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California Mosquito and Vector Control Association, Inc.

Volume 52 January 29 - February 1, 1984

SURVEILLANCE FOR ARTHROPOD-BORNE VIRAL ACTIVITY AND DISEASE IN CALIFORNIA DURING 1983

Richard W. Emmons[1], Marilyn M. Milby[2], Patricia A. Gillies[3], William C. Reeves[2], Edmond V. Bayer[4], Kathleen White[5], James D. Woodie[1], and Robert A. Murray[6]

Mosquito-borne western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) have remained largely quiescent since the 1950's when these two diseases caused significant and frightening epidemics in California's central valley region, which led to concerted efforts at mosquito control.

During 1983 one of these "sleeping dragons" awakened enough to remind us that they are not tamed or extinct. This time the lower Colorado River region was the focus of much of the activity and concern. Extensive and prolonged flooding of the region hindered mosquito control efforts and provided fertile ground for amplification of viral activity. This report briefly summarizes the surveillance activities and findings of various units of the California State Department of Health Services (CDHS), the University of California School of Public Health's Arthropod-Borne Virus Research Unit (AVRU), local mosquito abatement districts, county health departments, the California Department of Food and Agriculture, private physicians and veterinarians, and other persons and groups which participate in this endeavor. This is the 14th annual published report since this special effort and series of reports began in 1969.

During 1983 292 patients suspected of having encephalitis were tested serologically for WEE and SLE at the CDHS Viral and Rickettsial Disease Laboratory (VRDL) or at the five county public health laboratories which also provided this service (Table 1). In addition to the serologic diagnostic tests, eight human brain samples and three human cerebrospinal fluid samples were tested for arboviruses at the VRDL by inoculation into infant mice. As usual, no WEE or SLE viruses were isolated from suspected cases, but nine cases of SLE were identified, which were apparently infected in California (Table 2). Of interest are: (1) the involvement mainly of the lower Colorado River region; (2) the occurrence of three cases with presumed sites of exposure in areas of urban counties not usually considered as highly endemic areas (cases #6, 7 and 9); (3) the rather late onset of illness in several of the cases; and (4) documentation of a presumptive-positive case of encephalitis due to California encephalitis virus (CEV), the first such case detected in California since 1942-1943 when the virus and the disease were first recognized. The CEV case, however, occurred in a child recently exposed to mosquitoes in Wisconsin, an area known to be highly endemic for CEV and CEV disease. Four additional positive or presumptive-positive cases of SLE were detected serologically, but these were out-of-state patients for whom the VRDL merely provided diagnostic assistance, and they are not included in this report.

There were 39 clinically suspect equine cases of WEE from 21 California counties which were tested serologically during 1983, and brain samples from 12 suspect cases were tested for virus by mouse inoculation. No arboviruses were isolated, and only two presumptive-positive WEE cases were detected: (1) a one-year old horse from Blythe, Riverside County, with onset July 12,

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Table 1.-Number of Humans Tested Serologically for WEE and SLE by County and Month of Onset, 1983.

COUNTY		MONTH												
	Totals	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Unk
TOTAL	292	4	6	0	11	21	26	56	51	51	25	11	2	28
Alameda	1	0	0	0	0	0	0	0	0	0	0	1	0	0
Berkeley	2	0	0	0	0	1	0	0	0	0	0	0	0	1
Butte	7	0	0	0	0	1	0	3	1	0	1	0	0	1
Contra Costa	3	0	0	0	1	0	0	1	1	0	0	0	0	0
Del Norte	2	0	0	0	0	1	0	0	0	1	0	0	0	0
El Dorado	4	0	0	0	0	1	0	1	1	1	0	0	0	0
Fresno	56(a)	0	0	0	1	6	11	10	8	10	5	4	0	1
Humboldt	2	0	0	0	0	0	0	1	1	0	0	0	0	0
Imperial	13	0	0	0	0	0	0	4	5	3	1	0	0	0
Kern	5	0	1	0	0	1	0	0	1	2	0	0	0	0
Kings	4	0	0	0	0	0	1	0	0	1	1	0	0	1
Lake	1	0	0	0	0	0	0	0	0	0	1	0	0	0
Lassen	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Los Angeles	38(a)	0	0	0	5	3	5	3	8	5	4	1	0	4
Marin	1	0	0	0	0	0	0	0	1	0	0	0	0	0
Mendocino	2	0	0	0	0	0	1	1	0	0	0	0	0	0
Merced	6	0	0	0	1	0	1	1	1	0	1	0	0	1
Napa	3	0	0	0	0	0	0	2	0	0	0	1	0	0
Orange	14(a)	0	0	0	0	0	0	3	6	4	1	0	0	0
Placer	7	0	0	0	0	0	0	2	3	1	0	0	0	1
Plumas	1	0	0	0	0	0	0	0	0	1	0	0	0	0
Riverside	5	0	0	0	0	0	0	4	0	1	0	0	0	0
Sacramento	5	0	2	0	0	2	0	0	0	1	0	0	0	0
San Bernardino	21(a)	3	3	0	0	0	1	2	5	4	0	1	2	0
San Diego	36(a)	0	0	0	1	1	3	8	4	9	8	1	0	1
San Francisco	3	0	0	0	0	0	0	1	1	0	0	0	0	1
San Joaquin	3	0	0	0	0	0	1	0	0	1	0	0	0	1
San Luis Obispo	3	0	0	0	1	1	0	1	0	0	0	0	0	0
Santa Barbara	1	0	0	0	0	0	0	0	0	0	1	0	0	0
Santa Clara	2	0	0	0	0	0	0	2	0	0	0	0	0	0
Santa Cruz	9	0	0	0	1	1	0	4	0	2	0	0	0	1
Shasta	5	0	0	0	0	0	0	0	1	1	1	2	0	0
Solano	1	0	0	0	0	0	0	0	1	0	0	0	0	0
Sonoma	1	0	0	0	0	0	0	0	1	0	0	0	0	0
Stanislaus	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Tulare	1	0	0	0	0	0	0	0	0	1	0	0	0	0
Yolo	8	0	0	0	0	2	0	1	1	2	0	0	0	2
Out of State	2	0	0	0	0	0	0	1	0	0	0	0	0	1
No County	12	1	0	0	0	0	0	0	0	0	0	0	0	11

(a) Most of these tests were performed at the county public health laboratory.

1983 and no history of WEE immunization; and (2) and 18 year old horse from Los Angeles County, with onset about July 15. The second horse had not been out of the area since early May. It was vaccinated against WEE in July and December of 1982, but vaccine is known to be incompletely protective. A horse from the same ranch had WEE in 1979, documented in 1983 when serum samples which had been stored frozen during the interval were submitted for antibody tests.

The mosquito testing program again was aided by a grant to the University of California School of Public Health's AVRU from special U.C. Mosquito research funds, which allowed assignment of Ms. Patricia Boehme to the program. Mosquitoes were collected from 26 California counties and from the Arizona border of the lower Colorado River (Table 3). There were 161,245 mosquitoes in 3,417 pools which were tested for virus. As usual, sampling was concentrated in counties where viral activity was expected to be most intense and favored *Culex tarsalis*, the major vector for epidemic SLE and WEE. Future sampling, however, may need to include a larger proportion of *Culex pipiens*

Table 2.-Human cases of arboviral encephalitis, California, 1983.

Case No.	Disease	Age	Sex	Residence	Date of Onset	Probable Place of Contraction	Outcome of Illness
1	SLE	75	F	Winterhaven (Imperial Co.)	7/22/83	Imperial County (Colorado River Area)	Recovered
2	SLE*	3	M	Bard (Imperial Co.)	7/26/83	Imperial County (Colorado River Area)	Recovered
3	SLE	32	M	Needles (San Bernardino Co.)	8/29/83	San Bernardino Co. or Arizona (Colorado River Area)	Recovered
4	SLE	69	F	Escondido (San Diego Co.)	8/31/83	Riverside County (Colorado River Area)	Recovered
5	SLE	68	F	Blythe (Riverside Co.)	9/1/83	Riverside County (Colorado River Area)	Recovered
6	SLE	72	M	Vista (San Diego Co.)	9/6/83	San Deigo County (Area of Residence)	Died
7	SLE	73	M	Long Beach (Los Angeles Co.)	9/16/83	Riverside County (Riverside City) or Area of Residence	Severe residual neurologic effects but some improvement 5 months after onset.
8	SLE	28	M	La Mesa (San Diego Co.)	9/25/83	Imperial County or Arizona (Colorado River Area)	Recovered (not hospitalized)
9	SLE	59	F	La Puente (Los Angeles Co.)	10/4/83	Los Angeles Co. Area	Recovered
10	CEV**	4	F	Anaheim (Orange Co.)	7/31/83	Wisconsin	Recovered

* simultaneous infection with Echovirus type 11 also documented

** Presumptive-positive; convalescent serum sample not available for test.

complex mosquitoes, since these also can be effective vectors. The occurrence in 1983 of human SLE cases in areas of San Diego and Los Angeles counties where *Cx. pipiens* is widespread but *Cx. tarsalis* is rare emphasizes this point.

The viruses isolated from mosquitoes are shown in Table 4: 182 WEE, 53 SLE, 83 Turlock, 193 Hart Park, and 9 CE group. As usual, most viral activity was in the southern half of the state and all isolates were from *Cx. tarsalis* or *Aedes melanimon*. The geographic distribution of WEE and SLE viruses are depicted in Figures 1 and 2, respectively, and Figure 3 shows the distribution of all pools of *Cx. tarsalis* tested in 1983. To save space and expense, the detailed listing of the individual positive mosquito pools which was included in past reports has been left out of this report. The information was provided periodically in the 24 weekly bulletins issued during the past year to participants in the surveillance program. The final summary includes corrections for a few errors or incomplete data that occurred in the weekly reports.

Sentinel chicken flocks were located in 31 sites throughout the state (Table 5). The chickens were bled and tested monthly for SLE and WEE antibodies by the indirect immunofluorescence method. Initial positive findings were for WEE in Kern County (June 28) and WEE and SLE in Imperial County (June 29), but WEE showed the broadest geographic activity by this surveillance technique. The widespread presence of WEE in mosquitoes and its transmission to sentinel chickens was contrasted with the limited findings of human and equine WEE cases, whereas more narrowly focused SLE viral activity was correlated with a significant occurrence of human cases of SLE in the corresponding regions of the state.

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Health Services, all participating local Mosquito Abatement Districts, County Health Departments, the California Department of Food and Agriculture, private physicians and veterinarians, and all others who helped in the surveillance program. This program was supported in part by special funds for mosquito control research appropriated annually by the California Legislature.

Table 3.-Number of mosquitoes and pools tested during 1983 by the Viral and Rickettsial Disease Laboratory Section by county and species.

County	*Cx. tarsalis*		*Ae. melanimon*		Other Species			Totals	
	Mosq	Pools	Mosq	Pools	Mosq	Pools	(Species and No. of pools)	Mosq	Pools
Butte	3,115	63	520	12	-	-		3,635	75
Colusa	278	6	-	-	-	-		278	6
Fresno	203	5	26	1	73	1	(*Aedes vexans*-1)	302	7
Glenn	233	5	-	-	-	-		233	5
Imperial	21,527	466	-	-	191	9	(*Cx. pipiens*-9)	21,718	475
Inyo	92	3	68	2	45	1	(*Cx. erythrothorax*-1)	205	6
Kern	58,163*	1178*	8,251**	160**	1,225	26	(*Cx. erythrothorax*-5, *Cx. pipiens*-10, *Cul. inornata*-11)	67,639	1374
Kings	401	9	-	-	21	1	(*Cx. erythrothorax*-1)	422	10
Lake	234	5	9	1	-	-		243	6
Los Angeles	100	2	-	-	42	1	(*Cx. pipiens*-1)	142	3
Marin	208	4	-	-	57	2	(*Cx. peus*-1, *Cx. pipiens*-1)	265	6
Merced	1,303	29	137	3	-	-		1,440	32
Orange	111	3	-	-	174	4	(*Cx. pipiens*-4)	285	7
Riverside	17,118	360	-	-	2,321	53	(*Cx. peus*-32, *Cx. pipiens*-21)	19,439	413
Sacramento	6,883	149	-	-	100	2	(*Cx. erythrothorax*-2)	6,983	151
San Bernardino	8,755	180	-	-	-	-		8,755	180
San Diego	750	17	-	-	-	-		750	17
San Joaquin	487	11	-	-	-	-		487	11
Santa Barbara	212	5	-	-	500	10	(*Cx. pipiens*-10)	712	15
Solano	44	2	-	-	50	1	(*Cx. pipiens*-1)	94	3
Sonoma	580	12	-	-	44	1	(*Cx. peus*-1)	624	13
Stanislaus	1,502	47	127	3	78	5	(*Cx. pipiens*-5)	1,707	55
Sutter	1,301	30	-	-	-	-		1,301	30
Tulare	9,990	234	974	22	300	6	(*Cx. pipiens*-6)	11,264	262
Yolo	10,373	215	340	7	-	-		10,713	222
Yuba	909	19	-	-	-	-		909	19
Arizona	700	14	-	-	-	-		700	14
TOTALS	145,572*	3073*	10,452**	221**	5,221	123		161,245	3417

* Includes 144 pools (6,982 mosquitoes) of male *Cx. tarsalis*, Kern County

** Includes 15 pools (740 mosquitoes) of male *Ae. melanimon*, Kern County

Table 4.-Number of viral isolates from mosquitoes tested during 1983 by the Viral & Rickettsial Disease Laboratory Section by mosquito species, county and agent isolated (female mosquitoes except where labelled male)

Species	County	WEE	SLE	Turlock	Calif Grp	Hart Park	Total
Cx. tarsalis	Butte	-	-	-	-	4	4
	Imperial	20(0.9)*	19(0.9)	7	-	2	48
	Kern	115(2.2)	3(0.1)	43	-	142**	303**
	Los Angeles	-	-	1	-	-	1
	Marin	-	-	-	-	1	1
	Merced	-	-	1	-	1	2
	Orange	-	-	1	-	-	1
	Riverside	9(0.5)	23(1.3)	7	-	5	44
	Sacramento	1(0.1)	-	2	-	5	8
	San Bernardino	15(1.7)	6(0.7)	4	-	-	25
	San Diego	-	-	1	-	2	3
	San Joaquin	-	-	-	-	1	1
	Santa Barbara	-	-	-	-	1	1
	Stanislaus	-	-	1	-	-	1
	Sutter	-	-	-	-	1	1
	Tulare	8(0.8)	-	10	-	19	37
	Yolo	1(0.1)	-	4	-	9	14
	Arizona	2(2.9)	2(2.9)	-	-	-	4
Cx. tarsalis (Total)		171	53	82	-	193**	499**
Ae. melanimon	Fresno	-	-	-	1	-	1
	Kern	11	-	-	5**	-	16**
	Tulare	-	-	1	3	-	4
Ae. melanimon (Total)		11	-	1	9**	-	21**
TOTAL VIRUS ISOLATES		182	53	83	9	193	520

* minimum infection rate/1000 female mosquitoes tested

** includes 1 isolate from a pool of male mosquitoes

Table 5.-Serological conversions* to WEE and SLE viruses in sentinel chickens, California, 1983.

Chicken Flock Location	*Cx. tarsalis* Females per NJ Trap Night (seas. avg.)	Number (%) Chicken Positive WEE	SLE	Date 1st Positive(s)
Shasta, MAD office	1.4	0	0	-
Tehama, MAD office	6.6	0	0	-
Butte, Chico	0.4	0	0	-
Butte, Gray Lodge	161.1	0	0	-
Glenn, MAD office	15.2	1(5)	0	9-28
S-Yuba, Marysville	2.3	0	0	-
S-Yuba, Dean's	33.5	1(5)	0	9-26
Sac-Yolo, Elk Grove	4.6	0	0	-
San Joaquin, Escalon	14.7	0	0	-
Turlock, Victoria	6.8	0	0	-
Merced, Rogers Dairy	29.7	0	0	-
Fresno Westside, Mendota Refuge	36.1	9(69)	0	8-29
Consolidated, Friant Rd.	2.1	1(5)	0	9-23
Kings, Riverview Ranch	No data	4(31)	0	8-30
Tulare, Gilroy Foods	2.6	5(25)	0	7-26
Westside, Lost Hills	1.4	3(18)	0	9-27
Westside, Maricopa	15.1	6(75)	0	8-23
Delano, Teviston	1.0	0	0	-
Kern Wildlife Refuge	225.6**	7(30)	0	8-29
Kern, Buttonwillow	0.5	7(30)	0	7-26
Kern, F.C. Tracy	5.2	17(85)	0	6-28
Kern, Wasco	1.3	10(43)	0	8-29
Kern, Oildale	0.9	0	0	-
Kern, John Dale	15.6	18(86)	0	7-26
Northwest, Corona	1.3	0	0	-
Coachella Valley, Mecca	7.1	1(5)	3(14)	9-26
Orange, 20 Ranch Duck Club	5.4	0	0	-
San Diego, Lakeside	0.7	0	0	-
San Diego, Sorrento	15.9	0	0	-
Imperial, Palo Verde	62.8	12(60)	16(80)	6-29
Imperial, Corda Ranch	1.8	4(29)	12(86)	8-2

* indirect immunofluorescence tests
** CDC traps with dry ice

PANEL: EVALUATION OF ALTERNATIVE METHODS FOR CONTROL OF ADULT *CULEX TARSALIS* AND *ANOPHELES FREEBORNI* IN TWO AREAS OF CALIFORNIA[1]

INTRODUCTION

William C. Reeves[2]

My responsibility is to present a panel that was organized to bring you up-to-date on research in California on the control of adult *Culex tarsalis* and *Anopheles freeborni*. The end objective of the research is to develop effective procedures to control epidemics of encephalitis and malaria. Projects were carried out in 1983 in the Sutter-Yuba and Kern Mosquito Abatement Districts and represented a collaborative effort between mosquito abatement districts, several Departments of the University of California, the State Department of Health Services and the US Army Research and Development Command.

The projects reflected an increasing concern that we do not have effective means to control epidemics of mosquito-borne diseases. In California, good experimental field data that will serve as a basis for effective control of adult *Cx. tarsalis* and *An. freeborni* are amazingly limited. The same is true in other areas of the United States and elsewhere in the world.

Let me provide a brief perspective on the magnitude of the problem in California. Western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) have been and still are an epidemic threat in an area of at least 29,000 square miles in the Sacramento Valley, San Joaquin Valley and Southern California. Historically, malaria also was endemic in much of this area and cases are still being identified each year in immigrants and visitors to what are considered to be receptive areas. This 29,000 square mile area of California is the home for over 5,000,000 people and has many visitors each summer. Most of the populated sections of the area are within the boundaries of organized mosquito control agencies and their budget from local taxation exceeded $15,000,000 in 1983. Current encephalitis and malaria control programs are focused on the use of insecticides, biological agents and water management to minimize populations of *Cx. tarsalis* and *An. freeborni*. The programs have minimized the disease problem in recent years. However, if the present control efforts are unsuccessful for any reason, the last recourse is to control the infected adult female vectors so as to prevent their biting susceptible people. The epidemics of WEE, SLE and malaria in earlier years have taught us what can happen in our state and epidemics in other parts of North America have provided further information on the problems that will be encountered in control of epidemics. Dr. Emmons will report later in the program on the apparent resurgence of WEE and SLE in California in recent years and malaria continues to be reintroduced into potentially receptive areas of the state.

To put the present research into another frame of reference, I estimate that it would cost California $10,000,000 or more in emergency funds from government sources if we had to control adult *Cx. tarsalis* to prevent a WEE or SLE epidemic in the potential epidemic areas referred to above. The objective of the program would be to control the infected and transmitting adult vector population over this large area for 7-10 days. The vector population would have to be reduced to levels sufficiently low to break the chain of virus transmission from birds to people. The only alternative to adult control is to do nothing or to urge the people to protect themselves individually from mosquito bites during the critical hours when vectors are feeding most intensively which is a period of 1-2 hours beginning with sunset.

The above estimate of costs is based on a control program during a WEE epidemic in Manitoba, Canada in 1981. Minnesota had a similiar problem in 1983. Manitoba spent $2,000,000 to control 5,000 square miles to protect less than 1,000,000 people ($2.60 per person). They utilized large DC6B planes-- each is the equivalent of 70 conventional agricultural aircraft of the type readily available in California. They applied 200,000 liters of Baygon MOS, an insecticide not currently available in volume in California or licensed for widespread dispersal. Minnesota applied malathion to a large part of the state for *Cx. tarsalis* control and we know this insecticide would not be effective in California today due to genetic resistance of the *Cx. tarsalis* population.

I hope the preceding summary has alerted you to the importance of the following panel presentations. We have identified an important problem. The keynote is that we would like to be prepared to mount an emergency control program in the event of an epidemic. We cannot assure you that we have sufficient knowledge, effective insecticides or methods to control an epidemic. The research of the past two years does allow us to say that if everything was right--weather, equipment and insecticide--adult control could be relatively effective. However, experience also tells us that everything seldom is right at the time when an emergency vector control program must be instituted and we need to continue research to correct this deficiency.

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PESTICIDE SUSCEPTIBILITY OF *CULEX TARSALIS* IN KERN COUNTY CALIFORNIA, 1983

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In the development of methodology for the suppression of adult mosquito populations, care must be taken to ensure that the insecticide chosen is effective at the labeled rates and that the spray system adequately covers the target area. This is especially true for the Bakersfield area of Kern County, California where insecticide resistant *Culex tarsalis* Coq. annually transmit epizootic western equine encephalomyelitis adjacent to concentrations of a largely non-immune human population.

In Kern County, insecticide resistance or tolerance has been documented in laboratory bioassays of *Cx. tarsalis* to all available organophosphate compounds (Yoshimura et al. 1983), and has been associated with a long history of operational failures. The level of resistance was greatest in areas under intensive agriculture and diminished in undeveloped areas of the county. Recent laboratory bioassays have suggested that populations which traditionally have been considered susceptible to chlorpyrifos have developed greater tolerance to this compound and already have developed cross-resistance to the synthetic pyrethroid, resmethrin.

The present paper further documents organophosphate resistance in populations of *Cx. tarsalis* from Kern County and presents the results of 1983 laboratory bioassays and field exposures of this species to Baygon.

MATERIALS AND METHODS.-**Laboratory bioassays.** Female *Cx. tarsalis* of unknown age were collected in CO_2-baited miniature CDC traps and exposed in the laboratory to serial concentrations of technical grade insecticides using the filter paper method of Georghiou and Gidden (1965), with the exception that 2 ml of insecticide-acetone solution were applied to each filter paper. Specimens tested came from field areas used to evaluate aerial applications of Baygon (for protocols and description of study areas, see Reisen et al. 1984).

Field Exposures. Four field tests were conducted in 1983. For all tests, 0.2 lb Baygon 70% W.P. per gallon of GB-1356 larvicide oil was applied at the rate of 0.5 gallons per acre, yielding a dosage of 0.07 lbs AI per acre. Spray droplet size was monitored during each test using rotating magnesium oxide-coated slides. At Poso West, insecticide was applied by an Ayres Thrush equipped with 36 D-4-45 hollow cone nozzles pressurized at 50 psi. Aircraft speed and altitude were 140 mph and 30-50 feet, respectively. This system was previously calibrated to deliver droplets in the 120 μ size range. During the early morning September trials at John Dale, 36 D-6-45 hollow cone nozzles were pressurized at 60 psi, and calibrated to deliver droplets in the 140 μ size range. Altitude and speed were similar to the poso tests. For the afternoon trials at Breckenridge and John Dale, the insecticide was applied by a Bell UH-1 Helicopter equipped with an underslung Simplex Model 6800 Army Bucket Sprayer. 36 D-4-45 hollow cone nozzles were calibrated to deliver droplets in the 200 μ size range at 40 psi. Aircraft speed and altitude were 57 mph and 30-50 feet, respectively. The estimated swath width for all tests was calibrated to be 200 feet.

During each test, adult *Cx. tarsalis* from the target population and the Br 80 reference colony were placed in sentinel cages which were located within the spray zone along exposed transects, at CO_2-trap stations, and sequestered beneath vegetative canopy. Comparison sentinels were placed at CO_2-trap standards outside of the spray zone. During the evening sprays (Poso, Breckenridge and John Dale in August) sentinels remained in the field and mortalities were recorded 12 hours post-spray. For the morning spray at John Dale in September, sentinels remained in the field for one hour, were returned to the laboratory, and mortalities recorded six hours post-spray.

RESULTS.-**Laboratory bioassays.** In agreement with past observations, resistance to malathion appeared to be widespread in Kern County. The Breckenridge wild population (BrW) showed an LD_{50} of 11.3 μg/cm² and an LD_{90} of 203.3 μg/cm² (n=1 test), while the John Dale wild (JDW) population had LD_{50} and LD_{90} values of 46.5 and 1013.2 μg/cm², respectively (n=1). These LD_{90} values were extrapolated linearly, as actual 90% mortalities were not achieved at the highest dosage tested (314.7 μg/cm²).

