reasonably assess the composite response of vector populations of the State. The information learned through surveillance will be pertinent to selecting the insecticides to be used under emergency conditions.

Substantial amounts of OP insecticides are used each year in California to control larval and adult mosquito populations. As a matter of integrated pest management policy, insecticides should be used selectively and in a manner to minimize the development of resistance. Where appropriate OP insecticides will probably continue to be valuable in mosquito control.

An objective for 1985 is to increase the knowledge of resistance in *Cx. tarsalis* populations throughout the State. To accomplish this objective agency assistance and input are essential. *Cx. tarsalis* populations need to be tested from more areas of the State, and a greater number of populations per area need to be tested. Participating agencies are asked to search out and locate good *Cx. tarsalis* sources, and monitor them beforehand. This increases the number of bioassays that can be undertaken during the time spent at an agency. As much as possible, populations previously tested will be retested annually.

ACKNOWLEDGMENT.-I would like to extend my gratitude to agency managers for providing our program with assistance of their time and personnel. I am grateful to the technical staff of those agencies who supplied the data noted in the table.

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LABORATORY AND OUTDOOR EVALUATIONS OF NEW FORMULATIONS OF *BACILLUS THURINGIENSIS* SEROVAR. *ISRAELENSIS* AGAINST LABORATORY REARED MOSQUITOES (DIPTERA: CULICIDAE)[1]

Arshad Ali[2] and Jai K. Nayar[3]

ABSTRACT

Three granular (G) and one flowable concentrate (FC) new formulations of *Bacillus thuringiensis* serovar. *israelensis* (*B.t.i.*) were tested as larvicides of laboratory reared mosquitoes. In standard laboratory bioassays with the FC (ABG-6145 containing 600 *Aedes aegypti* (AA) International Toxic Units (ITU/mg), *Culex salinarius* was the most susceptible (LC_{50} = 0.11 ppm) followed by *Cx. quinquefasciatus, Cx. nigripalpus, Aedes taeniorhynchus, Ae. aegypti,* and *Wyeomyia mitchellii* (LC_{50} = 0.93 ppm). Each of the G formulations (ABG-6140, ABG-6138, and ABG-6141, containing 100, 200, and 300 AA ITU/mg, respectively) applied at 5 kg/ha against *Ae. taeniorhynchus* and *Cx. quinquefasciatus* maintained in outdoor concrete tanks gave 69-100% control of the two species. In laboratory tests conducted in laundry tubs, ABG-6140, 6138, and 6141 applied at 5.0, 2.5, and 1.67 kg/ha, respectively, gave similar levels of control (65 and 70%) of *Cx. nigripalpus* with ABG-6140 and ABG-6138, but ABG-6141 showed superior activity against *Cx. nigripalpus*. In the tubs, the G formulations were highly effective against *Ae. aegypti* at low application rates of 0.84 kg/ha (ABG-6141), 1.25 kg/ha (ABG-6138), and 2.5 kg/ha (ABG-6140). These new formulations of *B.t.i.* are additional useful tools for the biological control of *Aedes* and *Culex* species of mosquitoes.

INTRODUCTION.-Numerous laboratory and field studies have been conducted on the use of the microbial agent, *Bacillus thuringiensis* serovar. *israelensis* (*B.t.i.*), showing its larvicidal activity against at least 70 species in more than eight genera of mosquitoes distributed worldwide (Anonymous 1982). Species of *Anopheles* and *Wyeomyia*, generally, are the two genera in which the efficacy of *B.t.i.* is in question (Ali et al. 1984, Mulla et al. 1980). Due to the remarkable activity of *B.t.i.* against mosquitoes and some other aquatic pest and vector insects, some commercial producers have developed the biocide in different formulations of various potencies. The experimental and commercial formulations of *B.t.i.* produced since 1977 have primarily been wettable powders (WP) and flowable concentrates (FC). However, to meet some specific needs, a broad diversification of formulations (especially granular) has been attempted in the past two years to realize full potential of *B.t.i.*. At present, several new WP, FC, and different sized granules (G), pellets, briquettes, and other formulations with slow or sustained release properties are available for testing.

The objective of this study was to evaluate the efficacy of three recently developed G and one FC formulations of *B.t.i.* against mosquito larvae. The FC was tested in standard bioassay cups in the laboratory, while the G formulations were evaluated against laboratory reared larvae introduced into concrete tanks maintained outdoors, and into laundry tubs maintained in the laboratory.

MATERIALS AND METHODS.-Test Formulations: The G and FC *B.t.i.* formulations were manufactured by Abbott Laboratories, North Chicago, Illinois. The G formulations were ABG-6140 (lot no. 50-002BR), ABG-6138 or Vectobac-G (lot no. 51-004BR), and ABG-6141 (lot no. 50-003BR), and reportedly contained 100, 200, and 300 International Toxic Units (ITU)/mg against *Aedes aegypti*, respectively. The FC was ABG-6145 or Vectobac-AS (lot no. 63-010BA) containing 600 ITU/mg against *Ae. aegypti*.

Laboratory Bioassays: For bioassays, a mixture of late 3rd and early 4th instars of *Aedes aegypti, Ae. taeniorhynchus, Culex nigripalpus, Cx. quinquefasciatus, Cx. salinarius,* and *Wyeomyia mitchellii* were used. These species were maintained at the Florida Medical Entomology Laboratory at Vero Beach, Florida. The procedures of bioassays were generally the same as described by Mulla et al. (1982). Twenty larvae were placed in a 120-ml disposable cup containing 100 ml of tap water (pH 7.4). Distilled water (pH 6.9) was used for rearing *Wy. mitchellii* because of the possibility of its larval mortality in tap water (Nayar 1982). The FC, ABG-6145, was suspended in distilled water by using a commercial blender for 1 to 2 min to make a 1% stock suspension (wt/vol). The suspension was serially diluted by using magnetic stirrers for making 5-6 different concentrations, each of which was applied to 3 replicates (cups) in a test. Three untreated cups were maintained as control in each test. Each species was exposed to the FC on at

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least three different occasions when the stock suspension and serial dilutions were freshly prepared. After a 24-h exposure period in a controlled environment room (14 h photoperiod and 27 ± 2°C) larval mortality was recorded. The corrected mortality (against controls) of a species at different concentrations of ABG-6145 was subjected to log-probit regression analysis.

Outdoor Concrete Tanks: The G formulations, ABG-6140, 6138, and 6141, were evaluated on equal wt. basis against *Ae. taeniorhynchus* and against *Cx. quinquefasciatus* maintained in 2.25 X 0.75 m concrete tanks placed outdoors in July-August 1983. Each tank was filled to 15 cm with tap water. Twelve tanks were utilized for each species. Each formulation was applied at 5 kg/ha to three separate tanks, thus using nine tanks for treatments of each species while three tanks were maintained as controls. Approximately 2000-2500 first instars of each laboratory maintained species were introduced and allowed to develop to late 3rd and early 4th instars at which time the tanks were randomly treated by applying the required dosage of a formulation to the water surface. Two grams of food (a mixture of 60% hog liver and 40% yeast) were added to each tank every other day. A 400 ml dipper was used to collect 5 larval samples, one from each corner and one from the middle of each tank. These samples were taken immediately prior to treatment and at 1 and 2 days posttreatment. The living or dead larvae in each sample were counted and the living larvae were returned to the respective tanks. Measurements of ambient water temperature in one tank were taken by a remote recording thermograph; water pH in each tank was also measured.

Indoor Laundry Tubs: During the cool weather in January-April 1984, the three G formulations were evaluated on equal potency basis (i.e., no. of ITU/mg) against late 3rd and early 4th instars of *Ae. aegypti*, *Ae. taeniorhynchus*, *Cx. nigripalpus*, and *Cx. quinquefasciatus*. These tests were made in 12 laundry tubs maintained in the laboratory. Each tub (0.5 X 0.5 m) contained 15 cm deep tap water. To each tub, ca. 500-1000 first instar laboratory maintained larvae were introduced and were provided with 0.5 g of food every other day until development to 3rd and 4th instars. The tubs were utilized to expose (to *B.t.i.*) larvae of one species at a time. Against *Ae. aegypti*, ABG-6140, 6138, and 6141, were applied at 2.5, 1.25, and 0.84 kg/ha, respectively, while *Ae. taeniorhynchus*, *Cx. nigripalpus*, and *Cx. quinquefasciatus* each received 5.0, 2.5, and 1.67 kg/ha of ABG-6140, 6138, and 6141, respectively. Against each species, each formulation was applied to three replicated tubs while three control tubs were left untreated. The tubs were thoroughly cleaned at the termination of each test. The pre-, and posttreatment larval sampling procedures and the sampling frequency for each test was the same as adopted for the study in concrete tanks. A 14-hr photoperiod and 27 ± 2°C were maintained in the laboratory during each test. The percentage reductions of larvae due to treatments in each test were calculated according to the formula given in Mulla et al. (1971) and the resulting data were statistically analyzed by Analysis of Variance and Duncan's Multiple Range Test.

RESULTS AND DISCUSSION.-The LC_{50} levels of the species bioassayed indicated that *Cx.*

Table 1.-Susceptibility of laboratory reared late 3rd and early 4th instar mosquito larvae[1] to a flowable formulation (ABG-6145 or Vectobac-AS) of *Bacillus thuringiensis* serovar. *israelensis* in the laboratory, 1984.

Species	24-h lethal concentration (ppm)		
	LC_{50}	C.L.[2]	Slope
Aedes aegypti	0.32	0.25 - 0.39	3.40
Aedes taeniorhynchus	0.28	0.25 - 0.31	3.79
Culex nigripalpus	0.21	0.19 - 0.23	3.99
Culex quinquefasciatus	0.19	0.17 - 0.21	3.42
Culex salinarius	0.11	0.09 - 0.12	3.59
Wyeomyia mitchellii	0.93	0.83 - 1.04	3.37

[1]Maintained at the Florida Medical Entomology Laboratory at Vero Beach, Florida.

[2]95% confidence limits.

salinarius was the most susceptible (LC_{50} = 0.11 ppm) to ABG-6145, followed by *Cx. quinquefasciatus* (LC_{50} = 0.19 ppm), *Cx. nigripalpus* (LC_{50} = 0.21 ppm), *Ae. taeniorhynchus* (LC_{50} = 0.28 ppm), and then *Ae. aegypti* (LC_{50} = 0.32 ppm). The LC_{50} value of *Wy. mitchellii* was 3-8X higher than the two *Aedes* and three *Culex* species (Table 1).

Among the G formulations applied on equal wt. basis at 5 kg/ha against *Ae. taeniorhynchus* and *Cx. quinquefasciatus* in concrete tanks, ABG-6140 (containing 100 ITU/mg) was the least effective and gave a maximum of 84% and 69% control of *Ae. taeniorhynchus* and *Cx. quinquefasciatus*, respectively, after 2 days of treatment (Table 2). ABG-6138 (containing 200 ITU/mg) was equally effective against the two species causing >95% mortality of each species, while ABG-6141 (containing 300 ITU/mg) gave complete control of these species in the tanks. Thus, as should be expected, the formulation of highest potency of *B.t.i.* (ABG-6141) resulted in highest level of control of *Ae. taeniorhynchus* and *Cx. quinquefasciatus*, although the % reductions of each species affected by ABG-6138 and 6141 after 2 days of treatment did not differ statistically (Table 2).

The G formulations applied on equal potency basis at rates of 5.0, 2.5, and 1.67 kg/ha of ABG-6140, 6138, and 6141, respectively, gave similar levels of control of *Cx. nigripalpus* (65 and 70%) with ABG-6140 and ABG-6138 after 2 days of treatment. ABG-6141, however, showed superior activity against *Cx. nigripalpus* causing 91% larval mortality of this species (Table 3). The three formulations applied against *Cx. quinquefasciatus* at the same rates of ITU/mg as used for *Cx. nigripalpus*, gave 60-69% control of *Cx. quinquefasciatus* (Table 3) and 83-91% control of *Ae. taeniorhynchus* after 2 days of treatment

Table 2.-Evaluation of three granular formulations of different potencies of *Bacillus thuringiensis* serovar. *israelensis* applied at the same rate (5 kg/ha) against 3rd and 4th instars of *Aedes taeniorhynchus* and *Culex quinquefasciatus* reared outdoors in concrete tanks[1] (July - August 1983).

Formulation	Mean no. larvae/dip[2] pre-, and posttreatment (days) Pretreatment	1	2
		Aedes taeniorhynchus	
ABG-6140 (100 ITU/mg)	42.8	12.5 (67.5)[3] a	5.8 (84.1) a
ABG-6138 (200 ITU/mg)	39.3	1.8 (94.9) b	0.3 (99.1) b
ABG-6141 (300 ITU/mg)	50.3	0.0 (100) b	0.0 (100) b
Control	56.0	50.3	47.8
		Culex quinquefasciatus	
ABG-6140 (100 ITU/mg)	51.3	19.0 (46.3) a	10.8 (69.2) a
ABG-6138 (200 ITU/mg)	79.5	9.5 (82.7) b	2.3 (95.8) b
ABG-6141 (300 ITU/mg)	96.8	0.0 (100) c	0.0 (100) b
Control	48.3	33.3	33.0

[1]Each tank 2.25 X 0.75 m; water temperature ranged from 22-32°C; water pH 8.9 - 9.3 between tanks.

[2]400 ml water.

[3]Mean % reduction in parentheses. Means in a column under each species followed by the same letter are not significantly different from each other at the 5% level of probability (DMRT).

Table 3.-Evaluation of three granular formulations of different potencies of *Bacillus thuringiensis* serovar. *israelensis* applied on equal potency basis (i.e., no. of ITU/mg) against 3rd and 4th instars of *Culex nigripalpus* and *Culex quinquefasciatus* reared in laundry tubs[1] in the laboratory[2] (January - March, 1984).

Formulations	Rate kg/ha)	Mean no. larvae/dip[3] pre-, and posttreatment (days) Pretreatment	1	2
		Culex nigripalpus		
ABG-6140	5.0	21.3	7.8 (54.0)[4] b	6.0 (64.9) a
ABG-6138	2.5	22.5	9.3 (48.0) ab	5.3 (70.6) a
ABG-6141	1.67	21.3	3.8 (77.0) bc	1.5 (91.2) b
	Control	29.3	23.3	23.5
		Culex quinquefasciatus		
ABG-6140	5.0	42.3	26.8 (40.3) a	17.3 (60.7) a
ABG-6138	2.5	39.5	24.3 (42.0) a	16.3 (60.4) a
ABG-6141	1.67	37.5	17.0 (57.3) a	12.0 (69.3) a
	Control	36.3	38.5	37.8

[1]Each tub 0.5 X 0.5 m; water pH 7.4.

[2]Air temperature 27 ± 2°C.

[3]400 ml water.

[4]Mean % reduction in parentheses. Means in a column under each species followed by the same letter are not significantly different from each other at the 5% level of probability (DMRT).

(Table 4). In the tubs, the G formulations showed better activity against *Ae. aegypti* than against *Ae. taeniorhynchus*, causing 88-99% larval reductions of the former species at one-half the rate of each formulation used against *Ae. taeniorhynchus*. However, the magnitude of larval reductions of *Ae. aegypti* caused by the three formulations was generally similar and did not differ statistically (Table 4).

It is evident from these studies that the FC formulation ABG-6145 possessed an excellent larvicidal activity against *Ae. aegypti*, *Ae. taeniorhynchus*, *Cx. nigripalpus*, *Cx. quinquefasciatus*, and *Cx. salinarius*. The three G formulations generally were equally effective against mosquito larvae exposed to the formulations in laundry tubs and provided acceptable levels of control (60-91%) at low rates of 1.67 kg/ha (ABG-6141), 2.5 kg/ha (ABG-6138), and 5.0 kg/ha (ABG-6140) used against *Ae. taeniorhynchus*, *Cx. nigripalpus*, and *Cx. quinquefasciatus*. *Aedes aegypti*, however, was controlled 88-97% at even lower rates of 0.84 kg/ha (ABG-6141), 1.25 kg/ha (ABG-6138), and 2.5 kg/ha (ABG-6140). Thus, these *B.t.i.* formulations are useful additional tools for the biological control of mosquitoes. In the field, the G formulations would be particularly useful because of their ease of handling, ease of dispersal, reduced drift, better canopy penetration, and perhaps the potential of prolonged residual activity due to slow release as compared to WP and FC formulations.

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Table 4.-Evaluation of three granular formulations of different potencies of *Bacillus thuringiensis* serovar. *israelensis* applied on equal potency basis (i.e., no. of ITU/mg) against 3rd and 4th instars of *Aedes aegypti* and *Aedes taeniorhynchus* reared in laundry tubs[1] in the laboratory[2] (March - April, 1984).

Formulations	Rate (kg/ha)	Mean no. larvae/dip[3] pre-, and posttreatment (days) Pretreatment	1	2
			Aedes aegypti	
ABG-6140	2.5	40.0	6.5 (84.5)[4] b	2.0 (94.8) a
ABG-6138	1.25	21.8	8.3 (63.8) a	2.5 (88.1) a
ABG-6141	0.84	27.3	2.3 (92.0) b	0.7 (97.3) a
	Control	25.8	27.1	24.8
			Aedes taeniorhynchus	
ABG-6140	5.0	50.5	10.3 (78.1) a	4.5 (90.5) a
ABG-6138	2.5	38.3	13.3 (62.7) a	6.0 (83.2) a
ABG-6141	1.67	55.0	17.0 (66.8) a	8.3 (83.9) a
	Control	42.8	39.8	40.0

[1]Each tub 0.5 X 0.5 m; water pH 7.4.

[2]Air temperature 27 ± 2°C.

[3]400 ml water.

[4]Mean % reduction in parentheses. Means in a column under each species followed by the same letter are not significantly different from each other at the 5% level of probability (DMRT).

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RISK CATEGORIES OF BIOLOGICAL CONTROL ORGANISMS

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ABSTRACT

Assigning natural enemies to decreasing environmental risk categories, eg., terrestrial vertebrates> zoopathogens> phytopathogens> phytophagous arthropods> terrestrial scavengers> aquatic vertebrates and invertebrates> and arthropodophagous arthropods, may provide a realistic base for allocating research time to a particular group before importation. However, the amount of research necessary to select the best biological control candidates might be greater for the least environmentally hazardous categories.

Biological control successes might be maximized by placing the various natural enemy groups into different risk categories before proceeding with data based decisions for introduction. Although the full impact of natural enemies which have never been studied is impossible to accurately predict before establishment (Coppel & Mertins 1977, DeBach 1964, Ehler & Miller 1978, Miller 1983), and, therefore, involves empirical judgment, a focus on educated empiricism (Coppel & Mertins 1977, Ehler & Hall 1982, Legner 1984) may hasten the selection of better natural enemy species.

Risk Groups in Biological Control.-Biological control by imported natural enemies is one of the most potent techniques in our arsenal of pest management. However, because it is relatively permanent, mistakes cannot be readily corrected. Imported organisms, once established, are not easily extirpated; and in some instances, their elimination is impossible altogether, regardless of the amount of effort and funding. There is therefore, some risk involved in any biological control approach. However, risks are a companion to life itself, and any pest management strategy involves some degree of risk, with most alternatives to importation of natural enemies being much more formidable (Pimentel et al. 1984). Eliminating too much risk through government regulation is not advisable at it can have the paradoxical effect of making life more dangerous as well as more expensive and less convenient (Huber 1983).

The broad nature of biological control including manipulation of vertebrates, arthropods and pathogens, requires considerations of the risks to the environment and health of humans and domestic animals, and risks of making wrong choices which may preclude or adversely affect biological control at a later date.

Environmental risks are of especial concern in the biological control of weeds, where both arthropods and pathogens are candidates for importation. The use of vertebrates for biological control of terrestrial pests is loaded with potential risk and is rarely practiced.

Environmental risks are minimized when vertebrates such as fish are imported to restricted aquatic ecosystems for the biological control of noxious aquatic weeds, mosquito habitats or mosquitoes and chironomids. Such fish can be studied under natural conditions, but in isolation for adverse effects before being widely disseminated. Yet, there are still possibilities that undesirable unforseen behavioral and adaptive traits, such as spawn-feeding on other desirable fish species, or an extension of subtropical species into temperate climates (eg. *Gambusia* spp.) may be expressed once populations are allowed to establish broadly (Legner & Sjogren 1984).

The risk of making wrong choices of arthropodophagous natural enemies, especially host specific ones, does not pose obvious environmental threats, as the outcome of the establishment of an innocuous natural enemy is the pest density remaining at status quo; although there is some theoretical debate on that issue (Legner 1984). However, wrong choices could possibly preclude the achievement of maximum biological control and add to the list of failures, so that careful decisions are desirable (Legner 1984).

We might arbitrarily consider in descending order of environmental risk, the terrestrial vertebrates first, followed by zoopathogens, phytopathogens, phytophagous arthropods, terrestrial scavengers (e.g., scarab beetles), aquatic vertebrates and invertebrates, and finally arthropodophagous arthropods. It would be logical to screen the first group more thoroughly than the last.

Terrestrial vertebrates are more readily observed in their places of origin because of their size, so their attributes may be more easily viewed. However, they are also capable of becoming conspicuous additions to the general landscape, and without natural predators of their own, may have the capacity to soar in numbers in the areas of their introduction. Thus, they may pose the threat of becoming pests themselves because of their numerical abundance and often nonspecific feeding behavior, and the side effects this can have on native and other desirable fauna and flora in the ecosystem.

Phytophagous arthropods and phytopathogens have traditionally evoked the most thorough of preintroduction studies to safeguard desirable plant species in the areas of introduction (Legner 1984). Host-plant specificity is strongly emphasized. Past screening has been so successful, that among numerous importations of beneficial phytophagous arthropods and pathogens around the world, there has never been any widespread occurrence of harmful behavior shown by the organisms imported (Legner 1984).

Terrestrial scavengers include scarab beetles that remove excess cattle dung accumulated in grazing areas to improve pastures, and to control the symbovine flies, *Haematobia irritans* (L.), *Musca autumnalis* deGeer, and *Musca vetustissima* Walker, primarily. Although dramatic successes have been achieved in the removal of dung by the importation of several species of exotic scarabs in Australia, Hawaii, California and Texas, there apparently have been no widespread concurrent practical reductions of fly densities (Legner 1984a, M. M. H. Wallace, pers. commun.). In fact, in some instances fly densities may have actually increased in the presence of established dung-burying scarabs (M. M. H. Wallace, pers. commun.; Legner, unpub. data). Although laboratory and field experiments predicted practical fly reductions by the dung scattering activities of the scarabs, in pastures several forces may interplay under certain conditions to thwart experimentally based predictions. Elimination of predatory arthropods and increase of available larval breeding habitat could be two of the principal causative factors (Legner 1985).

Terrestrial organisms that alter large habitats, such as scarab beetles, are especially risky biological control candidates because their activity may overlap portions of the niche of other species, so that potential disruptive side-effects among organisms in different guilds exist. The outcome for future symbovine fly control may be undesirable in that some potentially regulative natural enemies, such as certain predatory arthropods, may now be difficult to establish in the disrupted habitat. In California and Texas, the predatory staphylinid genus *Philonthus* is severely restrained from colonizing the drier dung habitat created by *Onthophagus gazella* F. (Coleoptera: Scarabaeidae) activity (Legner 1985, Roth et al. 1983). Furthermore, various nongraminaceous weed species often invade in California irrigated pastures utilizing scarab beetles, so that mechanical pasture renovation again is required (Legner 1984).

Aquatic vertebrates and invertebrates involve the use of fish for biological aquatic weed and arthropod control, and Turbellaria and Coelenterata for arthropod control. The minnows, *Gambusia* and *Poecilia* species, are used worldwide in the biological control of mosquitoes (Legner & Sjogren 1984). However, the threat to endemic fish has caused widespread concern, so that alternatives in the use of native fishes are under consideration (Legner et al. 1975, Walters & Legner 1980). Because fish can be manipulated readily, the potential for resident species to increase their effectiveness as natural enemies is greater than with terrestrial organisms where widespread natural dispersion may have already covered most possibilities.

A group of cichlid fish has been imported from Africa to the southwestern United States for the biological control of mosquitoes, mosquito habitats and chironomid midges. The degree of control achieved by the three species imported varied in different parts of the targeted area. The fish species referred to are *Tilapia zillii* (Gervais), *Sarotherodon* (*Tilapia*) *mossambica* (Peters), and *Sarotherodon* (*Tilapia*) *hornorum* Trewazas, which were imported to California for the biological control of emergent aquatic vegetation that provides a habitat for such encephalitis vectors as the mosquito, *Culex tarsalis* Coquillett, and as predators of mosquitoes and chironomid midges.

Careful studies under natural, but quarantined, areas in California showed that the different fish species each possessed certain attributes for combating the respective target pests (Legner & Medved 1973). *Tilapia zillii* was best able to perform both as a habitat reducer and a midge and mosquito predator. It also had a slightly greater tolerance to low water temperatures, which guaranteed its survival through the winter months in the south, while at the same time it did not pose a threat to salmon and other game fisheries in the colder waters of central California. It was the superior game species and most desirable for eating.

Nevertheless, the agencies supporting the research (mosquito abatement and county irrigation districts) acquired and distributed all three species simultaneously throughout thousands of kilometers of irrigation system, storm drainage channels and recreational lakes.

The outcome was the permanent and semipermanent establishment of the two less desirable species, *S. mossambica* and *S. hornorum*, over a broader portion of the distribution range (Legner & Murray 1981). This was achieved apparently by a competitive superiority rendered by an ability to mouth-brood their fry, while *T. zillii* did not have this attribute strongly developed. It serves as an example of competitive exclusion such as conjectured by Ehler & Hall (1982). In the clear waters of some lakes in coastal southwestern California, the intense predatory behavior of *S. mossambica* males on the fry of *T. zillii* could be easily observed, even though adults of the latter species made a strong effort to fend off these attacks.

This was not too serious for chironomid control because the *Sarotherodon* species were quite capable of permanently reducing midge densities to below annoyance levels (Legner et al. 1980). However, for control of higher aquatic weeds, namely *Potamogeton pectinatus* L., *Myriophyllum spicatum* var. *exalbescens* (Fernald) Jepson, *Hydrilla verticillata* Royle, and *Typha* species, they showed no capability whatsoever (Legner & Medved 1973, Legner unpub. data). Thus, competition excluded *T. zillii* from expressing its maximum potential in the irrigation channels of the lower Sonoran Desert of California and in recreational lakes of southwestern California.

Ironically, as the *Sarotherodon* species were of tropical nature, they died out annually in the colder waters of the irrigation canals and recreational lakes, and *T. zillii* populations could have been restocked. However, the appraisals given during the first few years by nonacademic, technical, irrigation district and recreation personnel discouraged further trials. This caused a switch in attention to a potentially much more environmentally dangerous species, the white amur, *Ctenopharyngodon idella* (Valenciennes) and other carps (Legner & Fisher 1980, Legner & Pelsue 1983, Osborne 1982). Thus, the various problems associated with the delivery of irrigation water without effective biological weed control (Legner & Murray 1981) continue.

The substitution of *T. zillii* in storm drainage channels of southwestern California is presently impossible, as the *Sarotherodon* species are permanently established over a broad geographic area (Legner & Pelsue 1983, Legner et al. 1980). Whether or not such a substitution would be more desirable from a chironomid midge control standpoint is not known; but the fact remains that presently it cannot be tested.

Arthropodophagous Arthropods (Insecta and Acarina) are in a distinct category which usually defies accurate prejudgment of biological control potential. Theoretical guidelines based on laboratory studies and mathematical models are not always useful to judge performance in nature. The extremely small size of arthropodophagous arthropods, their high dispersal capacity, unique sex determination mechanisms, differential response to varying host densities and climate, distribution patterns and size, unreliable sample techniques, dependence on alternate hosts, and possibilities of rapid genetic change at the introduction site, make predictions of their performance highly uncertain (Legner 1984).

However, because risks to the environment posed by arthropodophagous arthropods are very low, as previously considered, the inability to predict their impact has not been a major obstacle to their deployment in successful biological control. At the same time, selection of these biological control agents for importation has not been wholly unsophisticated and lacking in scientific judgment as was recently suggested by van Lenteren (1980). There are valid scientific criteria for deciding probable good candidates, which are especially useful for eliminating those with little likelihood for success or which possess certain undesirable characteristics such as hyperparasitism (Legner 1984).

CONCLUSION.-Natural enemies for use in biological control should be categorized into separate risk groups. Arthropodophagous arthropods fit into the lowest risk category, but are the most difficult to study and to assess for potential success. Yet, progress is being made with increased attention to basic ecological and behavioral research.

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THERMAL CHARACTERISTICS OF AQUATIC HABITATS AT COYOTE HILLS MARSH: IMPLICATIONS FOR SIMULATION AND CONTROL OF *ANOPHELES* POPULATIONS

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ABSTRACT

Continuous records of temperature change within dense submergent pondweed, *Potamogeton pectinatus*, at the shallow Coyote Hills Marsh, Fremont, California, show persistent thermal stratification. Maximum diel temperature range was 15 to 34 °C at the surface, and 14 to 18 °C at 20 cm depth. Thermal relations among depth strata result from the influence of macrophytic plant structure on the direction and rate of heat flux. Epiphytic organisms that inhabit pondweed are subject to high environmental heat capacity and reduced water turbulence. We hypothesize that the rate of organismal development and the amount of phenological synchrony within or among epiphytic populations is influenced by temperature stratification. Routine measurement of temperature extremes at the water surface, when applied to empirical models of temperature change at various depths, is practical for monitoring the complex thermal relationships throughout the pondweed community.

INTRODUCTION.-During recent years, researchers from the University of California at Berkeley and the California State University at Hayward have used a variety of field and laboratory studies to develop an integrated control strategy for *Anopheles freeborni* and *An. occidentalis* at the Coyote Hills Marsh, Fremont, California. Results from these studies have also been used to configure and calibrate the Coyote Hills Vector Control Model, a computer-based simulation model of anopheline bionomics (Roberts 1982, Schooley et al. 1982, Roberts et al. 1983, Schooley 1983). Previous studies at this marsh have described plant and animal populations that interact directly with *Anopheles* larvae or have secondary influences on larval survivorship (Collins et al. 1983, Balling and Resh 1984, Collins and Resh 1984, Fleming and Schooley 1984, Garcia et al. 1984, Graham and Schooley 1984, Lamberti and Resh 1984, Page and Schooley 1984, Young et al. 1984, Collins and Resh 1985). Of these populations, pondweed, *Potamogeton pectinatus*, is especially important because it forms a dense canopy at the marsh surface that physically inhibits predation of mosquito larvae by insectivorous fish. Pondweed also supports invertebrate predators and a variety of possible prey species besides *Anopheles* mosquitoes (Collins et al. 1983).

The success of the Coyote Hills Vector Control Model depends upon the accuracy of predicted patterns of population dynamics for predators and alternative prey that influence survivorship of anopheline larvae. Since temperature is a primary determinant of the growth rates for these poikilothermic populations, realistic simulation of their bionomics will require information about the functional relationships between these populations and the thermal characteristics of their aquatic microhabitats. This paper is the result of a study designed to describe patterns of temperature variation in the pondweed environment.

A practical consideration of efforts to simulate natural systems that are thermally variable is the high cost of routine temperature monitoring, which is necessary to establish timetables for temperature-related phenological events and organismal development. These costs might be reduced, however, if patterns of temperature change can be predicted based upon records of temperature extremes, such as diel (i.e., 24 hr) maximum and minimum temperatures. Therefore, this study assesses the usefulness of these types of information for describing thermal relationships among aquatic microhabitats.

MATERIALS AND METHODS.-Water temperature at three depth strata was recorded continuously for one year (May 1983 to May 1984) using a Foxboro Model 40 Thermograph. Sensors were fixed to a floating bracket and thus maintained their positions relative to the water surface. The three depth strata were the interface between the water and atmosphere (i.e., the surface flux boundary) and 10 and 20 cm depths (Figure 1).

From mid-spring to late autumn, while the pondweed canopy was present the uppermost stratum corresponded to the "intersection line", which is the microhabitat of *Anopheles* larvae (Hess and Hall 1943). The lower two strata approximately corresponded to the middle and bottom of the canopy. As a result, all three sensors together measured the full range of environmental temperatures for the epiphytic community.

The temperature data were reduced to instantaneous hourly values and daily maxima, minima, and means of temperature for each stratum. Weekly mean values for each hour of the day were also calculated for each stratum. These data permitted the empirical description of temporal and spatial patterns of temperature change throughout the pondweed canopy. Regression analysis was used to examine relationships between maximum or minimum temperature at the water surface and sub-surface temperature ex-

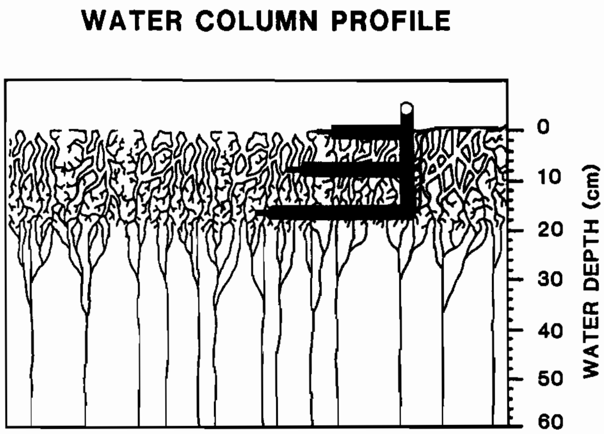

Figure 1.-Diagram of water column profile, showing pondweed canopy (canopy extends from water surface to 20 cm depth) and positions of three temperature sensors.

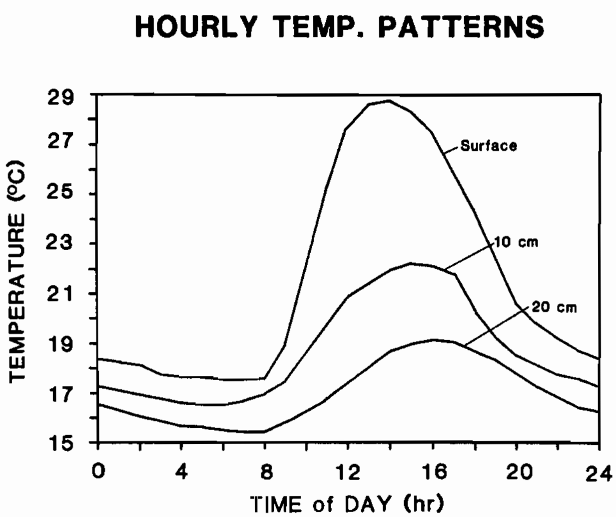

Figure 2.-Typical patterns of diel temperature change at three depth strata within the pondweed canopy. Temperature curves were derived from mean hourly readings for July and August 1982. Approximate times of sunrise and sunset were 0700 and 1930 hrs.

tremes.

Sine functions were fit to diel patterns of temperature change that were typical of the different depth strata within the pondweed. Such functions are commonly used to calculate day-degrees (i.e., heat accumulated per day within temperature thresholds for physiological development) and to predict day-degree rates, based upon short series of instantaneous temperature measurements (Wilson and Barnett 1983). We used sine function approximations to compare rates of day-degree accumulation among the depth strata. We further attempted to determine if the thermal relationships among aquatic microhabitats at different depths, variations in day-degree requirements, or thermal preferences of epiphytic organisms could affect the survivorship of *Anopheles* larvae in the Marsh.

RESULTS.-Typical summertime patterns of hourly temperature change among the three depth strata (Figure 2) reveal the following thermal characteristics of submergent vegetation of the Coyote Hills Marsh:

1. temperatures were always different at all three strata;
2. diel temperature range and daily temperature maxima and minima decreased with depth;
3. the simultaneous rate of heating (i.e., the positive slopes of the temperature curves at any given time) decreased with depth;
4. nighttime rates of cooling were similar throughout the canopy profile;
5. daily maximum temperature occurred at successively later times of day at deeper strata. The time lag for thermal maxima was about 1 hr per 10 cm increase in distance below the surface of the canopy.

Significant positive correlations were found between daily temperature extremes at the water surface and at the 10 and 20 cm depths (Table 1). The values of the coefficient of determination (i.e., r^2 values) decreased only slightly with increased distance between depth strata.

The results of day-degree calculations demonstrate an exponential decrease in heat absorption in relation to depth below the canopy surface (Table 2). Since this relationship was similar throughout the period of canopy development, the distribution of day-degrees among the depth strata also remained fairly constant (Table 2).

DISCUSSION.-Small ponds and shallow marshes (i.e., those with a maximum depth less than 1 m) that do not support submergent vegetation generally exhibit daily isothermy or loss of thermal stratification (Wallen 1950, Young and Zimmerman 1956, Butler 1963). Near the surface of the shallow Coyote Hills Marsh, a dense floating canopy of pondweed prevents isothermy and stratification persists throughout most summer

Table 1.-Results of linear regression analysis showing correlations between maximum and minimum temperatures (°C) at the canopy surface and at 10 and 20 cm below the surface in submergent vegetation. All the correlations are significant (i.e., P-values less than 0.05).

Comparison	Equation	r^2
max surface (X) vs max 10 cm (Y)	Y = 3.4 + 0.6X	0.96
max surface (X) vs max 20 cm (Y)	Y = 4.3 + 0.5X	0.90
min surface (X) vs min 10 cm (Y)	Y = ¯0.4 + 0.9X	0.96
min surface (X) vs min 20 cm (Y)	Y = ¯2.2 + 1.0X	0.94

Table 2.-Seasonal day-degree (°C) relationships among three depth strata.

Stratum	Day-Degrees May	Aug	Oct	Stratum Ratio	May	Aug	Oct
surface	86	98	98	surface/10 cm	1.5	1.4	1.4
10 cm	57	69	70	surface/20 cm	2.0	1.9	1.8
20 cm	41	53	54	10 cm/20 cm	1.4	1.3	1.3

days. The persistence of a temperature gradient during summer and autumn is the most obvious thermal feature of the pondweed environment.

Over a diel cycle, beginning near sunrise, solar heating of the surface produces a downward hear flux via conduction. Most incident short-wave radiation is intercepted very near the dense canopy surface, such that heat accumulates there faster than it is dissipated, and surface temperatures increase rapidly. After the sun passes its zenith, the surface begins to cool in response to decreases in solar inputs; however it remains warmer than the subsurface strata or atmosphere. This results from the high heat capacity of the canopy environment, relative to the open water without vegetation. Surface cooling produces a bi-directional heat flux, with heat loss being directed upward to the atmosphere and downward to cooler depths. This bi-directional heat flux at the surface causes it to cool rapidly and to transfer heat to lower strata, which therefore continue to increase in temperature. Turbulent mixing within the uppermost few centimeters, however, as can result from evaporative cooling at the surface (Woodcock 1941), was not apparent. Since heat flux between strata is proportional to their absolute difference in temperature, these differences tend to equilibrate in the absence of new heat inputs (Oke 1978). Thus at night, when the vertical profile of temperature throughout the canopy is linear (i.e., equal increments of temperature change correspond to linear increases in depth below the canopy surface), the rates of heat loss from different strata tend to be similar. Since the canopy has high heat capacity, the strata tend to cool slowly and fail to achieve isothermy (i.e., a uniform distribution of heat) prior to sunrise.

Shading and heat absorption by the canopy surface causes the sub-canopy water column and the marsh bottom to remain cool throughout the summer. The resultant density boundary between the lower depths of water and the canopy bottom, as well as the hydraulic resistance of the dense vegetation, might be sufficient to prevent the two strata from mixing. This is supported by casual observations of turbidity and wind-generated currents at mid-column that were not detectable within the canopy. Since open water beneath the canopy tends to be cool but turbulent, it probably exports heat conducted from the lowermost canopy strata. These features in combination prevent reverse stratification (i.e., increasing temperature with depth) and tend to maintain a sharp thermocline between the pondweed canopy and sub-canopy environment. Significant correlations between the maximum and minimum temperatures for the various strata suggest that weekly values of temperature extremes at one stratum can be used to predict or monitor thermal conditions throughout the pondweed community. For example, based upon simple measurements of thermal maxima and minima at the water surface, adjustments in the amplitude of sine function approximations of the diel patterns of temperature change can be made. Consequently, numerous and relatively inexpensive max-min thermometers can be used to improve understanding of thermal variability throughout the Marsh.