Chlorpyrifos exposures yielded LD_{50} and LD_{90} values of 0.07 and 0.27 μg/cm², respectively, in BrW adults (n=1). Two tests with chlorpyrifos conducted against a Kern River population (KRW- located about 15 miles SW of Bakersfield, California) gave pooled LD_{50} and LD_{90} values of 0.03 and 0.94 μg/cm², respectively. By comparison, the JDW population exhibited pooled LD_{50} and LD_{90} values of 0.95 and 7.34 μg/cm² respectivley (n=2), 27 times higher than the BrW population and eight times higher than the KRW population at the LD_{90} level.

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In sharp contrast to these results, the response of *Cx. tarsalis* to Baygon was very consistent regardless of the geographical origin of the population tested: Poso West (PWW), pooled LD_{50} = 1.8 µg/cm², LD_{90} = 2.7 µg/cm² (n=4); BRW pooled LD_{50} = 2.2 µg/cm², LD_{90} = 3.2 µg/cm² (n=2); JDW pooled LD_{50} = 1.7 µg/cm², LD_{90} = 2.7 µg/cm² (n=4). The Br80 reference colony had comparable LD_{50} - LD_{90} values of 1.7 and 2.3 µg/cm², respectively (n=2).

Field exposures. The estimated amounts of Baygon slurry delivered averaged within three percent (range -6 to +16 percent) of the desired 0.5 GPA output for all trials. Volume mass diameters (with ranges) averaged 118 (102-162), 193 (132-262), 203 (118-305) and 143(94-185) microns for the tests at Poso West, Breckenridge, John Dale (Aug.) and John Dale (Sept.), respectively.

Average mortalities of Br80 and wild sentinels exposed at CO_2-trap standards differed less than three percent at all test locations. Pooled results of Br80 and wild sentinels showed a fairly consistent pattern among the three groups of sentinels, with the highest overall mortalities occurring at exposed transects (98%), followed by trap standard (89%) and sequestered sentinels (58%). Average mortalities for exposed transect sentinels (Br80 and wild) ranged from 93% at Breckenridge to 100% at John Dale (Sept.), indicating good coverage of the spray zone. Mortalities of sentinels exposed at CO_2-trap standards (Br80 and wild) ranged from a mean of 82% at John Dale (Aug.) to 98% at Poso West (only completely exposed trap standards were counted here; the remainder were situated under heavy canopy and were counted as sequestered). Mortalities ranged from 52% at Poso West to 63% at John Dale in September for sentinels sequestered under canopy. Comparison sentinel mortalities averaged 9,3,9 and 4 percent for the tests at Poso, Breckenridge, John Dale (Aug.), and John Dale (Sept.), respectively.

DISCUSSION.-The variability of susceptibility to organophosphate insecticides in Kern County renders these compounds unreliable for the emergency suppression of adult *Cx. tarsalis* populations in an epidemic situation. The results of bioassays presented in this paper represent average $LD_{90:50}$ ratios of 14.3 for chlorpyrifos (n=5) and 19.8 for malathion (n=2), indicating considerable heterogeneity of response to these compounds. This ratio for Baygon was 1.5, and mortality with this compound at the LD_{90} level varied no more than 1.4 times for the populations tested.

The successive decline in average mortality between transect, trap station, and sequestered sentinels was attributed to the amount of vegetative canopy protecting each group. Overall mortalities of field sentinels indicated that the target populations were highly susceptible to the labeled rates of Baygon when directly exposed to spray particles. These results sharply contrasted those of Yoshimura et al. (1983) who reported average mortalities of 45% at the same CO_2- trap stations and 22% among sequestered sentinels when JDW *Cx. tarsalis* were exposed to Dursban applied at the labeled rate; simultaneously exposed Br80 sentinels showed 91% mortality at the trap standards. Laboratory bioassays by Yoshimura et al. (1983) indicated the JDW population contained a mixture of homozygous susceptible, heterozygous and homozygous resistant genotypes. Both 1982 (Yoshimura et al. 1983) and 1983 (Reisen et al. 1984) studies indicated that laboratory adult bioassays using the filter paper method were predictive of the response of the target population under field conditions.

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A MANAGER'S VIEW OF THE NEED FOR AN EFFECTIVE AREA-WIDE ADULTICIDING TECHNIQUE

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In an area that has financial limitations and 155,000 acres of rice, an effective as well as economical means of mosquito control is necessary to thwart the threat of the arbovirus diseases, western equine and St. Louis encephalitis and to prevent secondary infections of malaria, let alone reduce the mere annoyance of mosquitoes.

Since 1974, the Sutter-Yuba Mosquito Abatement District has had 307 cases of malaria diagnosed, four of which are presumed to have been secondary cases. Each case prompts three spray treatments. In a rural environment the acreage treated each time is 1280 to 2560. In urban areas the acreage is 1/16 the size of the rural setting.

All suspected cases of encephalitis, though rare in the last 20 years, are treated in a like manner.

Often the habitats to be sprayed are not of the type that are the easiest to spray; like a rice field, which with a wide open expanse usually has no significant objects that will deflect the volume of air that is carrying the insecticide. However, young orchards may have some deflection characteristics. Often the kill will extend for ¼ mile or more. In our area there are few orchards that are planted greater than ¼ mile square, and if the plantings are larger, service roads usually bisect the area to be treated.

Old peach orchards which are common in the District provide a very difficult environment to treat. The canopy provides a "roof" and the lower limbs with leaves cause great deflection. Often pesticides only penetrate a few yards (0-25) particularly when the wind velocity is less than one mile per hour.

The ultimate in dense habitat in the District is what is locally known as "the jungle". This is a riparian area in designed flood-plains. It is several miles long with no cross roads and only 100 to 200 yards wide. Since the wind parallels the long axis, even with the best wind conditions the control is not good.

The rice field habitat, though expensive, can be sprayed by aircraft or ground ULV techniques, but urban-suburban, old orchard, and jungle areas can not be effectively and/or economically sprayed by our presently known techniques, hence the need for a better method to control adult mosquitoes that are vectors of disease.

ORGANOPHOSPHATE INSECTICIDE RESISTANCE IN MOSQUITO POPULATIONS IN SUTTER AND YUBA COUNTIES, CALIFORNIA

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INTRODUCTION.-Organophosphate insecticide resistance levels were monitored for populations of adult *Culex tarsalis* Coquillett and *Anopheles freeborni* Aitken collected in Sutter and Yuba Counties, during the summer and fall, 1983. These 2 mosquito species are of concern in control efforts not only because of abundance but disease transmission potential as well (Reeves et al. 1981). Other works concerning the susceptibility of mosquitoes to insecticides in California have largely dealt with larvae (Gillies 1964 and Zboray and Gutierrez 1979). Some tests have been done with adult populations (Reeves et al. 1982 and Yoshimura et al. 1983), but none have been reported representing the areas of concern to the Sutter-Yuba Mosquito Abatement District.

Much of the control efforts are aimed at adult populations of *Cx. tarsalis* and *An. freeborni*, which are sprayed in the late evening or early morning hours. Primarily, malathion is used and is applied by nonthermal aerosol generators. This method has been used for approximately 15 years, and was brought about by economic reasons and the high levels of resistance encountered in larval populations (Gillies et al. 1974). It was felt that tests to survey the present status of insecticide resistance in adult populations of concern were necessary in order to enhance control strategies and hopefully avoid the development of high levels of resistance, as was the case with larval populations 10 years earlier.

MATERIALS AND METHODS.-Mosquito collections. Collection sites for *Cx. tarsalis* and *An. freeborni* were located in areas that had been sprayed frequently by the District for adult mosquito control and had been sprayed by others for agricultural purposes. The area was divided into quadrants to aid in the grouping of data in relation to spraying activities (Fig. 1). Collection sites were designated as being in area number 1, 2, 3 or 4. Most sites were near rice fields, or in areas known to produce large enough numbers of mosquitoes for trapping purposes.

Adult *Cx. tarsalis* were collected using miniature CDC light traps baited with dry ice. Traps were put out in the evening and were picked up the following morning. Non-collapsible light trap bags made from ice cream cartons and stocking-net material were used. Collected mosquitoes were allowed to emerge from the trap bags into 1 $ft.^3$ aluminum screen cages in order to minimize injury from handling. *An. freeborni* adults were collected using both CDC light traps and mechanical aspirators. Mosquitoes were usually tested within 2 or 3 hours after trap retrieval, but if tests were delayed, cotton pads soaked in 10% sugar water or water-soaked raisins were provided as a carbohydrate source.

Mosquitoes tested. Tests were performed on 2-5 day old colonized *Culex pipiens* L. adults, colonized *Cx. tarsalis* adults (Br80 Colony) and field collected *Cx. tarsalis* and *An. freeborni*. Ages of field collected mosquitoes were not determined. Rearing procedures for the *Cx. pipiens* colony are described by Gerberg et al. 1969 and Petersen and Willis 1972. The *Cx. pipiens* colony was originally established in this country ca. 20 years ago, and is of indeterminate origin. It is maintained in order to facilitate the laboratory rearing of the mermithid nematode, *Romanomermis culicivorax*. The Br80 Colony was first established in 1980 (Reisen et al. 1981) in Kern County, California. Br80 Colony *Cx. tarsalis* and most of the *An. freeborni* collections for tests conducted herein were supplied by University of California, Davis, personnel, in conjunction with another study.

Bioassay methodology. All mosquitoes were tested to determine insecticide resistance levels using a bioassy method described by Georghiou and Metcalf (1961) and Georghiou and Gidden (1965). Insecticides used were high purity technical grade samples of malathion (Cythion®) and chlorpyrifos (Dursban®). Insecticides were dissolved in acetone that had been dehydrated with anhydrous sodium sulfate crystals. A range of 4 or 5 insecticide concentrations was used to determine LC values. Insecticides were applied at the rate of 2 ml per filter paper (Whatman glass microfibre filters, GF/A, 9.0 cm diam.). Three (3) to 4 papers per concentration were treated. Control papers were treated with acetone only.

Filter papers were allowed to dry for ca. 5 minutes and were then rolled and placed inside 2.5 x 9.5 cm glass shell vials. Adult mosquitoes were removed from the screen cages using a mechanical aspirator and anesthetized using carbon dioxide. The anesthetized mosquitoes were then placed on a portable chill table (kept at 10-15°C) and transferred to vials containing insecticide-treated filter papers. Between 10 and 20 mosquitoes were placed in each vial, depending on the number collected. All tests were performed using adult female mosquitoes except 2 tests with the *Cx. pipiens* colony, in which both males and females were used. Each vial was capped with a piece of nylon netting held in place with a rubber band. After an exposure period of 1 hour in the vials, the test insects were transferred to clean holding cups made from 0.5 pt. paper cans with clear plastic lids. A piece of cotton soaked in 10% sugar water was stapled on the inside sur-

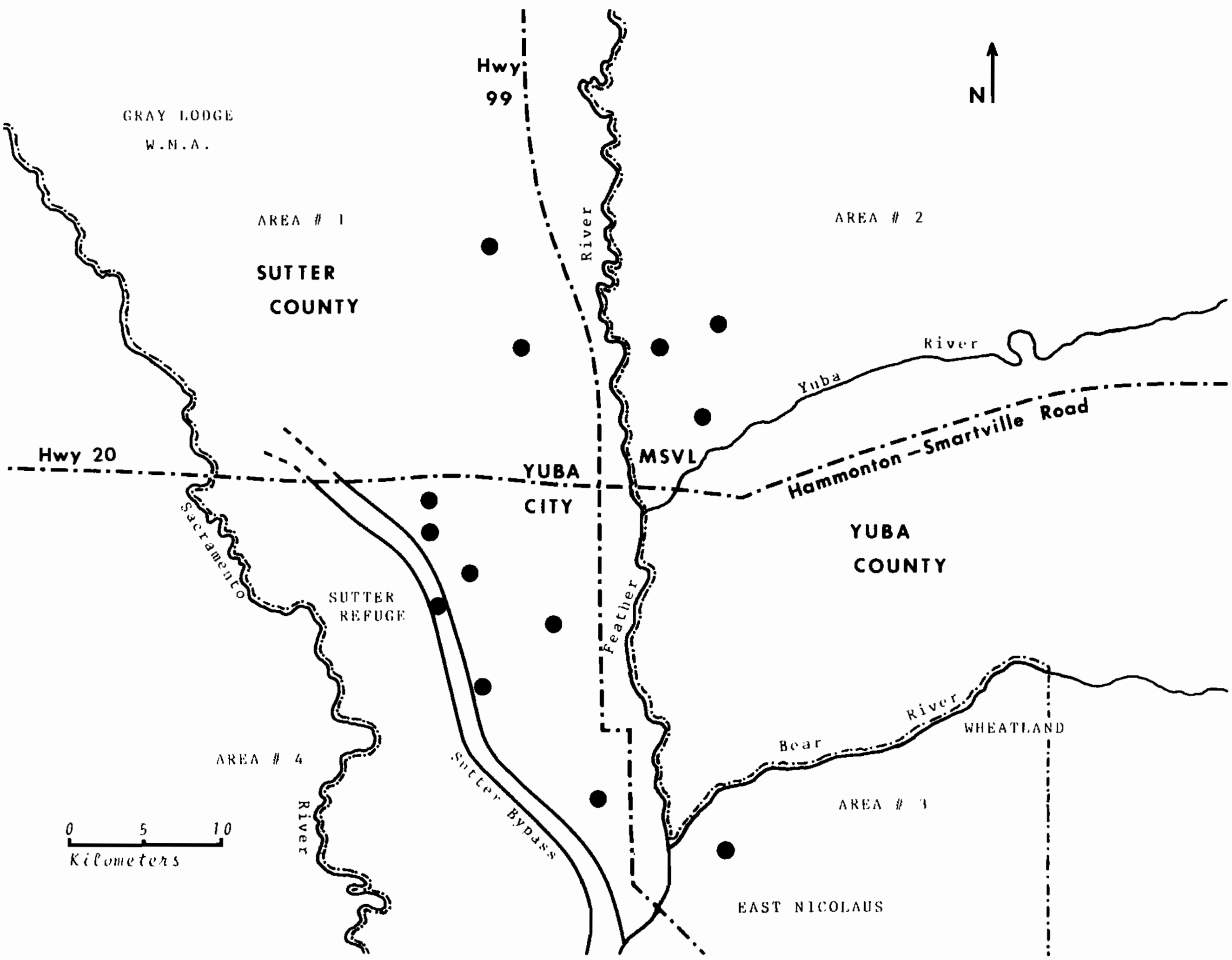

Figure 1.-Map showing distribution of collection sites and division of area into quadrants.

face of each lid. Holding cups were placed in a darkened room at room temperature and remained there for a period of 24 hours. After 24 hours, the number dead was recorded for each cup. Mortality data were analysed using probit analysis (SAS 1979) to obtain LC values and slope estimates. Tests having control mortalities greater than 10% were considered invalid and were not included in this report.

RESULTS AND DISCUSSION.-Bioassays. Insecticide susceptibility levels for the *Cx. pipiens* colony against malathion and chlorpyrifos are reported in Table 1. Colony *Cx. pipiens* were more susceptible to chlorpyrifos than to malathion, as was the case with all groups tested. Dosage mortality lines were steep, with high slope estimates, indicating the homogeneous manner in which the colony responded to malathion and chlorpyrifos.

Susceptibility data for *Cx. tarsalis* against malathion are given in Table 2. LC_{50}, LC_{90} and LC_{95} values for *Cx. tarsalis* were considerably higher than colony *Cx. tarsalis* (Br80), as indicated by resistance ratios. Slope estimates were much lower, indicating heterogeneous populations comprised of many resistant or tolerant individuals. When LC_{50} values were compared to light trap catch numbers, a positive correlation was found to exist between the 2 sets of data, denoting a seasonality of response to malathion (Fig. 2). As the number of mosquitoes increased or decreased, so did LC_{50} values. This was possibly due in part to physiological changes in the mosquitoes, and apparently occurred independent of insecticide selection pressure from spraying activities. This did not appear to be the case with *An. freeborni* populations tested. Spearman rank-correlation coefficients showed that a positive correlation existed for *Cx. tarsalis* but not for *An. freeborni* (α = .05). Future data sets will be analysed in the same manner in order to detect trends useful for the enhancement of mosquito control tactics.

Table 3 shows the susceptibility levels of *Cx. tarsalis* to chlorpyrifos. The levels are very

Table 1.-Baseline susceptibility mortality rates for the Sutter-Yuba Mosquito Abatement District colony of *Cx. pipiens* adults exposed to malathion and chlorpyrifos on glass fiber filter papers-summer, 1983.

Test Date	Insecticide	Males or Females	Control Mortality (%)	LC values in μg/cm² LC_{50}	LC_{90}	LC_{95}	Slope
July 11	malathion	Both	1.7	3.5	7.2	8.8	1.8
July 18	"	Both	1.7	5.9	9.6	11.1	2.6
Aug. 18	"	Females	0.0	4.2	9.2	11.5	1.6
Sept. 30	"	Females	0.0	4.0	5.5	6.0	4.1
Aug. 17	chlorpyrifos	Females	1.7	.05	.12	.16	1.5
Sept. 29	"	Females	0.0	.05	.11	.14	1.5

Table 2.-Susceptibility of *Cx. tarsalis* adults collected[1] in Sutter and Yuba Counties, CA, exposed to malathion on glass fiber filter papers-summer and fall, 1983.

Collection Site	Area	Test Date	Control Mortality (%)	LC values in μg/cm² LC_{50}	LC_{90}	LC_{95}	Slope	Resistance[2] Ratio LC_{50}	LC_{90}
Sheppard	1	Aug. 3	0.0	16.8	2073.9	8118.3	.27	2.80	162.02
Sheppard	1	Sept. 22	0.0	7.8	162.6	385.0	.42	1.26	5.53
Gollenbusch	1	Sept. 8	0.0	20.7	229.5	454.0	.53	3.34	7.81
Vickrey	2	June 23	5.1	3.7	48.9	101.5	.49	.62	3.82
Vickrey	2	July 20	2.5	14.2	179.9	369.6	.50	2.37	14.05
Hill & P. V. Ranch	2	Aug. 3	0.0	25.7	1670.1	5451.9	.31	4.28	130.48
Hill & P. V. Ranch	2	Sept. 20	10.0	4.1	364.0	1301.1	.28	.66	12.38
Barker	3	July 12	5.0	20.8	76.2	110.1	.98	3.47	5.95
Dean	4	July 7	10.0	8.0	198.8	494.4	.39	1.33	15.53
Dean	4	July 26	1.7	30.4	577.3	1329.8	.44	5.07	45.10
Crowhurst	4	July 7	6.7	15.6	110.3	191.9	.66	2.60	8.62
Crowhurst	4	July 27	0.0	40.7	339.6	619.8	.60	6.78	26.53
Crowhurst	4	Aug. 18	0.0	3.4	238.7	795.3	.30	.55	8.12
Crowhurst	4	Sept. 13	10.0	19.8	2111.6	7931.6	.27	3.19	71.82
Melani	4	July 12	0.0	12.7	59.1	91.3	.83	2.12	4.62
Melani	4	Sept. 22	3.3	10.2	501.0	1509.3	.33	1.65	17.04
Sutter Refuge	4	July 26	0.0	33.9	309.4	578.9	.58	5.65	24.17
Sutter Refuge	4	Aug. 11	3.3	59.5	792.2	1650.5	.49	9.92	61.89
Sutter Refuge	4	Sept. 1	0.0	86.5	570.3	973.5	.68	13.95	19.40
Amarel	4	Sept. 8	0.0	10.4	234.9	568.2	.41	1.68	7.99
Brubeck	4	Sept. 15	10.0	7.0	962.0	3890.5	.26	1.13	32.72
Br80[3]		July 26	0.0	6.0	12.8	15.9	1.70		
Br80		Sept. 1	10.0	6.2	29.4	45.7	.82		

[1] Using CDC miniature light traps baited with dry ice.
[2] Resistance Ratio= $\frac{LC_x \text{ wild population}}{LC_x \text{ susceptible pop.}}$

[3] *Cx. tarsalis* colony, University of CA, Davis, established 1980, Kern County, CA.

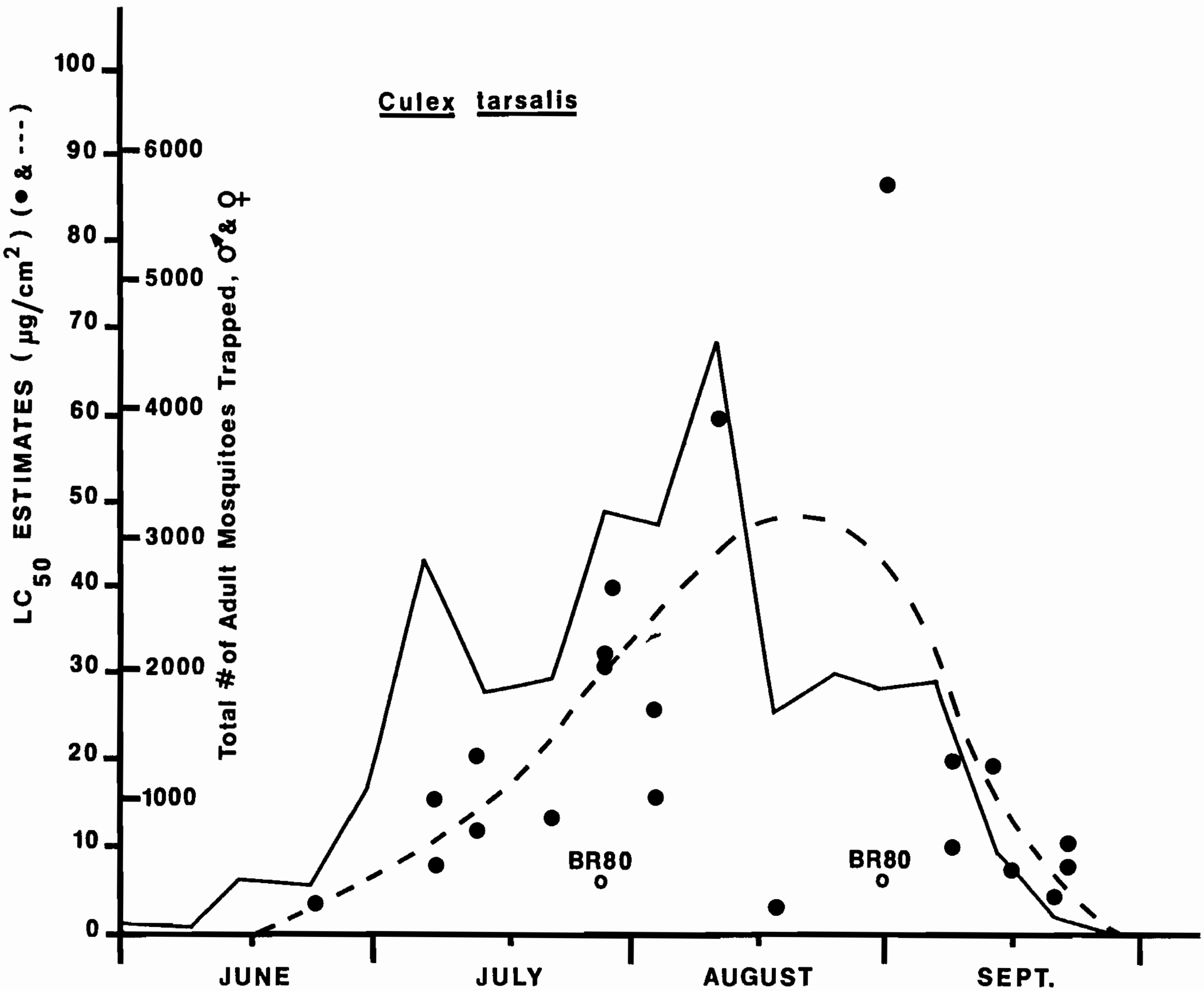

Figure 2.-Comparsion of LC_{50} values (malathion against *Cx. tarsalis*) and light trap catch numbers, by month.

Table 3.-Susceptibility of female *Cx. tarsalis* adults collected in Sutter and Yuba Counties, CA, exposed to chlorpyrifos on glass fiber filter papers-summer and fall, 1983.

Test Date	Collection Site	Area	Control Mortality (%)	LC values in µg/cm² LC_{50}	LC_{90}	LC_{95}	Slope
Aug. 17	Sutter Refuge	4	0.0	.11	.31	.41	1.30
Sept. 22	Melani	"	0.0	.01	.12	.25	.52

Table 4.-Susceptibility of female *An. freeborni* adults in Sutter County, CA, exposed to malathion and chlorpyrifos on glass fiber filter papers-summer, 1983.