The differences in the thermal characteristics of the aquatic microhabitats at the Coyote Hills Marsh may have ecological significance. Daily heat regimens that are persistent, and therefore predictable, might be more important that the annual or seasonal cycles of heating and cooling for populations of aquatic invertebrates that are mobile or have short generation times. In this regard, the relationship between day-degrees and depth below the plant canopy surface is probably most important. Although the rate of day-degree accumulation for any particular stratum varied slightly during the period of canopy development, the distribution of day-degrees among the strata remained almost constant (Table 2). This provided a basis for formulating the following series of hypotheses regarding the vertical distribution and phenology of epiphytic organisms at the Coyote Hills Marsh:

1. a population that occurs vertically throughout the floating pondweed canopy will have asynchronous development;

2. populations with different thermal preferences will be vertically segregated;

3. segregated populations with similar day-degree requirements will have asynchronous development;

4. some organisms will migrate through the canopy to regulate their body temperatures.

Any of these organismal responses to the presence of a thermal gradient might be expected to influence predator-prey relationships. For example, synchronous development of multivoltine predator populations could cause cyclic patterns of prey survivorship, or predation rates could be nearly uniform in time because of asynchrony within or among predator populations. Certainly, an upward movement of predators or a downward migration of alternative prey could benefit a mosquito control program, since either type of faunal re-distribution would tend to increase the consumption of mosquito larvae.

ACKNOWLEDGMENTS.-These studies are the result of cooperative efforts among personnel of the University of California at Berkeley, the California State University at Hayward, the East Bay Regional Park District, and the Alameda County Mosquito Abatement District. We thank S. S. Balling, J. W. Feminella, and G. A. Lamberti for field assistance. We also thank J. W. Feminella and E. P. McElravy for assistance with the manuscript. Support for these studies was provided by the University of California Mosquito Research Funds and the Alameda County Mosquito District.

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FACTORS THAT LIMIT THE ROLE OF IMMATURE DAMSELFLIES AS NATURAL MOSQUITO CONTROL AGENTS AT COYOTE HILLS MARSH

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ABSTRACT

The community of macroinvertebrates associated with pondweed, *Potamogeton pectinatus*, at the Coyote Hills Marsh, Fremont, CA, was sampled bi-weekly from June to November 1983. The epiphytic predator guild was dominated by the immature stages of three species of damselflies: *Enallagma civile, E. carunculatum* and *Ischnura cervula*. Mean density for all three species combined sometimes exceeded 1200 naiads per m^2. Although larval *Anopheles occidentalis* dominated the neuston (peak densities exceeded 500 larvae per m^2), mosquito larvae always composed less than 15% of the identifiable organisms in the foreguts of damselfly naiads. The diet of these predators was composed mostly of sestonic microcrustacea and epiphytic chironomid larvae, and reflected the relative abundances of these prey types in the pondweed environment. Damselfly microdistribution, age-specific feeding habits, phenology, and the architecture of the habitat that supports the naiads reduces their importance as mosquito predators at Coyote Hills Marsh.

INTRODUCTION.-Immature damselflies (Odonata: Zygoptera) that inhabit aquatic vegetation are generally regarded as predators of mosquito larvae (Hinman 1934, Laird 1947, Jenkins 1964). The inference that damselflies can benefit mosquito control programs (e.g., Geiger and Purdy 1919, Collins and Washino 1982) has been based upon observations of predation of mosquitoes by damselfly naiads in laboratory aquaria (e.g., Twinn 1931, Lee 1967, Notestine 1971), the apparent dominance of invertebrate predator guilds by these naiads (e.g., Miura et al. 1981), and the implicit assumption that damselflies and mosquitoes coexist in in the same microhabitat. When alternative prey densities are reduced, consumption of mosquito larvae by damselflies and other invertebrate predators is expected to increase (e.g., Bence and Murdoch 1982). As a result, negative correlations between densities of naiads and mosquitoes have been attributed to predation by damselflies (e.g., Sailer and Lienke 1954, Miura et al. 1978).

However, some experimental efforts to quantify predator influences on mosquito survivorship have indicated that damselflies and other invertebrate predators do not appreciably reduce mosquito densities under field conditions (e.g., Mulla 1961, Washino 1969), and that invertebrate predation alone might be insufficient for mosquito control (Jenkins 1964). Possible reasons for invertebrate predator inefficacy include phenological asynchrony between predator and prey populations (e.g., Washino 1969, Notestine 1971, Bay 1974), the consumption of invertebrate predators by insectivorous fish (Hoy et al. 1972, Farley and Younce 1977, Bence and Murdoch 1982), and the tendency for invertebrate predators, especially Odonata, to be general or opportunistic consumers (Corbet 1980).

Recent results of field and laboratory studies that were designed to calibrate a computer-based simulation model of *Anopheles* bionomics at Coyote Hills Marsh, Fremont, CA, suggested that predation by immature damselflies might significantly affect anopheline larval survivorship (Collins et al. 1983, Lamberti and Resh 1984). Therefore, a detailed investigation of damselfly diet, energetics, and bionomics was initiated. This paper presents the preliminary finding of that study in the context of an integrated mosquito control program.

MATERIALS AND METHODS.-The Coyote Hills Marsh is a shallow, flood-control impoundment designed so that it also benefits wildlife and recreation interests (Collins et al. 1983). The Marsh was colonized by pondweed, *Potamogeton pectinatus*, in 1977 and since then has supported large populations of *Anopheles* mosquitoes. From mid-spring to autumn, the pondweed forms a dense canopy of vegetation at the marsh surface. The floating canopy can function for *Anopheles* larvae as a refuge from fish predation (Collins et al. 1983) while also supporting a diverse assemblage of epiphytic invertebrates (Lamberti and Resh 1984).

The pondweed community was sampled every two weeks from June to November 1983, which was the time period for canopy development and senescence. A pull-up type sampler was designed for this study to collect all the invertebrates and pondweed occurring together beneath a small area (i.e., 0.06 m^2) of the marsh surface (Balling and Resh 1984). Fifteen samples were taken on each sample date from a region of the marsh that supported an extensive and dense pondweed canopy. Based upon these samples, the phenologies and temporal patterns of density for the dominant species of macroinvertebrates, including damselflies, mosquitoes, and alternative prey species (i.e., potential damselfly prey besides mosquito larvae) were described.

The invertebrate predator guild of the pondweed community was dominated by three species of damselflies: *Enallagma carunculatum, E. civile,* and *Ischnura cervula*. The alternative prey were dominated by epiphytic midge larvae

(Diptera: Chironomidae), including *Paralauterborniella subcincta*, *Dicrotendipes nervosus*, *Cricotopus* spp., *Coryneura* spp., and several species of Tanytarsini, but also included several species of microcrustacea (Collins et al. 1983, Lamberti and Resh 1984). During this same sampling period, damselfly emergence was monitored by enumerating the exuviae that accumulated each week on standardized emergence substrata. The emergence data provided an estimate of the cohort production interval for the damselfly guild (i.e., the average generation time among the three damselfly species) and a relative measure of their abundance as final instars.

The number of size classes of damselfly naiads (i.e., the average number of instars among the component species of the damselfy guild) was chosen based upon the frequency distribution of naiad head widths. Graphical analysis of these data revealed twelve distinct aggregations of head width measurements, which were assumed to represent instars for the average cohort. Our determination of twelve instars is supported by other studies of damselfly life history (Corbet 1980).

Damselfly diets were described based upon the enumeration of prey items contained in the foreguts dissected from three hundred middle- to late-instar naiads (i.e., size classes 6-12). Among smaller naiads, no identifiable prey items were found. Since only the final instars of *E. civile* and *E. carunculatum* can be used to distinguish these closely related species (Garrison 1984), no species-specific dietary analysis was attempted. For the guild as a whole, no obvious differences in diet were apparent among individuals of any of the larger size classes.

Secondary production (i.e., the amount of animal tissue elaborated per unit time) was estimated for mosquito larvae and immature damselflies using the Hynes-Coleman method (Benke 1984). Appropriate values of production efficiency (i.e., the ratio of production to consumption) for Zygoptera were taken from the literature (e.g., Fischer 1966, Klekowski 1970, Lawton 1970). These data can be used to estimate the amount of consumption required to account for observed production as follows:

$$\text{production X efficiency}^{-1} = \text{consumption.}$$

This estimate is improved when age-specific efficiencies that are adjusted for temperature conditions or food quality are applied to the equation. For this preliminary study, however, we assumed that an average value from the literature would pertain equally well to the different species of damselflies and their prey.

RESULTS AND DISCUSSION.-A comparison of temporal patterns of density for immature damselflies and *Anopheles* larvae (Fig. 1A) suggests either a functional predator-prey response, whereby the density of damselflies increased at the expense of the mosquitoes, or phenological asynchrony, which caused the predators and potential prey to attain peak densities at different times during the sampling period. Another

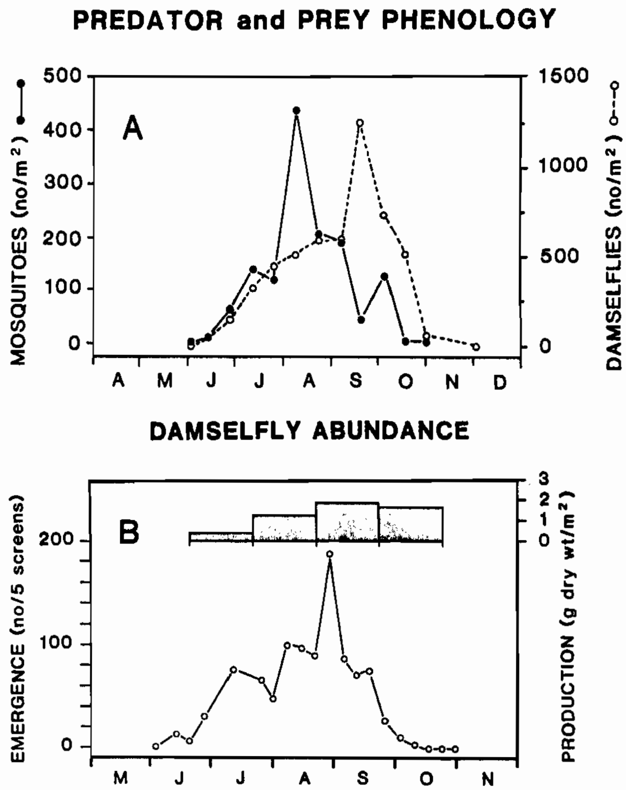

Figure 1.-(A) Temporal patterns of density for mosquito larvae and damselfly naiads; (B) Damselfly abundance, as indicated by emergence and secondary production.

explanation for the observed patterns might involve an undisclosed source of mortality that differentially affects mosquitoes and damselflies and causes them to appear segregated in either space or time.

The relative merit of these possible explanations for temporal segregation of the damselfly guild and larval *Anopheles* population is not apparent because bionomic comparisons do not provide sufficient evidence of ecological interactions. Furthermore, functional relationships between populations of mosquito larvae and their predators can be obscured when life history information about different species is combined or if adequate taxonomy to separate species is not available. For example, the three damselfly species at Coyote Hills Marsh have different temporal patterns of emergence, which in combination produce a series of emergence peaks for the guild (Fig. 1B). These species might also have different diets, but graphical analysis could not distinguish interspecific relationships from other sources of variability in diet for the damselfly guild as a whole. Thus, obvious patterns of abundance or phenology among predator and prey guilds might not have ecological significance.

A comparison between the amount of secondary production of *Anopheles* larvae and the amount of consumption by the damselfly guild

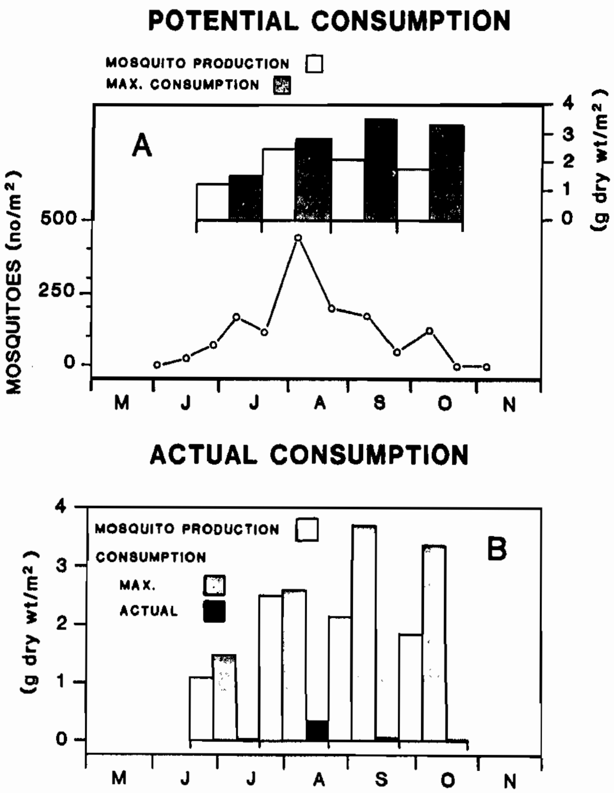

Figure 2.-(A) Mosquito production and maximum possible consumption of mosquito larvae by damselflies, in relation to seasonal changes in mosquito density; (B) Seasonal patterns of secondary production of mosquito larvae, in relation to maximum potential and actual consumption of mosquito larvae by damselflies.

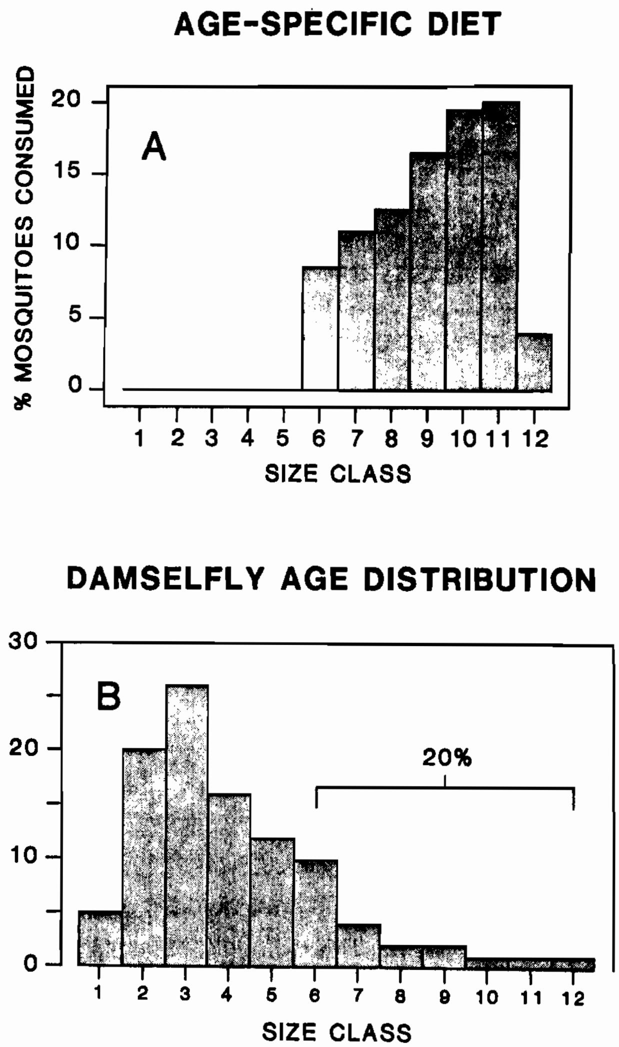

Figure 3.-(A) Relationship between the age or size of damselfly naiads and their consumption of mosquito larvae; (B) Distribution of naiads among age classes. This graph shows that damselfly size classes 6-12 comprise only 20% of the total naiad population.

would suggest that the naiads potentially can consume all the mosquitoes in the Marsh, even during periods of peak mosquito density (Fig. 2A). To achieve this potential as mosquito control agents, the damselflies would have to prey on mosquitoes exclusively. However, our analysis of the damselfly diet indicates that not more than about 15% of the food requirements of the naiads was provided by their consumption of mosquitoes (Fig. 2B).

Several factors can explain why immature damselflies are limited as predators of *Anopheles* mosquitoes at Coyote Hills Marsh. First, prey selection based upon predator and prey size can reduce that proportion of the mosquito population that is consumed by damselflies. Other researchers (e.g., Lee 1967, Notestine 1971) have observed that although late-instar damselflies will consume all instars of mosquitoes, the larger mosquito larvae and pupae tend to be more evasive and are not consumed by small damselflies. At Coyote Hills Marsh, mostly small larvae (i.e., first and second instars) were consumed by damselflies (Lamberti and Resh 1984) and this consumption can be attributed to large naiads (Fig. 3A), which only composed about 20% of the total naiad population (Fig. 3B). The final-instar naiads, which remain near the water surface in preparation for emergence and would therefore be in a position where encounters with mosquito larvae would be most likely, represent a developmental stage that generally does not feed.

Second, *Anopheles* larvae might be insufficiently available as prey to be a major component of the damselfly diet. An examination of monthly changes in dietary composition for the damselfly predator guild reveals that the naiads mostly consumed the dominant sestonic and epiphytic invertebrates. For example, during the summer and early autumn, the naiads consumed mostly epiphytic midge larvae; in late autumn the sestonic microcrustacea were the dominant dietary components (Fig. 4A). These changes in dam-

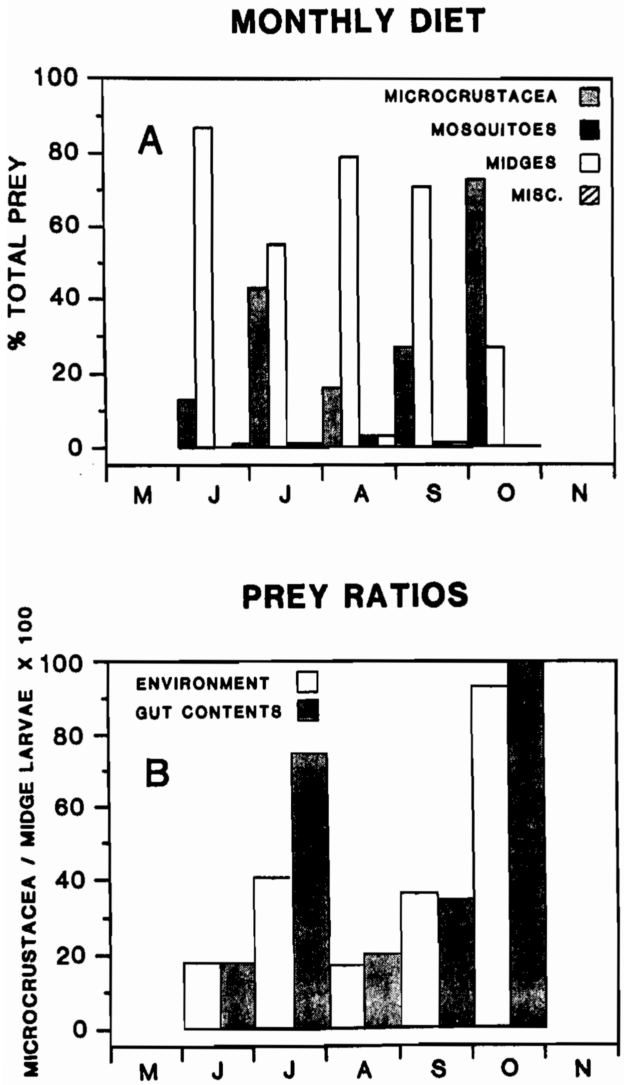

Figure 4.-(A) Temporal patterns of diet for damselfly size classes 6-12; (B) Comparison of the relative abundances of dominant prey types in damselfly foreguts and natural habitat.

selfly diet reflect changes in relative abundance of midge larvae and microcrustacea in the habitat (Fig. 4B), which strongly suggests that immature damselflies are opportunistic predators and that *Anopheles* larvae were always much less available than alternative damselfly prey.