Test Date	Collection Site	Area	Insecticide	Control Mortality (%)	LC values in µg/cm² LC_{50}	LC_{90}	LC_{95}	Slope
July 25	Sutter Refuge	4	malathion	0.0	7.2	12.6	14.7	2.3
July 26	"	"	"	1.7	5.8	8.3	9.2	3.6
Aug. 11	"	"	"	3.3	6.9	8.5	9.1	5.9
Aug. 18	"	"	"	2.2	5.3	6.8	7.4	5.0
Sept. 1	"	"	"	0.0	4.8	6.4	6.9	4.6
Sept. 1	"	"	"	0.0	4.1	5.3	5.7	5.1
Aug. 17	Sutter Refuge	4	chorpyrifos	3.3	.11	.14	.15	5.8
Aug. 31	Vanderford Airstrip	"	"	0.0	.14	.28	.34	1.9

Table 5.-Approximations of amount of insecticide applied (malathion, pounds of actual toxicant) in the vicinity of collection sites, Sutter and Yuba Counties, CA, over a four year period for mosquito control.

Area #	1980	1981	1982	1983	Totals (four years)
1	1396.8	1568.0	436.0	1004.8	4405.6
2	535.7	1751.7	381.8	839.8	3509.0
3	9.4	46.5	0.0	113.9	169.8
4	45.6	1214.6	1055.4	880.7	3196.3
Yearly Totals	1987.5	4580.8	1873.2	2839.2	

low, with relatively large slope estimates. No tests were performed using Br80 *Cx. tarsalis* against chlorpyrifos. Most of the adulticiding in the District is carried out using malathion, which may explain the lack of tolerance to chlorpyrifos.

Susceptibility levels for *An. freeborni* against malathion and chlorpyrifos are reported in Table 4. Again, *An. freeborni* were much more susceptible to chlorpyrifos than malathion and were similar to the *Cx. pipiens* colony in their level of response to insecticides. LC values did not rise and fall in relation to light trap catch numbers, but showed a steady decrease through the summer and fall, independent of population fluctuations (discussed above).

Spraying activites. In Table 5, the amounts of malathion used by year and over a 4 year period are shown. The heaviest spraying occurred in 1981. Over a 4 year period, area 1 was sprayed the most, followed by areas 2 and 4. Area 3 required the least amount of spraying for adult control. Sites providing highly tolerant *Cx. tarsalis* were located in areas 1, 2 and 4. Mosquitoes sampled from area 3 proved to be much more susceptible to malathion, but more tests are needed to clearly establish a trend in that area.

Along with the amounts of malathion used by the District, the effect of agricultural pesticides probably played a significant role in the development of resistance. In the past, malathion was widely used as an agricultural pesticide, but is not used to any great extent in the 2 county area today (Bagley, 1984). Other factors such as immigration or dispersal of mosquito populations sampled contributed to the difficulty in evaluating the effect of spraying activites.

During the 4 year period analysed, the heaviest spraying in the District occurred in the months of August and September, with most spray activities ending by October. An exception in the records took place in 1981, when the heaviest spraying occurred in July, gradually decreasing and ending in November. Much of the late season spraying was directed towards *An. freeborni* populations with spraying for *Cx. tarsalis* taking place earlier in the season. Clearly, the *An. freeborni* populations tested were exposed to the largest amount of malathion via spray activities, yet showed little or no tolerance to the chemical. On the other hand, *Cx. tarsalis* populations were highly tolerant. This may indicate the possible influence of other sources of pesticides and chemicals on the insecticide resistance levels of *Cx. tarsalis*.

The importance of monitoring resistance levels in populations of mosquitoes, especially those of public health importance, has been well documented. In addition, awareness of the occurrence of resistance levels in problem mosquito species may help determine control tactics. At this District, the monitoring of resistance levels in populations of *Cx. tarsalis*, *An. freeborni* and other mosquito species will be continued using the filter paper bioassay and field cage tests to further determine control failures in the field. Tests involving other insecticides besides malathion and chlorpyrifos will be performed, and resistance levels will be compared to other variables such as spray activities and the seasonality of response to insecticides.

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THE IMPACT OF LOW VOLUME AERIAL APPLICATIONS OF PROPOXUR ON THE RELATIVE ABUNDANCE AND AGE STRUCTURE OF *CULEX TARSALIS* POPULATIONS IN KERN COUNTY, CALIFORNIA, DURING 1983

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In 1982, the Arbovirus Research Unit of the School of Public Health, University of California, Berkeley, and the Kern Mosquito Abatement District initiated collaborative research to evaluate the impact of aerial applications of adulticides on *Culex tarsalis* Coq. Results indicated that adult relative abundance could be reduced significantly if the target population was susceptible and exposed to the spray (Yoshimura et al. 1983), and the population addition rate was relatively low (Reeves et al. 1983). However, experimental adult suppression on the San Joaquin Valley floor in 1982 was limited seriously by resistance to chlorpyrifos, resmethrin, and perhaps bendiocarb (Yoshimura et al. 1983). With the insecticide arsenal depleted, evaluations in 1983 emphasized the use of a low volume formulation of propoxur for the four planned experimental applications which included: a late afternoon spray of a foothill site by fixed wing aircraft, late afternoon sprays of a foothill and a valley site by an experimental underslung helicopter unit, and an early morning spray of the same valley site using fixed wing aircraft.

MATERIALS AND METHODS.-**Insecticide application.** Baygon 70% wettable powder was suspended in Witco-Golden Bear larvicide oil and sprayed at the rate of 0.5 gal/acre. Fixed wing applications were made by the Kern Mosquito Abatement District's Ayres Thrush Commander, while helicopter applications were made by the U.S. Army using the Simplex Army Bucket Sprayer suspended beneath a Bell UH-1 helicopter. Efficacy of the Baygon to females from the target population and adults from a reference laboratory strain was described under both laboratory and field conditions by Parman et al. (1984).

Evaluation. Adulticide applications were evaluated using the 23 day protocol developed during 1982 (Reeves et al. 1983) with spraying done on days 9, 12, and 15. Host-seeking females were sampled by 16 or more CDC miniature light traps augmented with 1-2 kg of dry ice (CO_2T). Traps were arranged along N-S and E-W intersecting transects, with four comparison traps positioned one or more miles outside of the border of the spray zone and 12 traps positioned within the spray zone. Additional host-seeking females were collected at the valley site by a CO_2-baited time segregated sampler (TSS) which separated nightly catches into hourly samples (Meyer et al. 1984). Suppression of host-seeking female abundance to a threshold value of less than 30 females per trap night was considered necessary to interrupt virus transmission (Reeves et al. 1983).

Sentinel mosquitoes from a reference laboratory strain (Br80) and from the target population were positioned at each light trap standard to bioassay spray coverage and document kill under field conditions. In addition, adult mosquitoes were dusted cohort-specific fluorescent colors and were released in the center of the spray zone four and one days before the first spray. Marked mosquitoes were considered to be unrestrained sentinels and control was implied from recapture patterns following the two releases. When available, distinctively marked females were released at comparison standards to study patterns of infiltration into the central spray zone.

Resting mosquitoes were collected from small and/or large walk-in red boxes concurrently with light trap pick-up. At Poso West, additional resting adults were collected from eight pipe traps, and egressing adults were taken in ten cone traps placed over the entrances to rodent burrows.

The reproductive age of the target population was monitored by determing the parity status of 10 host-seeking females from each of four central spray zone and four comparison zone traps and 40 resting females collected from shelters in the spray and comparison zones.

Percent population reduction was calculated using Mulla's formula (Mulla et al. 1971) which compares changes in mosquito abundance within the spray zone pre- and post-treatment to concurrent changes in abundance within the comparsion zone.

Areas sprayed. Two areas semi-isolated by surrounding arid grassland in the Sierra-Nevada foothills and one site on the floor of the San Joaquin Valley were sprayed in Kern County, California, during 1983.

Mosquito populations at the Poso West foothill site developed in rivulets created by oil field effluent. A total of 800 acres including the riparian Poso Creek habitat was treated by fixed wing aircraft during late afternoon on 17, 20 and 23 June. Trap placement was modified by access and a comparison trap could not be positioned to the east. Additional CO_2-traps were placed in the spray zone along Poso Creek and at McVans, an ecologically similar, but unsprayed, comparison area located 5 km to the north of the spray zone.

The second foothill site, Breckenridge Road, consists of three relatively parallel canyons. Oil field waste-water disposed by percolation in ponds and by sprinkling on the canyon walls creates an environment suitable for the production of a large *Cx. tarsalis* population. The 60 acre central canyon was sprayed by helicopter during late afternoon on 22, 25, and 28 July. Trap placement at Breckenridge was modified by the hilly terrain with eight traps positioned in the sprayed central canyon and three traps placed in each of the two unsprayed comparison canyons.

The John Dale Ranch study area is a mixed agricultural habitat typical of the southern San Joaquin Valley floor and was selected for study because it has a consistent pattern of mosquito abundance and virus activity. The heavily vegetated Mosesian's duck club in the center of the study area served as a settlement area for mosquitoes that immigrate from peripheral breeding sites. An 800 acre area was sprayed by helicopter during late afternoon on 16, 19 and 22 August. Four comparison traps were positioned within 2.5 km from the spray zone. Additional mosquito collections were made by TSS's situated at Mosesian's duck club and Costerisan's farm headquarters, 4 km to the NW. A comparison "walk-in" red box was placed at Costerisan's.

The presence of fodder crops approaching harvestable stage forced us to shift the John Dale spray zone 1.6 km to the E for the early morning fixed-wing spray. The new spray zone encompassed the Los Pobrecitos gun club which consisted of reclaimed and irrigated pasture. An 0.8 km extension to the west included 160 acres from the previous spray zone. The flooding of Mosesian's and Los Pobrecitos duck clubs prior to the start of hunting season led to mosquito breeding within the spray zone. A total of 950 acres was sprayed just after sunrise on the mornings of 13, 17 and 20 September.

RESULTS.-**Fixed wing, afternoon spray at Poso West.** Sentinel mortality at standards within the spray zone remained greater than 95% indicating good coverage during each spray. Recapture rates among three cohorts released in the center of the spray zone decreased markedly after each spray indicating good kill within the spray zone.

The number of females resting in red boxes and pipe traps and egressing from rodent burrows declined significantly after the first spray and remained low during the spray and postspray periods. The relative abundance of host-seeking females within the spray zone decreased from a mean of 90 *Cx. tarsalis* females per trap night to 24 during the spray period. Relative abundance remained below 30 females per trap night at traps 1-4 during the spray period, but again exceeded this threshold value by 30 June, six days postspray. The relative abundance of females at traps within the spray zone was correlated significantly with female abundance at traps within the comparison zone ($r = 0.67$, $n = 14$), but not at the more distant McVans comparison area ($r = 0.45$, $n = 7$). The percent reduction of host-seeking females at sprayed trap sites based on comparison traps was only 35%, while the percent reduction based on McVans traps was a more realistic 89%. It appeared that a significant reduction of the population in the spray zone also caused a concurrent reduction of abundance in the adjacent unsprayed comparison zone.

Adulticiding reduced the parity rate of host-seeking females from 44% prespray to 23% postspray. A concurrent decrease in age structure was not observed at comparison traps. The parity rate among resting females was 27% prespray, decreased to 3% postspray, and was still only 9% 2 wks postspray. The parity rate among trapped females recovered more rapidly than the parity rate among resting females. Exposures of field-collected larvae and pupae at one breeding site indicated that the spray did not kill immatures. Thus, the shift in adult age structure was attributed to the elimination of older parous females by the spray and the continuous recruitment of nullipars by emergence within the spray zone.

Helicopter, afternoon spray at Breckenridge. The kill of sentinel females from the target population averaged 83% at light trap standards in the spray zone. Mortality was low at some peripheral standards that were poorly sprayed. Mortality at standards in the unsprayed canyons was 3%, indicating that the spray did not drift out of the central canyon. Recapture rates among released females declined after the initial spray. In addition to the adults released in the center of the sprayed canyon, ca. 12,000 females were released in each of the two comparison canyons concurrent with release 2. Recapture patterns among marked dispersives indicated considerable mixing among the populations of all three canyons. The recapture rate of emigrants from the central canyon declined markedly following the first spray.

The relative abundance of females in the sprayed canyon decreased significantly from 620 females per red box collection and 1,864 females per CO_2 trap night prespray to 87 and 157, respectively, during the spray period. The relative abundance of females trapped in unsprayed canyons to the south and north concurrently decreased in abundance and was correlated over time with abundance in the sprayed canyon, $r = 0.83$ and $r = 0.95$, respectively. The percent reduction in the central canyon was estimated to be 73% for both resting and host-seeking females using female abundance in the southern canyon for comparisons. A more marked reduction of 97% was estimated for the less dispersive resting males. However, postspray abundance in the sprayed canyon remained well above the threshold of 30 females per trap night considered to be necessary for virus transmission. Persistent abundance was attributed to a high rate of recruitment by emergence and immigration from the unsprayed canyons.

Parity rates among resting and host-seeking females in the sprayed canyon were 36 and 26% prespray and 4 and 1% postspray, respectively. The virtual elimination of older, parous females indicated that infected mosquitoes would be removed and transmission possibly interrupted even though relative abundance remained above the desired threshold level.

Helicopter, afternoon spray at John Dale. Sentinel mortality was more than 80% within the spray zone and less than 25% in the comparison zone. Survival within the spray zone was limited to sites protected by vegetative canopy. Sentinel mortality at comparison and some spray standards was caused by a dust/wind storm on 16 August. The effectiveness of the spray was confirmed by a decline in the recapture rate of marked mosquitoes released 4 and 1 days before spray 1.

Cx. tarsalis female abundance within the spray zone decreased significantly from 243 females per red box collection, 234 females per CO_2 trap night and 310 females per TSS trap night prespray to 38, 81, and 58, respectively, during the spray period. Female abundance at the central sprayed traps never declined below 30 females per trap night. Female abundance at comparison zone traps declined coincidentally with abundance at spray zone traps, r = 0.83. In contrast, female abundance at red boxes and at the TSS at Costerisan's were not correlated over time with abundance within the spray zone, r = 0.31 and -0.13, respectively. Reduction was 44% when sprayed traps were compared to comparison traps, but was 84 and 86% when resting and TSS collections within the spray zone were compared to those at Costerisan's.

Parity rates of females from red boxes, CO_2 traps or TSS collections were not changed by spraying. High parity rates also occurred in females from comparison zone traps as well as from red box and TSS collections at Costerisan's. High parity rates were attributed to elevated autogeny rates observed in females reared from pupae collected from an irrigated pasture (80%) and from sewer farm run-off (82%).

Fixed-wing, early morning spray at John Dale. Coverage was adequate (>90% sentinel mortality) except for the center of Los Pobrecitos on 17 September which was left unsprayed due to the presence of hunters. Good kill within the spray zone also was indicated by the postspray decline in the recapture rate of released mosquitoes. Concurrent with release 2, 3400 to 4400 distinctively marked females were released at each comparison standard. The recapture patterns of dispersives indicated that infiltration into the spray zone occurred mostly downwind from W to E.

Cx. tarsalis relative abundance within the spray zone decreased from 249 females per red box collection, 212 females per CO_2 trap night, and 244 females per TSS night prespray to 140, 109 and 153, respectively, during the spray period. Abundance of host-seeking females collected at traps never was reduced to below the 30 females per trap night considered necessary to maintain virus transmission. Abundance at red boxes, traps and the TSS within the spray zone was not correlated significantly with abundance at comparison zone traps (r = 0.43) and at red boxes (r = -0.17) and the TSS (r = -0.49) at Costerisan's.

Percent reduction of females collected by spray and comparison zone traps, and resting and TSS collections within the spray zone and at Costerisan's were 45, 27 and 75%, respectively. The percent reductions achieved by morning applications by fixed-wing aircraft were less than the percent reductions achieved by afternoon spraying by helicopter.

The parity status of females collected by different methods varied over time, decreasing in red boxes and TSS collections at John Dale and Costerison's, and at comparison zone traps, but increasing at spray zone traps. Decreases in parity were attributed to a general increase in the emergence rate from breeding sites created by duck club flooding and not necessarily to the selective elimination of older females by effective spraying.

DISCUSSION.-Late afternoon aerial applications of Baygon wettable powder in oil significantly suppressed adult relative abundance at both foothill and valley habitats. The central spray zone at all three study areas included favorable adult resting habitat that formed a settlement area for adults emerging from peripheral breeding sites. Suppression of the adult population in the spray zones concurrently reduced host-seeking female abundance at comparison traps positioned up to 2.5 km outside the spray zone, but not at traps positioned 4 or more km away. These findings may begin to delineate the effective spatial relationships among *Cx. tarsalis* populations.

Significant adult suppression by spraying caused a marked shift in the female population age structure at semi-isolated foothill sites. The Baygon formulation did not control immature *Cx. tarsalis*. Adult relative abundance at sprayed traps rebounded to prespray levels four to seven days after the last spray. However, the parity rate among resting females remained unchanged for up to 2 wks postspray at Poso West and Breckenridge. Thus, although adult female abundance recovered rapidly, the resulting population age structure was unfavorable for arbovirus transmission.

Early morning applications of Baygon by fixed wing aircraft did not provide as good control as afternoon treatments by helicopter. Operationally both sprays provided good coverage of the spray zone and achieved good kill among exposed sentinels. *Cx. tarsalis* egress from diurnal resting sites during dusk and thus may have contacted persisting spray droplets or residues shortly after late afternoon applications. In contrast, adults move into diurnal resting sites shortly after sunrise which reduces their chances of contacting spray droplets after an early morning spray. Theoretically, the best control would be expected if applications could be made

1-2 hrs after sunset when *Cx. tarsalis* activity rhythms would expose adults to insecticides. However, applications at this time were impractical by available aircraft.

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AERIAL AND GROUND APPLICATIONS OF MALATHION FOR CONTROL OF ADULT *ANOPHELES FREEBORNI* AND *CULEX TARSALIS* IN SUTTER COUNTY, CALIFORNIA

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INTRODUCTION.-Renewed interest in adulticiding as a mosquito control measure reflects an increasing concern that we do not have effective means to control mosquito-borne disease or pest outbreaks. This concern is further discussed by Reeves (1984). In Sutter County, California, adulticiding with ultra-low volume (ULV) applications of malathion is the method currently used to control *Anopheles freeborni* and prevent secondary transmission of imported malaria, especially in situations involving the large local populations of Punjabi immigrants. In cooperation with the Sutter-Yuba Mosquito Abatement District, evaluations of the efficacy of ground and aerial applications of malathion for control of *An. freeborni* and *Culex tarsalis*, a vector of western equine and St. Louis encephalitis, were conducted in 1982 and 1983.

METHODS.-In 1982, ULV malathion was applied by mosquito abatement ground vehicles over a 40 sq. mi. area southwest of Yuba City in Sutter County, California. The spray area was planted primarily with rice with some row crops and orchards. Applications were made once weekly on Thursday nights from 22 July to 9 September. Road access limited routes of spray vehicles to an interval of ca. 1 sq. mi. The surrounding, nonspray area was used as a comparison zone and was planted with row crops, orchards, and rice. Nine walk-in red boxes (6'x4'x4') were located in the spray area and 5 were placed in the comparison zone. Resting mosquitoes were collected from red boxes once weekly on Tuesday mornings.

Three times during the study, mosquitoes were also collected on Wednesday, Thursday and Friday mornings. Sentinel cages with laboratory-reared and field-collected *An. freeborni* and *Cx. tarsalis* were used during three of the weekly sprays to monitor spray drift and estimate effectiveness at various distances from the spray vehicles. An additional sentinel cage test was conducted in an orchard environment to determine the impact of heavy vegetation on movement of the spray.

In 1983, an evaluation of the Army's helicopter slung pesticide dispersal unit (HSPDU) was conducted in the Sutter National Wildlife Refuge. The 1 square mile spray zone consisted of a central strip of dense, riparian vegetation bordered on either side by habitat suitable for mosquito breeding. The overstory of the forested area was cottonwood (*Populus*) and the understory consisted primarily of willow (*Salix*) and box elder (*Acer negundo*).Rice fields made up the remainder of the spray area, along with fields flooded by the refuge management to provide waterfowl habitats. The area surrounding the spray zone was similar in nature and was used as the nonspray, comparison area.

A total of 20 walk-in red boxes (6'x4'x4') were positioned in the spray zone. Most were placed in, or along, the riparian vegetation, and the remainder were placed in more open areas closer to potential mosquito breeding sites. CO_2--light traps were placed near 12 of the spray-zone red boxes. Four walk-in red boxes and four CO_2-light traps were positioned in the surrounding, comparison area, 1 mile from the border of the spray zone.

Calibration procedure for the HSPDU was similar to that followed in Kern County (Schaefer 1984). The major difference lay in the use of malathion as an 8 lb/gal formulation applied at a dosage rate of 0.5 lb/acre. The HSPDU discharged malathion through two Beecomist nozzles each fitted with a sleeve with 40 micron openings. Based on an assumed swath width of 200 ft. and a ground speed of 57 mph, a discharge rate of 90 fluid oz/min/nozzle was required to give the desired dosage rate of 0.5 lb/acre. A ground calibration determined that 60 psi was adequate to give the required discharge rate.

Field calibration was conducted using a 400 ft. transect with sampling stations at 50 ft. intervals. Organophosphorus susceptible mosquitoes in sentinel cages and oil sensitive papers were placed at each station. At four of the sampling stations, silicone coated slides on rotating spinners were used to collect data on droplet size. On each of the four calibration runs, the helicopter flight path was perpendicular to the test line, crossing the center of the line at an altitude of 50 feet. Sentinel mosquitoes showed high mortality over at least 200 ft. on each of the test runs, and 200 was used as the operational swath width for subsequent field applications. Droplet sizes were slightly larger than expected, with many droplets well over 100 microns.

During 2 of the applications in the study site, an 800 ft. transect with sentinel cages every 25 ft. was established to monitor spray coverage. Mortality among sentinel mosquitoes was generally high, but there were some gaps in coverage. These gaps may have resulted from the helicopter straying slightly off course during spray runs. Flagmen for the helicopter were located only at either end of the 1 mi runs. Because mortality of caged mosquitoes is generally higher than mortality of the wild population, it is likely that even in the open areas in which the transect was located a portion of wild mosquitoes were not killed.

Two trials of the HSPDU were conducted using the 23 day protocol developed by Reeves et al. (1983). The first trial included evening sprays on 2, 5, and 8 August. The second trial included dawn sprays on 26 and 29 August; a third spray was cancelled due to technical problems.

Sentinel cages were used during each spray to monitor spray coverage and penetration of the spray into the dense, riparian vegetation.

Over 35,000 *An. freeborni* dusted with various colors of fluorescent pigment were released in and around the spray zone to monitor survivorship and movement of mosquitoes. Mosquitoes were released at sunset in the center of the spray zone on 29 July, 1 August, 22 August and 25 August. A release in the nonspray, comparison area was made on 25 August. Mosquitoes collected from red boxes and light traps on subsequent days were checked under a UV light for the presence of marked individuals.

Laboratory bioassays using the treated filter paper technique of Georghiou and Gidden (1965) were used to monitor insecticide susceptibility. *An. freeborni* and *Cx. tarsalis* from the spray zone were tested before spraying began, between trials, and at the conclusion of all spraying.

All mosquito collections were returned to the lab for processing. Samples were first checked under a UV light for the presence of individuals marked with fluorescent dust. Mosquitoes were then identified as to species and sex, and the trophic states of all females were noted. Periodically, a subsample of female *An. freeborni* were dissected using the technique of Polovodova (Detinova 1962) to determine the gonotrophic age structure of the population. Midguts of dissected mosquitoes which were freshly blood-engorged were frozen and saved for later analysis of host feeding patterns.

RESULTS.-1982 - Ground application. Abundance of *An. freeborni* and *Cx. tarsalis* is summarized in Figures 1 and 2, respectively. The ratio of mosquitoes collected in the spray and the comparison areas was used as an indicator of mosquito abundance. A drop in this ratio or control index (CI) indicated a relative decrease in the spray zone population. The relative abundance of *An. freeborni* in the spray zone showed a decline, but only after four weekly applications of malathion. From mid-August on, the spray zone continued to decline for the remainder of the study. The impact of the spray on the *Cx. tarsalis* was apparently insignificant. The population levels in the spray and comparison zones were erratic, but, in general, the relative number in the spray zone increased over the length of the study.

Sentinel cage results suggested that *Cx. tarsalis* was relatively resistant to malathion. Mortality of both *Anopheles* and *Culex* was high in sentinel cages near the routes of the spray vehicles and decreased as distance from the spray increased. Within 0.1 mi. all sentinel *An. freeborni* were killed; at 0.5 mi. about 90% of the sentinels died. Mortality of *Culex* decreased rapidly, however, and at 0.5 mi only 10-15% of the sentinel *Cx. tarsalis* were killed.

Parity of the *An. freeborni* ranged from 0 to 43%, but was generally about 10-25%. On five of the days that parity determinations were made, there was a significant difference between parity rates in the spray and the comparison zones. On four of these occasions, parity was significantly higher in the spray zone. Only once was there a significantly higher parity rate in the nonspray comparison zone.