Third, although both predators and prey were very abundant within the same samples, actual encounters between damselfly naiads and mosquito larvae might be uncommon. Since the damselfly species at Coyote Hills Marsh typically do not stalk their prey (Corbet 1980), the rate of predation for any particular naiad will depend upon the density of prey nearby and prey mobility. Furthermore, although *Anopheles* larvae have a high affinity for positive menisci (Renn 1943) that characterize the plant-air-water interface or the "intersection line" (Hess and Hall 1943), they are only associated with a very small portion of the total plant surface that damselfly naiads can inhabit (Fig. 5). In fact, at the Coyote Hills Marsh, where a lush (i.e., 20 cm thick) canopy of submergent pondweed occurs, close examination of the microhabitats of *Anopheles* larvae and damselfly naiads suggests that their populations are spatially segregated.

Since aquatic plant architecture seems to influence the ratio of intersection line to total macrophytic surface area, some aquatic vegetation might be more suitable than pondweed for interactions between mosquito larvae and epiphytic predators. For example, emergent vegetation such as rice, which represents a littoral environment that is structurally less complex than pondweed and which may provide less microhabitat below the water surface, might be more conducive to predation by damselfly naiads at the intersection line.

Summary of Findings.-Life history characteristics of the immature damselflies and *Anopheles* larvae at Coyote Hills Marsh reduce the efficacy of damselflies as natural mosquito control agents. The more important limitations are:

1. phenological asynchrony between the damselfly guild and *Anopheles* larvae;
2. phenological asynchrony among the damselfly species, such that peak densities of different damselfly populations do not coincide and maximum possible predator densities might not be attained;
3. variable spatial distributions of predator and prey within the same microhabitat, such that aggregations of predators and their potential prey might not coincide;
4. minimal overlap between the damselfly and mosquito microhabitats;
5. opportunistic or non-selective predation by damselflies in regard to type of prey, but selective predation in regard to size or age of prey;
6. selective predation in regard to predator size or age, such that damselflies that consume mosquito larvae represent a small proportion of the predator population;
7. greater availability of alternative prey species, even during periods of peak density of *Anopheles* larvae.

These limitations do not preclude the possibility that damselfly naiads influence mosquito densities, but that the degree of influence does not approach the levels desired for vector control. Further research is being conducted to determine whether habitat modifications can improve the role of damselflies as mosquito control agents at Coyote Hills Marsh.

ANOPHELES AS EPIPHYTIC PREY

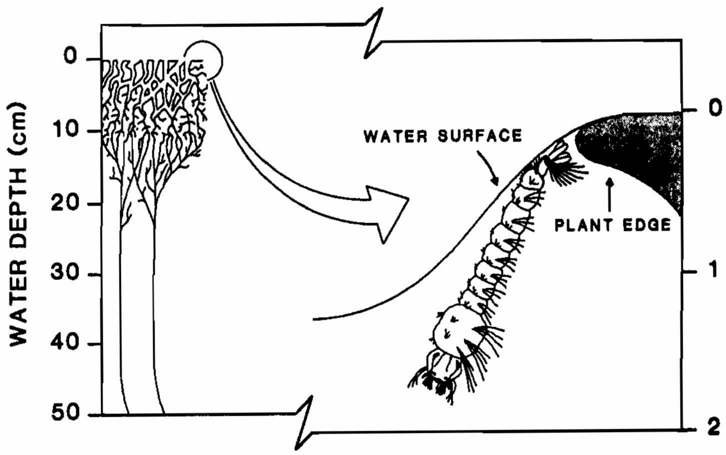

Figure 5.-Detail of the *Anopheles* microhabitat that shows its restriction to the canopy surface and a magnified image of an anopheline larva characteristically entrained within a meniscus at the air-plant-water interface (i.e., the intersection line).

ACKNOWLEDGMENTS.-These studies are the result of cooperative efforts among personnel of the University of California at Berkeley, the California State University of Hayward, the East Bay Regional Park District, and the Alameda County Mosquito Abatement District. We thank S. S. Balling, J. W. Feminella, and G. A. Lamberti for field assistance. We also thank J. W. Feminella and E. P. McElravy for assistance with the manuscript. Support for these studies was provided by the University of California Mosquito Research Funds and the Alameda County Mosquito Abatement District.

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COMPARATIVE LARVIVOROUS EFFECTS OF MOSQUITOFISH AND SACRAMENTO BLACKFISH IN EXPERIMENTAL RICE FIELDS

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ABSTRACT

To test the hypothesis that juvenile, native minnows could provide mosquito control comparable to the mosquitofish (*Gambusia affinis*) in rice field habitats, mosquitofish and Sacramento blackfish (*Orthodon microlepidotus*) were stocked in three and five, respectively, experimental rice paddies (122 m x 3.5 m) at the UCD Rice Research Facility. In addition, 6,000 to 12,000 *Culex tarsalis* mosquito larvae were mass cultured and weekly stocked into the paddies over an eleven week period. Three mosquito larvae samples, by dipping method, in all rice paddies within two weeks of paddy draining in September and direct fish counts immediately after paddy draining showed that mosquitofish had depleted mosquito larval numbers significantly greater ($P < 0.025$) than either Sacramento blackfish, unstocked controls, or hitch (*Lavinia exilicauda*) which were stocked in one paddy. However, mosquitofish populations had grown to contain 8-10 times the number of fish compared with the minnow paddies. Thus, a strong negative correlation ($r = -0.83$) was calculated between total fish in a paddy and total mosquitoes in each paddy. Although young-of-the-year Sacramento blackfish seemed to thrive as well as the one and two year old juvenile minnows in the rice paddies, all minnow populations showed 1% per day mortality rates through the rice growing season. One sample of other invertebrates in each paddy, using dipper, showed no clear correlations with fish species stocked.

INTRODUCTION.-The mosquitofish, *Gambusia affinis*, is used widely for mosquito control. However, this species' seasonal use as a biological controller of mosquitoes has limitations. One of the primary limitations is the number of mosquitofish available when they are needed. As an example, insufficient numbers of *Gambusia affinis* are available in late spring for widespread stocking in California rice fields, a problem which has been recognized for some time (Hoy and Reed 1970, Coykendall 1975). One solution to this problem, mass culture of mosquitofish, has two main limiting factors. Male and female must first be mated for internal fertilization of ova. Secondly, the fecundity of these livebearing fish is limited (Krumholz 1948).

An alternative solution to biological control of mosquitoes in California might be the juveniles of native minnows. Our previous field studies on *Gambusia* show that juveniles of several minnows are already present in rice fields. These juveniles come in with the irrigation water from surrounding drainages. Two species with especially good potential in California rice fields are the Sacramento blackfish, *Orthodon microlepidotus*, and the hitch, *Lavinia exilicauda* (Cech and Moyle 1983). Both of these species have larvivorous food habits and substantial fecundity as large, egg-laying adult fish (Moyle et al. 1982). The present study took place during the 1984 rice growing season when the following question was posed. Can juveniles of native minnows provide mosquito control comparable to the mosquitofish in experimental rice paddies? Our hypothesis was that they could provide comparable control. The field studies were designed to supplement concurrent laboratory studies on these species' prey preferences and metabolic food demands.

MATERIALS AND METHODS.-Twelve rice paddies 122 meters by 3.5 meters were constructed at the UCD Rice Research Facility. Each paddy had its own screened water inflow and screened outflow box. The screens consisted of 6 mm hardware cloth and 1.5 mm plastic window screen as inserts. In spite of these screening efforts, some very young Sacramento blackfish were discovered in some paddies near the end of the study. Three of the paddies were each stocked with 93 mosquitofish. Three were stocked with 93 2-year-old Sacramento blackfish and two were stocked with 93 young-of-the- year Sacramento blackfish, and finally one was stocked with 93 one- and two-year-old hitch. Three paddies were not stocked, as controls. Battery operated thermographs were situated at the upper, middle, and lower sections of one of the interior paddies with the thermister located in sparse rice vegetation in each case. Because the juvenile Sacramento blackfish and hitch cannot reproduce in the rice paddy habitat, available young-of-the-year blackfish were subsequently stocked in the blackfish paddies during the rice growing period. Ninety-three more Sacramento blackfish were added to each Sacramento blackfish paddy three weeks after the initial stocking and 500 more young-of-the-year fish, seven weeks after initial stocking. All Sacramento blackfish were cultured in a concrete-lined pond on the UCD campus. In addition, 6,000 to 12,000 *Culex tarsalis* mosquito larvae were weekly mass cultured and stocked into the paddies over an eleven week period. Three mosquito larvae samples, by the dipping method in all rice paddies within two

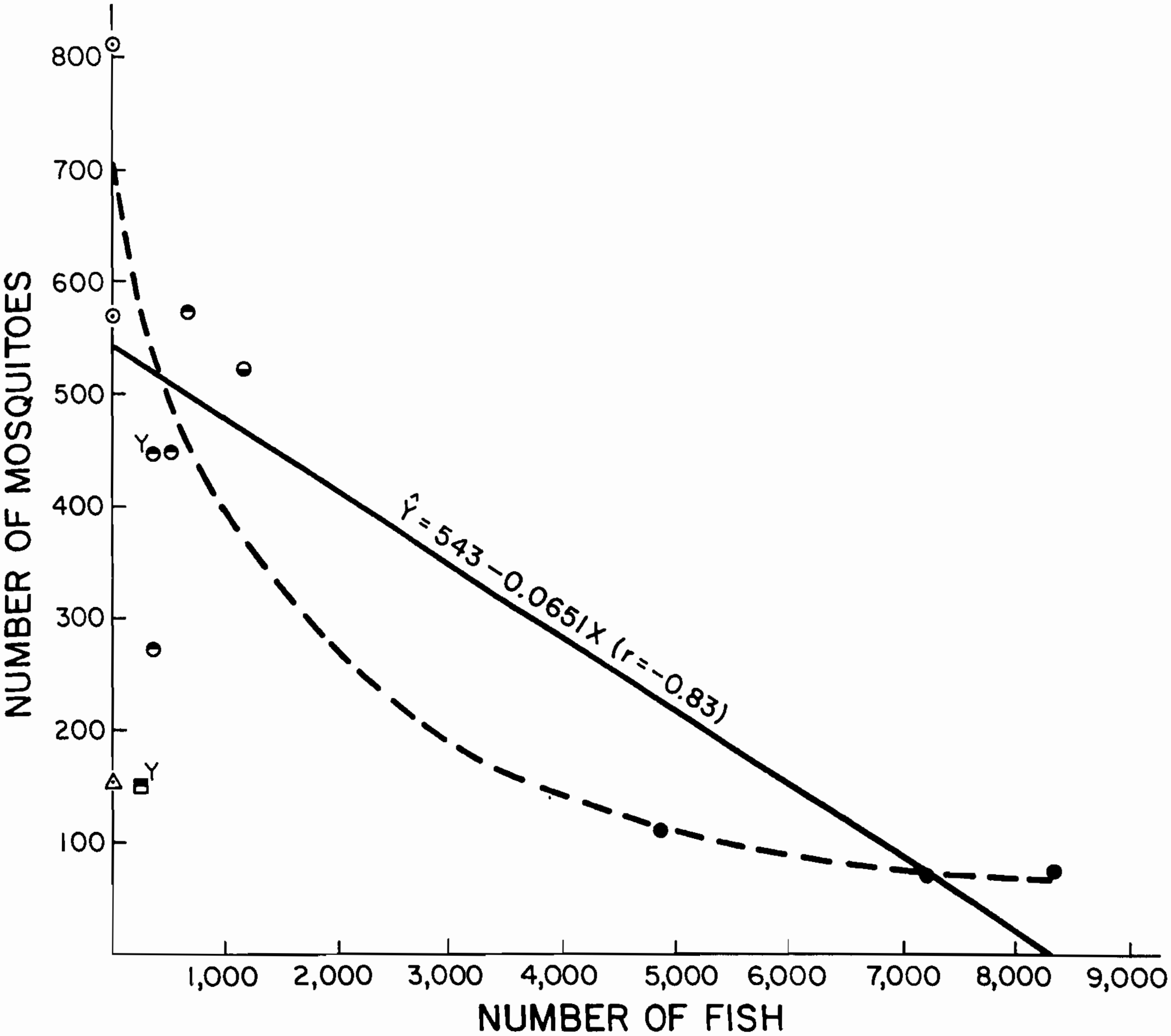

Figure 1.-Relationship between total number of mosquitoes; sampled on September 8, 12, and 15, 1984; and total number of fish; sampled on September 18 and 19, 1984; in each of twelve experimental rice paddies. Solid line is least squares linear regression fit. Dashed line is possible hyperbolic function fitted by eye. Key: control paddies ⊙, hitch paddy = ◒, Sacramento blackfish paddies = ⊖ (young-of-the-year fish denoted by Y), and mosquitofish paddies = ●. The two paddies at the end of the field (△, ▬Y) were excluded from analyses (see text).

weeks of paddy draining in September, and direct fish counts immediately after paddy draining, provided data to test the hypothesis.

RESULTS.-Mosquitofish reduced larval numbers significantly greater ($P < 0.025$, t-test) than either Sacramento blackfish, hitch or controls. However, mosquitofish populations had grown to contain 8-10 times the number of fish compared with the various minnow paddies (Fig. 1). Consequently, a strong negative correlation ($r = -0.83$) was calculated between total fish in a paddy and total mosquitoes sampled in each paddy. However, a lack of data at intermediate fish densities prohibited confidence in a true linear relationship between larval abundance and fish abundance. A hyperbolic function (dashed line fitted by eye in Fig. 1) may also represent an adequate description of the data. The two paddies at the ends of the rice field were excluded from this analysis because the easternmost paddy tended to dry partially near the adjacent road while the westernmost paddy apparently received pesticide overspray from an adjoining experiment.

Because young-of-the-year were distinguishable from two-year-old Sacramento blackfish, and from the very young immigrants which were able to come into the paddies through the inflow screens, we could calculate mortality rates for the young-of-the-year and two-year-olds by knowing the number stocked, the stocking dates, and the final counts. Interestingly, each of the minnow paddies revealed mortality rates of 1% per day. These relatively small numbers of minnows remaining in the hitch and Sacramento blackfish paddies did not significantly reduce mosquito larvae numbers compared with control paddies (t-test, $P > 0.05$). Mosquito numbers in the hitch paddy were statistically indistinguishable from those in the Sacramento blackfish paddies. However, difficulties in obtaining sufficient hitch precluded establishment of more than one hitch paddy.

In addition to mosquito larvae, aquatic invertebrates were quantified by counts from the dipper samples. T-test analysis showed no general trends among *Gambusia* stocked paddies versus Sacramento blackfish and hitch stocked paddies versus control fields regarding the aquatic invertebrates (Table 1). The sole exception to this trend might be a significantly lower aeshnid abundance in the *Gambusia* paddies compared with the controls ($P < 0.05$, t-test). A large temperature variation was noted down the length of the rice paddies. The mean temperature for the last three sampling days was 16.4°C at the upper station near the water inlet, 21.3°C in the middle of the paddy, and 25.5°C at the lower end.

DISCUSSION. - Initial sampling problems plagued the study. An acceptable dipping technique was finally established which minimally disturbed adjacent mosquito larvae. Thus, the last three samples were, comparatively, very representative of the mosquito populations. The difficulties in obtaining hitch allowed use of young-of-the-year Sacramento blackfish in two paddies. This showed us that young-of-year blackfish can thrive in rice field environments, an unexpected finding (Cech and Moyle 1983). Laboratory studies show that a respiratory homeostasis at lower dissolved oxygen tensions also characterizes the young Sacramento blackfish, (Cech and Linden, unpubl.) which may contribute to the survival of these young fish.

Information on invertebrates is preliminary. Invertebrates were identified and enumerated from dipper samples on only one sampling day. Because the dipper samples were primarily designed

Table 1.-U.C.D. EXPERIMENTAL RICE PADDIES INVERTEBRATE SAMPLE.

			NUMBER OF INSECTS IN 50 DIPS ON 8 IX 84 SAMPLE IN RICE PADDIES IDENTIFIED BY NUMBER AND FISH SPECIES											
			Gambusia			Blackfish & Hitch						Control		
ORDER AND FAMILY WITH (GENUS)	FEEDING GUILD	POSSIBLE SAMPLING BIAS WHILE DIPPING	3	4	10	YOY 1	2	7	YOY 8	9	H 11	5	6	12
EPHEMEROPTERA - Mayflies														
Baetidae	Collectors, gatherers, scrapers		3	0	13	0	2	5	0	3	17	2	5	1
ODONATA - Dragonflies & Damselflies														
Coenagrionidae	Engulfers - predators*	On bottom	1	3	6	3	5	1	1	2	16	5	3	2
Aeshnidae	Engulfers - predators*	On bottom	2	3	3	1	11	13	10	22	22	17	14	6
Libellulidae	Engulfers - predators*	On bottom	0	1	0	0	0	0	0	0	2	1	0	1
HEMIPTERA - True Bugs														
Belastomatidae	Piercers - carnivores*	Quick swimmer	2	1	0	0	0	1	0	2	1	3	0	1
Veliidae	Piercers - carnivores*		0	0	0	0	0	0	0	1	0	0	0	1
Corixidae	Piercers - carnivores*	Dive and stay on bottom/quick swimmer	0	1	0	0	0	0	0	0	0	0	0	1
Notonectidae (*Buenoa*)	Piercers - carnivores*	Dive and stay on bottom/quick swimmer	0	0	0	0	3	1	0	1	0	6	0	0
Gerridae	Piercers - carnivores*	On surface/skate away	0	0	0	0	0	0	0	0	0	1	1	0
Hebridae (*Merragata*)	Piercers - carnivores*		0	0	0	0	0	0	1	0	0	0	0	0
COLEOPTERA - Beetles														
Dytiscidae (*Bidessus*)	Piercers - carnivores*	Quick swimmer	0	1	0	0	0	0	0	0	0	0	0	0
(*Hygrotus*)	Piercers - carnivores*	Quick swimmer	0	2	0	0	0	1	0	1	0	1	0	0
(*Laccophilus*)	Piercers - carnivores*	Quick swimmer	1	1	2	0	0	3	1	1	4	1	1	0
Hydrophilidae (*Tropisternus*)	Larvae = engulfers-predators* Adults = collectors, gatherers*	Larvae on bottom	7	0	0	0	0	3	0	0	2	1	0	0
(*Hydrophilus*)	Larvae = engulfers-predators* Adults = collectors, gatherers*	Larvae on bottom	3	0	0	0	0	0	0	0	0	1	0	0
DIPTERA - Flies														
Ceratopogonidae	Engulfers or collectors*		22	4	6	1	0	32	3	19	30	9	4	12
Chironomidae	Engulfers or collectors*		13	0	5	0	0	6	1	5	26	4	7	5
Tipulidae	Shredders - herbivores		5	0	1	0	1	4	0	4	1	1	0	1
Ephidridae	Engulfers or collectors*		1	0	1	0	0	2	0	6	0	2	0	3
Stratiomyidae	Collectors, gatherers		0	0	1	0	0	1	0	1	0	0	0	0

*Possible predators on mosquito larvae

YOY = Young of the year Sacramento blackfish paddy; H = hitch paddy.

for mosquito larval sampling, many benthic and fast-swimming invertebrates were not captured in the dipper samples (Table 1). For example, when the fish were sampled from the drained paddy, notonectids seemed, subjectively, to be more abundant in the minnow and control paddies compared with the *Gambusia* paddies. A cylindrical-type sampler for the entire water column and benthic surface would certainly be warranted for future invertebrate samples.