The orchard sentinel test indicated that the ground applied malathion was unable to penetrate dense vegetation. In the young orchard (ca. 5% canopy cover), mortality occurred at all distances tested. Mortality was highest near the spray route (i.e., over 90 percent to 100 meters) and decreased as distance from the spray route increased. At 400 meters, the longest distance tested, mortality averaged 35 percent among the three test cages.

Mortality in the old orchard that had ca. 70% cover with extensive branching was negligible in the cages closest to the spray (i.e., average 5 percent at 10 meters), and all mosquitoes survived at all the other distances tested. The absence of mortality in the old orchard suggests that, under the conditions of the test, the orchard foliage prevents penetration of the spray. The orchard apparently encloses an envelope of stagnant air with little mixing with the outside environment.

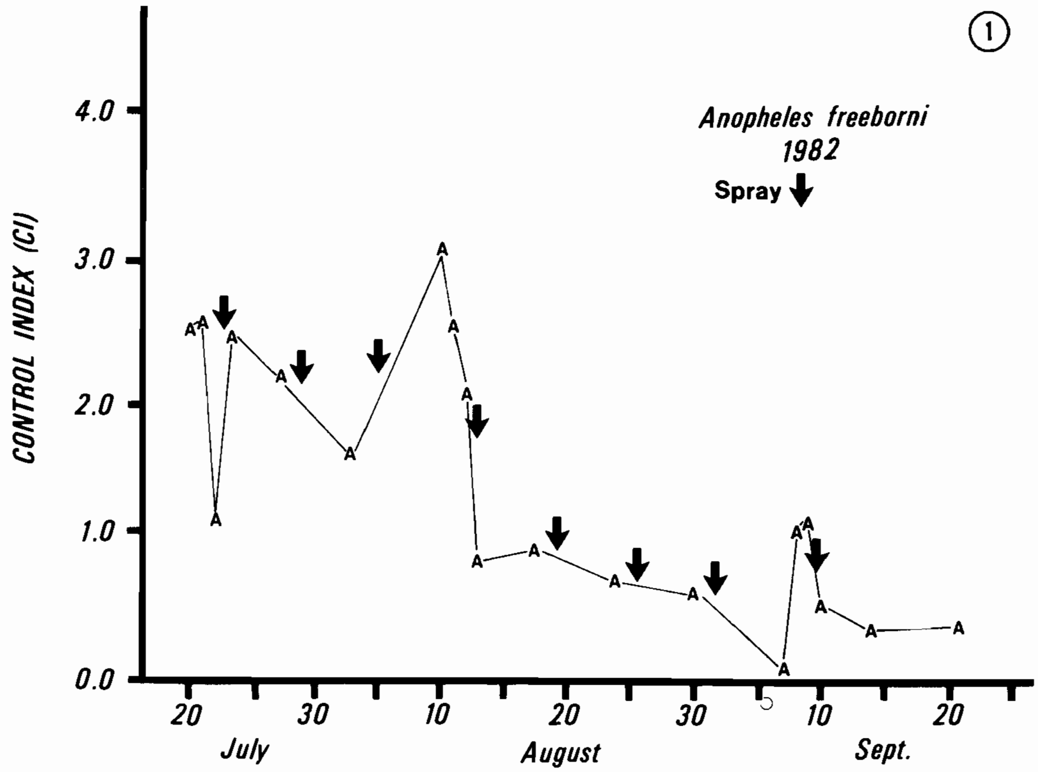
The fate of the ultra-low-volume (ULV) fog in the old orchard test was not determined. It may have settled in the road cut at the point of application or turbulent flow may have pulled the spray up and over the tops of the trees or forced it along the road and out to the sides of the orchard.

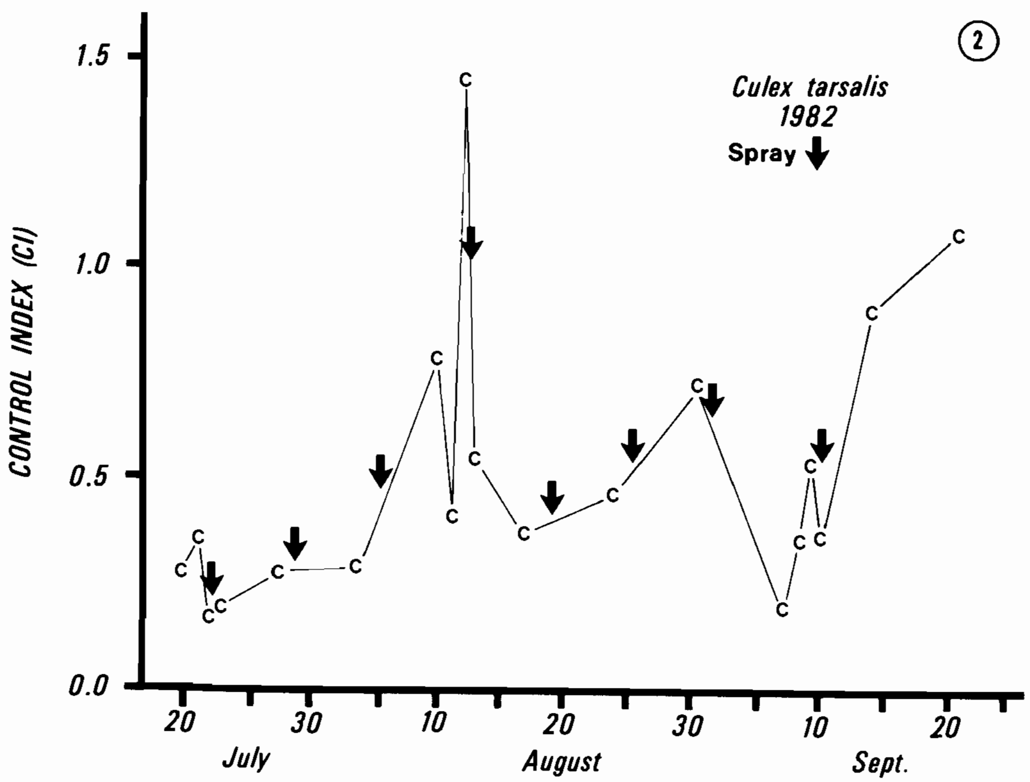
Wind speed during the test was extremely light (0 to 1 mph); an increase could significantly alter the spread of insecticide through the orchard. Additional tests are necessary to determine the effect of wind speed and to map wind flow patterns in an old orchard.

1983 Aerial application. Mosquito abundance during the two 1983 trials is summarized in Figs. 3-6. As in the 1982 study, the control index was used to compare relative numbers in the spray and comparison zones.

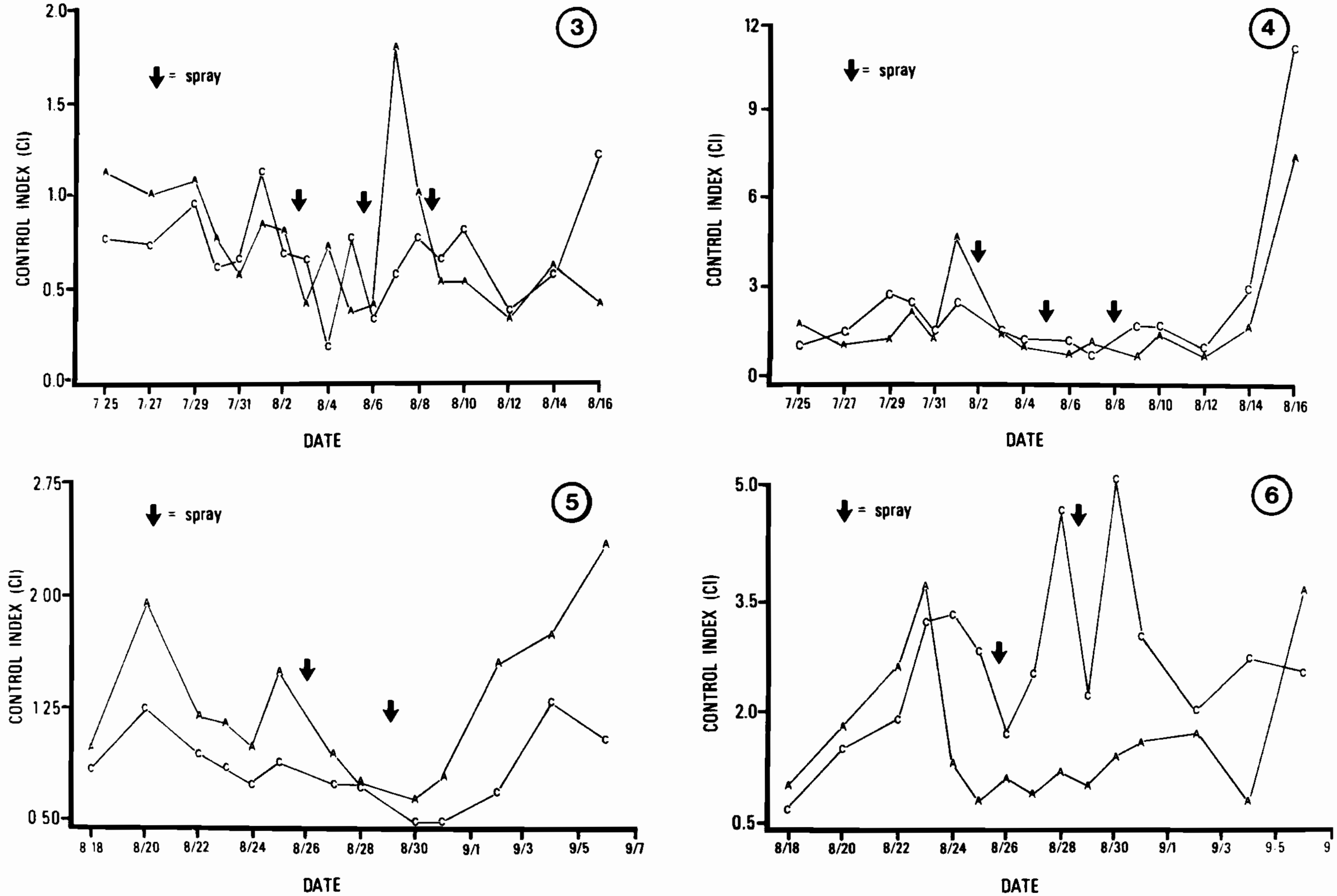
During the first field trial (i.e., 25 July-16 August), the population of *An. freeborni* was low, and it was difficult to detect an appreciable decline due to the malathion application. During the second field trial (i.e., 18 August - 6 September), collections from light traps and red boxes gave evidence of control of both *An. freeborni* and *Cx. tarsalis*. The decline in mosquito numbers in the spray zone was only transient, however, and the populations quickly recovered to pretreatment levels.

Sentinel cage data indicated that aerial application of malathion can be very effective in areas with no vegetation cover; mortality approaching 100% was seen in most sentinel cages in





Figures 1-2. Summary of *Anopheles freeborni* (A) and *Culex tarsalis* (C) collected from red boxes during the 1982 evaluation of ground applications of malathion. Points represent the ratio of spray zone to comparison zone collections. 1. *Anopheles freeborni*. 2. *Culex tarsalis*.



Figures 3-6. Summary of *Anopheles freeborni* (A) and *Culex tarsalis* (C) collected during the 1983 evaluation of aerial applications of malathion. 3. Red boxes, first field trial; 4. CDC miniature light traps with CO_2, first field trial; 5. Red Boxes, second field trial; 6. CDC miniature light traps with CO_2, second field trial.

open areas. In areas with light or moderate cover, however, mortality was appreciably reduced. Mortality of sentinels sequestered in heavy vegetation was no higher than that experienced by sentinels in the comparison area. The inability of spray to penetrate thick, vegetative cover was demonstrated during the 26 August spray. A transect of sentinel stations with cages in the open, on the edge of, and in dense vegetation suffered 93, 7 and 0% mortality of caged *An. freeborni*, respectively. In general, field-collected *Cx. tarsalis* showed less mortality than did *An. freeborni*. This trend consistent with results of 1982 sentinel cage tests and laboratory tests for resistance which are reported below.

Parity in the mosquitoes from the spray zone was significantly greater than in those from the comparison zone (i.e., 11.8 vs 2.5%) on 1 August, one day prior to the beginning of spray operations. There were no significant differences on subsequent days. Parity during the two trials ranged from 1.9 to 17.5%.

The number of marked individuals which were recovered was insufficient to make statements concerning the survivorship of *An. freeborni* in the field environment. The recapture patterns do, however, provide insight into the dispersal capability of this mosquito. In a few cases, female mosquitoes released at sunset were collected the following morning as far as 1.5 mi. from the point of release. More of the marked mosquitoes were recaptured to the north of the release point than to the south. This probably reflects the influence of wind which was generally from the south during the study.

Results of the bioassay for susceptibility to malathion were covered in detail by Case (1984). Spray zone *Cx. tarsalis* were much more resistant to contact exposure to malathion (LC_{50} of 570 µg/cm for the final, post-spray bioassay) than were the *An. freeborni* (LC_{50} of 6.36 µg/cm). There was no significant change in the susceptibility of either over the course of the study.

DISCUSSION.-In the 1982 study, abundance of *An. freeborni* in the spray zone began to decline only after four weekly applications of malathion. Perhaps the population was responding to continued insecticide pressure. A change in the behavior of the mosquitoes may have increased their exposure to the insecticide or the population decline may have resulted from mosquito migration out of the spray area. Migration of nullipars away from breeding sites in preparation for overwintering could also have influenced the parity rates in the population which remained.

The resistance of *Cx. tarsalis* to malathion indicated by sentinal cage results and the failure to demonstrate population suppression in 1982 was confirmed by laboratory bioassays in 1983. Resistance of this mosquito to several compounds is well documented in other areas of the Central Valley (Womeldorf 1984).

The failure to demonstrate longer-lived suppression of *Anopheles* and *Culex* in the 1983 study may have been due to immigration of mosquitoes into the spray zone and/or failure to kill mosquitoes sequestered in heavy vegetation. Mark-release-recapture estimates of dispersal (i.e., as much as 1.5 mi. overnight) indicate that *An. freeborni* has the ability to invade the spray zone and mask the impact of control measures. Visibility restrictions required helicopter spray operations be conducted during pre-dark or post-dawn hours. Unfortunately, this may have been when many mosquitoes were resting in heavy vegetation and therefore protected from the spray. Ideally, sprays should be applied during periods of peak activity when mosquitoes are in open areas in search of mates, hosts, or oviposition sites.

Both ground and aerial applications of malathion may be effective when conditions are optimal. Operational limitations (e.g., road access and visibility restrictions), the inability of either method to effectively penetrate vegetation, and possible insecticide resistance, however, may limit the success of these applications in many real world situations. Additional research is necessary to identify materials and methods which will give adequate control under a wide variety of conditions including some of which were encountered in this study.

ACKNOWLEDGEMENTS.-This work was supported in part by grant U.S. Army DAMD 17-83-M-9821, Research and Development Laboratory, Fort Detrick, Frederick, Maryland, and Special Mosquito Augmentation Fund, University of California. We thank the following for their effort on the project: E.E. Kauffman and his staff at Sutter-Yuba MAD, D.J. Womeldorf and his staff, Vector Biology and Control Branch, State Department of Health Services, M.M. Milby, School of Public Health, University of California, Berkeley, Commander S. Husted, U.S. Navy Disease Vector Ecology and Control Center.

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PANEL: CALIFORNIA MOSQUITO AND VECTOR CONTROL ASSOCIATION, INC. -- HOW, WHY, AND WHAT

INTRODUCTION

Marvin C. Kramer

California Mosquito & Vector Control
Association, Inc.
197 Otto Circle
Sacramento, California 95822

This panel will address itself to the how, why, and what of the California Mosquito and Vector Control Association, Inc. Two giants of mosquito control in California have been asked to tell you the how and the why. These two gentlemen represent 76 years of experience in mosquito control. Their contribution is measured not just in length of service, extensive as it is, but more importantly in the quality of that service. They have been pioneers of ideas and approaches, and have exhibited a leadership that has prompted mosquito control workers not only in California but world-wide to seek their counsel.

With specific reference to the California Mosquito and Vector Control Association, Inc., they have had a deep and abiding concern. They have analyzed problems correctly, have had courage of their convictions, and have campaigned with vigor and success in bringing about necessary changes, with disregard for personal popularity in the instances in which their conclusions met with less than universal favor.

The following brief introductions highlight their relationship to California Mosquito and Vector Control Association, Inc.; they are by no means complete for either of them.

Howard Greenfield started his career in 1948 as a research assistant with the California Department of Public Health, moved on to the Merced County Mosquito Abatement District as entomologist in 1949, and in 1951 was hired as manager of the Northern Salinas Valley Mosquito Abatement District, where he served with distinction until his retirement in 1982. He was president of the Association in 1957 and served on committees in CMVCA and in AMCA during every year of his 31 years as manager of the Northern Salinas Valley Mosquito Abatement District, frequently as chairman.

Dick Peters' work experience began under Title 6 in the late '30's in Solano County. Monterey County beckoned in the early '40's and shortly thereafter he started a long and illustrious career with the California Department of Public Health, interrupted only by service to his country during World War II as a mosquito control expert who taught classes in malaria control in the South Pacific, and by trips to various parts of the world as special consultant for the World Health Organization and A.I.D. Dick has been president of CMVCA and of the American Mosquito Control Association, Inc. He has served on numerous committees in both CMVCA and AMCA, frequently as chairman, and has been a member of the Board of Trustees of the Contra Costa MAD for a number of years.

HOW THE CMVCA WAS BORN

Howard R. Greenfield

When Jack Walker, Assistant Chief of the Bureau of Vector Control (now retired), presented his author-title index (Volumes 1-24, 1930 - 1956), at the 25th Annual Conference in 1957, he wrote in the Foreward, "The writing of a history of the California Mosquito Control Association is a task yet to be undertaken". In the absence of such a reference certain comments are believed desirable here in order to orient readers wishing to use the index which follows:

The first "Conference of Superintendents and Trustees of the Mosquito Abatement Districts in California", was held December 16, 1930, at Agricultural Hall, University of California, Berkeley, at the invitation of Professor William B. Herms, Head of the Division of Entomology and Parasitology.

Now having established the date when the first conference was convened, I believe the events that preceded the first conference are important enough to be retold, and the people involved to be identified.

Much of the information I am going to present came from one of the most unlikely publications I can imagine - The Transactions of the Commonwealth Club of California, San Francisco, Volume XI, No. 1, March 16, 1916, entitled, "The Malaria Problem". It was a committee report submitted before the general membership of the Commonwealth Club of California.

The membership of the committee was as follows:

Dr. Ebright, Chairman, President of the State Board of Health

Dr. Ray Lyman Wilbur, Chairman, Section on Public Health, President of Stanford University

Professor William B. Herms, Associate Professor of Parasitology, University of California

Dr. Karl F. Meyer, Associate Professor of Tropical Medicine in the University of California

Dr. George H. Whipple, Director of the George Williams Hooper Foundation for Medical Research.

I believe the make-up of this committee - the President of the State Board of Health, the President of Stanford University, two Professors from the University of California and the Director of the Hooper Foundation, clearly indicates the great concern the people of California had about malaria and how it could be controlled.

Dr. Ebright in his remarks stated that malaria was unknown in California prior to 1850 and he conjectured that the disease was brought in by immigrants from the Mississippi Valley, the Isthmus of Panama, and from Italy. He also stated that it cost the State about $2,820,000 yearly, and that was in 1916 dollars!

Professor Herms in his remarks, said that malaria control was synonymous with mosquito control and that he was very gratified that the state legislature had passed the Mosquito Abatement Act in 1915.

Of course Professor Herms should be gratified, since he had toiled for many years prior to the passage of the Mosquito Abatement Act in the Central Valley as the representative of the University of California and also at the request of the Southern Pacific Railroad Company trying to educate the public about the need to control malaria. Southern Pacific provided Professor Herms with a railroad car to travel the length of the Central Valley and also strongly lobbied for the passage of the Mosquito Abatement Act. Southern Pacific very early recognized that if their land holdings in the Central Valley were ever to be developed (in excess of 10,000,000 acres), malaria had to be controlled.

Professor Herms in an address before the Bakersfield Booster Club, said that with the passage of the Mosquito Abatement Act, several districts had been organized - one in Marin County, one in San Mateo County, Bakersfield was to follow with a fifty square mile district and ten mile square district in Springville, Tulare County.

With the passage of the Mosquito Abatement Act in 1915, seven districts were organized and operating by the year 1920. They were:

1.	Marin/Sonoma	1915
2.	Three Cities	1916
3.	Los Molinos	1917
4.	Pulgas	1917
5.	Dr. Morris	1917
6.	Durham	1918
7.	Shasta	1919

Progress was slow but steady and by 1930, twenty districts had been organized and eight more were in the mill. Professor Herms, by this time, was recognized as the authority on mosquito control in California and it was he who recognized that a need existed to bring together those people in the firing line or in the trenches who had accumulated some 15 years experience in fighting mosquitoes to exchange information. Thus was born the forerunner of our present organization.

Now with this brief history of events etc., that led to the convening of the first conference of mosquito abatement officials, let's see who the participants were:

Prof. W. B. Herms-University of California, College of Agriculture

Prof. H. J. Quayle-University of California, College of Agriculture

Prof. W. M. Hoskins-University of California, College of Agriculture

Prof. C. G. Hyde-University of California, College of Civil Engineering

Edward Stuart-Sanitary Engineer in charge of Malaria Control State Board of Health

Managers (Superintendents):	Noble Stover
	R. E. Hackley
	J. C. Dickey
	Fred Rush
	A. M. Emerick
	W. P. Menefee
	H. F. Gray
Trustees:	John Freitas
	R. M. Sheldon
	E. C. Crowley
	Oliver Sancomb
	L. B. Nevin
	T. A. Keiser

Others present, (and I find this very interesting), were:

M. S. Robertson-Curtiss-Wright Flying Service

H. C. Riker-Fairchild Aerial Service

L. L. Funk-Aerial Photography

Mr. Barnhill-Growers Chemical Co., LTD

Professor Herms in his opening remarks welcomed the group and presented a brief history of mosquito control in California and suggested the group select a chairman and a secretary. Noble Stover was selected as chairman and Harold Gray as Secretary.

Papers were presented and after each, the chair opened the meeting for general discussion or questions about the information presented.

During one of the discussions, the idea of the group having a clearing house for information was explored and it was finally agreed to leave the matter in the hands of Professor Herms.

The conference ended with those in attendance stating that the meeting had proven to be an excellent way to exchange information, etc., and that the conference should be continued with permanent organization. Upon motions, duly seconded, the above named officers were elected for an annual term.

The meeting was so successful that the group suggested that two meetings be held in 1931. The meeting was adjourned at 4:45 p.m. to reconvene in April, time and place to be determined by the officers of the Association. The April meeting was not held and the conference did not reconvene until December 15th, 1931 at Agricultural Hall in Berkeley.

In conclusion, this seems to be how the California Mosquito and Vector Control Association was born; and as you have probably noted, the one name that is found throughout this narrative is Professor Herms. It is no wonder that he became known as the father of mosquito control - loved by many and revered by all. Professor Herms recognized the need to exchange information among mosquito control workers for the programs to be successful and that need is even more important today, some fifty-four years later.

WHY THE CMVCA WAS FORMED:

WHAT TRUSTEES MIGHT AIM FOR

Richard F. Peters[1]

My recollection of the California Mosquito Control Association dates back to the mid-thirties while an undergraduate student in entomology at the University of California, Berkeley. Meetings were held in the auditorium of Agriculture Hall on campus from the time the Association began in 1930. Professor W. B. Herms, then Chairman of the Department of Entomology served as host of the annual meetings. At every meeting until 1949 he provided the attendee's, mostly Superintendents but also a sprinkling of Trustees, with a greeting, followed by an overview of the world-wide status of mosquito-borne disease. Recitals were made by many of the superintendents, who related their individual practices and problems. Each, operating by his lonely was eager to hear what the others were doing, working within the powers granted by the Mosquito Abatement District Act. Harold Farnsworth Gray, Engineer, Alameda County Mosquito District, of course, dominated the early meetings.

It must be appreciated that at the outset most mosquito abatement districts were tiny and widely scattered throughout the state. There was no link between them and transportation at first was by horse and buggy until automobiles gradually became available. Shasta County had five separate districts. Tehama County had one in the area of Los Molinos. Butte County had two small districts covering Durham and Oroville. The city of Merced and outlying area constituted another. The Delta District contained only Visalia and environs. Then, came the Dr. Morris - Mosquito Abatement District, named after the physician who took the initiative in forming the district, covering only Bakersfield and its environs. Santa Barbara County had a small district in the vicinity of Carpinteria.

Los Angeles county was represented only by the Ballona Creek and Compton - Creek MAD's. The Coachella Valley MAD was then devoted only to *Hippelates* eye gnat control. In the bay area, San Mateo county had three small districts, the Pulgas, Three Cities and Matadero which bordered on Santa Clara County. Marin County, which had the first district formed in California in 1915, included San Rafael and its outlying marshes. Sonoma County had only taken coverage of its mosquito problem around the city of Sonoma. Napa and Solano Counties showed wisdom by creating county-wide districts. Contra Costa County began with its district covering only the northern part of the county. Alameda County at first also included only the cities bordering its marshes.

The annual association meetings also served as a forum to discuss and offer opinions on mosquito species behavior. Considerable tension existed between Superintendents of the neighboring districts in the bay area concerning mosquito flights and invasions which could not be explained. Both salt marsh species, *Aedes squamiger* and *Aedes dorsalis* are capable of traveling many miles and their unexpected appearances throughout the bay area gave rise to much speculation, charges and counter-charges concerning their origin. This situation was finally resolved by district consolidation in San Mateo County and more extended coverage in the other counties. A similar situation was later to develop among the districts in the Central Valley, where *Aedes nigromaculis*, the pasture mosquito, and *Anopheles freeborni*, the malaria mosquito, both also capable of flying considerable distances, vexed the managers of neighboring mosquito abatement districts for many years.

Until the state subvention to local mosquito control agencies began in 1946 and with it the rapid expansion in number of agencies and area of the state covered, the association was largely a means for annual information exchange. The impact of this growth and the introduction of DDT, a completely new control technology, provided a need for more and closer communication among everyone concerned. A new element of professionally and technically trained personnel was also coming into existence among the local agencies. In 1951, the CMCA incorporated as a non profit agency, adopted a constitution and by-laws and demonstrated its intention of standing on its own feet. Undoubtedly an element of concern existed in the minds of some local agencies that the Association was a means of protecting local autonomy from those "monsters" called State and Federal government. The pattern of association utilized by the Local Health Officers and Directors of Environmental Health - - a conference structure, influenced the founders of the revised CMCA. However, those two groups had something in common - - an M.D. and M.P.H. for the Health Officers and Sanitation training and background for the Sanitarians. The CMCA had a wide assortment of background characterizing its administrators which didn't lend to a conference structure. Also, the CMCA recognized the need to provide for trustee involvement and in subsequent years as Trustee Corporate Board was created for this purpose.

Since the trustees govern the policies of districts, particularly with regard to use of local

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[1]Retired Chief, Bureau of Vector Control, California Department of Health Services; currently Trustee, Contra Costa Mosquito Abatement District.

funds, the role and functions of the Trustee Corporate Board of the Association became virtually the same relating to the Association in behalf of every district in the state. Unfortunately, however, only token awareness exists among trustees statewide that they are an integral cog in this Association. Attest the few trustees attending this meeting. The problems facing mosquito control in California today are primarily fiscal, brought on by the impact of Proposition 13. Accordingly, this is primarily a trustee matter. Our managers can't solve this one alone. If our problems were technical or operational we could expect they could be effectively dealt with by them.

Our district in Contra Costa County happens to be marginal with respect to funding. We have cut back our program since Proposition 13 to a point where only the primary mosquito producing sources can be covered. At one stage, some years ago our district approached being a vector control district like Orange County, Northern Salinas Valley and Delta MAD's in that we also controlled domestic flies, gnats and yellow jackets. We now have diminished our staff and mosquito control coverage to a point where our Board recently debated whether to hold an election like Alameda, San Mateo, San Joaquin and Turlock MAD's did to boost our budget to a more comfortable level. We concluded that we wouldn't hold an election at this time since that approach is shakey and there must be a better way to obtain more certain long term funding. In order to start the ball rolling toward this end our Board wrote the California Mosquito and Vector Control Association the following letter:

Frank Pelsue, President-CMVCA
California Mosquito and Vector Control Association, Inc.
Southeast Mosquito Abatement District
9510 South Garfield Avenue
South Gate, California 90280

Dear Dr. Pelsue:

It has been five years since the passage of Proposition 13, and it is very apparent that long term financing is still a major concern of mosquito abatement districts in California. To date, legislative progress has been insufficient with limited, piecemeal programs and supplemental financing through AB 8 legislation which continues to be in jeopardy.

Although a Governor's task force on local government financing has been created to review solutions to this chronic problem there are no assurances mosquito control will be favorably considered.

Contra Costa Mosquito Abatement District urges the CMVCA to become involved in this latest process to ensure a fair hearing in potential long term solutions and would like to support the Association in anyway it could. It behooves each District to actively pursue financial legislation until satisfactory ends are met. To this end, we would appreciate knowing what organized plan the Association has to help member Districts by outlining just what is and could be done by individual Districts.

A copy of this letter is being sent to the local legislators whom we trust would be willing to assist in long term solutions.

Sincerely,

H. Roger Willis, President
Board of Trustees

cc: R. Heim
M. Kramer

CB:cjp

Dr. Pelsue replied, citing passage of S.B. 628, the Emergency Mosquito Funding Bill, (which will be covered in detail during the afternoon session today), but indicated that no plan presently exists within the CMVCA to deal with this unmet need of California mosquito control. He expressed hope that all local control agencies would "become involved with the development of a plan" needed for "achievement of permanent funding for vector control agencies".

Ladies and Gentlemen, securing adequate funding is a trustee oriented matter which deserves our statewide attention. I understand the author of Proposition 13 didn't intend for such health services agencies as mosquito abatement districts to be crippled by that proposition. It happened that way in the inevitable political shuffle of limited local funds remaining available to Boards of Supervisors. Local augmentation of districts budgets is not prospective of solving the long range needs of mosquito abatement districts. Do you realize that there are more than 300 trustees in the state who could become active in bringing this predicament to the attention of our Senators and Assemblymen? I would wager that every Board of Trustees has one or more members who are on close speaking terms with the local legislators.

What is needed, of course, is a plan fortified with appropriate legislation, by which to restore adequate funding to local vector control agencies. It is suggested that such an undertaking be the main effort of the Trustee Corporate Board during 1984, rallying statewide participation and support from the 300 plus latent trustees. It can be done, but it won't be if we sit on our hands and wait for someone else to do it.

This Association, properly backed and represented by its trustees could be a powerful force in achieving the statewide needs of vector control agencies. Although we directly represent our respective districts as agents of our cities and counties as private citizens, we indirectly relate to every phase of public life-commercial, industrial, agricultural environmental, recreational etc. Through these relationships we can reach our legislators and inform them of our fiscal predicament. An Association can be much more powerful than government in influencing legislative action.

WHAT CMVCA PROVIDES

Marvin C. Kramer

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As every conscientious member of the Board of Trustees should be, you are interested in what your district receives from the California Mosquito and Vector Control Association in return for the dues that are paid. Association activities can be categorized as committee work; central clearing house for receiving and disseminating information; sponsor of conferences, seminars, workshops, demonstrations, and cooperative negotiations; parent organization of the joint powers agency; promoter of intergovernmental programs aimed at reduction of restrictions and improved effectiveness of districts; and publisher.

Committee work is the mainstay of the Association. Individual members have strengths, but no one person has great strength in all of the complex requirements of mosquito and vector control. Members with demonstrated proficiency in a given area are grouped together in a committee. With the variety of charges among the various committees, all approaches for effective mosquito control are addressed. Examples of benefits that accrue to Association members are the accomplishments of the Legislative and Central Office Committees. The Legislative Committee was instrumental in securing $3.25 millions from the State for supplemental financial support of mosquito and vector control districts. This program was administered by the Vector Biology and Control Branch of the California Department of Health Services; allocations were made to all mosquito and Vector Control agencies who applied for funds.

Another accomplishment of the Legislative Committee was successful promotion in 1983 of legislation to provide for funding of emergency encephalitis control by mosquito and vector control districts or county health departments, and for early surveillance by the Vector Biology and Control Branch of the California Department of Health Services. The committee is currently making plans for promoting state help on a permanent basis for mosquito and vector control districts.

The Central Office Committee has reviewed operation of the office, has made in-depth studies of objectives and procedures, and has made recommendations for a more cost-effective approach. Part of this was an overhaul of procedures for making publications camera-ready and for printing, and the mechanics of this include purchase of a computer and word processor.

Other committees are Budget and Executive; Computer; Control, with subcommittees Biological, Entomological, and Physical Control; Continuing Education; Cooperative Planning; Environment; Equipment; Fiscal Review; Historical Archives; Integrated Pest Management; Local Arrangements; Program; Publications; Research; Training; and Ways and Means. Each of them is making an important contribution and if we had more time at this meeting we could enumerate their accomplishments, but there is time only for the highlights of the various categories of Association activities.

The central office of the Association is a continuing, identifiable physical location which has continuing personnel to maintain relationships with many organizations. As the central clearing house for receipt and dissemination of information, the Association office prepares and distributes questionnaires for the annual yearbook and for fiscal information; maintains mailing lists for essential distribution of communications and publications to members and to purchasers of publications, prepares minutes of Board meetings and distributes them to members; duplicates and sends out agenda for meetings, announcement of conferences, committee meeting schedules, and Bio-Briefs; and answers correspondence from a variety of organizations and individuals regarding mosquito control in California.

Also, the pooling of ideas helps to develop a uniform pattern of operations for districts, and allows district personnel to avoid mistakes of others and to share information on procedures that are successful. As Howard Greenfield has mentioned, representatives of the California Department of Public Health; the University of California, Berkeley, College of Agriculture and College of Engineering; mosquito abatement districts; flying services; an aerial survey company; and a chemical company met in conference in December, 1930. The Association has had an annual conference continuously since except for three years during World War II. The attendees at the first conference heard papers on and held discussions of management of duck club properties; county budget laws; relation of sanitary engineering to mosquito abatement work; the use of airplanes for observation of mosquito sources; mapping and spraying; highway construction and maintenance in relation to mosquito abatement; the use of *Gambusia* and sticklebacks; the need for new legislation that is favorable and the importance of watching for legislation that is unfavorable; the desirability of a central clearing house; control of mosquitoes in salt marshes; and the use of algicides.

The staging of the annual conference requires attention to many details. Advance preparations include the designing, duplication and mailing of the call for papers, registration cards and invitations, pre-registration forms, preliminary program, and notification of speakers; the printing of tickets; procurement of ribbons, awards and name badges; negotations and contract with the hotel; and sale of exhibit space. At the conference there are the registration desk and message board, collection and recording of fees paid, and the myriad last minute adjustments. Following the conference we audit and pay the bills, and print the Proceedings and Papers.

On a less structured time table, we arrange seminars or workshops for such subjects as management, source reduction, calibration of equipment, resistance of mosquitoes to insecticides, mosquitofish culture, wildlife management-mosquito suppression, or emergency control of encephalitis.

Membership in the California Mosquito and Vector Control Association made it possible for districts to form the joint powers agency which has saved the districts thousands of dollars in workmen's compensation payments.

In recent years a number of organizations, in vigorous pursuit of their own objectives, have placed restrictions upon mosquito abatement districts which would have resulted in disruption of the critical timing of mosquito abatement efforts had they been allowed to stand. The Association represents 46 agencies which cover more than 30,000 square miles and protect more than 15 million people. Its voice carries more impact than one individual district in dealing with governmental agencies, the legislature, or individuals. The Vector Biology and Control Branch and the CMVCA have been successful in securing a general permit which allows districts to by-pass the restrictions.

As a publisher, CMVCA produces Proceedings and Papers of annual conferences, a Yearbook, Bio-Briefs, guides (mosquito, fish, fly, gnat), mosquito notes, and the trustee manual, to record the information developed and to provide reference materials.

In support of all of the above, we have the routine, on-going, constant chores to maintain the office in order to provide the services.

If an individual district were to contract for these services in the open market, the price would be much more than the ¼ of 1% of its actual expenditures for operations it now pays to the Association. Each district has a responsibility to its tax-paying constituents to provide the most efficient and effective program possible, and ¼ of 1% of expenditures for operations toward this end is justifiable, desirable, and productive.

THE ROLE OF THE C.M.V.C.A.

Claude L. Watson

East Side Mosquito Abatement District, 300 Santa Fe Avenue, Modesto, CA 95355

How many of us tend to forget the importance of our C.M.V.C.A. and the Central Office? Many of us need to be reminded from year to year of what the C.M.V.C.A. and its Central Office actually does for our Mosquito Control Programs.

Most of our formal and informal information by C.M.V.C.A. members is compiled by the Central Office. This valuable information unifies and carries the voices of different individuals of the results of their experiments and projects to all of us. This information has proven to be of great value to many of the agencies throughout California and The United States, as well as to some foreign countries.

The knowledge and professionalism that is gained by all C.M.V.C.A. members is well worth the effort of keeping the association strong and its Central Office in good operating condition.

The C.M.V.C.A. was formed in 1930 and has risen to the high standards it is today. It is recognized nation wide with participants attending the conferences throughout the United States and foreign countries. These participants give reports and present papers with valuable information that is compiled and distributed by our Central Office to all member Districts as well as other organizations. Committees are formed within our Association and constantly do studies to enhance our knowledge of all aspects of technology, such as legislation awareness, mosquito control methods (biological, chemical and physical) plus many more tasks to improve our programs.

The challenge to keep this valuable information flowing will be up to all of us. So, let's keep our Association strong, by our support. The expense we all share, will pay us dividends many times over in the future.

THE HISTORY OF MOSQUITO CONTROL

IN CALIFORNIA - OPERATIONAL

Howard R. Greenfield

In California, the first recorded effort to control mosquitoes was in Marin County near San Rafael in 1903, under the direction of Professor C. W. Woodworth.

In 1904, the Burlingame Improvement Club requested Professor Woodworth to conduct a campaign to control salt marsh mosquitoes in their area. He assigned his assistant, Professor H. J. Quayle, under whom the control work was begun. However, there was a vast difference between the control work performed in San Rafael and that done in the Burlingame area.

In San Rafael, the main control technique was larvaciding using oil, whereas in the Burlingame marshes, extensive ditching and diking techniques were performed. Both campaigns were successful.

The first anti-malarial mosquito control campaign was set up in Penryn, Placer County in 1910, under the direction of Professor Herms and concluded on October 1st of the same year. The costs of that campaign ran as follows:

1.	Field & Laboratory Equip.	$ 30.83
2.	Materials (360 gals. oil, etc.)	35.20
3.	Freight	4.27
4.	Salaries of Field Agents	458.70
5.	Expenses of Expert (Prof. Herms)	129.15
6.	Expense of Field Agents	39.70
7.	Incidentals	17.90
	TOTAL:	$715.75

One interesting aspect in the Penyrn campaign, aside from demonstrating that malaria could be controlled by limiting mosquito production, was that the field agents and Professor Herms were temporarily sworn in as Assistant Health Officer (Prof. Herms) and Sanitary Inspectors (field agents). This action was taken to provide legal access to private property during the campaign.

Other campaigns followed, which I won't describe here, and they were all very successful, providing additional support for the justification of conducting mosquito control activities in the State of California. Although the State of New Jersey passed its' Mosquito Control Act in 1906, California didn't pass its' Mosquito Abatement Act until 1915, although an earlier attempt was made in 1911, but was vetoed by the Governor.

With the passage of the Mosquito Abatement Act, districts were formed and by 1920, seven (7) districts were formed and operating. Who can give me the name of the very first district to be formed in the State of California? Marin/Sonoma in 1915!

It was 1930 when Professor Herms, with the aid of Professor Quayle, determined there existed a great need to bring together, the trustees and superintendents of mosquito control districts, the State Board of Health and the University of California, to discuss the progress of fifteen (15) years of organized mosquito control in California.

Many of the subjects we will be discussing at this conference, such as, biological control, legislative needs, chemical and physical control, and budget needs, were also on the agenda at that first meeting fifty-four (54) years ago.

The success of that first conference was so obvious to those in attendance that when the suggestion was made to meet again the following year, it was met with unanimous approval.

In the seventeen to eighteen (17-18) years that followed, the University of California annually hosted the Conference of Mosquito Control Districts. The Association grew steadily, committees were appointed, officers elected and an executive committee was developed - with the goal of dissemination of information to all those interested in mosquito control. By 1946, California had thirty (30) organized mosquito control districts.

In 1947 the State Health Department, having recognized that returning veterans of World War II presented a mosquito-borne disease hazard to the State, formed the Bureau of Vector Control to administer $400,000 per year to provide assistance to local mosquito control districts.

With the formation of the Bureau of Vector Control, an incredible development in mosquito control took place. New districts were formed (13 new districts in five years) and demands were made to improve administrative, technical and operational techniques.

To meet those demands, the University of California increased its efforts toward basic mosquito control research. Mosquito control districts upgraded their staffs, (employing biologists and entomologists), and began to provide funding for operational research in their own areas.

These actions in turn, increased tremendously the acquisition and dissemination of information between districts, the State Health Department and the University of California and consequently the Association of Mosquito Control Districts became more deeply involved in gathering, publishing and providing that information to all agencies interested in mosquito control technology.

The California Mosquito Control Association, in trying to meet the ever increasing demands being placed on it, decided to become a corporation under the laws of the State of California in 1951.

It is interesting to note that for fifteen (15) years - the development years, the Association's Secretary-Treasurer's office was located in the State Health Department. In 1948, it was moved to Turlock, then to Merced, Alameda County, and to Kern County, before it again settled in Turlock for seven years. Then in 1960, the office of

Secretary was moved to the Delta Mosquito Abatement District where it remained for twenty (20) years under the supervision of Don Murray.

It is my judgment that because the Delta Mosquito Abatement District Trustees were willing to provide for the physical needs of the Association, space, secretarial duties, phone, files. etc., Don Murray was able to (with Board of Directors approval) expand greatly those services expected by individual districts. Those services included producing publications by the Association, which involved printing and disseminating statistical and research data, administrative information etc.

It must be noted also, that during this twenty (20) year period, the financial position of the Association was improving to such a degree that ultimately there were sufficient reserve monies available for investments in T. bills.

Regardless of the financial security the Association was enjoying, changes in its structure were being discussed. Questions were being asked; is there a need for a central office? Should the association hire a full-time Executive Secretary? Should there be less emphasis or more emphasis given to the printing facilities? Strangely, there were even questions raised as to the need for the CMVCA.

Even more strange, these questions were being raised at a time when disaster was confronting all special districts in the State - Proposition 13. With the passage of Proposition 13, income to mosquito control districts was almost cut in half. It became clear that the CMVCA had to play a more active role in contacting legislators and overall, becoming a more active part in the legislative process. Thus the decision was made to move the Central office to Sacramento, hire an Executive Secretary to analyze and reorganize the priorities and goals of the Association. This is now being accomplished.

No one can speak about operational mosquito control on a district by district basis, because no district in this State is identical to another. However, the one common goal that is shared by all districts is to control disease vectors which cause human health problems. How this is done depends on the Boards of Trustees, the Managers and the availability of information from the California Mosquito and Vector Control Association. For within this Association's files, lies years of research mapping the history and future of mosquito control in California.

DEVELOPMENT OF MOSQUITO CONTROL IN CALIFORNIA THROUGH THE WINDOW OF THE DEPARTMENT OF HEALTH SERVICES

Richard F. Peters[1]

It is my hope that this "see through" presentation will not prove too "panefull". The title was suggested by Jack Fiori, Program Chairman, but I suspect John Combs, Historical Committee Chairman, had something to do with getting him to interrupt the bliss of my retirement, obliging me to reflect deeply into the past. Jack initially suggested that I reference my remarks strictly to the Bureau of Vector Control, but that governmental entity was created in 1947 and the relationship of the Department of Public Health to mosquito control began over a quarter of a century earlier; hence the more extended coverage.

Shortly after California gained statehood the need for a health hierarchy became evident. The legislature accordingly created a state Board of Health composed of a representative citizenry and empowered it to function through its secretary. This pattern of government continued virtually unchanged until the early years of the twentieth century when growing population, industry and commerce dictated that a Department of Public Health, staffed with key personnel, be established to conduct the growing responsibilities of the Board of Health.

The first environmental staff included: (1) A State Sanitary Engineer - - C. G. Gillespie, who became Chief, Bureau of Sanitary Engineering, served the state for around forty years in this capacity. (2) food and drug inspection - - M. P. Duffy became Chief, Bureau of Food and Drug and Cannery Inspection and served the state over fifty years. (3) sanitation activities - - E. T. Ross became Chief, Bureau of Sanitary Inspection, which program eventually also performed plague surveillance. Mr. Ross retired with well over thirty years of service.

The first individual associated with mosquitoes who related to the Department was none other than Professor W. B. Herms, U. C. Berkeley, named a Consultant in the mid teens by the Board of Health, to provide counsel to the Board on the control of malaria. He and his assistant, S. B. Freeborn, were engaged in anti-malaria studies and mosquito control demonstrations in Placer and Butte counties aided by an engineer named Harold Farnsworth Gray. During the twenties, Harold Gray served the Department as its northern California District Health Officer.

In the early years of this century, malaria was a significant health problem in the southern United States and in California, causing the U. S. Public Health Service to undertake anti-malaria activities. During the early 1920's two engineers, Stewart and Lennart, were dispatched to California to assist the Department in furnishing anti-anopheline consultation to local agencies, including the few mosquito abatement districts in existence at the time. A biologist, W. C. Purdy, also spent a year in California studying *Anopheles* occurrence in the Sacramento Valley rice fields. It was Purdy who postulated the deterrent impact of blue-green algae upon mosquitoes, based upon his observations. These U.S.P.H.S. personnel are credited with having introduced the mosquitofish, *Gambusia affinis*, into California, unbeknown to the Department of Fish and Game, first placing them in the Sutter Fort lily ponds.

The California Mosquito Control Association was born in 1930, with mid-wifery provided chiefly by Professor Herms, Harold Gray the Engineer-Superintendent of the newly created Alameda County-Mosquito Abatement District, and Nobel Stover, Superintendent, Contra Costa-Mosquito Abatement District #1. The Department participated in the first activities of the Association through the services of E. A. Reinke, Senior Sanitary Engineer, Bureau of Sanitary Engineering, who was its Secretary Treasurer until 1938. In 1939, S. F. Dommes Bureau of Sanitary Engineering continued in this capacity, he being the first Department employee assigned full time to mosquito control activities. Sid remained only a year in mosquito control, departing for a promising career opportunity in industrial hygiene engineering. He subsequently was appointed Oakland's Trustee on the Board of the Alameda County-Mosquito Abatement District where he has served for many years.

Returning to the early 1930's, as an acceptant senior citizen, I well remember a most depressing depression which spawned an era of government by alphabet. The federal W.P.A. gave rise to a C.W.A. and S.E.R.A. in California. The State Emergency Relief Act provided matching state funds during the thirties for local public works agencies. Mosquito abatement districts were eligible to participate in drainage and related projects. This proved a boon to a number of the small struggling districts, helping them to survive the depression.

In 1940, C. G. Gillespie, Chief, Bureau of Sanitary Engineering invited a young Department entomologist attached to the sanitation program of the Monterey County Health Department (who in addition to his other duties performed mosquito control) to become the state's first Mosquito Control Officer, the first non-engineer allowed in

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[1]Retired Chief, Bureau of Vector Control, California Department of Health Services, currently Trustee, Contra Costa Mosquito Abatement District.

the Bureau of Sanitary Engineering. His name: Richard F. Peters. Duties consisted of furnishing statewide technical assistance to local mosquito control agencies, performing mosquito surveys for local jurisdictions and responding to requests for mosquito control information from citizens, service organizations and various other interests. A close working cooperation was developed with Professor Herms and two of his graduate students, Thomas H. G. Aitken and William C. Reeves in the identification and documentation of mosquito species occurrence throughout the state. Mosquito surveys in the Porterville and Riverside areas brought me in close contact with Arthur F. Geib, then with the Tulare County Health Department and Ted G. Raley, then serving the Riverside City Street Department, controlling its acute mosquito problem.

Then came December 7, 1941 with our entry into World War II and the need to protect our military and essential defense areas in California from mosquitoes and mosquito-borne diseases. Again, the alphabetical agencies emerged! The U.S.P.H.S. created an M.C.W.A. (Malaria Control in War Areas) program hinged to the W.P.A. with all of its cumbersome and inflexible eligibility criteria and operational requirements. Virtually all of my time in the first half of 1942 was devoted to extra-military mosquito surveys, facilitating control operations in eligible areas and training federal arrivals to California in mosquito control technology. Ultimately, convincing the U.S.P.H.S. that encephalitis, above and beyond malaria, should be the key criterion for local funding eligibility, proved to be one of the most significant but difficult accomplishments. On July 1, 1942 Uncle Sam made me a 1st Lieutenant, Sanitary Corps, Army of the United States. First duty was Camp Beale near Marysville. Yes, I had recently completed a mosquito survey of that area and understood its control needs. Was I assigned by the Army to utilize this knowledge? Certainly not, the critical need at Camp Beale Station Hospital was for a Mess Officer, my assignment. 1st Lt. Robert F. Portman from Colorado was assigned to mosquito control. On February 9, 1943, I departed the U.S. on an emergency war mission, to spend nearly three years on anti-malaria activities in Australia, New Guinea and the Philippines.