Although the results regarding our hypothesis were equivocal as ultimate fish numbers were confounded with fish species type, the relative control effectiveness of mosquitofish and Sacramento blackfish can be compared by calculating estimated fish stocking requirements. Recent calculations of Sacramento blackfish metabolic food demand (Cech and Linden, unpublished) compare well with previous determinations for mosquitofish for the same temperatures and dissolved oxygen tension (Cech, Massingill, and Wragg 1980). From the estimated mean number of *Culex* larvae stocked per paddy (5,962) on the last two stocking dates, the ratio of small (1st and 2nd instars) vs. large (3rd and 4th instars) larvae stocked (1.20:1), the *Culex*: *Anopheles* ratio sampled on the last three sampling dates (1.86:1), and the mean water temperature on those dates (21.3°C), a total of 232 *Gambusia* or 197 Sacramento blackfish would be needed to devour the estimated resident mosquito larvae in only one day. Assumptions inherent in this calculation include that wild *Culex* numbers in the rice paddies during September samples were insignificant, that these predatory fish eat nothing but mosquito larvae, and that no energy is needed for the fishes' movements, growth, or reproduction. Data from other rice paddies, which were not stocked with mosquitoes, at the UCD Rice Research Facility during these September sampling dates indicate that wild *Culex* larvae were, indeed, rare (Blaustein, pers. comm.). Although mosquitofish prefer *Culex* larvae to other similar-size prey in rice fields (Linden and Cech 1983), their willingness to take other prey invalidates the first assumption and underestimates the number of fish needed for control. Similar laboratory studies on Sacramento blackfish prey preferences are currently underway. On the other hand, activity (Cech, Mitchell and Castleberry, in prep.), growth (Wurtsbaugh and Cech 1983), and reproduction (Cech, Wurtsbaugh, and Vondracek 1981), also require energy in mosquitofish, invalidating the second assumption and overestimating the number of fish needed for control. Also, observations of the *Culex* culture laboratory of the present study by Northrup and Washino (1981) on *Anopheles freeborni* place the mosquito larval residence and development time in the rice field closer to a week than a day. The mean residence time of the sampled *Culex*, i.e., stocking date to sampling date, was 2.3 days also tending to overestimate needed fish numbers. These calculated fish numbers represent about 1/29 the actual *Gambusia* numbers in the *Gambusia* rice paddies and only about 2/5 the actual Sacramento blackfish numbers remaining in their paddies. Thus, the significantly larger number of fish distributed throughout the mosquitofish paddy effected significant larval predation compared with the unstocked control paddies. This result is in accord with Hoy, O'Berg and Kauffman's (1971) conclusion that many fish are needed to control mosquitoes in rice fields. We hope to test if comparable numbers of native juvenile minnows (and fish to larval ratios) can provide significant control in future research projects.

Plans for the 1985 field season include more, but smaller paddies in which larger, separate populations of juvenile minnows and smaller, separate populations of mosquitofish will be maintained. In addition, some paddies will be stocked with mosquitofish plus minnow combinations. We believe that this research program is critical to long-term economical biological control of mosquitoes in California rice fields and will lead to abatement district procedures suitable for use of alternative fishes in rice fields.

ACKNOWLEDGMENTS.-We acknowledge the support of the University of California Mosquito Research Program. We were assisted by the special help of Dr. R. Fontaine, Dr. N. Rishikesh and Mr. L. Blaustein. Invaluable field help was rendered by Margo Erdmann and John Chiu. This manuscript was typed by Donna Raymond. Facilities of the UCD Institute of Ecology, Experimental Ecosystem and Department of Zoology, were essential in the rearing of the mosquito larvae, juvenile hitch, and juvenile Sacramento blackfish, respectively. Susan Palchick identified the invertebrates.

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SUSCEPTIBILITY OF THE CLEAR LAKE GNAT, *CHAOBORUS ASTICTOPUS*, TO THE HYPHOMYCETE FUNGUS *TOLYPOCLADIUM CYLINDROSPORUM*

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ABSTRACT

An aqueous conidiospore suspension of the hyphomycetous fungus *Tolypocladium cylindrosporum* was bioassayed against larvae of the Clear Lake gnat, *Chaoborus astictopus* (Diptera: Chaoboridae). A mortality of 73.9% occurred after 9 days exposure to a 5×10^7 spores/ml concentration. Infection reduced pupation by more than 30% after 3 days. After death hyphal development was extensive, primarily near the crop and the posterior hydrostatic organs. Aerial tufts of hyphae formed on the larvae remaining at the surface after death. These tufts produced conidiospores infective to other *C. astictopus* larvae.

INTRODUCTION.-The Clear Lake gnat, *Chaoborus astictopus* Dyar and Shannon (Diptera: Chaoboridae), is an economically important pest in some areas of California (Cook 1967). At times during the late spring and summer months, adults of this non-biting species swarm so thickly around lights that outdoor activities become impractical. This nuisance can interfere with tourism and decrease land values near recreational lakes.

In the past, response to the problem involved applications of chemicals to decrease larval populations, and *C. astictopus* has developed resistance to some chemical pesticides (Dolphin 1959, Apperson et al. 1978). Currently, biological agents are being investigated as alternatives or supplements to chemical treatment.

A hyphomycete fungus, *Tolypocladium cylindrosporum* Gams, is being investigated as a possible microbial insecticide for use against the gnat. *T. cylindrosporum* is a demonstrated pathogen of some mosquitoes (Pinnock et al. 1973, Soares 1979, Soares et al. 1979), but its efficacy against Chaoboridae has not been studied. The results of a bioassay challenging *C. astictopus* larvae with an aqueous suspension of conidiospores of *T. cylindrosporum* are presented here.

METHODS AND MATERIALS.-The fungus inoculum was prepared from colonies of the California strain of *T. cylindrosporum*, maintained at the Lake County Mosquito Abatement District laboratory on plates of Sabouraud Dextrose Agar medium (B.B.L.®) incubated at approximately 22°C.

Parafilm®-sealed colony plates (2 to 4 weeks old) were opened and the aerial conidiospores flushed into a beaker by repeated rinsings with deionized water. A clean microscope slide was used to facilitate removal of the spores. The resulting slurry was stirred for 5 minutes using a magnetic stir bar. The spores were separated from hyphal clumps by filtering through Whatman® No. 1 filter paper, and cleaned by repeated centrifugation and resuspension in deionized water. Conidiospore concentration in the cleaned spore solution was determined using an improved Neubauer hemocytometer (A.O.® Brightline).

Ten active fourth instar *C. astictopus* larvae (collected by Ekman dredge from Upper Blue Lake, Lake County, California) were added to each of fifteen 200 ml glass petri dishes containing 180 ml of deionized water. No food was provided for the gnat larvae, so only ten were used in each dish to impede cannibalism.

A 20 ml volume of *T. cylindrosporum* conidiospore suspension was added to each treatment dish without stirring. Three repetitions of each of three treatment concentrations (5×10^6, 1×10^7, 5×10^7 conidiospores/ml) were tested. Higher and lower concentrations were not tested, based on the results of preliminary screening. Six control repetitions received 20 ml of untreated deionized water to give a total volume of 200 ml in all treated and control dishes. Water temperature was maintained at 17 ± 1°C during the bioassay.

Larval mortality and pupation was assessed at 48 hr intervals, beginning 24 hrs after initial exposure and continuing for 9 days. Larvae were considered dead if incapable of movement or if repeated probings with a collecting pipette elicited only sight twitching motions. Pupae were considered as survivors. All pupae and dead larvae were removed at each counting. Dead larvae were examined at 40x phase contrast and 200x brightfield for signs of mycosis. Hyphae and spores from gnat cadavers were transferred to Sabouraud Dextrose Agar. Colonies that formed were used as sources to infect more *C. astictopus* larvae to test the recycling ability of the fungus.

Cumulative percent mortalities for all treatments and controls were compared for each day using a one-way ANOVA. Where a significant F-value occurred differences between individual means were examined using the Student-Newman-Keuls multiple range test (Zar 1974) at $p < 0.05$. Cumulative percent pupation was evaluated identically.

RESULTS AND DISCUSSION.-The cumulative percent mortality over time for each group is shown in Table 1. No significant differences were observed between the treatments and controls until the third day after exposure. Beginning on Day 3, mortality at the highest concentration (5×10^7) of *T. cylindrosporum* was significantly greater than that for larvae in both the control and low concentration (5×10^6) groups. On Day 5 and thereafter, both 10^7 treatment mortalities were significantly greater than control, with mortality in the 5×10^7 group reaching 73.9% by Day 9. Mortality in the control group reached 18.3%, in part due to cannibalism, which was observed among the larvae. This behavior may also account for a part of the mortality observed in the low (5×10^6) concentration group, which was never significantly different than control.

Figure 1 graphically depicts the cumulative percent pupation for all the groups for each day of the experiment. Maximum pupation reached 50%, occurring in the control larvae after 9 days. Pupation in the 5×10^6 treatment was slightly, but not significantly, less at 46.7%. Minimum pupation on Day 9 was 10.0%, occurring in the 1×10^7 treatment, with pupation in the 5×10^7 treatment not significantly greater at 16.1%. A comparison of mortality and pupation during the bioassay reveals a potentially important consequence of fungus infection; although there was only a 9.4% difference in mortality between the controls and the 5×10^7 group on Day 3 (Table 1), the difference in pupation was greater than 30% (Figure 1). Many infected larvae were unable to pupate. This suggests that *T. cylindrosporum* would be an effective treatment in pupating populations, offsetting the lengthy time required to achieve 50% mortality (>7 days; see Table 1).

Periodic examination of dead treated larvae confirmed the presence of mycoses. In nearly all larvae exposed to the two highest concentrations (1×10^7 and 5×10^7), fungal hyphae were observable. There was at least one confirmed mycosis among larvae from the lowest treatment concentration (5×10^6), but none from the controls. The appearance of larvae killed by *T. cylindrosporum* was striking. Masses of fungal hyphae could be seen throughout the translucent bodies of the larvae. Hyphal growth was especially abundant in two body regions: the thorax near the crop and the anterior hydrostatic organs, and adjacent to the posterior hydrostatic organs.

After death hyphae ramified throughout the bodies of the larvae, eventually growing outwards through the cuticle. Dead fungus-infected *C. astictopus* larvae that remained floating at the water's surface developed aerial tufts of coni-

Table 1.-Mortality (%) of 4th instar *C. astictopus* exposed to an aqueous suspension of *T. cylindrosporum* conidiospores[1].

Days after exposure	Concentration (conidiospores/ml) 0.0	5×10^6	1×10^7	5×10^7
1	0.0^{a}	0.0^{a}	3.3^{a}	0.0^{a}
3	0.0^{a}	0.0^{a}	3.3^{a}	9.4^{b}
5	5.0^{a}	3.3^{ab}	16.7^{bc}	18.9^{c}
7	11.7^{a}	10.0^{ab}	26.7^{bc}	47.2^{c}
9	18.3^{a}	20.0^{a}	60.0^{b}	73.9^{b}

[1]Means in the same row not followed by the same letter are significantly different ($p < 0.05$, Student-Newman-Keuls tests).

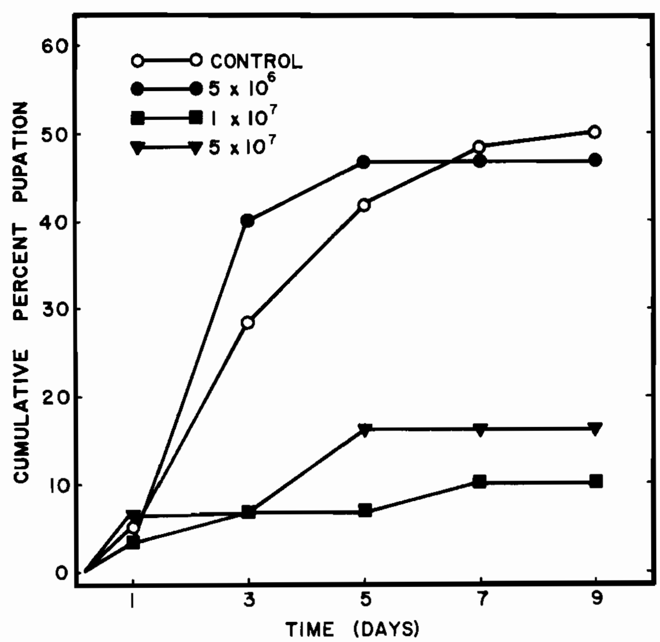

Figure 1.-Cumulative percent pupation of 4th instar *C. astictopus*; untreated controls and treated larvae exposed to three concentrations (conidiospores/ml) of *T. cylindrosporum*.

diospore-forming hyphae. When transferred by sterile inoculating needle to Sabouraud Agar medium, spores from these tufts formed normal *T. cylindrosporum* colonies. In addition, exposure to these spores infected and killed other *C. astictopus* larvae, with a mycosis identical to that previously recorded. This potential for recycling is encouraging, because the fungus would be a better control agent if a single dosage resulted in recycling and amplification of the disease in the pest species.

ACKNOWLEDGMENTS.-We thank N. L. Anderson, L. W. Davidson, and J. Haberthur for their help and support in this study.

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ABUNDANCE AND DISTRIBUTION OF IMMATURE *CULEX TARSALIS* AND *ANOPHELES FREEBORNI* IN RICE FIELDS OF THE SUTTER-YUBA M.A.D.: I. INITIAL SAMPLING TO DETECT MAJOR MOSQUITO PRODUCING RICE FIELDS, AUGMENTED BY ADULT LIGHT TRAPPING

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INTRODUCTION.-During the 1984 growing season, approximately 143,000 acres of rice were grown within the boundaries of the Sutter-Yuba Mosquito Abatement District. *Culex tarsalis* and *Anopheles freeborni* utilize the rice field habitat for breeding purposes and were the 2 mosquito species of concern in this study. Both of these mosquito species are responsible for the transmission of diseases of public health importance and can occur in numbers large enough to warrant extensive monitoring and control efforts. An efficient method is needed to determine where, when and how effective control methods for rice field mosquitoes should be used. To accomplish this, several studies have been conducted to determine densities and spatial distributions of *Cx. tarsalis* and *An. freeborni* larvae in rice. Many of these studies have shown that larval densities can differ dramatically from 1 field to the next, with relatively few fields being responsible for the majority of mosquito production (Miura et al. 1983 and Washino and Westerdahl 1981).

The primary objective of this study was to determine which rice fields, if any, in the study areas produced unusually high numbers of mosquito larvae as compared to numbers of larvae found overall. In addition, trapping for adult *Cx. tarsalis* and *An. freeborni* in the larval sampling areas was conducted to aid in the determination of species abundance and distribution. Other studies have been conducted which compare larval data to light trap data. Collett et al. (1964) showed a positive relationship between adult and larval collections and felt that conclusions reached concerning mosquito populations were more reliable if both sampling methods were used.

It is hoped that data from this study will help establish guidelines, such as threshold levels, for *Cx. tarsalis* and *An. freeborni* larvae in rice fields of concern to the Sutter-Yuba M.A.D. This report presents preliminary aspects of an on-going study, as it will be continued to further test the hypothesis that certain rice fields always produce more mosquitoes than others.

MATERIALS AND METHODS.-Two study areas were chosen in rice growing areas of the Sutter-Yuba M.A.D., one in Sutter County and the other in Yuba County. Rice fields ranged in size from 15 to 448 acres. Collection of data concerning the fields, such as the direction of irrigation water flow, contoured or laser leveled fields, variety of rice planted, agricultural chemicals applied, planting, flooding and drainage dates, number of years in rice and other environmental and cultural factors was initiated by this study. The Yuba County study area, located approximately ½ mile NE of Marysville, CA, contained 3283 acres of rice and 208 larval sampling sites, or 6 sites for every 100 acres of rice. The Sutter County study area, located approximately 5 miles west of Yuba City, contained 3937 acres of rice and 298 larval sampling sites, or 8 sites for every 100 acres of rice. Adult sampling was conducted in both counties in areas chosen randomly from within the 2 larval study areas. Each adult sampling area consisted of approximately 900 acres of rice.

Larval sampling sites were located along edges of fields, usually accessible by roads. The sampling procedure followed at each site is shown in Figure 1. This method made it easy to tell which direction to walk in the field and how far to go. Standard white plastic dippers (473 ml) were used in sampling. Three dipper samples were taken at each of 8 stations, giving a total of 24 dips/site. At the first and eighth stations, 3 dips were taken along the edge of the field. At stations away from the edge, 1 dip was made on each side and in front of the individual sampling. The genus and stage of each immature present/dipper sample was recorded and the sample returned. At each site, water temperature, water depth and visual clarity was recorded, along with the height of the rice and the type of stand (open, sparse, average, dense). A record of the time of day was made before and after each site was sampled. The 2 study areas were sampled alternately (Table 1), which allowed 3 sampling periods per study area. This resulted in many sites with few replicates.

Data were analysed to obtain mean values of mosquito larvae/dip/field and associated variances. Sample periods were compared to detect significant changes in larval densities, and numbers and ratios of fields producing ≥ .08 larvae/dip were calculated. The threshold level of .08 larvae/dip was chosen as it represented finding at least 2 larvae/sample site, and was slightly more discriminating than the previously reported level of .1 larvae/dip (Stewart et al. 1983).

Adult mosquitoes were sampled weekly from 6/21 to 10/10, 1984, using CDC light traps baited with dry ice. Each trap was suspended from a pole which was secured in the ground along the edge of a rice field. Trap sites in both study

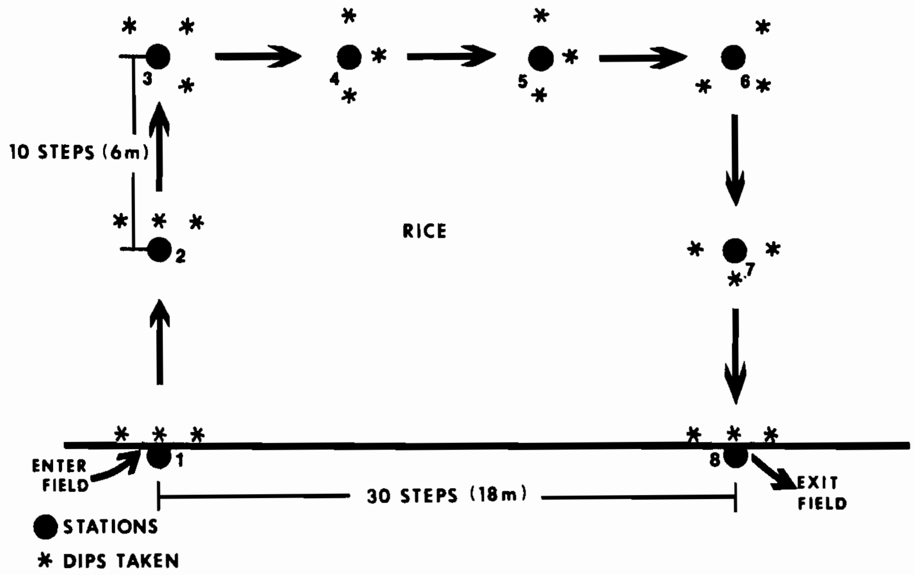

Figure 1.-Sample pattern used for *Cx. tarsalis* and *An. freeborni* in rice, summer, 1985, Sutter-Yuba M.A.D.

Table 1.-Sampling schedule, ricefield larval survey, 1984.
* No. of days sampling took place.

	Sampling Dates		
Sample Period	**1**	**2**	**3**
SUTTER CO.	7/2 - 7/13 (8)*	7/24 - 8/3 (9)	8/13 - 8/24 (8)
YUBA CO.	6/19 - 6/28 (7)	7/16 - 7/23 (5)	8/6 - 8/10 (5)

areas were located along roads running north and south through rice fields. All traps were set out in the late afternoon and picked up the following morning, running 1 night per week for a total of 14 weeks in Sutter County, and 16 weeks in Yuba County. Collections were taken to the laboratory where they were counted and identified. Only female mosquitoes were tabulated. Data were analysed to determine differences in numbers of adult *Cx. tarsalis* and *An. freeborni*. In addition, adult light trapping data were compared to larval sampling data in order to detect significant relationships, if any.

RESULTS AND DISCUSSION.-In the Yuba County study area, it was found that a moderate number of fields harbored the majority of *Cx. tarsalis* larvae sampled. For *An. freeborni*, approximately half of the fields sampled were responsible for the majority of larvae found. The highest numbers of *Cx. tarsalis* larvae occurred during the first sampling period (6/19 - 6/28). Seven out of the 22 fields sampled during that time were responsible for 89% of the larvae found, with 21% of the flooded acreage in the study area having larvae occurring in numbers ≥ .08/dip. For *An. freeborni*, the greatest numbers of larvae were found during the last sampling period (8/6 - 8/10). Twelve out of the 22 fields sampled were responsible for 90% of the larvae found, with 64% of the flooded acreage in the study area having larvae ≥ .08/dip. For *Cx. tarsalis*, during the second and third sampling periods, 99% and 100%, respectively, of the acreage sampled had 0 or < .08 larvae/dip. For *An. freeborni*, during the first and second sampling periods, 98% and 73%, respectively, of the acreage sampled had 0 or < .08 larvae/dip.

In general, the same trend occurred in the Sutter County study area (Table 2). For *Cx. tarsalis*, highest numbers of larvae were found during the first sampling period (7/2 - 7/13). Eleven fields out of the 47 sampled were responsi-

Table 2.-Comparison of sample averages obtained from each study area for both *Cx. tarsalis* and *An. freeborni* larvae, showing similarities in results between the 2 areas.

Percentages Calculated From Sample Means	Yuba County Study Area		Sutter County Study Area	
	Cx. tarsalis	*An. freeborni*	*Cx. tarsalis*	*An. freeborni*
Percentage of fields sampled/ study area having ≥ .08/dip	32%	54%	23%	55%
Percentage of larvae found/ study area located in fields sampled having ≥ .08/dip	89%	90%	77%	92%
Percentage of flooded acreage sampled/study area having ≥ .08/dip	21%	64%	25%	63%

ble for 77% of the larvae found, with 25% of the flooded acreage having larvae occurring in numbers ≥ .08/ dip during that time period. For *An. freeborni*, the greatest numbers of larvae were found in the third sampling period (8/13 - 8/24). Twenty-six fields out of the 47 fields sampled during that time were responsible for 92% of the larvae found, with 63% of the flooded acreage in the study area having ≥ .08/dip. For *Cx. tarsalis*, the second and third sampling periods (7/24 - 8/3 and 8/13 - 8/24) had 85% and 92%, respectively, of the acreage sampled with 0 or < .08 larvae/dip. For *An. freeborni*, the less active sampling periods (7/2 - 7/13 and 7/24-8/3) showed that 68% and 59% of the acreage sampled had 0 or < .08 larvae/dip, respectively.

These data indicate a difference between larval sampling periods for each mosquito species in both study areas. A one-way ANOVA performed on the average number of mosquito larvae/field/sample period showed that all 3 sample periods differed significantly for *An. freeborni* and *Cx. tarsalis*, $\alpha = .05$. These differences are to be expected, as numbers of larvae will fluctuate over the summer. In addition, one-tailed t tests were performed on the same means, comparing one with the other. For *Cx. tarsalis* in both study areas, the sample means for the first sample periods were significantly higher than those for the second and third periods. For *An. freeborni* larvae sampled in both study areas, means for the third sample periods were significantly higher than those for the first and second periods. As a result, not only should the time of season be taken into consideration when sampling, but differences between the seasonal occurrence of different species should be realized.

These data indicate opposite population trends for the 2 species over the summer, with the highest number of *Cx. tarsalis* occurring in June and the highest number of *An. freeborni* occurring in August. In general, the same trend was observed in both study areas, as far as the larvae were concerned. This was not the case however, with adult mosquitoes sampled.

In both Sutter and Yuba counties, numbers of adult mosquitoes trapped at each site were compared using Spearman's rank correlation coefficient, in order to detect any possible patterns of distribution. In Yuba County, the resulting r_s values indicated a somewhat homogeneous distribution of adult *Cx. tarsalis* and *An. freeborni*. However, in the Sutter County study area, no specific pattern of occurrence for either species was found.

Numbers of adult *Cx. tarsalis* in Yuba County reached a maximum in early July with > 1700/trap night. Numbers of *An. freeborni* were highest in late July with 950/trap night. The Sutter County study area showed a similar pattern, with *Cx. tarsalis* being highest in mid-July with 375/trap night, and *An. freeborni* in mid-August with 80/trap night. More *Cx. tarsalis* were trapped in the 2 study areas than *An. freeborni*. This may be due to the possibility that *An. freeborni* are not attracted to CDC light traps as well as other mosquito species (Milby et al. 1978 and Endicott and Washino 1982). After their respective peaks, both species decreased in number in a similar manner throughout the remainder of the summer.

In comparing adult light trapping data to larval sampling data, opposite results were found. While more adult *Cx. tarsalis* than adult *An. freeborni* were trapped, more *An. freeborni* larvae than *Cx. tarsalis* larvae were sampled in the 2 study areas. In addition, when comparing numbers of larvae found in the Yuba County study area to numbers found in the Sutter County study area, no significant difference was found for either species. However, many more adults were trapped in the Yuba County study area than in the Sutter County study area, both species combined. These findings may again be partially due to the possibility that adult *An. freeborni* are not attracted to CDC light traps as well as other mosquito species. Results obtained from adult light trapping indicated that other methods would be necessary to successfully associate larval and adult activity in the study areas.

CONCLUSIONS.-Because of the similarity of findings in both study areas, the following observations were made concerning the distribution of *Cx. tarsalis* and *An. freeborni* larvae among rice fields within the boundaries of the Sutter-Yuba M.A.D. At the beginning of the growing season, immature *Cx. tarsalis* were the most abundant of the 2 mosquito species. By the end of the season, or by mid-August, numbers of *Cx. tarsalis* had dropped significantly. For *An. freeborni*, numbers immatures found were significantly lower at the beginning of the growing season and were the most abundant of the 2 species by mid-August. Results obtained involving the collection of adult *Cx. tarsalis* and *An. freeborni* indicated that other methods would have to be used in future studies to associate larval and adult activity and distribution.

Larval sampling data indicated that a minority of the rice fields sampled had larvae of either species occurring in numbers $\geq .08$/dip. This study will be continued to order to determine if this situation occurs in the same fields each season. Knowledge of this type would greatly enhance the operational aspects of any rice field mosquito control program. In addition, more information will be obtained in order to establish other guidelines, such as threshold levels for *Cx. tarsalis* and *An. freeborni* larvae in rice fields of concern to the Sutter-Yuba M.A.D.

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IMPROVED *ROMANOMERMIS CULICIVORAX* PREPARASITE PRODUCTION

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INTRODUCTION.-The mermithid nematode *Romanomermis culicivorax* has been extensively studied as a biological control agent of larval mosquitoes over the past decade. More recently, in a number of field experiments, positive results have prompted mosquito abatement districts to focus on *R. culicivorax* as a method of controlling mosquitoes (Kerwin and Washino 1983, Kimball and Kauffman 1984). To date, a few mosquito abatement districts have successfully established rearing facilities and have incorporated nematodes with existing control methods.

Operationally, nematodes can be applied as preparasites or post parasites. Post parasites appear to be the more practical method, since applications can begin in early spring, before the onset of the mosquito season. In this stage, the nematodes will enter the soil, mate, lay eggs, and depending on ambient temperatures, eggs will mature in 3-12 weeks, at which time the eggs will begin to hatch. Later into the mosquito season, host seeking preparasites may be applied to sources where mosquito larvae have become established. During the winter months, nematodes are lab reared to replenish the supply of cultures for the following season. Some of these cultures may spend six to seven months in storage before they are needed for field use.

In previous studies, the following has been determined concerning culture storage:

1. Premature hatching of nematode eggs can occur from excess moisture in a culture (Cupello et al. 1982);

2. To the other extreme, if not carefully monitored, dessication of a culture can cause a partial or total loss of nematodes in a culture (personal experience);

3. Prolonged storage of a culture results in low yields of preparasites (Petersen 1978).

These factors pose a problem for mosquito abatement districts attempting to store numerous cultures during the winter months. Cultures must be checked periodically, and misted when they appear dry. Usually, by the time a culture appears dry, damage has already begun, and direct misting involves the risk of premature egg hatching and consequently the loss of preparasites. These steps are labor intensive and increase rearing costs dramatically. Idealistically, when a culture is placed in storage, it should require no further maintenance until time for flooding.

This study focuses on the egg stage of the nematode life cycle, and was designed to improve preparasite yields and increase culture longevity by ensuring optimum storage conditions. To achieve this, a few minor modifications in standard rearing techniques were made.

MATERIALS AND METHODS.-New cultures were started by filling a 32.5 x 21.5 x 6.5-cm plastic Tupperware container with medium coarse (216) sand to a depth of 2.5 cm. Dechlorinated tap water was added to the same level, or just below, so there was no free standing water above sand level. Premeasured amounts of post parasites were placed on top of the sand. After the post parasites had entered the sand, excess water was removed. A precut piece of plywood with a mounted handle was used to drain the cultures. The board was cut to fit snug inside of the container, resting just above the sand. Once the board was in place the culture was tilted to a 90° angle and drained until only a few drops of water remained. The board was then carefully removed, a piece of paper toweling placed on top of the culture, and the container was sealed. Cultures were stored in a room maintained at 25.5° - 27° C.

RESULTS AND DISCUSSION.-The first modification made was the type of container used. By using an air tight container, moisture was retained in all cultures throughout the test period, with no maintenance required. By using the board for draining, all free standing water was removed, eliminating one factor of premature egg hatching. The purpose of the paper toweling was to absorb any droplets of water that might fall onto the sand from condensation formed on the lid of the container, also a factor in premature egg hatching. Mold occurred on the paper towels in all cultures tested, but with no apparent adverse effects. Lowering the water to sand level when starting a culture seemed to expedite the post parasite's entry into the sand. Within 3-4 days, virtually all post parasites had completed entry and the culture was drained and stored, compared to standard techniques of draining after three weeks (Petersen 1972).

Table 1 indicates the number of preparasites obtained per culture in relation to culture age. Preparasites were obtained from all cultures tested. Petersen suggests that cultures aged 11-14 weeks produce the highest yields of preparasites (Petersen 1978). In this study, the average preparasite yield was 1460×10^3 (84×10^3 - 3600×10^3). In cultures aged 13-23 weeks, 62% produced over 1000×10^3 preparasites. Overall, 57% of the 21 cultures produced over 1000×10^3 preparasites, 33% over 2000×10^3, and 19% over 3000×10^3. The lowest single yield (84×10^3) occurred in a culture that was 11 weeks old. The maximum yield (3600×10^3) was produced by three separate cultures. Their ages were 15, 18

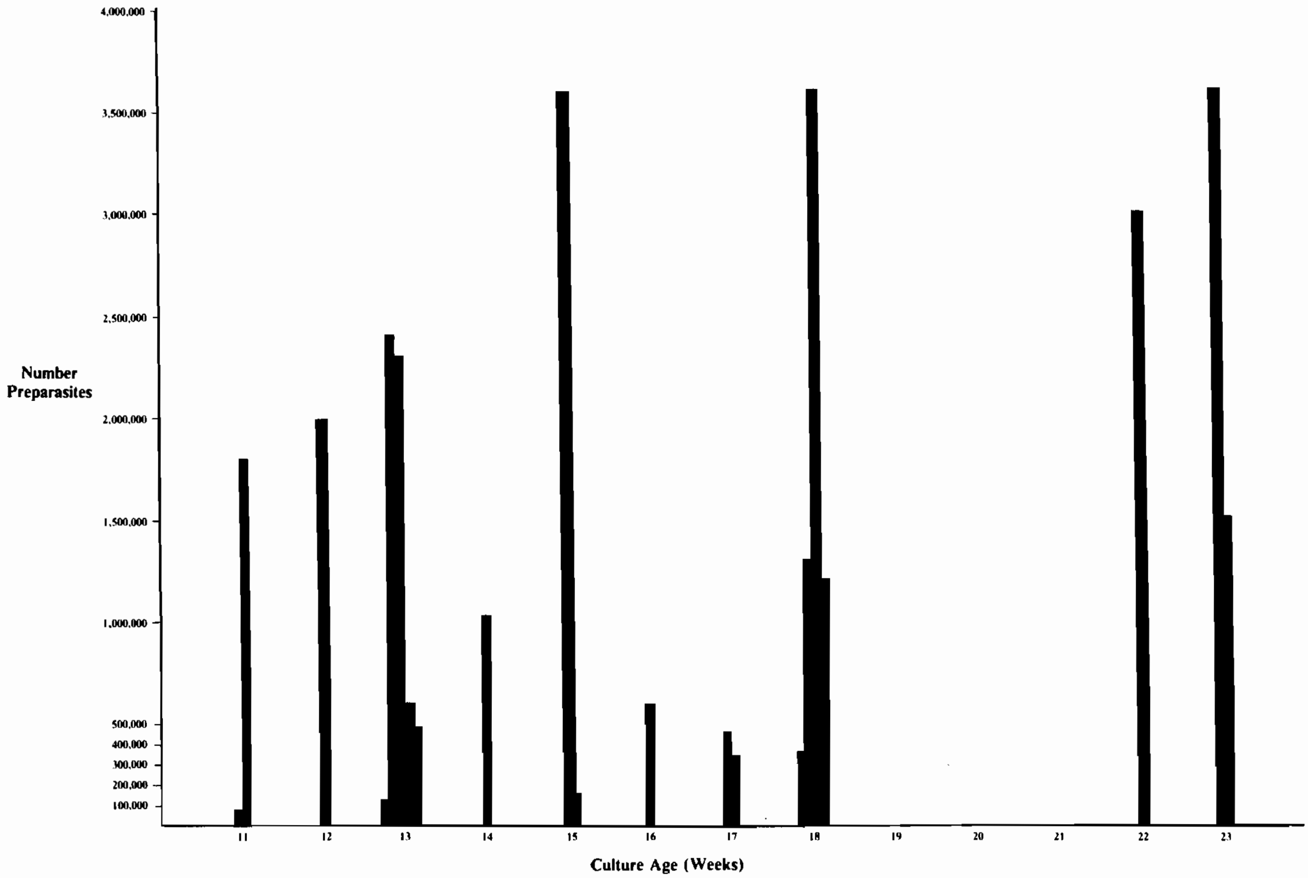

Figure 1.-Number of Preparasites in Relation to Culture's Age.

and 23 weeks. Nematode eggs were observed in all cultures yielding low numbers (below 500 x 10^3) of preparasites. Although their numbers were not recorded, they were often greater than the number of preparasites counted. This is an indication that egg-laying was not completed and/or eggs had not reached maturity. There were no dead preparasites observed in any of the cases.

CONCLUSIONS.-This study has helped us to better understand the storage requirements of *Romanomermis culicivorax*. It has shown that by using proper water management, a substantial number of preparasites can be obtained from cultures stored over a long period of time. Hopefully, this will be an aide to mosquito abatement districts rearing this nematode for field operations.

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INFLUENCE OF FLOW RATE ON *GAMBUSIA AFFINIS* GROWTH RATE AND WATER QUALITY

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ABSTRACT

Results of both trials indicated there was no significant difference within stocking rates, in survival, growth rates, ammonia or nitrate levels. This may have been a result of low temperatures during the studies, not enough difference between flow rates or a combination thereof. Water quality results were of significance and can serve as a basis for determining lethal and sublethal concentrations for *Gambusia*. These fish had high rates of survival at NH_3 and NO_3 levels seen to be toxic to other fish.

INTRODUCTION.-*Gambusia affinis* has long been recognized as a significant predator of mosquitoes (Krumholz, 1948) and is still the most widely used larvivorous fish in mosquito control (Hass & Pal, 1984). In California it is utilized in both urban and rural mosquito abatement districts, however, early spring availability is inconsistent due to limited natural supply. Outdoor pond cultures have had inconsistent yields and natural watershed areas such as duck clubs are subject to environmental stress such as pollution and bird predation. Thus, current limitations in its application as a biological control agent can often be attributed to limited availability. As a consequence, research efforts have been made to provide a more reliable supply of these fish when needed.

Gambusia is suitable for aquaculture for the following reasons. It is small in size, has a short generation time with multiple broods, a short interbrood interval and a wide temperature tolerance with optimal growth at 25-30°C. The greatest limitations for intensive culture are a low fecundity (10-400 per brood) and internal fertilization requirements (Krumholz, 1948; Stearns, 1977). To date, several attempts have been made to design an aquaculture system that can satisfactorily produce *Gambusia* in large numbers (Coykendall & Kauffman, 1984; Downs & Beesley, 1983; Gall, 1983; Reynolds, 1977). Although none have been entirely successful, each attempt has shed new light on factors which are most limiting on *Gambusia* production.

Because of the variety of systems previously studied, both outdoor (Reynolds, 1977) and indoor raceways (Drazba & Gall, 1980) there are no established design criteria for intensive culture of *Gambusia*. Spotte (1970) outlined the factors which influence the carrying capacity of a closed aquarium system in which he correlated the carrying capacity with filter particle size, rate of filtration and filter volume. Consequently, the following experiments were conducted to test the effects of flow rate through an inboard filter bed on growth and survival of *Gambusia*. This information would then be applied to the design of a permanent system for intensive culture of *Gambusia*.

MATERIALS AND METHODS.-The fish culture system used is the same described by Hoy et al. (1984), comprised of 12 rearing tanks located inside a fiberglass greenhouse which utilized passive solar heating supplemented with a 100,000 BTU propane forced air heater. The rearing tanks were constructed with 12.5mm plywood, 2.44 x 2.44 x 0.41m deep. Each tank was lined with a 20ml vinyl sheet and rested on the asphalt pavement. Tanks were filled with water 15.25 cm above the top of the filter for a total volume of 1180 liters. Water loss from evaporation and/or leaks was replaced weekly, and averaged 17% of total volume.

The biological filter in each tank consisted of a 76mm layer of crushed rock (6 x 12mm) covered by a 76mm layer of Lapis Luster #3 aquarium sand (Fig. 1). The two layers were separated with a layer of muslin to prevent the sand from clogging up the bottom intake pipes.

Water was recirculated within each tank by eight vertical air-lifts on each side of the tanks, connected to eight return pipes (37mm) on the bottom of the tanks. Pressurized air was delivered to all of the tanks from a high volume, low pressure 1.5 h.p. electric blower capable of delivering ca. 900 CFM at a head of 25cm of water. Air flow was controlled by restricting air flow into supply manifolds and/or by changing the submergence of the air lines. Air lifts were 76mm in diameter with the outfall 18cm from the top of the filter. Each air lift pipe had a 12.5mm airline placed inside it where forced air came in the tanks from the blower. Water percolated through the biofilters into the return pipes and in turn flowed back to the air lifts (Fig. 1).

Flow rates were measured by collecting the volume of water delivered from the 16 air-lift pipes per tank in a 5 second interval. This was measured weekly and submergence of air-lines altered to maintain flow rates of either 1.3 and 2.0 L/sec/m3. These two flow rates were the maximal range achieved by the system when all twelve tanks were supplied with air.

Water quality tests were conducted weekly using a Hach Drel/4 water quality kit. Samples

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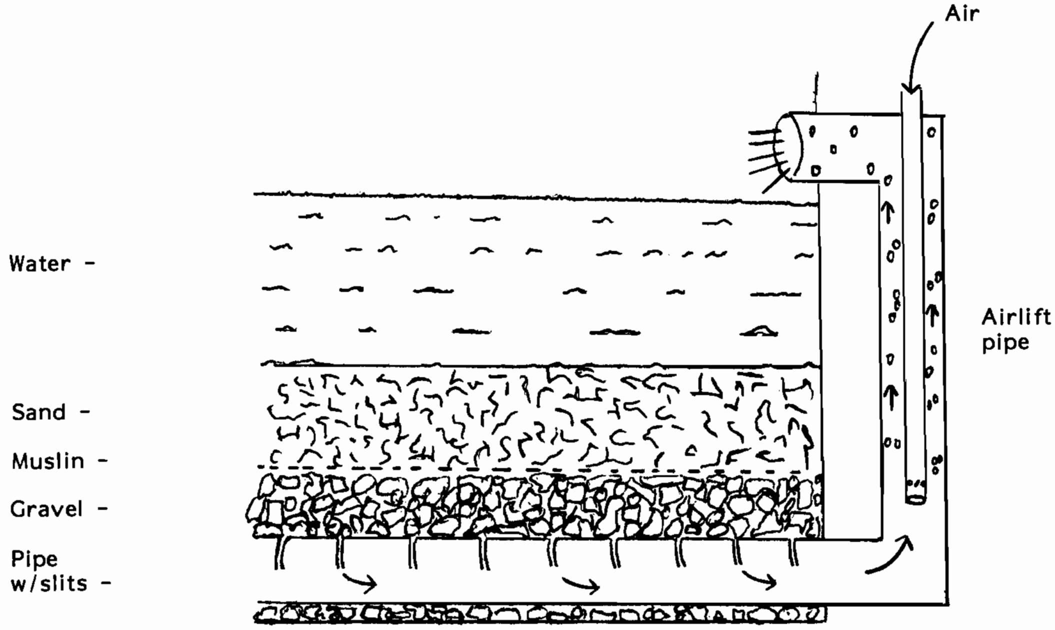

Figure 1.-Cross section of a culture tank showing the air-life design.
* arrows show direction of water flow

were analyzed for NH_3, NO_2, NO_3 and D.O. Measurements of pH were taken on an irregular basis. Temperature was recorded continuously with a remote reading thermograph (Taylor Instruments).