I returned to California in January, 1946 to find that during the early part of my absence, Art Geib had covered my post and during the latter period a Sanitary Engineer, Oscar Blumberg, had carried on. He gladly relinquished my previous functions to me, preferring water and sewage engineering. I also found myself part of a newly created Division of Environmental Sanitation, with Frank M. Stead, formerly an engineer with the Los Angeles County Health Department the Chief. His role was that of supervising the three previously mentioned Bureau Chiefs who collectively possessed over a hundred years of Department experience. His dynamic leadership was to be conspicuous in the vector control picture until his retirement in 1967. Art Geib in 1946 was the newly named manager of the Dr. Morris Mosquito Abatement District in Bakersfield, later to become the Kern County-Mosquito Abatement District. Ted Raley was a Principal Engineering Aid with the M.C.W.A., located in Marysville, later to become manager of the newly formed Sutter-Yuba-Mosquito Abatement District. G. Edwin Washburn was an M.C.W.A. entomologist, soon to become manager of the newly created Turlock-Mosquito Abatement District. 1946 marked one of the most dramatic years in California Mosquito Control history. Great concern characterized the California scene regarding the public risk from introduced mosquito-borne disease in the setting of a rapidly increasing state-wide mosquito problem. New strains of malaria, encephalitis and dengue threatened to accompany military returnees to California, posing epidemic hazard to the public. The Department, with invaluable assistance from Drs. K. F. Meyer, W. B. Herms, W. C. Reeves and S. B. Freeborn prepared a "Report on the Disease Bearing Mosquito Hazard in California", which caused the legislature, meeting in extraordinary session, to appropriate $700,000 for use by the Department in meeting this emergency. $400,000 was earmarked for subvention to local mosquito control agencies and $300,000 was provided the Department to enable it to perform the needed administrative, technical, operational, epidemiological and laboratory services. The impact of this legislative action was tremendous in stimulating the growth of mosquito control in California.

The status of mosquito control in 1946 must be appreciated. There were 25 local control agencies collectively covering only 5,000 square miles with the budgets of all barely totalling $500,000. People, their lives disrupted by the war, were swarming to California, mostly choosing to settle in the suburbs proximal to mosquito production. Irrigated agriculture was booming. DDT, a new miracle insecticide was becoming available which gave promise that it might well obliterate mosquitoes. Certainly the public was now in prospect of undisturbed outdoor living in full protection from mosquitoes and mosquito-borne diseases.

In fiscal year 1946-47 a Mosquito Control Section was created within the Division of Environmental Sanitation. Arve H. Dahl, an engineer with the M.C.W.A. program in Hawaii was recruited to serve as Chief. I was appointed a Senior Mosquito Control Specialist and assigned to supervise the biological activities. Others recruited as Mosquito Control Specialists included entomologists: Harvey I Magy, Robert F. Portman and W. Donald Murray; and Robert W. Jones III, Public Health Engineer. Don Murray departed the program in the Fall of 1946 to accept a teaching post in Illinois, to return to California a year later as manager of the Delta Mosquito Abatement District. His departure provided the Section with the opportunity to obtain the services of John R. Walker.

In order to assure objective professional administrative and technical management and operation of this new state-local program, a Vector Control Advisory Committee was created to serve the Department. Representation at the outset included Dr. K. F. Meyer, Dr. W. C. Reeves, Dr. S. B. Freeborn, Harold F. Gray (CMCA), a local Health Officer, a U.S.P.H.S. engineer and a local Director of Environmental Sanitation. This committee recommended criteria of eligibility for subvention and state-local mosquito control programs, policies, content and emphasis. A similarly structured Advisory Committee has continued to provide valuable recommendations to the Department to the present day. 1947 brought about an amalgamation of the personnel of the Bureau of Sanitary Inspection with the Mosquito Control Section to give rise to the Bureau of Vector Control, providing with this development for a full scope of vector control program. The Communicable Disease Center, with the dismemberment of the M.C.W.A. activities had also reinforced our staff, assigning entomologists D. C. and Ernestine Thurman, Richard P. Dow and Basil G. Markos and engineer William P. Warner. The U.S.D.A. also subsequently assigned an entomologist, C. M. Gjullin to conduct mosquito control studies. Earl W. Mortenson made his debut in the Bureau. Perhaps the most significant development of the year was the incorporation into the State Administrative Code of the Standards and Recommendations, prepared by the Bureau, governing subvention to local mosquito control agencies. These standards placed primary emphasis upon "the progressive reduction of mosquito sources", secondary emphasis upon larviciding, with tertiary recognition given to adulticiding. Regrettably, this order of emphasis was not commonly heeded, in the late 40's and early 50's, with larviciding and adulticiding receiving underserving priority. Qualifications for local management and technical guidance were spelled out in the standards and were carefully observed for the most part, accounting for today's wealth of sound local management.

The Bureau's mosquito control activities included surveys, ecological studies, control demonstrations, training of local personnel, vector identification and program evaluation, all conducted in close cooperation and collaboration with the CMCA. A close working relationship developed at the out-set between the Bureau and Dr. W. C. Reeves' encephalitis studies and several personnel were provided to that program for an extended period. A Year Book containing mosquito control administrative, technical and operational inserts was developed in concert with the CMCA. The section survey mapping and inspection - treatment recording systems were jointly developed with the CMCA. MOSQUITO BUZZ, a mimeographed California mosquito control news-sheet monthly was disseminated to all local control agencies. It even once carried the description of a new mosquito species, *Aedes bicristatus*, E. B. Thurman. The BUZZ, was succeeded in a few years by California Vector Views, a more sophisticated and comprehensive vector control journal which continued until the economy crunch of the current decade. My primary duty during this period was providing assistance to local jurisdictions, service organizations and miscellaneous interests in forming and expanding mosquito abatement districts. From 1946 to 1950 the 25 agencies doubled, increasing in total area by about 500%, with corresponding budgetary increases.

1948 found Bob Portman departing the Bureau to become the first manager of the newly formed Butte County Mosquito Abatement District. That opening allowed for Russell E. Fontaine to join the Bureau as our Sacramento Area Representative, providing thereafter for his being referred to as "Senator". Howard R. Greenfield made his start in mosquito control with the Bureau, soon going local.

1949 a year of joint meeting of the CMCA with the AMCA, found Thomas D. Mulhern of New Jersey visiting California, liking what he saw and returning in the heat of that summer to stay for a quarter of a century as a member of the Bureau. Tommy performed a wide variety of services as the Bureau's Technical Consultant on mosquito control.

1950 was a year of transition. Arve Dahl departed California for a new career opportunity in Washington, D.C. Mosquito resistance to DDT manifested and serious larviciding failures became commonplace. Every possible explanation was assigned to the failures, including operator goof-offs, formulation economies and too old toxicant, until documentation of resistance left no doubt. The need to invest a portion of the subvention funds into applied research aimed at developing more reliable control alternatives became evident and a cooperative CMCA - BVC research effort took shape. An ecologically oriented applied research project began in the Turlock-Mosquito Abatement District and culminated several years later in a Fresno research facility, obtained through negotiation for war assets buildings. Harvey I. Scudder followed D.C. Thurman as our research supervisor.

1951 provided me attainment of my personal goal. I became Chief, Bureau of Vector Control, which position I had the privilege to hold until my retirement in 1978, after forty one years of public service. Some years later, a position of Assistant Chief was created and everyone in the Bureau was fortunate to have John R. Walker move up to serve in this capacity until 1978, when he also retired to his Oregon homeland. Jack provided selfless professional and technical support to every aspect of the program. His literary talent gave the written word of our entire staff more clarity and readibility, including that of the Chief.

1952, was a year of horror which remains indelible to the present day. The 1951-52 winter snow pack exceeded 200% of normal throughout the mountain systems of the state. A mild spring with extended rains left water filled depressions everywhere. Rivers and creeks flooded outlying areas. Everything was conducive to *Culex tarsalis* propagation and adult longevity.

Encephalitis cases, both horse and human, manifested early and continued throughout the summer. Over 800 human cases were reported, more than half being laboratory confirmed, with fifty deaths. Over forty counties had horse or human cases or both. After what seemed an eternity of waiting, an emergency was proclaimed and funding was authorized for an anti-encephalitis program. Circumventing the routine state protocol for procurement of equipment, materials, and services proved virtually impossible. Finally, by early summer, everything came together. All local mosquito control agencies were provided supplemental funding for an all-out effort against *Culex tarsalis*. Every Bureau staff member, augmented by personnel from the U.S.P.H.S. and locally recruited individuals was assigned mosquito control duty in strategic locations throughout the state. Marvin C. Kramer received his introduction to California mosquito control in this effort called *OPERATION TARSALIS*. Marv, subsequently sampled local posts in the Tulare and Alameda County Mosquito Abatement Districts, joined the Bureau in the mid 50's, served with distinction, retiring in 1976. Control consisted largely of adulticiding with DDT by air and ground, with strategic larviciding where indicated. In truth, for the most part, the effort was too little too late. We learned considerable from this gruelling experience, however, and everyone concerned, state and local, resolved never again. I am relieved to be able to say that California hasn't since had a repeat of that horrible experience, although several years have had similar settings for potential outbreaks and they received deserved extraordinary anti-encephalitis local and state effort. This doesn't necessarily portend that another major epidemic won't happen and it certainly permits no complacency.

The obvious need following this travesty was to institute a state-wide surveillance and reporting system that would hopefully alert everyone to a future potential epidemic. Accordingly, a state-local system was created incorporating findings of early larval distribution and density, adult occurrence by light trap capture and mosquito virus recovery and has been voluntarily performed by local control agencies in cooperation with Bureau staff every year since to the present.

The 1950's found organophosphorous insecticides replacing DDT and its relatives, only to have OP mosquito resistance manifest within a few years. The Bureau, seeking to augment its program to enable it to cope with this dilemma was rewarded with an administrative review by the State Department of Finance and a technical review by the Communicable Disease Center. It passed both reviews with flying colors and moderate program increase followed. Don J. Womeldorf and his Fresno State contemporaries were welcomed aboard. A. Ralph Barr was appointed Supervisor of Vector Research reinforced by a fine staff whose activities resulted in many productive accomplishments. Surveillance of mosquito resistance to pesticides became a major statewide bureau activity.

The 1960's saw the Bureau's research funds transferred to the University of California. This disappointing development we accepted with regret but not necessarily agreement. Greater program emphasis of the Bureau was placed upon mosquito source reduction and newly acquired Bureau engineers provided close support to local agencies on specific projects. A political misadventure of the Nixon administration happened--the Environmental Protection Agency came into being. The Bureau responded, establishing a statewide vector control training and certification program encompassing all local vector control personnel. This training program endures to the present day and has verified that the competence of vector control workers in California is second to none.

The 1970's found growing federal and also state interference in mosquito and other vector control programs spurred on by the "environmentalists". The Department moved its administrative headquarters from Berkeley to Sacramento, requiring a number of program and personnel adjustments. A devastating development called proposition 13 wracked the very foundation of the Mosquito Abatement District Act which had so ably served California mosquito control since 1915. Recovery from the fiscal impact of this shattering development still faces the agencies comprising this Association. The solution will not come easily, but I am certain that with a combined state-local effort addressed to this matter and other unmet needs of California mosquito control the outlook can be optimistic.

In closing, I wish to pay tribute to my former Department and particularly the past and present staff of the Bureau of Vector Control (Vector Biology and Control Branch if you will) for their profound professional service to California mosquito control. There never has been a more dedicated or more competent group of people in the public service.

TRUSTEE CORPORATE BOARD REPORT

Roland W. Finley, Chairman
Trustee Corporate Board

The major emphasis of the Trustee Corporate Board [TCB] throughout 1983 was to rebuild and re-establish interest and participation in the Board. Quarterly meetings at Monterey, South Lake Tahoe and Long Beach were settings for meetings which saw some increased attendance and renewed interest in the Board structure and objectives. Trustee attendance and participation in Board matters, however, remains a strong concern.

At the request of some Districts, the subject of increased "in lieu" expenses for trustees was considered by the Board. Diverse feelings on this topic resulted in no recommendation, however the matter was referred to the California Mosquito and Vector Control Association [CMVCA] Legislative Committee for later consideration at the time the California law is recodified.

Dr. Robert H. Brown, Consolidated MAD, was appointed by the chair to fill the one year directorship on the Vector Control Joint Powers Agency [VCJPA] reserved for the Chairman of the TCR or his appointed representative. Trustees were well represented on the Board of Directors of the VCJPA with Warren Hall, Orange County VCD serving as Chairman of the Board and Roland Finley, Chairman of the TCB, San Mateo County MAD, serving as Director from the Coastal Region.

Since many trustees remain somewhat unfamiliar or uninformed concerning the CMVCA as an organization, efforts were made throughout the year to familiarize them with the parent organization. The values of membership, educationally, politically and perhaps financially, to the individual district and to mosquito abatement throughout California, were addressed as well and the problems, mostly financial, which currently face the organization and threaten both its effectiveness and existence. These subjects are to be further addressed through a panel discussion to be held at the State Conference in Long Beach in January 1984. Increased understanding, cooperation and mutual assistance between these two segments of the CMVCA is a continuing goal.

We look forward to 1984 with the increased confidence that the Board will continue it upward thrust in attendance and interest and will contribute to its one objective -- better and more cost effective mosquito control for the people of the State of California.

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

January 1983-January 1984

William C. Hazeleur[1]
Shasta Mosquito Abatement District
Post Office Box 331, Redding, CA 96099

RESEARCH.-Jack Hazelrigg, Chairman. In an attempt to fund mosquito research that meets the needs of California mosquito control, the C.M.V.C.A. Research Committee met in March 1983 at University of California at Davis and reviewed the mosquito research proposals for the 1984-85 year. After the review, a report of the Committee's recommendations was prepared and shared with the University's committee which also reviews mosquito research proposals.

ENVIRONMENT.-Fred Roberts, Chairman. The Committee continued to work on the Rare and Endangered Species Data Base (computerized) and continued to coordinate and work with the Co-operative Planning Committee on its Cooperative Agreement with wildlife agencies.

The California Department of Fish and Game is providing written papers for publication in the proceedings to inform managers and entomologists on methods to access the Natural Diversity Data Base. Hopefully, this will provide districts with the knowledge necessary to access the Data Base to determine where rare and endangered species exist in the districts.

BUDGET AND EXECUTIVE COMMITTEE.-Frank Pelsue, Chairman. The Committee is charged with the responsibilities of preparing agendas for the Board of Directors' meetings, preparing annual budgets, reviewing Association expenditures and recommending the dues schedule for corporate members based on the needs of the Association.

The Committee discharged its duties in a responsible manner during the past year, doing an outstanding job. The Association's budget increased slightly over the previous year, from \$55,719 to \$57,583 for 1984. This represented a 3.3% increase over the 1983 budget.

The Association was able to keep the corporate dues at the same rate as 1983 with slight modification. The dues for 1984 were 0.25% of the actual expenditures of the operating budget for the 1982-83 budget year rather than the proposed 1983-84 operating budget. The 1983 dues were based on 0.25% of the 1982-83 operating budget. The other modification consisted of increasing the budget cap from \$2,750 to \$3,000 for those districts with operating expenditures that would have caused their dues to exceed \$3,000 based on .25% of their operating expenditures.

This Committee feels we now have the Association on a firm financial footing, with the future looking exceeding bright.

PUBLICATIONS COMMITTEE.-John Combs, Chairman. The Publications Committee met five times and enjoyed a very busy and productive year. The Committee continues to work closely with the Central Office Committee and with the Association's Executive Director and staff.

Among its accomplishments in 1983 have been: an enlargement of the Committee's charge; recommendation of the I.B.M. "Displaywriter" for the Central office; approval of Yearbook format and distribution; acceptance by Prof. Reeves of the task of authoring a thirty-year sequel to his monograph "The Epidemiology of Arthropod-borne Viral Encephalitides in Kern County, California, 1943-1952"; approval for recognition of the contribution of Dr. Wesley Nowell in compiling a comprehensive index of the Association's Proceedings and Papers.

The Committee is deeply appreciative of the efforts of its editors, and particularly of our Editor-in-Chief, C. Donald Grant of the North Salinas Valley M.A.D.

There are two new undertakings for 1984. The first will be to provide the Board with sufficient information to determine whether or not the Association should provide the membership with a newsletter. The second task will be the adaption of our publishing procedures to computerization.

WAYS AND MEANS.-Gil Challet, Chairman. There were no major items submitted to this Committee for consideration.

INTEGRATED PEST MANAGEMENT.-Gilbert Challet, Chairman. A policy was presented to the C.M.V.C.A. Board of Directors. It is this Committee's recommendation that this committee be dropped in 1984.

CHEMICAL CONTROL.-L. Lino Luna, Chairman. The Chemical Control Committee accepted the mandate to pursue the development of a series of Chemical Control Notes similar to the format of the Mosquito Notes. The Committee has reviewed its first draft of the Notes and is in the process of streamlining them. It is hoped that the task is completed in a successful manner by the end of 1984.

The Committee also discussed and implemented a request from the California Department of Food and Agriculture to have every mosquito control agency include the "Number of Pesticide Applications" on their Monthly Pesticide Use Reports.

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

Don Womeldorf's assistance on this item is most certainly appreciated and acknowledged.

ENTOMOLOGY AND BIOLOGICAL CONTROL.-Chuck Hansen, Chairman. A project underway is the draft development of a series of Bio-Notes, patterned after the Mosquito Notes, which will depict various predators and parasites in our operational program. The mermithid nematode, mosquito fish, guppy and backswimmer will serve as prototypes for this project.

The development of a color slide series on the predators and parasites encountered by field personnel which can be used as a training guide is a project under consideration.

Projects underway by the Entomology subcommittee are the draft development of a midge write-up, a Bti single sheet handout and a series of handouts on SLE, WEE and canine heartworm.

The Committee will attempt to draft recommendations which will hopefully improve current systems and greater standardization and reliability in the collection of light trap data as it appears there is a wide variance in reporting methods from district-to-district.

EQUIPMENT COMMITTEE.-Claude Watson, Chairman. Of significant importance is the development by the East Side M.A.D. of a Closed Loading System for handling wettable powders.

COOPERATIVE PLANNING.-Charles Dill, Chairman. This past year the Committee has conducted meetings and corresponded with State and Federal Wildlife agencies, the legal staff of the State Department of Health Services and with Earl Mortenson in order to complete a final draft of the "Proposed Cooperative Agreement with Local Mosquito Control Agencies and State and Federal Wildlife Agencies".

There have been many changes, mostly dealing with the format, since the original draft was submitted for comment to the various C.M.V.C.A. regions. The Committee believes that the document they now have will enable them to achieve their goals, without compromise, and will eliminate costly delays experienced in the past when working in areas of this type. Copies of the draft will be submitted to regional representatives of the C.M.V.C.A.; and upon reaching a consensus with the C.M.V.C.A., the Committee can formally and officially meet with State and Federal agencies involved.

The Chairman wishes to extend his thanks to all members of the Committee and to the C.M.V.C.A. Board of Directors for their support. Special thanks is given to Earl Mortenson for his tremendous amount of work on developing the concept and all the re-writing that was necessary to produce the current draft.

COMPUTER COMMITTEE.-Chuck Beesley, Chairman. A two-day workshop was held in April, 1983 at the Contra Costa M.A.D. One dozen districts attended together with staff from the Department of Health Services, V.B.C.B. This was a practical/operational workshop which included discussion and comparison of programs - both written and purchased by districts.

A list of programs on hand at each district has now been compiled for reference. This has been particularly helpful for districts wishing to compare or troubleshoot programs. The list is available upon request from the chairman of the Computer Committee. A brochure of operational programs written by districts is currently in progress.

A computer demonstration was held at the 1984 C.M.V.C.A. conference which included computer programs being run and written copies of district print-outs on administrative and operational programs. Districts using computers are Alameda, Contra Costa, Marin/Sonoma, San Mateo, Solano, Butte, Fresno, Sacramento/Yolo, Sutter-Yuba, San Joaquin, Fresno Westside, Kings, Madera, and Orange.

PHYSICAL CONTROL.-Cal Rourke, Chairman. Preparation of a "field guide" on physical control is an on-going project on which the Committee continued to work. The introduction to the guide has been written and approved. All members of the Committee are continuing to take pictures, many of which will be used in the guide. The Committee plans to complete this project in 1984.

AD HOC TRAINING-Don Womeldorf, Chairman. The Committee completed its charge to develop a plan for a continuing education program designed to maintain a high level of proficiency in certified mosquito control technicians. Recommendations accepted by the C.M.V.C.A. Board of Directors included: (1) Establishing a Continuing Education Committee under C.M.V.C.A. auspices to develop and oversee a mandatory continuing education program for employees of C.M.V.C.A. members; and (2) Establishing a series of Regional Accreditation Committees and carry out the program.

LEGISLATIVE.-Allen Hubbard, Chairman. Special projects in 1983 included the passage of Senate Bill 628, recodification of the laws relating to mosquito and pest abatement districts and making recommendations to the C.M.V.C.A. Board of Directors about legislation.

Legislation that could have an impact on mosquito abatement districts, as well as local government, was reviewed. In addition the Committee formed and maintained a close liaison with Ralph Heim, the C.M.V.C.A. lobbyist.

WHAT'S HAPPENING IN THE DEPARTMENT OF HEALTH SERVICES[1]

Patricia A. Rauscher, Chief Deputy Director

ABSTRACT

The Department of Health Services has been reorganized under the current Administration. There are several Divisions including Environmental Health under a Deputy Director in charge of Preventive Health Services. Other Deputy Directors head Medical Care Services, Toxic Substances Control, External Affairs and the Office of Legal Services. In addition, some units answer directly to the Director's office.

The Governor's budget for fiscal year 1984/85 emphasizes education as well as construction and renovation of public buildings. The budget for health and welfare will increase about 2% overall. This will allow the Department to continue to provide the services needed by the residents of the State of California and to better assist the counties as well.

Regarding vector control, we will be able to continue to provide vector control services at their present level of activity. Any increase would have to come about by redirecting other resources. The Department is encouraging implementation of the Emergency Mosquito Abatement Funding Act of 1983. We will be available to assist each of you in implementing that Act. A legislative proposal currently being studied would authorize the Department to control infestation of exotic vectors into the state. Such importation would be allowed only if would not endanger the public health and safety.

The Department is very interested in legislation dealing with toxic substances control, especially in relation to cleaning up toxic waste disposal sites. The subject of indoor air pollution is receiving attention and the Department hopes to fund research in 1984 to look into the cause of illness related to indoor air pollution.

Other proposed legislation would establish a new partnership with counties. The Public Health Enhancement Program would allow counties to take control of five major preventive health services. Administrative savings would go to the counties as increased funding.

The Department wants to continue to maintain communication with the California Mosquito and Vector Control Association. You are invited to contact me if you have any questions or problems.

[1]Presented at annual meeting, California Mosquito and Vector Control Association, January 30, 1984.

CALIFORNIA CONFERENCE OF DIRECTORS OF ENVIRONMENTAL HEALTH PRESENTATION

Richard L. Roberts, Secretary
135 Palmyra Street, Auburn, California 95603

I bring you warm greetings from CCDEH and the California Association of Environmental Health Administrators. Both President Dennis Otani, Director of Environmental Health, Kings County, and Bill Norman, Director of Environmental Health, Merced County, send their regrets that they were unable to attend and asked me to pass along their regards. As your program indicates, the Director's Conference has a Vector Control Committee and Bill Norman is the chair; and serves well in this capacity, as he was instrumental in forming the Merced County MAD and still serves as a founding trustee.

I will briefly discuss at least five forums where the CMVCA and local environmental health agencies have an opportunity for interface and cooperative ventures.

First - Several years ago, two wise leaders of our respective organizations decided that there should be an exchange of presidents to speak at our respective annual conferences. This has been an effective means to better understand the mission of public service we both have and how our respective services can interrelate for mutual public health benefit. I'm sure if you ask your President, Frank Pelsue and President Elect, Jack Fiori they will tell you the director's conference last fall at the Asilomar was both educational, interesting, and entertaining.

Second - The California Conference of Local Health Officers' Environmental Health Committee, chaired by Sonoma County Health Officer, Bob Holtzer. Your representative to that Committee, Bill Hazeltine, has been very effective in assisting the Health Officers to establish various environmental health positions, especially on recodification of the state Health and Safety Code for MAD/VCD's and PAD's. Bill works quite well with the health officers, the directors of Environmental Health and the other public health officials who serve on the Committee.

Third - The CCDEH Vector Control Committee formerly chaired by yours truly and now most capably chaired by Bill Norman. This committee established a task force two years ago which includes several members from CMVCA and the SDOHS/Vector Biology & Control Branch to develop a local land use guide checklist for vector control.