Gambusia were fed four times daily at 5% of tank biomass with a 50:50 mixed ration of "Basic Flake" and Sutter-Yuba fish meal (Coykendall, 1977). Trials were conducted in the fall and winter of 1984 under conditions of diminishing photoperiod. Supplemental lighting from fluorescent lights on an automatic timer maintained a 14:10 L.D. cycle. Mortality was recorded daily.

All fish for the experiments were obtained from a local stock pond. The tanks in the first trial were each stocked with 908 grams (tare weight) of *Gambusia* while 2724 grams per tank were stocked in the second trial. In both trials, total weights of the fish were weighed at the start and finish. Subsamples of 100 female *Gambusia*/tank were also weighed at the beginning and end of each trial, in which large females (>2 gm.) were excluded from the subsample. These weights were taken to better assess actual growth of fish for it is known that mature males and large females are no longer in a growth phase (Krumholz, 1948). Additionally, this would exclude mortality from influencing growth results.

Twenty one day growth tests were conducted, with the first trial at the low stocking rate and the second trial at the high stocking rate. In both trials, a total of ten tanks were utilized, allowing for five replicates at each flow rate, the remaining two tanks were used for holding additional fish.

Average daily growth as discussed was calculated using wet weights, rather than dry weights, in the formula: G = 100 (logW2-logW1)/t where W = initial (W1) and final (W2) weights of fish, t = number of days (Wurtsbaugh & Cech, 1983).

RESULTS AND DISCUSSION.-At both stocking rates, there were no significant differences in average daily growth rates of mosquitofish between the two water flow rates (Table 1). At the low stocking rate, fish grew at 0.5 to 0.6% of body weight per day at a mean temperature at 20.5°C. Fish survival in this experiment was >98%. At the high stocking rate, negative growth (declines in total fish biomass per tank) of -0.3 to -0.4% of body weight per day was measured at a mean temperature of 16.5°C. Fish survival in this second experiment was 99%. Comparison of female subsamples showed growth rates to be higher. At the low stocking rate average daily growth was 2.0 and 1.4% at the low and high flow rates respectively. At the higher stocking rate average daily growth rates were seen to be 1.0 and 0.4% at these respective flow rates. Again, there was no significant difference within trials.

It has been previously reported that increasing the flow rate through a filter bed increased the rate of ammonia removal (Haug & McCarty, 1972; Forster, 1974). This was not evident in either trial (Table 2). The two stocking rates were calculated to be 50 and 132% of the carrying capacity of the filters. It was also thought there would be a correlation between growth rates, water quality and flow rates but this was not the case. Consequently increasing the flow rate

Table 1.-Effect of flow rate thru a filter bed on weight of fish (gm.) - totals.

Trial	Flow rate (L/sec/m³)	Initial weight	Final weight ($\bar{X}$±SD)	Growth (%/day) (±SD)	Survival %	Biomass (gm/L)	Average temp (°C) (±SD)
1	1.3	908	1007±128	0.5*±.56	98	.82	20.5±5.5
	2.0	908	1033±80	0.6*±.37	99	.82	20.5±5.5
2	1.3	2724	2497±454	-0.4*±.97	99	2.31	16.5±4.5
	2.0	2724	2551±236	-0.3*±.47	99	2.31	16.5±4.5

* Growth rates not significantly different ANOVA ($p<.01$)

Table 2.-Effect of flow rate thru a filter bed on NH_3, NO_2 and NO_3 levels (mg/L).

Flow rate (L/sec/m³)	Weight of fish (gm.)	NH_3 avg.	NH_3 range	NO_2 avg.	NO_2 range	NO_3 avg.	NO_3 range	Survival %
1.3	908	.27*	.10-.48	.039	.023-.063	27*	0-77	98
2.0	908	.23*	.13-.45	.081	.033-.165	38*	0-88	99
1.3	2724	.83*	.27-2.81	.284	.040-.990	113*	31-255	99
2.0	2724	.78*	.32-1.65	.156	.030-.380	154*	53-334	99

* NH_3 and NO_3 avgs. not significantly different ANOVA ($p<.01$)

Table 3.-Effect of flow rate thru a filter bed on weight of fish (gm.) subsample results.

Trial	Flow rate (L/sec/m³)	Initial mean weight (±SD)	End mean weight (±SD)	Growth (%/day)
1	1.3	.44±.06	.67±.04	2.07*
	2.0	.51±.11	.68±.09	1.44*
2	1.3	.51±.07	.63±.09	1.01*
	2.0	.57±.38	.61±.06	0.39*

* Growth rates not significantly different ANOVA ($p<.01$)

should have improved water quality, if not growth, particularly at the higher stocking rate. The only trend evident was an increased level of metabolites at the higher stocking rate, but the lower temperatures experienced during this time nullify any conclusions.

Analysis of water quality revealed levels of ammonia at the low stocking rate were in the same range reported to have inhibited or retarded growth in trout sac fry and juvenile channel catfish (Colt & Tchobanoglous, 1978). Ammonia levels at the high stocking rate trial were in the range considered toxic to some fish (Colt & Tchobanoglous, 1976). Similar comparison of nitrite at the high stocking rate revealed levels known to be toxic to rainbow trout (Russo & Thurston, 1977). From this, we concluded that levels of ammonia of 0.7-.8 mg/1 and nitrites of 0.15 mg/1 could result in no growth of *Gambusia*. More testing needs to be done to confirm this.

CONCLUSIONS.-The effects of flow rates on growth of *Gambusia* were clouded by the difficulties in maintaining optimal temperatures (25°C). Consequently the initial goals were not met. Temperatures during the first trial were adequate for growth of fish but there was no significant difference in growth at the two flow rates. Growth was essentially halted in the second trial, a result of the low temperatures and high metabolic waste levels. The higher rate of water flow was not sufficient enough in either trial to speed up ammonia conversion in the biofilters, nor was nitrate oxidation improved by increased flow rates.

Gambusia survival was very high throughout the trials, indicating above normal tolerance to toxic levels of NH_3 and NO_2. Although we were unable to further elucidate the relationships between carrying capacity and rate of filtration, the high rate of survival indicated good potential for intensive culture. This is of great value. Whether fish are being held for growth, fry production or overwintering, it is critical to be able to hold large numbers because of their limited fecundity and short life span. From an operational viewpoint this is an asset, for less time is devoted to maintaining water quality although monitoring is essential. With this in mind, we will design an intensive culture system to hold large numbers of *Gambusia* for growth, fry production and overwintering.

ACKNOWLEDGMENTS.-This study is the result of prior research by Dr. James Hoy on Methodology for Operational-scale Mosquitofish Production and has been funded by the University of California Mosquito Research Funds. Cooperative support has been provided by Alameda and Sutter-Yuba Mosquito Abatement Districts.

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INTENSIVE AQUACULTURAL PRODUCTION OF MOSQUITOFISH IN A LARGE SOLAR GREENHOUSE - A PROGRESS REPORT

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INTRODUCTION.-At last year's Annual Conference I reported some promising preliminary results that were achieved by incorporating a small solar greenhouse for the overwintering and enchanced reproduction of mosquitofish, *Gambusia affinis* (Baird & Girard). Consequently, we believed a larger-scale pilot production facility was justifiable, so a standard commercial greenhouse was purchased and erected at our District headquarters. With the exception of electrical wiring, all labor was performed by our own personnel. By the end of July we were able to introduce gravid female mosquitofish for an initial shakedown test of the fry production system we designed and installed in the greenhouse. Following are descriptions of the basic construction, all modifications incorporated to convert this structure into a passive solar configuration and our mosquitofish culture system. This building was oriented on an east-west alignment and sited approximately one meter (3.3 feet) below the surrounding ground elevation to help shelter it from winter winds.

BASIC CONSTRUCTION.-Most newer commercial greenhouses are manufactured quite similarly in that they have metal framing and plastic exterior coverings. Framing for our unit consisted of galvanized steel pipe and formed sheet metal. Fiberglass-impregnated plastic glazing covered the exterior walls of the greenhouse; however we selected an inflated 'bubble'-film roof instead of using the same corrugated plastic also for the roof. Optional features included a thermostatically-controlled roof vent and an air cooling and recirculation fan jet. Supplied to this greenhouse were raw well water, softened well water, compressed air, natural gas and electricity. When completed the structure measured 25.6m x 6.4m x 2.4m eave height (84' x 21' x 8').

PASSIVE SOLAR ENHANCEMENTS.-Energy costs for heating and cooling have risen dramatically in the last decade; therefore it was absolutely necessary to maximize our use of solar energy and energy conservation measures to enable our intensive production system to be as cost effective as possible. Techniques commonly employed in passive solar greenhouses were incorporated into this building after we completed its basic construction.

First, the south walls and both ends of the building received additional full glazing on the inside of the greenhouse walls to prolong solar energy retention. Second, for further heat retention the north wall and roof vent were insulated with foil-faced polyisocyanurate foam core sheathing, which provided additional R-11 and R-8 insulative values, respectively. Third, sixty-six 208 liter (55-gallon) water-filled, plastic drums were stacked along the north wall to capture solar energy for release at night and on cool days. Fourth, additional foam core sheathing having insulating values up to R-17 was placed over all sandy floor areas below walkways and tanks to minimize heat losses to the cooler earth. Fifth, a 2,270 liter (600-gallon) elevated water reservoir was installed to capture additional heat and lessen direct well water use. Lastly, all tanks constructed by our personnel were painted dark brown to help absorb solar energy.

INTENSIVE PRODUCTION SYSTEM.-Our fish culture system begins with two quarantine tanks, each having a volume of 1500 liters (400 gallons). These are receiving containers used to temporarily hold female fish that are brought into the greenhouse until we are certain they are ready to spawn and are not harboring any infectious diseases or parasites. If necessary, medications can be administered at this time to these isolated fish. Once we determine the fish are ready to spawn, we size the females with bar graders to obtain females approaching parturition and place these fish in a sorting tank from which we hand select those very likely to spawn immediately. These gravid females are individually placed into 660 screened berry baskets, which have been partially immersed on submerged support trays set into either of two shallow spawning tanks having a combined volume to 3760 liters (994 gallons). Window screen covers are placed over the baskets to prevent females from jumping into the open waters of each spawning tank. The fry are usually expelled within a few hours to a few days and most are able to escape through the screening of the baskets into the open water area of the two spawning tanks. After virtually all females have released their young, we net out the fry and enumerate them photographically. Several thousand fry are usually collected at each spawning and are soon transferred to one of several isolation cages located in our two deeper nursery tanks. They will be maintained here until they are needed for field or cultural use. Spawned females are returned to a holding tank to await subsequent pregnancies or removed for distribution to the public. The volume of the holding tank plus each of the two identical nursery tanks is 12,763 liters (3,372 gallons).

Water exhausted from all tanks comprising the spawning, nursery and holding functions is gravity-plumbed to a biological filtration unit. From there filtered water is recirculated and sprayed back into the individual culture tanks by way of a pedestal sump pump. The biological filtration system operates through the action of both aerobic and anaerobic bacteria which convert toxic un-ionized ammonia present in fish excretions into comparatively harmless nitrate compounds. The aerobic phase of this conversion process utilizes an partially-immersed rotating drum containing 25.4mm(1") Koch plastic media; while the anaerobic phase consists of a stationary submerged bed of identical plastic media. Thus far, this biofilter has been able to maintain water quality such that un-ionized ammonia has been kept well below toxic levels. The complete intensive culture system, including the biofilter encompasses a total volume of 18,208 liters (4,811 gallons).

AQUACULTURAL PROGRESS.-Last summer our system produced 11,767 fry in a three-week test run with the range of production between 1,133 and 6,172 fry per spawning. Production decreased markedly in late August with the beginning of what later became a serious disease problem. Spawning operations had to be terminated, the fish removed and the whole system sterilized. Subsequent examination by a State fisheries pathologist revealed bacterial infection and several parasites. Blood samples indicated possible equipment-related toxicities as well. As all plastic materials were bioassayed prior to use in the system, any metal object became suspect. The galvanized steel axle in our biofilter was quickly replaced with one of stainless steel. Hopefully, all toxic materials were removed from the system. More nutritionally-complete feeding regimes were also investigated at this time.

This winter, water temperatures in the unheated tanks have dropped to a December low of 7.2°C(45°F), with a mean daily range of 11 to 13.1°C(51.8-55.6°F). Yuba City has been experiencing extended atypical cold weather in conjunction with foggy, overcast skies; consequently, solar energy input has been minimal this month (January 1985). We installed supplemental electrical heating and provided an extended photoperiod for some fish stocked at the beginning of December in one of our quarantine tanks in an attempt to bring them into spawning condition. Some fish are now beginning to show gravid spots indicative of pregnancy; however, it would be very speculative at this time to predict the actual spawning date. We have recently purchased a remote thermograph to improve temperature monitoring so we may better assess the thermal advantages and characteristics of our passive solar design. Currently we are still outfitting our system and making modifications to existing equipment.

COST CONSIDERATIONS.-The basic cost for the standard greenhouse was $8,655. Added to this were the charges by our electrician, which amounted to $1,570 and the costs for passive solar modifications and enhancements which was $2,522. Thus the complete cost for the empty, but solar-enchanced greenhouse totaled $12,747. The intensive culture system, including tanks, plumbing supplies, pumps and other support equipment has added another $2,513. Combining all charges results in a final figure of $15,260. These figures were based upon outlays occurring through October 1984. Expenditures since then have been fairly minor and have been more closely allied with day-to-day operational costs than with the greenhouse itself or its intensive culture system.

THE UTILIZATION OF PLANT INGREDIENTS AS AIDS IN MOSQUITO CONTROL

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ABSTRACT

Two species of aquatic plants, *Elodea nuttali* Planch and *Spirogyra nitida* Dillw, a sea weed, *Macrocystis pyrifera* L. and a terrestrial sage brush, *Artemisia cana* Pursh, were evaluated for biological activity against *Culex quinquefasciatus* Say.

In field studies, growing *Elodea* plants were found to inhibit the populations of mosquito larvae in ponds.

Organic and aqueous extracts from the four plants were tested on natural populations of mosquitoes in Southern California ponds. Organic extracts from *Artemisia* and *Macrocystis* were found to be highly toxic to mosquito larvae. Toxic extracts from *Artemisia* were found to be soluble in nonpolar solvents and have an acidic property.

Extracts from *Artemisia, Macrocystis* and *Elodea* also acted as mosquito-repellent substances.

INTRODUCTION.-Studies on the interaction between mosquitoes and plants have provided possibilities for the use of plant substances in mosquito control. Mosquitoes breed in a variety of habitats and the larval distribution in these habitats is affected by the kinds of plants growing in the habitats. These effects are produced through a selective discrimination of the ovipositing females and by the effects of plant toxins on the mosquitoes.

Field observations on natural and seminatural ponds in Riverside County, CA., revealed the absence of immature stages of *Culex quinquefasciatus* in ponds containing certain plants; whereas mosquitoes were abundant in ponds lacking such plants (Sherif and Hall 1984). Some species of blue-green algae are known to inhibit mosquito reproduction in rice fields (Gerhardt 1954) and laboratory studies have shown that certain substances derived from algae are lethal to mosquito larvae (Judd and Borden 1980).

In this study we evaluated the effects of different plant extracts as well as the effects of naturally growing plants on different stages of mosquitoes under natural and laboratory environments.

METHODS AND MATERIALS.-*Elodea* and *Spirogyra* were obtained from natural ponds in Riverside County, CA. Each plant species was planted in three ponds, each having a surface area of approximately 100 sq. ft. Three ponds were left plant free to serve as controls. Five egg rafts of *Cx. quinquefasciatus* were placed into each pond. Starting 4 days after the eggs were introduced, weekly larval counts were made for the next 16 weeks. Using a standard 400 ml dipper, 5 samples were collected, 4 from each corner, and one from the middle of each pond.

For extraction purposes plants were collected, washed with tap water, dried at 60°C, and powdered. Seventy g of each plant were refluxed separately for 8 h with absolute methanol and petroleum ether (3:1 vol/vol). The methanol petroleum ether extract was chromatographed on a neutral aluminum (350 g) column. One and one-half liters of each of the following solvents were used in the following order; petroleum ether, ethyl acetate, and methanol. The eluents were evaporated to dryness. Each of the three residues from each plant was dissolved in acetone (15 ml) and assayed at different concentrations (0.5 and 0.9 ml/100 ml water) to determine if they were inhibitors to mosquito larvae. Twenty first-stage mosquito larvae were placed in 100 ml water in glass bowls. Larvae were fed a (3:1) mixture of rat chow and Brewer's yeast. Calculations were made on the number of mortalities in treatment and control cultures.

To test the attractancy of the plant extracts, 50 pairs of adult mosquitoes were placed in each cage. A 10% glucose solution was available to adults at all times and blood was offered for three nights, by placing restrained chicks in the mosquito cages.

Oviposition bioassays were conducted in adult mosquito cages using six small plastic cups (7 x 7.5 cm), 3 containing 50 ml of distilled water and 3 containing distilled water plus a plant extract. After discontinuing blood feeding, the cages were examined daily, and all egg rafts were removed and counted.

RESULTS AND DISCUSSION.-Figure 1 shows the results of the weekly larval sampling in the ponds. While the presence of *Elodea* did not repress *Cx. quinquefasciatus* breeding, there was a marked reduction in *Cx.* larvae in ponds containing *Elodea*. This finding suggests the presence of toxic or metabolic by-products produced by the *Elodea* which are toxic to mosquito larvae. This inhibition was much less for *Spirogyra*.

The toxicity of methanol petroleum ether extracts for *Artemisia, Elodea, Macrocystis,* and *Spirogyra* against first-stage larvae of *Cx. quinquefasciatus, Cx. tarsalis,* and *Ae. aegypti* are presented in Table 1. *Elodea* was found to be more toxic to *Cx.* species than to *Ae. aegypti*.

Table 1.-Evaluation of the methanol-petroleum ether extract from *Artemisia, Elodea, Macrocystis* and *Spirogyra* against first-stage larvae of three species of mosquitoes in the laboratory.

	Mean % mortality in larva (L), pupa (P) and % adult emergence (E) in plant extracts (ppm).											
	30			60			90			120		
	L	P	E	L	P	E	L	P	E	L	P	E
CULEX QUINQUEFASCIATUS												
Elodea	73a	0	27b	83ab	0	17bc	90b	0	10c	100a	0	0
Macrocystis	73a	0	27b	80b	0	20b	97a	0	3d	100a	0	0
Artemisia	76a	0	24b	96a	0	4c	100a	0	0	100a	0	0
Spirogyra	0	0	100a	06c	0	94a	12c	0	88b	15bc	0	85b
Control	2b	0	98a	0	0	100a	1d	0	99a	3c	0	97a
AEDES AEGYPTI												
Elodea	19b	1b	80c	65a	2b	33c	90a	3a	7c	100a	0	0
Macrocystis	10c	3a	87b	36ab	7a	57c	100a	0	0	100a	0	0
Artemisia	17b	0	83bc	35ab	0	65bc	73b	0	27b	84c	0	16b
Spirogyra	3d	0	97a	5bc	0	95ab	10c	0	90a	10c	0	90a
Control	0	0	100a	2c	0	98a	2c	0	98a	4c	0	96a
CULEX TARSALIS												
Elodea	77a	0	23b	76b	7a	17b	83b	7a	10c	100a	0	0
Macrocystis	70a	0	30b	80b	0	20b	93a	0	7c	100a	0	0
Artemisia	64a	3a	23b	99a	0	1c	100a	0	0	100a	0	0
Spirogyra	0	0	100a	7c	0	93a	10c	0	90b	15b	0	85b
Control	1	0	99a	2c	0	98a	2d	0	98a	1c	0	99a

Means followed by the same letter in the columns are not significantly different from one another (Duncan's multiple range test P = .01).