The checklist has been approved by both associations and is being effectively used by local entities. This task force will now take the next step to develop land use guideline standards for mosquitoes and other vectors. A lot of work - but it will be extremely helpful to local environmental specialists and planners as we review and approve local projects. Frank Pelsue, Gil Challet, Cal Rourke, Fred Roberts, Earl Mortenson, and Al Quintana have helped in this effort. Incidentally, Bill Norman says he will be calling a task force meeting soon. Bill's committee also has a charge from our conference to develop the ways and means to provide advanced vector training for our local environmental health specialists and sanitarians. I hear that the CMVCA has a Mosquito Control Certificate Continuing Education Program. We would like to know more about it.

Fourth - The SDOHS Vector Control Advisory Committee. Once again representatives of both of our associations meet with representatives of the SDOHS, other state agencies, academia and industry to develop recommendations to the State Director of Health pertaining to statewide vector issues.

Fifth and Finally - At the local level. We are all on the public health team. There is more than enough vector control work for everybody and there is every reason for the local MAD, VCD or PAD to work with and coordinate with the local environmental health agency and, vice versa. Beyond mosquito control, environmental health agencies must deal with the other vectors that plague our rural, suburban and urban areas: rats, flies, cockroaches, lice, fleas, ticks, spiders, wasps, etc.

For example, in October, 1979 the Vector Biology and Control Branch, Department of Health Services, conducted a survey of local environmental health agencies to obtain information on all vector control needs. One survey question was to rank the importance of different categories of vector problems encountered by the local agencies. In their response, the domestic rat problem was ranked most often as the most important vector problem of the seven categories of vector problems listed.

Following, in 1980, another survey was conducted. The answers received were based primarily on information derived from agency experience and citizen complaint response for urban rats. This survey was conducted in cooperation with the Vector Committee of the Directors Conference and with the aid of the California Agricultural Commissioners Association.

There was an excellent response from these local agencies. Forty-six of the 58 counties in the state indicated that they have a domestic rat problem reported by either the local environmental health agency or the County Agriculture Commissioner.

Thirty-two out of 49 local environmental health agencies reported having a domestic rat problem. Of those 32 having problems, 13 indicated a problem with roof rats, 4 reported having a Norway rat problem, and 15 said they had a problem with both roof rats and Norway rats. The problem is increasing!

Local county funding for Environmental Health Agency vector control activities has been drastically reduced over the past several years due to competition for local county/city funds. For at least three reasons:

(1) It is not a mandated program - at least service level is not.
(2) There is very little opportunity to collect fees for services, and
(3) Vectors are perceived by many as a low health risk.

Yet, in my county (San Bernardino), almost half of our citizen nuisance and health complaints are vector related - - approximately 2,500/year. We are looking for unique and innovative ways to establish stable funding mechanisms for Vector Control; and, I know other health agencies are doing the same. We need advice and your help to satisfy the needs of our local communities.

Tomorrow, on your program, Lyle Stotelmyre, our Supervisor of the DEHS Insect & Rodent Vector Control Section, is presenting a paper titled, "1984 - Brave New World For California's Newest District". He will describe how we went about getting this district formed in order to provide for a stable funding mechanism. It is anticipated that the district will contract with the County, Department of Environmental Health Services, to provide the control activities. We had our first Board of Trustees meeting - January 16, 1984.

In conclusion, we can site many examples of where MAD, VCD and PAD programs are well coordinated and mutually supported with the local environmental health agency. If this is not occurring in your area, I encourage you to take the first step. Invite the local environmental health officer to visit with you, share information, attend your trustees' meetings as a health consultant, and generally become better acquainted.

We appreciate this time on your program. Your program indicates a most educational and productive meeting - I congratulate you.

CMVCA President-elect Bill Hazeleur - We look forward to you attending our Conference next fall. Bring your cards, dice and money.

THE VECTOR CONTROL TECHNICIAN CERTIFICATION PROGRAM IN CALIFORNIA-- 1974 TO 1984

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Nineteen eighty-four marks the tenth anniversary of the Vector Control Technician Certification Program administered by the California Department of Health Services' Vector Biology and Control Branch (VBCB). The first examination for certification in mosquito control was given in California on April 5, 1974. This year, on May 18, 1984, we will give our fifteenth statewide certification exam.

The certificates we issue qualify a person (while working on the job in a public health vector control program) to use or supervise the use of pesticides that are classified for restricted use in accordance with the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), as amended, and related State laws and regulations. Our program is officially recognized by the U.S. Environmental Protection Agency (EPA) and the California Department of Food and Agriculture (CDFA).

The idea of certifying vector control technicians originated in California before the 1972 amendments to FIFRA that gave certification a Federal mandate. Instead, it began here because of actions taken by the California Mosquito Control Association in the early 1970's (Mulhern, 1975). At that time there was an extreme amount of concern about the growing problem of pesticide resistance in mosquitoes in many areas of California. As one way of dealing with this problem, the CMCA and its member agencies proposed that as training program be developed by the State Health Department to teach some other approaches to mosquito control which could provide effective and safe alternatives to chemical control as it then existed.

A series of training workshops was conducted in 1971 by the then Bureau of Vector Control and Solid Waste Management (BVCSWM, later re-designated VBCB). The course syllabi were combined into a draft manual called the <u>Comprehensive Mosquito Control Training Manual</u> which was distributed for review and comments in January 1972. After two revisions, this manual became the <u>Training Manual for California Mosquito Control Agencies</u>, Revised 1975. It is published by the CMVCA and is in use today as the basic training manual for certification in mosquito control.

Also in 1972, CMCA President Ronald L. Wolfe established a joint CMCA-BVCSWM Committee on Certification of Operational Personnel. The committee submitted a report at the Board of Directors' meeting in May 1972 calling for a certification program to be administered by the Bureau. Its recommendations and guidelines closely anticipated the standards that were later

Table 1.-California vector control technician certification examinations: persons examined and results, 1974-1983.

EXAM NO.	DATE MO/DY/YR	PERSONS EXAMINED / % PASSED TOTAL	SEC A	SEC B	SEC C	SEC D	CERTIFICATES ISSUED MOSQ*	TERR*	VERT*	TOTAL
01**	04/05/74	603 / 92.0					555			555
02**	10/18/74	107 / 66.4					71			71
03**	05/23/75	128 / 71.1					91			91
PILOT	02/19/76	31	31 / 96.8			31 / 96.8			30	30
04	04/09/76	428	428 / 86.9	105 / 58.1		355 / 93.5	58		313	371
05	11/05/76	164	118 / 66.9	87 / 79.3		73 / 72.6	65		54	119
06	05/27/77	405	357 / 91.9	195 / 83.6		299 / 93.6	160		285	445
07	11/04/77	58	44 / 84.1	49 / 75.5			43			43
08	05/05/78	465	150 / 62.0	127 / 63.0	377 / 89.4	76 / 65.8	81	334	52	467
09	02/23/79	347	161 / 77.6	103 / 70.0	249 / 86.3	165 / 80.0	47	187	103	337
10	02/29/80	319	216 / 77.7	103 / 55.3	186 / 68.3	176 / 78.4	58	127	131	316
11	05/23/80	166	84 / 61.9	147 / 55.8			81	1	5	87
12	05/15/81	278	169 / 76.3	144 / 55.6	140 / 67.9	147 / 63.3	77	94	94	265
13	05/21/82	213	115 / 76.5	108 / 60.2	130 / 58.5	110 / 76.4	65	79	88	232
14	05/20/83	271	165 / 75.2	137 / 49.6	136 / 68.4	128 / 64.1	66	94	83	243
	TOTAL	3983 exams given to 2359 individuals					1518	916	1238	3672
	ACTIVE as of 12/83	1366***					871	745	897	2513
	INACTIVE as 12/83	993					647	171	341	1159

* MOSQ=Mosquito; TERR=Terrestrial invertebrate; VERT=Vertebrate.
** Early 105-question combined exam for certification in mosquito control.
*** 1239 certified and 127 not certified.

mandated by the EPA in its regulations in October 1974.

A tremendous boost was provided to our program by over $70,000 of Federal assistance obtained from the EPA during 1974-75 (Hansgen, 1976). This money helped us (1) to revise Community Pest and Related Vector Control, Second Edition 1975, our other basic training manual which is published by the Pest Control Operators (Association) of California, (2) to develop and produce a series of 19 self-study units incorporating synchronized slide/tape presentations, reading supplements and practice quizzes, and (3) to purchase audio-visual equipment which can be loaned to local agencies to view the self-study units.

The program reached its present form in 1978. Since then, at least once each year, we administer a 4-part written exam, with 50 multiple choice questions in each part. Exam answer sheets are graded on a Scan-Tron grading machine at the CDFA in Sacramento. All applicants must pass Section A, the Core Section, to be certified for any of the three certificate specialties. Then they must also pass a specialty section: Section B to be certified for mosquito control, Section C for terrestrial invertebrate vector control, or Section D for vertebrate vector control. The passing score for each section is 35 correct answers, or 70 percent.

Fourteen (14) statewide exams and one pilot exam have been conducted since 1974 (see Table 1), for a total of 3983 exams given to 2359 individuals (the difference is because many have taken the exam on more than one date). A total of 3672 certificates has been issued in the three specialties. At the end of 1983, our files, which are updated annually, showed 1239 certified technicians active, that is, currently employed in public health and vector control agencies. Of those, 871 are certified for mosquito control, 745 for terrestrial invertebrates, and 897 for vertebrate vector control.

It should be noted, however, that not all these certificates are held by technicians who are actually applying pesticides in operational vector control programs. Many certificates have been issued to Registered Sanitarians and other employees of county environmental health agencies. A policy decision was made early in our program to open the training program to them also, and to allow them to take the exams, even though the training and exams are specifically intended and designed for technician-level personnel in operational vector control programs.

Of the 871 certified mosquito control technicians active at last count, 409 (47%) are employees of agencies that are corporate members of CMVCA. Adding the certified technicians in a few other operational programs outside CMVCA, such as Imperial, San Diego and Santa Clara counties, should bring that figure up to around 50%. Employees of CMVCA member agencies also hold 148 certificates for terrestrial invertebrate vector control (20% of the total) and 142 certificates for vertebrate vector control (16% of the total).

The administration of our program was significantly improved in 1982 when VBCB acquired a Radio Shack TRS-80 Model II microcomputer. The certification records of all active technicians who had ever taken our exam were entered onto a Profile + filing program. The computer greatly facilitates the locating and updating of records, allows us to quickly produce printouts to send to local agencies for records updating and to report exam results, and it even fills in the blanks on our certificates. It also is used as a word processor for printing exam notices and other correspondence and to print mailing labels.

The VBCB continues to assign a full-time Training and Certification Coordinator to oversee this program. The position's charges are to coordinate future exams, to assist the CMVCA with the continuing education program that it is developing, and to coordinate updating revisions of the manuals and other study materials. In particular, we plan some substantial changes in the organization and content of the basic manuals during the next couple of years, and we look forward to continuing our close cooperation with the CMVCA and its members in making this program a success.

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USE OF CARD READERS TO INPUT FIELD DATA

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ABSTRACT

Two computer programs were written in Basic, for the TRS-80, Model II, utilizing a Chatsworth Data OMR-2000/RS232 Card Reader to input field data for storage on magnetic media. A considerable amount of time was saved when using card reader input over keyboard input. Copies of the programs are available through the CMVCA Computer Committee.

INTRODUCTION.-Orange County Vector Control District (OCVCD) receives more than 10,000 service requests each year. Each one of these requests must be recorded, answered, and summarized. Using multipart hand-written forms, it took a minimum of 3.25 days each month to sort and produce a monthly summary report. The summary contained only the vector groups which initiated the service requests (mosquitoes, midges, flies, rats, or fish) in the zones in which the work was performed. To obtain any other information, such as what kind of sources produced the vector problems, would require that all the forms be hand sorted again.

OCVCD wanted all of the information stored in a way that the information could be sorted and summarized as quickly as possible. Computers are capable of achieving this, but it still takes as much as one minute and 12 seconds to input the necessary data for a 45 character record when using the keyboard. Multiply this by 10,000 or so, and it takes 200 hours, or about 2.1 days per month just to input the data. This is a savings of only 1.15 days per month.

Optical card readers can process a card in about two seconds. That is a good deal less than one day per month to record all, not just part, of the data. The 10 minutes that the computer takes to summarize the data and print the report is not even worth counting.

HARDWARE DESCRIPTION. The unit purchased by the District is a Chatsworth Data Corporation, OMR-2000/RS232 Card Reader that is connected to their TRS-80, Model II by the way of the computer's RS232 serial port.

The OMR-2000 is an optical mark sense card reader. It will read cards that are marked with No. 2 pencils (or equivalent), or can read punched or pre-printed data just as easily. The reader will hold about 120 cards at a time and process cards that are from 5 to 12 inches long. Like most card readers, the cards must be 3.25 inches wide and be made of card stock that is 0.007 of an inch thick. That means you have to buy general purpose preprinted cards, or have the cards specially printed. The print registration tolerances are extremely close, within 1/64th of an inch, and the ink must not have even a trace of carbon in it. There are several printing companies in So. California that can do this kind of work. Names and addresses of these companies will be provided upon request.

The cards are read by the machine as a series of columns that are divided into 12 channels. This can be seen by holding the card horizontally, with the black timing marks at the bottom. Each timing mark represents one column, while each number on the column represents a channel. There are 12 channels on each column that read, from the timing mark, as 9, 8, 7, 6, 5, 4, 3, 2, 1, 0, 11, 12). The card reader will read the data channels only when it encounters a timing mark. This allows cards to be designed with areas for written information that will not be included in the data input.

The practical concern of how the cards should be marked is always important and can "make-or-break" the card reader system as an effective method of data input. Experience has shown that the easiest and most effective pencil marks are the ones forming a diagonal slash from one corner of a number box to the opposing corner. It is very important to stay within the confines of the box. Otherwise the card might be improperly read. It is also vitally important that no marks be made in between the timing marks, because the card reader would then insert extra columns into your data string causing the computer to interpret the data incorrectly. Also, the reader will always read 12 channels on every card, therefore marks of any kind should not appear in the vicinity of unused channels. Lastly, dirt and oil smudges will frequently be read as extraneous data, so the cards should be kept clean.

SOFTWARE DESCRIPTION. As always, the longest part of the project was developing the software that would handle the data in the way the OCVCD wanted it processed. The things that needed to be done were:

1. Create a file in which the data records could be stored.
2. Command the card reader to read a card.
3. Input the card's data into the computer memory.
4. Check the data to see if they are valid. (this is optional, but highly recommended).

5. Change the data into a form that can be put into long term storage.
6. Store the data onto floppy disks.

Two programs were developed that would fulfill the above criteria. Each program stores the data in a different manner and is designed to be used for somewhat different purposes. The first program (CARDRD1/BAS) stores each record separately and is intended for those applications requiring special report formats, such as the District's monthly Cause of Service Request Report. The other program (CARDRD3/BAS) stores records in groups of three, in a form that can be processed by Profile II, Radio Shack's data base management software.

Both programs use the same method to command the card reader to read a card. The "Host" function, that comes with the operating system of the Model II, is turned on (SYSTEM"HOST ON" Command) which makes the computer think that the card reader is a remote keyboard terminal. One of the two sets of switches located on the side of the card reader is set in a pattern corresponding to the binary code of a specific control character; in this case a "Control Q" for which the Basic command is "PRINT CHR$(17)". Whenever the card reader receives this control character, it reads a card. The card data are transferred to the computer's memory via the basic command "INPUT CD$".

A "Do File" with the file name of "READER" is used to turn on the "Host" function. One peculiarity of this programming method is that since the memory locations used by the "Host" function and the "Do File" partially overlap, the "Do File" must contain a statement that reloads Basic.

Listings of both programs and the "Do File" will be made available upon request. Magnetic copies of these programs will be added to the CMVCA Computer Resource Disk. Both will be available through the CMVCA Computer Committee.

CONCLUSION.-The use of card readers has the potential of effecting considerable savings in the time required to input field data. Also, the potential for typographical errors is greatly reduced because the data do not pass through the intermediate step of keyboard input. Card readers become truly cost effective in applications where there is a large volume of data, or when there are a number of different applications requiring rapid data input. The programs described in this paper can be easily modified to accommodate a wide variety of field data input applications.

EMPLOYMENT RELATIONSHIPS OBJECTIVES

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Managers would like to think of themselves as being perceptive, having keen judgment, capable of sizing up a situation and knowing just what to do about it.

And employees are people who are diligent, discerning, show good judgment and clearly understand instructions.

But, the enlightened will agree with H.G. Wells' observation that "no human being is altogether noble nor altogether trustworthy nor altogether consistent and not one is altogether vile."

So for the bedeviled managers that are beset or plagued by problems or worries or doubts and indecision, I have some suggestions for you. I feel quite safe in doing this as I understand that half of everything told to a person is forgotten in thirty minutes and the other half wasn't heard.

It has been my experience that experience is what you get when you were expecting something thing else. I am in excellent company as British Statesman Disraeli noted, "What is anticipated seldom occurs: And what we least expect generally happens." This is the reason one must determine the objective of any project and have an adaptable, flexible and above all tentative plan. Remember those three words, they can be applied to many situations. In fact, tentative is as good as sending it to a committee. For example, a tentative suggestion is one that can be withdrawn as the situation clears up or a tentative program can be changed and hopefully improved as time goes by.

Once a firm position has been taken, it is difficult to change one's mind no matter how strong the evidence to the contrary. Hunches are not enough, it is important to ascertain facts by examination, trial and error, experiment, or any other means possible. There are alternative methods of arriving at the same results.

Don't be afraid of appearing inconsistent as changes are justifiable due to arising circumstances. However, confusion about one's ultimate goal can be detrimental to attaining success or progress. If you are in doubt about what to do, it is usually a good idea to wait, defer, even procrastinate. Procrastination has been said to be the art of keeping up with yesterday.

But don't delay decisions until you are at the mercy of deadlines, don't insist upon too much information, and be forced into reaction. Set your priorities. Follow through.

The most common problems--accidents, turnover, absenteeism and grievances--are often related to poor morale. Managing for better morale aims at two related goals, happier employees and improved production.

As every child and successful manager knows, each person is an individual and responds differently to whatever. But yet these individuals are to be treated fairly. An employee's attitude towards his earnings, supervision, hours, fringe benefits, fellow workers, his prestige and status and other working conditions will determine his morale.

It must be recognized that an employee must be paid adequately. The pay must accurately reflect the duties and responsibilities of the position and be justified by acceptable comparison methods such as salary surveys and classification studies. A standard salary schedule with salary ranges for all positions helps establish an orderly and methodical procedure for handling salary concerns.

When an employee is requested to do something he is not being paid for or does not have the ability to carry out or is not within reasonable limitations of his assigned job, expect difficulties. Think about the people involved whenever possible rather than just the work. Realizing a worker's feelings on his work situation may not ensure high morale but it certainly will not do any harm.

You have to be willing to take the time to observe the people you work with in order to "treat them right." Everyone does not react alike to standard politeness and kindness. The "treatment" needs to be adjusted to the mood and sentiments of the individual. People, being individuals, refuse to be treated in a uniform way.

People who are upset do things impulsively. Emotions drive them--not intelligence. A sense of proportion is absent, little things are magnified, really important matters will be overlooked or forgotten.

Although an employee's errors, inefficiences and maladjustments are higher than that of a machine, the employee is still more valuable. The machine is easily replaced. Develop and use sound methods and certain procedures for solving employee problems. Avoid wondering what you did right or wrong. A hit or miss solution is hardly likely to be successful again or even remembered. Problems when ignored do not go away, they get worse.

Employees will know they are appreciated if their problems are handled as quickly and fairly as possible. If employees are complaining about "The District" they are actually talking about the treatment they have received from you and/or their supervisor. Therefore, choose the best possible people for your supervisory staff. It will be these people who will reflect your concepts on personnel matters.

You cannot afford to run out of supervisory talent anymore than you can afford to deplete supplies. Without someone to share the workload, a manager will be a frazzled wreck and may even make some mistakes. He needs all the help he can get.

The core of any manager's responsibility to his organization lies in decision making. And the first decision to make is to whom to delegate duties. Choose wisely, a delegated duty is still your responsibility.

Developing supervisory personnel is not done over night, but the training improves present job performance, promotion is less difficult and provides temporary relief during absences and permanent replacement if the need arises. Failure to make use of subordinates' abilities may dull their initiative and undermine their confidence and proficiency. If the manager feels that he can do the job better, he has done a poor job of training.

Delegating means you tell your people what you want done, but not <u>how</u> to do it. You are interested in results, <u>not</u> minor details. Too close supervision restricts both you and your employees. Expect mistakes, but when the error is realized, correct it but don't agonize over it--move on. This procedure saves time and aspirin.

Provide your employees with an effective training program. If the job is to be done adequately, it takes more than an employee and an instruction manual. In handling people, it is important to differentiate between those quick to get the point and those who require patient explanation. Patience is the key to effective training. Being impatient doesn't speed things up. It produces disorder and delays, makes employees nervous, rushes them so they become less capable of getting out the work and may cause resentment and a feeling of being unjustly pressured. Be patient when giving instructions, in setting time limits, and improving work habits. Often times slower will lead you to your goal quicker. Remain objective, try to see it from the employee's viewpoint. Tell the employee the reason for doing a job a certain way. When he knows the reason, the method will be easier to remember.

Don't lose contact with the work force or you will lose your chance to motivating your people to their best performance. You can lose an employee without him ever leaving the payroll. He puts in his time, but never lives up to his potential.

Keep the work load distributed fairly, even if some quality is sacrificed. Don't take the attitude that an employee is paid to do his job well and forget to show appreciation and recognition when a good job is done. Remember whenever possible the praise should be public but criticism is <u>always</u> private.

Check your communication system, there may be an information gap. Establish good communications with your workers. Tell your employees in advance about policy or operation changes that will affect them. Provide information and guidance on matters affecting employees' security. It helps to keep rumors down and builds their confidence in you. Ask for suggestions, the man on the job is in the best position to give you ideas for improvement. Discussing changes does much to overcome resistance; it gains cooperation.

Keep an open mind and an open door. Encourage complaints. They aren't all bad. Let your people know when you will be available to hear their complaints. No matter if the complaint is trivial, it is significant to the complainant. Sometimes the complaint isn't really what is bothering the employee. It is not easy to recognize and express less logical reasons as it is to express concrete ideas. The better workers usually complain about things that prevent them from making more money or accomplishing more. The adequate worker usually complains about things that concern his personal comfort and is looking for recognition. The way the complainant, not necessarily the complaint, is handled will affect his productivity and morale. Complaints are symptoms and are to be considered early warning signals.

Rules and regulations must be known and applied uniformly. You will get no respect, if you try to retain your employees' goodwill by failing to enforce rules and regulations. Nothing influences employment relationships quite as much as the inequitable distribution of rewards or punishments. Don't give special privileges unless a special situation warrants it and everyone understands it.

Employees are responsible for performing the work and conducting themselves within established regulations. But everybody carries some responsibility for employee discipline. Hastily applied discipline may make waste of employee morale, supervisory reputation, and administrative time. Defer discipline until individual circumstances have been considered. Talk to the employee to be sure he knew the rule and had no good reason for breaking it. Ignorance may be no excuse but willful violation brings harsher penalties. Documentation of disciplinary action is a legal and practical necessity. Discipline is to encourage effective work and sensible behavior on the job. Most people are willing to accept discipline they consider fair--but they will never accept punishment. Discipline should help employees to help themselves. Make sure your employees understand the established disciplinary procedure. Arbitrary regulations or lack of due process will create feelings of helplessness and will encourage grievance complaints where the grievance procedure exists and abet unionization where it does not.

Discipline is effective only when it is generated by the employee's desire to cooperate. The employee cooperates to keep his job and/or to make things easier for himself. Lasting discipline is always self-imposed and self-enforced.

In the case where the employee fails to respond to prior corrective measures, suspension or discharge may be necessary. Keep in mind that the worker is responsible for his present situation. In fairness to the employee and to avoid morale and legal repercussions, these decisions should be planned in advance and made with care.

When discussing personal or job related problems with an employee, don't advise. Listen well, keep it private and confidential. Don't jump to conclusions or interrupt. Relate the personal problem only to how it affects job performance. Refrain from using sarcasm and speaking in a loud and commanding voice. Do not vent your anger and frustrations. The involved employee's reaction may be resentment and open angry rebellion that may be difficult to deal with. The manager must maintain control of himself in order to control the situation. Stay in control, it is better to control your temper than to let it control you.

There is more to influencing people than capturing their attention. You have to handle objections without forfeiting goodwill; win arguments without creating hostility.

Positive incentives on the job make restrictive regulations unnecessary. A survey indicated the most important fringe benefits to workers were health benefits and sick leave. Review rules, eliminate those that do not contribute to your objectives. The purpose for the rule should do the following:

1. Maintain worker safety
2. Avoid equipment breakdown
3. Control expenses
4. Prevent disruption of work flow

Rules which have no apparent reason are irritating. However, before changing a policy be sure it is an improvement.