Macrocystis was also more toxic to the *Cx.* species. The data in Table 1 also suggest that the pupal stage of *Cx. quinquefasciatus* is more resistant to the plant extracts than the pupal stage of *Ae. aegypti*.

At concentration of 120 ppm all plant extracts, except those of *Spirogyra*, resulted in 100% mortality. At lower concentration (30 ppm), *Elodea* and *Macrocystis*, when added to cultures of the *Culex* species, resulted in 73% mortality compared to 76% by *Artemisia*. *Ae. aegypti* larvae were more resistant to all plants, since a lower mortality of 19%, 10% and 17% was caused by *Elodea, Macrocystis,* and *Artemisia* extracts respectively.

The toxicity of the three fractions from the four plants against first-stage larvae of *Cx. quinquefasciatus, Cx. tarsalis,* and *Ae. aegypti* is shown in Table 2.

The petroleum-eluted fractions of *Artemisia* and *Macrocystis,* and the methanol-eluted fraction of *Elodea* were found to have the greatest larvicidal activity.

Exposure of the first-stage larvae to the petroleum ether fraction of *Artemisia* resulted in 100% mortality compared to 90, and 15, by extracts (0.9 ml/100 H_2O) from *Macrocystis* and *Elodea*. Controls had 2% mortality. *Artemisia* produced the most toxic ethyl acetate fraction. At 0.5ml/100 ml H_2O it resulted in 20% mortality compared to 0% by *Macrocystis* and 5% by *Elodea*. Complete mortality was reached in three days by the petroleum ether fraction of *Elodea*, while 5 days were required to cause 100% mortality by the ethyl acetate fraction of *Artemisia*.

Upon elution with methanol, *Elodea* was found to be the most toxic, causing a mortality of 100% within 3 days compared to 15% and 20% by *Artemisia* and *Macrocystis* at the highest concentration used.

The plant extracts induced a variety of morphological aberrations in the treated larvae. The majority of the treated larvae died as larvae exhibiting various abnormalities.

Table 3 gives the results of cage oviposition trials. When gravid *Cx. quinquefasciatus* females

Table 2.-Evaluation of different eluent-fractions from the plants: *Artemisia, Elodea* and *Macrocystis*.

Extract Concentration	Mean percent mortality in Larvae (L), Pupae (P), Adult (A) and percent Emergence (E). 0.9 ml/100 ml H_2O				0.5 ml/100 ml H_2O			
	L	P	A	E	L	P	A	E
ARTEMISIA								
Petroleum ether	100a	0	0	0	100a	0	0	0
Ethyl acetate	10c	10a	5a	75c	20c	5a	0	75b
Methanol	15b	0	0	85b	0	5a	5a	90a
ELODEA								
Petroleum ether	15b	15a	5a	65c	10b	5a	5ab	80c
Ethyl acetate	15b	0	0	85b	5c	0	10a	85b
Methanol	100a	0	0	0	100a	0	0	0
MACROCYSTIS								
Petroleum ether	90a	0	0	10c	10a	10a	0	80c
Ethyl acetate	10b	10a	10a	70b	0	5ab	5a	90b
Methanol	15b	5b	0	80b	10a	0	0	90b
CONTROL	2d	0	0	98a	2d	0	0	98a

Means followed by the same letter in the same column for each plant are not significantly different from one another (Duncan's multiple range test P = .01).

Table 3.-Cage oviposition tests with *Culex quinquefasciatus*, using containers with and without methanol-petroleum ether extracts from *Artemisia, Elodea, Macrocystis, Spirogyra* and plant-free containers.

Test No.	No. Gravid Females	Egg rafts deposited — Treatments: *Artemisia*	*Elodea*	*Macrocystis*	*Spirogyra*	Control
1	50	0	1b	5a	9a	10a
2	50	0	0	0	5a	18a
3	50	1b	0	2b	11a	15a
4	50	0	2bc	1bc	12a	5ab
TTL		1	3	8	37	48

Means followed by the same letter are not significantly different from one another (Duncan's multiple range test P = .01).

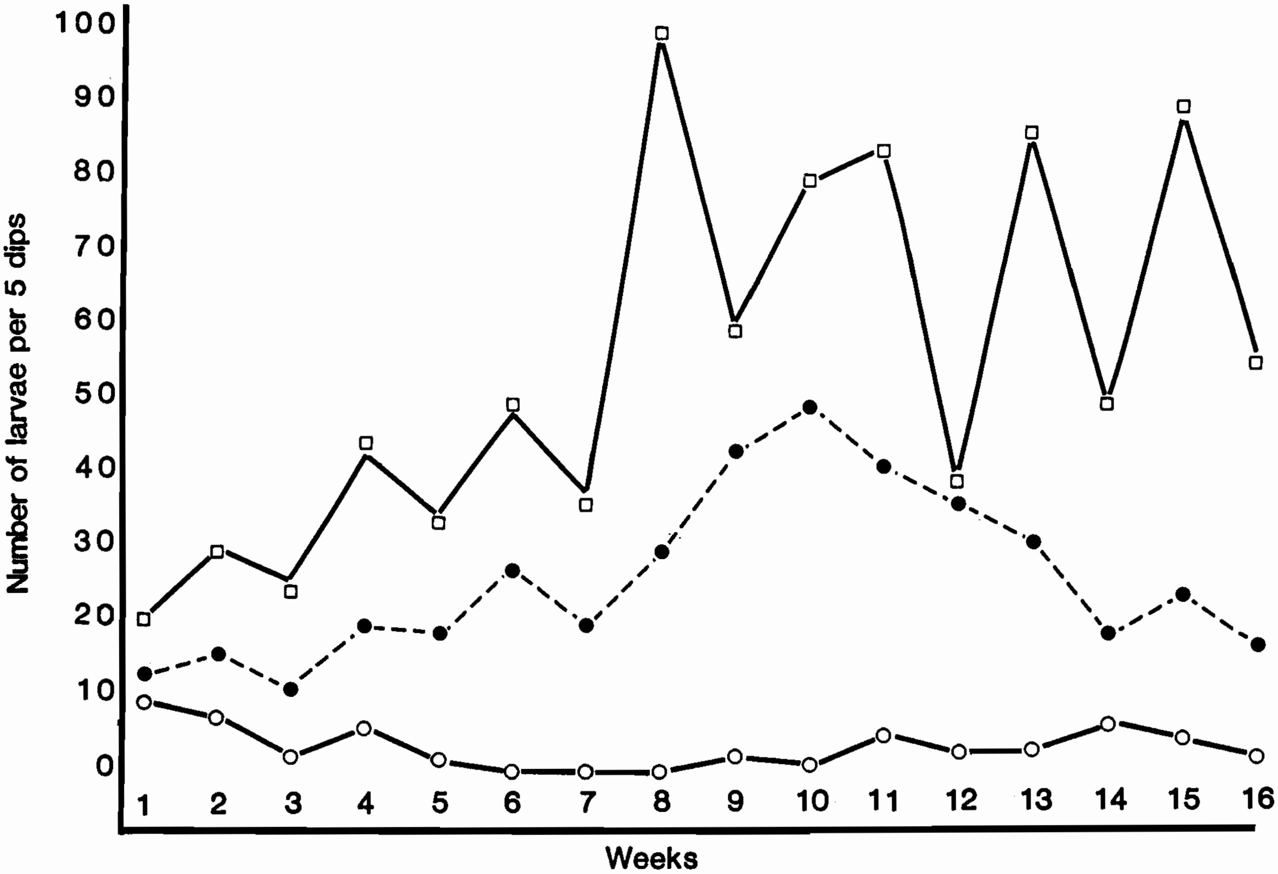

Figure 1.-Weekly larval survey for *Culex quinquefasciatus* in ponds supporting the growth of *Elodea* ○, *Spirogyra* ●, and plant free ponds □, in Riverside County.

were placed in a cage with five oviposition containers, containing methanol petroleum ether extracts of *Artemisia, Elodea, Macrocystis, Spirogyra* or no extract most of the eggs were deposited in the plant extract-free containers, thus showing that plant extracts also acted as mosquito repellent substances.

These results thus suggest that inhibitory compounds released by plants, if found to be selective and non-persistent in the environment, appear promising for use in mosquito control.

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THE INFLUENCE OF CONSTANT VERSUS FLUCTUATING TEMPERATURES ON LARVAL *CULEX TARSALIS*

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ABSTRACT

Culex tarsalis larvae derived from field-collected egg rafts were reared outdoors in a 40 cm deep shaded pond or a 10 cm deep unshaded pond. Daily water temperatures, measured 1 cm below the surface, remained constant in the deep pond, but fluctuated as much as 16°C in the shallow pond. The mean temperature in the shallow pond was consistently about 6°C higher than in the deep pond throughout the summer. Six cohorts of mosquitoes were reared from May through September at intervals of about 4 weeks.

No significant differences were found between ponds in the proportion of larvae surviving to adults, female autogeny rates, or wing lengths of adults. The average time from 1st instar to adult emergence ranged from 8 days at 31°C to 16 days at 17°C. Males developed faster than females, and both sexes always emerged faster from the shallow pond than from the deep one. However, when mean emergence times were adjusted to account for higher mean temperatures in the shallow pond, the temperature-adjusted mean emergence times were equal. Normalized cumulative developmental curves, fitted to a Weibull function, were identical for the 2 ponds.

The conclusion from these analyses is that mean water temperature determined the rate of larval development, whether that temperature was constant or fluctuating. This knowledge will facilitate the development of simulation models to describe the population dynamics of this species.

ACKNOWLEDGMENT.-This research was funded by Research Grant AI-3028D from the National Institute of Allergy and Infectious Diseases, Biomedical Research Support Grant 5-S07-RR-05441 from the National Institutes of Health and by special funds for mosquito research allocated annually through the Division of Agriculture and Natural Resources, University of California.

PATTERNS OF FRUCTOSE FEEDING BY *CULEX TARSALIS*

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ABSTRACT

A total of 75% of 350 female and 68% of 251 male *Cx. tarsalis* collected resting in walk-in red boxes during morning reacted positively for fructose using the cold anthrone reaction. Females imbibed fructose throughout life, since no significant differences were found in the percentage of females that were fructose positive regardless of insemination status, metabolic state, follicular developmental stage or parity. The reproductive status of females collected at melon-baited traps was not significantly different from that of fructose positive females collected resting in red boxes, indicating that nectar feeding occurred daily.

Host-seeking females imbibed nectar just after sunset and during early morning. The proportion of fructose positive host-seeking females collected in a time-segregated CO_2 -baited sampler increased during early evening (sunset-2000 hrs) and morning (0300 hrs-sunrise). The increase in fructose positivity during morning occurred after the number of host-seeking females decreased. These data indicated that females may imbibe fructose after ingesting a blood meal, but before ingressing into diurnal resting sites. Thus, adult control schedules need not be restricted to periods of host-seeking activity, since nectar feeding mosquitoes would also be exposed to insecticide applications.

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[1]This research was funded by research grant AI-3028d from the National Institute of Allergy and Infectious Diseases, Biomedical Research Support Grant 5-S07-RR-05441 from the National Institutes of Health, and by special funds for mosquito research allocated annually through the Division of Agriculture and Natural Resources, University of California.

A STUDY OF A SEMI-ISOLATED POPULATION OF *ANOPHELES FREEBORNI* NEAR SHERIDAN, CALIFORNIA

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ABSTRACT

A study of a population of *Anopheles freeborni* was conducted in a small valley at the edge of the Sierra Nevada foothills near Sheridan, California. Approximately 460 acres in the valley were planted to rice, and the nearest rice fields outside the study site were about 2 miles away. The selection of the semi-remote location was guided by a desire to study a population not exposed to routine insecticide treatment and to limit emigration of mosquitoes out of the study area.

Eighteen walk-in red boxes (6x4x4') were positioned in the study area, and collections of resting mosquitoes were made once daily from 13 August through 7 September (except on the mornings of 1-3 September). Additional collections were made several times before and after the period of daily sampling. The sex, species, and trophic status of all mosquitoes was determined, and a subsample of female *An. freeborni* was dissected to determine parity. Abdomens of some blood-engorged mosquitoes were saved for later analysis of host selection. A mark-release-recapture using approximately 37,500 fluorescently-marked *An. freeborni* and a release of marked and unmarked mosquitoes in a tent at the study site were conducted to estimate dispersal, survivorship, and the effect of fluorescent dust on the mosquitoes.

Predominant species collected during the study were *An. freeborni, Culex tarsalis,* and *Culex peus*. Other species collected included *Culex pipiens, Culiseta particeps, Culiseta inornata, Aedes melanimon, Aedes sierrensis, Anopheles punctipennis, Anopheles franciscanus,* and *Orthopodomyia signifera*.

Analysis of gontrophic age-grading data for *An. freeborni* (Table 1) showed a significant difference between parity rates of empty and bloodfed mosquitoes. Presumedly, the lower rate among the empty mosquitoes reflects the time required for maturation and mating prior to the first feeding as well as the shift to production of nulliparous, diapausing mosquitoes in early fall. A drop in the percent bloodfed *An. freeborni* from mid-August through the end of the study also reflects the shift from a reproductive to a diapausing population. The mark-release-recapture and the in-tent release studies failed to provide adequate data for estimates of survivorship.

Analysis of a small number of bloodmeals has been completed, and results indicate a high percentage of feedings occurred on bovines, horses, rabbits, or dogs. To date, no human feedings have been detected.

Table 1.-Summary of age grading data for *Anopheles freeborni* collected near Sheridan, CA, 19 July - 13 September 1984.

Parity	Trophic Status: Empty Frequency (Column %)	Trophic Status: Bloodfed Frequency (Column %)
Nulliparous	3647 (90.1)	1416 (63.5)
1-Parous	393 (9.7)	764 (34.2)
2-Parous	7 (0.2)	44 (2.0)
3-Parous	2 (0.05)	7 (0.3)
Total	4049	2231

WHO IS EATING WHOM? AN EVALUATION OF AN ENZYME IMMUNOASSAY FOR PREDATOR PREY ANALYSIS

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ABSTRACT

Increased resistance of important vector species to chemical control measures has stimulated efforts to develop a program employing biological control and integrated pest management. Both strategies rely on knowing which predators are consuming the target species. Documenting predation in field situations is also important for confirming selectivity by the predator, protecting nontarget species and determining the amount of control exerted by the predators. Predator studies are also important in answering basic ecological questions.

Numerous methods have been utilized in the past to study predation. Laboratory studies provide some information but cannot reveal what is happening in field situations. Dissection and examination of predator gut contents has several limitations. This method is applicable to insects with chewing mouthparts but not with a predator with sucking mouthparts which has no distinguishable prey parts in its gut. This method is also limited by the small size of many predators and their prey.

The use of serological tests, most frequently the precipitin test, have been restricted by their lack of specificity and sensitivity. This is especially important when trying to distinguish between species of mosquitoes or between mosquitoes and chironomids and other closely related dipterans.

We have adopted an Enzyme Immunoassay to detect larval antigens in saline extracts of putative larval predators. This method allows for the examination of large sample sizes and does not depend on the morphological preservation of the prey. It can also detect small amounts of prey antigen in the presence of excess amounts of predator antigen; however, the assay is qualitative in the sense that it cannot distinguish between predators fed on 5 larvae 3 hours prior to preservation from predators preserved an hour after feeding on a single larva.

This assay was evaluated using 3 aquatic predators of *Culex tarsalis* Coquillett larvae. We examined sensitivity, cross reactivity and classification agreement in a blind test.

We were able to detect nanogram quantities of *Culex* antigen. There was no cross reactivity between *Culex* and *Anopheles* antigens. A blind test was done with Notonectidae, Belostomatidae and Coenagrionidae. These predators were fed known number of *Culex* larvae, fed *Anopheles* larvae or starved for 24 hours. Feedings were done in Davis and then assayed as unknowns in Berkeley. The assay was evaluated as to its ability to detect fed (positive reaction) and starved (negative reaction) predator.

Sensitivity,

$$\frac{\text{true positives}}{\text{true positives + false negatives}} = 90\%,$$

and specificity,

$$\frac{\text{true negatives}}{\text{true negatives + false positives}} = 96\%,$$

were both high for the Notonectidae tested. Sensitivity was lower (69%) with the Belostomatidae tested but specificity remained high (100%). It is important to note the low number of false positives in both cases, which gives the investigator great confidence in positive results from the assay.

The first 2 predator groups assayed have sucking mouthparts. The last group evaluated, Coenagrionidae, which have chewing mouthparts, also had high sensitivity (94% and specificity (100%).

These data substantiate the feasibility of this assay for predator-prey studies as long as the limitations of the method are considered in the interpretation of results from field-collected material.

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PRODUCTION AND FIELD EVALUATION OF *LAGENIDIUM GIGANTEUM* AND *ROMANOMERMIS CULICIVORAX*

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ABSTRACT

During 1982 and 1983 approximately 20,000 m of linear transect in commercial rice fields near Sutter, California, were treated with the post-parasitic stage of the mermithid nematode *Romanomermis culicivorax*. During 1984 the persistence of nematode activity in these fields was evaluated using sentinel mosquito larvae. Indigenous larval density was too low to allow assessment of parasite activity based on infection levels in the native population.

Recycling of the nematode was documented at the highest levels (7 to 34% sentinel larval infection) along four transects of a field treated in 1982 and fallow in 1983. This demonstrated that some of the eggs laid by *R. culicivorax* may be highly resistant to desiccation. Parasite activity along the 19 transects monitored in 1984 documented sentinel infection levels ranging from 0 to 34% (20 to 25 sentinel cages per transect, 20 II-instar *Culex pipiens* per cage). In several cases high levels of sentinel infection were correlated with a relatively high density of indigenous larvae, but no definite relationship was established.

Liquid culture techniques for the sexual oospore stage of *Lagenidium giganteum* have been refined and adapted to 12-liter fermentation production. Basal growth media consists of Ardamine pH (an inexpensive autolyzed yeast extract), glucose, corn or wheat germ oil, a crude preparation of cholesterol, and small amounts of calcium and magnesium. Current yields in fermentation production are 5×10^7 oospores/liter, approximately one-half that which can be obtained in smaller scale (100-1000 ml) shake culture.

Conditions affecting the maturation and germination of *L. giganteum* oospores, which are a dormant stage in the life cycle of the organism, are not well-defined. Dehydration and a several month maturation period appear to be necessary for activation. Germination of a given culture of oospores is asynchronous, with initial germination initiated 2 to over 25 days following rehydration and continuing for several months. Less than 1% of a culture of 3×10^5 oospores are actively germinating as monitored morphologically at any given period of time.

To evaluate the stability of *L. giganteum* oospores, several 1977 collections of spores produced *in vivo* and stored at room temperature in sand or dirt were rehydrated. After 25 days of quiescence these oospores initiated germination, which proceeded at relatively high levels as monitored by sentinel mosquito infection until the test was terminated 6 weeks later. This demonstrates the long-term storage potential of these dormant spores.

Abortion of activated oospores was a major problem encountered during early 1984. This problem was mitigated by vigorous blending of the dehydrated spores in water followed by filtration and further washing until the filtrate was relatively clear.

It is estimated that approximately 200 liters of fermentation culture media will be needed to treat one hectare of mosquito breeding sites. Aerial field trials planned for the summer, 1985, will provide further data on the efficacy of *L. giganteum* oospores for mosquito control under field conditions.

PUBLICATION POLICIES AND INFORMATION FOR CONTRIBUTORS

"THE PROCEEDINGS" is the Proceedings and Papers of the California Mosquito and Vector Control Association, Inc. One volume is published each year. Intended coverage by content includes papers and presentations of the Association's Annual Conference, contributions and meritorious reports submitted for the conference year, and a synopsis of actions and achievements by the Association at large during the preceding year.

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