Don't hold meetings to make a public example, when time is short, when it interferes with the work schedule, or for no good reason. In fact, don't change anything or do anything except for a good reason.

Remember: communicate with your employees; let employees know what the rules are and what is expected of them; let employees know how they are doing; apply your policies fairly and equally; have adequate wages and decent benefits; and develop good supervisors and back them up.

Cooperation, communication and coordination assure a successful operation. Only with the active participation by all concerned can goals be met.

The basis of this report was derived from the following sources:

--Executive Action Series: Bureau of Business Practice: Waterford, Connecticut, 1970.
--Personnel Management and Safety: University of California, 1983.
--California Association of Employers: Employer-Employee Relations Guide, 1983.
--Turlock Mosquito Abatement District Personnel Rules and Regulations, 1983.

Although this report is general, there is much specific information available for any particular aspect of employer-employee relationships.

PANEL: ALTERNATIVE METHODS TO CONTROL MOSQUITO SOURCES

PHYSICAL CONTROL IN SACRAMENTO COUNTY-YOLO COUNTY MOSQUITO ABATEMENT DISTRICT

Calvin D. Rourke

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Over the years we have heard a variation of definitions of source reduction or physical control, as it is now more commonly referred to. Whatever the wording is that you are familiar with, it pretty much boils down to a term that basically says that we would like to "physically alter an existing or potential mosquito problem in order to minimize or eliminate its potential for mosquito production". This is a rather simple statement and it is very easily understood, but just how simple is it to convince an individual that it would be to his advantage to rid his property of his mosquito source? And how easy is it for him to understand that in the long run he will be money ahead?

After a thorough examination of the mosquito problem, these questions can be answered, but in the process there arises a whole host of questions. Questions that must be throughly investigated and intelligently answered to help the responsible person to better understand the whole picture of mosquito control and better appreciate the service we are trying to offer. A few of those questions might go something like this:

1. Is the problem caused by lack of adequate drainage or is it a problem of water management, or are there other factors?

2. If it is a drainage problem, is there a suitable nearby outlet that can be utilized? If an adjacent property is involved can we impose on that property owner to construct a drain? Or are there several ranches that can be joined with a common drain to an adequate outlet? Any or all of these options should be considered. I mention these options because our district constantly uses combinations of them.

3. Is an engineering survey required? If it is only a matter of cleaning an existing ditch to accelerate the drainage, a survey is generally not required. If the problem is a little more complicated, a survey is a must, for it will tell me whether the environmental changes I am requesting are basically sound.

4. Is it possible that the farmer could qualify for a conservation practice through the agricultural stabilization and conservation service to receive financial assistance?

5. Are there any underground facilities or hidden hazards in the immediate area that have to be located? Puncturing a high pressure gas line with a backhoe could have devastating results. Severing the transcontinental telephone cable that traverses our district could cost thousands of dollars per hour to get it repaired.

6. What happens if the farmer says he can not afford to participate? I don't like to sound like a used car salesman, but if we do the job, we can give him up to twelve months to pay the bill without a finance charge. At the rate we charge, it's difficult for him to turn the offer down.

In contrast to many districts the S./Y.-M.A.D. owns and operates its source reduction equipment. The primary piece of equipment is a backhoe. We charge a nominal fee for this which in the past has been adequate for equipment replacement. If the owner of a mosquito problem does not have the necessary equipment to correct the problem, an offer is made to use district equipment.

Personal contacts are made with the person responsible for a mosquito problem. We believe in this approach because many questions can be resolved at the very beginning. We try to point out that although the initial cost may seem high, the long-term benefits, no doubt, will offset the cost particularly if our board deems it necessary to charge the owner for future chemical control to keep the mosquito population at a comfortable level. In the thirteen years we have used this program, we have never been lacking for a project to work on. Hundreds of acres of sources have been eliminated at a great savings to the district.

There have been occasions, of course, when we have not completely achieved the goal we desired. Let me mention two instances when it was necessary to resort to an alternative method to achieve mosquito control.

Case number one: After several years and many discussions with a large, well-known food processing company to improve their drainage system and set up a maintenance program on their overland flow system to dispose of the effluent, our recommendations were disregarded. We finally advised them that they would be billed for all subsequent mosquito control. This has amounted so far to several thousand dollars.

Case number two: Our district entered into a cooperative agreement with the owner of a 40 acre ranch to improve the drainage and install a return flow system. The system was also designed so the winter water would drain into a community drain. The agreement form, signed by the owner, states that he would install a sump pump for the purpose of reusing his summer waste water. When we completed the project, not only didn't he carry out his part of the bargain, but some of the drain ditches were blocked, reverting the fields back to their original mosquito producing state. The owner has been advised that under provisions of the Public Health & Safety Code, we will be charging him for future mosquito control.

In both of these instances the charging for chemical control cannot be substituted for physical control. We view it as a temporary means to help motivate the responsible individual until the time that environmental modification is implemented.

In special cases another alternative to physical control is to use the services of the Water Quality Control Board. This board is extremely short on personnel and it sometimes takes a while for them to inspect the problem that we bring to their attention, but once they do, their action is swift and very positive. Consider the case with one of our dairymen that was running dairy drain water into a neighbor's property, across a county road and into a natural drain. Water Quality called an onsite meeting. The meeting was attended by the dairyman, Water Quality Control, County Road Department, the Dairy Inspector, Department of Fish and Game, and the President of the Dairymen's Association, and ourselves. Needless to say, the case against the offender built up so conclusively that he had no alternative but to correct the problem.

Other districts have different policies and, of course, different methods of solving similar problems, but all districts have a great deal in common when it comes to solving some of the "potential" problems. We all have the opportunity to comment on the environmental impact reports that are required for practically every environmental change that is being planned by developers and many governmental agencies. Some of these developers are not aware that part of their development could actually produce mosquitoes. We should take advantage of this opportunity to bring to their attention that we are in opposition to their plan if it is obvious that a mosquito problem may arise. We should also offer an alternative plan that we are confident will not create a problem. They are learning and they are complying with our requests, but we have an obligation to keep reminding them with our written comments.

For those districts here today that are contemplating going into a physical control program, let me say that it is exasperating work, but the end results are most rewarding and long lasting. I have seen some very dramatic reductions in mosquito populations from a well planned project. It gives me a great deal of satisfaction to look at a completed project knowing full well that I have not only helped improve that person's property culturally, but I have helped him eliminate one of his problems.

SOURCE REDUCTION PROCEDURE IN SAN JOAQUIN COUNTY

MOSQUITO ABATEMENT DISTRICT

Edward P. Leipelt

San Joaquin County Mosquito Abatement District
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In the beginning of the year 1980 the San Joaquin County Mosquito Abatement District upon consolidation changed its approach regarding major sources that have a history of mosquito development.

By just sending a source reduction letter once a year to mosquito producers brought very poor results which was the practice at one time in the San Joaquin County Mosquito Abatement District. Manager Jack Fiori has incorporated new ideas and procedures that must be followed at all times when making a source reduction contact.

First, there is an on site inspection by the manager and source reduction personnel so both men have an opportunity to exchange their views on the existing problem.

After a decision is reached between the manager and his assistant on what to do, an introductory letter or first letter is mailed to the property owner or lessee.

The contents of the first letter explains the district can no longer carry on an unlimited spray program on property under their control because of unwarranted expenses and resistance of mosquitoes to chemical control.

The letter requests that the person contact our office within ten days to arrange a meeting on their property to discuss this mosquito problem which is a public health nuisance.

No costs are mentioned at any time relating mosquito control on their property because our objective is to reduce or eliminate the source regardless of size.

The purpose of meeting on their property is to avoid misunderstandings of all parties involved as too many mistakes can be made in an office from drawing maps and having various discussions. Besides, not everyone can relate to maps and directions.

At no time will the district accept a "no" answer from the person in relation to reducing or eliminating the problem.

If by chance the person does not respond to our first letter, a second letter is sent to them which merely repeats itself, but at this time states that the responsibility of the mosquito source is that of the property owner and a legal abatement agency such as ours could enforce Article 4 Sections 2270 - 2292 of the California State Health and Safety Code. Most people will then contact our office for an appointment at their convenience.

The manager and his assistant will then meet with the property owner or lessee at the time requested by them on their property.

I feel that this is the key to success when making a source reduction contact because:

1. They are talking with management.
2. There is no time lost in additional meetings with someone else before a final decision can be made. It can be made right then.
3. If there is district financial participation, which is a practice in our district, we can then make a satisfactory agreement with the property owner.
4. Estimating the cost of the job is always made prior to making a source reduction contact.

The manager can state that if the job is estimated at $500.00 for example, he can assure the farmer that it will cost no more than that amount of money and if it does cost more the district will absorb any expense over this amount. This is a very convincing statement. This approach has been very successful in the past, because people have had an estimate given to them but were very much surprised that in the final analysis it far exceeded the original estimate.

If the district does not receive a response on our second letter, we then send a third and final letter, certified, for proof of receipt.

In this third and final letter the district still emphasizes that we hope to cooperatively resolve their mosquito problems, and if not, we then state the alternatives:

1. Cite them into the district attorney's office of San Joaquin County and request a warrant for their arrest under Section 372 of the Penal Code for creating a public nuisance.
2. Cite them in under the State Health and Safety Code Article 3.5 Section 2858 ordering them to abate the nuisance for pay a $500.00 per day fine until it has been abated.

We once again ask that they contact our office within ten days so that we can cooperatively resolve mosquito problems on property under their control. If there is no response to the third letter, the manager then requests the district attorney to make arrangements to cite the person into the district attorney's office within five days after receiving the citation by mail. When both parties meet in the district attorney's office the manager then stipulates the conditions for correcting the mosquito source on their property.

If all attempts fail to correct the problem on their property a warrant for the persons arrest is made up by the district attorney and a date is set for a hearing in municipal court. As of this time we have never had anyone plead innocent.

The judge usually asks for a time frame when corrections can be made and if this is satisfactory to the San Joaquin County Mosquito Abatement District. The person in most cases receives a two year probation period if the problem is corrected and that if this reoccurs they will be immediately brought back into court and subject to a fine, imprisonment, or both. To this date the district has never had to go past this point of action.

This district prefers to use the penal code because it is much faster and brings better results. The State Health and Safety Code does require much more time and effort by the district.

By using the penal code the district's Board of Trustees would not have to be the enforcing body.

The San Joaquin County Mosquito Abatement District has taken two dairymen to court for just completely disregarding some of our requests and not keeping promises made by them on numerous meetings on their property.

Even after taking them to court both dairymen appear to be friendly to district personnel.

One dairyman appeared in Sacramento in a dispute with an irrigation district and water quality control which could have been avoided if he would have taken the advice of the mosquito abatement district.

Sometimes our requests appear to be a burden on the farmer but in the future it becomes an asset. This is where a good source reduction program benefits the farmer and also helps the district. All of the procedures mentioned in this talk are now a permanent practice of the San Joaquin County Mosquito Abatement District.

APPROACHES IN SOLANO COUNTY MOSQUITO ABATEMENT DISTRICT

Dennis Beebe, Manager

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The methods for reducing potential mosquito sources may be as different and unique as the areas that produce them. The following methods were tried and found to be successful by the Solano County Mosquito Abatement District.

The first situation dealt with a slaughter house and meat packing plant in the northern part of the District. The area that produced large populations of mosquitoes (*Culex* sp.) consisted of holding ponds which trapped the waste water generated by the daily operation of the packing plant. Large amounts of water are used daily.

After several years of applying organophosphate insecticides to these ponds, resistance developed in the mosquito populations there. Due to the proximity of this mosquito source to a city, immediate action was of the utmost importance.

The initial procedure in evaluating the extent of the problem involved observations of the plant's operation. It was noted that the water from the packing plant was discharged into a primary pond, from there it could be transferred into several other ponds. No set pattern had been established for the transference of water between ponds. A maintenance program for the ponds, i.e. weed control, etc. was virtually nonexistant. Consultations with representatives from the California Department of Health Services, Vector Biology and Control Branch, and the Solano County Department of Public Health were held before formulating final recommendations for the solution of existing problems. A meeting was then arranged with the management of the packing plant.

The recommendations presented by the District were favorably received by the management of the packing plant and were immediately implemented. The District's recommendations were as follows:

1. Replace the discharge pipes leading from the primary pond to the secondary ponds with weir boxes. This would allow the water level to remain constant and at the same time promote the formation of a thick layer of organic debris or crust over the top of the pond. This in turn would eliminate the open water in this pond.
2. A weed abatement program would have to be maintained, specifically around the perimeter of each pond.

The foregoing recommendations were followed by the packing plant and have virtually eliminated mosquito production at this site.

The second type of situation involved problems created by livestock ranchers using irrigated pasture for forage to fatten livestock. With the advent of the irrigated pasture, ranchers were able to fatten livestock and make a profit from land that had previously remained unutilized throughout most of the summer. Unfortunately an extremely intolerable situation was created by the majority of these irrigated pastures. Along with prosperity came pestilence in the form of a mosquito. The mosquito densities became so severe that livestock were driven from the fields and workers could no longer perform their duties. Eventually a meeting was arranged between the ranchers and the District to determine possible solutions for the problem. It was decided that all surveillance would be done by the District on a routine schedule. Insecticide application by means of fixed wing aircraft would be done on an "as needed" basis. The ranchers and the District would share on an equal basis the total cost of the insecticide and it's application, provided the ranchers take whatever measures possible to reduce the standing water in their irrigated pastures.

This agreement was reached by representatives of the ranchers and the District many years ago with the only binding formality being a handshake between gentlemen. Today this agreement is still in effect, with the result being a reduction by 70% of the acreage of irrigated pastures that still require the aerial application of insecticide to control mosquitoes in the Solano County Mosquito Abatement District.

PHYSICAL CONTROL PROGRAM IN DELTA VECTOR CONTROL DISTRICT

Howard Mathews

Delta Vector Control District
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The philosophy of the Delta District is that there are five constituent parts of our integrated vector control program. These component methodologies are educational, physical, biological, legal and chemical.

Physical control we define as the accomplishment of a carefully executed physical alteration of an existing or potential aquatic habitat in order to minimize or eliminate its potential for vector production.

It is generally recognized that for most mosquito-producing sources requiring control "physical-control" is, whenever practicable, the methodology of choice. The manner in which districts achieve this result, however, varies greatly. And, our physical control program at Delta has changed over the years. A large part of this change was evolutionary, but part of it was necessitated by a sharp drop in the level of funding provided to the District.

I started working at Delta in 1950 as a field operator. I worked three years as an operator before the district started a source reduction program. At that time the district hired George Whitten, a recent graduate in Agronomy from the U.C. Davis campus. His assignment was to help organize and direct our source reduction effort. I was asked to join the program because of my lifelong background working on ranches and having operated a wide variety of heavy equipment.

The district bought a crawler-tractor and backhoe, and George and I started making contacts with growers that we felt had serious field mosquito problems. At first the operational personnel did not have much confidence in our source reduction program, but as soon as we got a little experience and won the respect of some growers, we gradually began to overcome that problem.

Then came the era of resistance in the early 1970's. Our principal field larvicide just was not killing mosquitoes. The manager, Don Murray, decided that this provided an opportunity to quit relying so much on chemical control. The airplane was grounded and subsequently sold. Not only did the direction of the source reduction effort change, our whole district program became integrated as never before, and legal documentation on all problem sources became critical.

We started sending detailed maps of breeding areas and met with growers in the field. If these contacts with district personnel did not achieve the desired cooperation the growers were invited in to discuss their mosquito problems with our Board of Trustees.

On one occasion, we held seven hearings in one night. These meetings were almost always friendly in nature, but the board expected results; this made our contact work much easier - we now had the growers' attention - and they respected our program and our willingness to assist with source reduction equipment.

At this same time the district started a fly and "other vectors" program. Surveillance, physical control and legal abatement were used almost exclusively. Routine chemical treatment was never contemplated.

The aftermath of Proposition 13 forced another major readjustment of our program. Since the constitutional amendment, we have been forced to park our heavy equipment. We do, however, still have a strong weed control unit. We have increased our educational effort. We do much more planning and assist the cities and Tulare County through such things as site plan review on new development. Our most recent project has been to help in the development of a new U.C. Extension publication on dairy waste-water management.

In conclusion, farming practices have really improved since we first started. Use of our own equipment isn't as necessary, even though it means losing some of our "hands on" experience and field savvy. Our zone operators are making contacts with growers in the field, as are the foreman and manager. I think that at this point, even without our equipment, we will continue with a very strong physical control program.

Robert Peters stated at our 1975 Conference that our source reduction programs had been ridiculed by such comments as: "The ballpoint pen is the must used tool in source reduction." Although, personally, I have loved working with the heavy equipment and am proud of all we accomplished with it, it may well be that in physical control from now on the pen will, indeed, be considered "mightier that the plow."

SOURCE REDUCTION VIA LEGAL ACTION

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Ultimately, mosquito abatement district personnel come across an owner and/or a person in charge of a property that is producing so many mosquitoes that it defies normal public relation techniques. It is then often felt by the district personnel that legal action may be appropriate; and the questions asked are:

1. What do we do now?
2. How do we go about it?

The following is an overview of the statutes and the Sutter-Yuba Mosquito Abatement District experience with this procedure. Due to legal requirements and good public relations, numerous visits are made to the property in question even to the view that it may be unnecessary. The sequence of visits is as follows:

1. The mosquito control technician goes to the field foreman and explains the problem, the foreman then verifies this information by an on-site visit.
2. After reporting to the general foreman, the field foreman draws a map of the pasture and follows the next irrigation closely enough to identify the exact areas causing the problems; and he has the general foreman verify the production of larvae or pupae during this irrigation.
3. The general foreman then notifies the manager of the situation and the manager has the source reduction foreman or the general foreman confront the owner about the District's finding and request his cooperation.
4. If no progress is made, the manager has the secretary type up an abatement notice, which requires a legal description of the property, the general area where the nuisance exists, and the owner's name and address. This is obtained from the county assessors office.
5. The notice is the pivotal document and the statute sections are followed quite closely (See California Health and Safety Code 1978, Division 3, Chapter 5, Article 4, Sections 2274-2289). The notice states that:

   a. A public nuisance exists (describe the nuisance), and the location of the nuisance on the property, (ie) NW¼ of SE quarter of Section 31, Township 17N, Range 2 East.

   b. Direct the owner to abate the nuisance within a specific time by destroying the larvae or pupae that are present.

   c. Direct the owner to perform within a specific time, any work necessary to prevent the recurrence of breeding in the places specified in the notice.

   d. Inform the owner that failure to comply with the requirement of subdivision (B) shall subject the owner to civil penalties of not more than $500 per day for each day the nuisance continues after the time specified for abatement of the nuisance in the notice.

   e. Inform owner that before complying, the owner may appear at a hearing before the district board at a time and place stated in the notice.

6. The notice is served upon the owner or upon the owner's agent.
7. The notice is served by any person authorized by the District Board in the same manner as a summons in a civil action.
8. If the property belongs to a person who is not a resident of the district and there was no tenant or agent of the owner upon whom service can be made, and who after a diligent search can not be found, the notice may be served by posting for a period of 10 days and by mailing a copy to the owner addressed to his address as given on the last completed assessment roll of the county in which the property is located.
9. At the time of the hearing the District Board shall determine whether the initial finding is correct and shall permit the owner to present testimony in his behalf. If, after hearing all the facts, the board makes a determination that a nuisance exists on the property, the board shall order compliance with the requirements of the notice or with alternative instructions issued by the board. Failure to comply with the order of the board issued pursuant to this section shall subject the owner to civil penalties as determined by the discretion of the board which shall not exceed $500 per day for each day such order is not complied with.
10. Any recurrence of the nuisance within 30 days of the time specified for abatement of the nuisance may be deemed to be a continuation of the original nuisance.
11. In the event that the nuisance is not abated the board may abate the nuisance by destroying larvae and pupae and by taking appropriate measures to prevent the recurrence of further breeding.
12. The cost of abatement shall be repaid to the district by the owner of the property.
13. When the nuisance occurs on property of any agency, the district shall notify the agency of the nuisance. The notice is almost the same as in the case of private ownership, as are the other sections concerning serving a notice, abatement, etc. If the agency thinks that the order to prevent recurrence of the breeding is excessive or inappropriate for the intended use of the land or if the agency determines that a nuisance does not exist, the agency may appeal the decision of the board to the State Director of Health Services within 10 days subsequent to the hearing. The director shall decide the matter on

appeal and convey his decision to the agency and district within 30 days of the receipt of the appeal. The decision of the Director is final and conclusive. If the control of the nuisance is performed by the district, the cost for such control is a charge against, and shall be paid from the maintenance fund or from other funds for the support of the agency. Any agency and a district may enter into contractual agreements to provide control of nuisances.

14. All sums spent by the district to abate the nuisance or prevent its recurrence, when notice of the lien is filed and recorded shall become a lien upon the property on which the nuisance is abated.

15. The lien is filed in the office of the county recorder of the county in which the property is situated within one year after the first item of expenditure by the board, or within 90 days after completion of the work, whichever occurs first.

16. An action to foreclose the lien shall be commenced within six months after filing and recording of the notice of lien.

17. The lien provision does not apply to the property of any county, city, district, or other public corporation.

This explanation is not necessarily complete and is for discussion purposes only. It is not to be considered as legal advice. Please seek the opinion of your attorney.

INTEGRATED PEST MANAGEMENT OF AN URBAN SWAMP

Frank W. Pelsue

Southeast Mosquito Abatement District
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Harbor Regional Park is a recreational area in South Central Los Angeles that contains a lake that in recent years has evolved into an urban swamp. The area, at one time, was a swamp that was part of the natural drainage of South Central Los Angeles. In fact, it was the southern most extension of this drainage system often forming a lake after extensive winter rainfall.

Historically, Southeast Mosquito Abatement District had experienced mosquito problems in the spring normally after winter rains but as the summer warmed up, much of the water would recede out of the shoreline vegetation and form a central lake with little or no vegetation in the water, thus eliminating mosquito breeding or at least providing greater access for mosquitofish to work the shore and control any mosquito breeding that might occur.

Consequently, the District seldom experienced any major outbreaks of mosquitoes during the summer months. However, the City of Los Angeles decided to embark on a program of developing the lake into a recreational lake with boating and fishing. To accomplish this, the lake had to be deepened. Prior to this excavation, the lake was essentially ringed with vegetation in the form of bulrush, *Scirpus* sp. and willow, *Salix* sp. that would be inundated with water in the spring and dry out in summer. In 1972, the area was drained and attempts were made to deepen the lake. The lake was deepened slightly, but could not be deepened to the desired depth as set out in the construction specifications. The lake was then filled with water and maintained at an elevation that inundated the bulrushes continuously throughout the summer. This permitted bulrushes to increase their numbers and provided suitable cover for mosquito breeding to occur essentially year round.

In 1973, SEMAD began experiencing outbreaks of mosquitoes in the summer, receiving numerous service requests from residents and businesses that surround Harbor Park. Investigations revealed that although there was a high mosquitofish population, they were unable to control the large larval population of mosquitoes that was found due to the increase in vegetation and the reduction in light able to get down into the water so that the mosquitofish could see the mosquito larvae. We undertook a spot treatment larviciding program and also did some adulticiding with very little success.

In 1973, we corresponded with the Los Angeles City of Recreation and Parks pointing out the magnitude of the problem and our recommendations for reducing or mitigating the mosquito breeding in Harbor Lake. They responded saying that they would initiate a vegetation management program. This talk bantered back and forth until 1975 when the problem really peaked. That year in August, we collected an average of 423 mosquitoes per trap night, causing numerous service requests. The problem was as bad as it had ever been, so SEMAD called the Recreation and Parks Department in for a hearing. At the hearing, Recreation and Parks agreed to attempt a vegetation-management program. They did not have enough money to remove sufficient amounts of bulrushes to open the water up so they purchased a used "Sprite" vehicle to cut paths in the vegetation opening it up to fish and allowing light in.

The paths were cut in the spring of 1976 and during that summer there was a significant reduction in mosquito service requests and light trap counts and the District only performed one pesticide treatment, whereas the year previously we performed four treatments receiving 48 service requests. See Table 1 for comparison. In 1978, trails were again maintained, and we experienced essentially no significant increase in mosquitoes.

Table 1.-Comparisons of service requests and average adult females per trap night during mosquito season with source reduction activities.

Year	Service Requests Total Area	Harbor Lake	#Pesticide Treatment	Females/ Trap Night
1976	71	48	4	1.28
1977*	27	8	1	0.50
1978	49	2	0	0.58
1979	20	2	0	0.41
1980	27	3	0	0.35
1981	46	19	3	0.45
1982	57	38	3	2.23
1983*	27	2	1	0.69

*Trails cut through tules to allow fish to forage more efficiently.

In 1978, proposition 13 was passed by the voters, causing a reduction in budgets for many governmental agencies including the Los Angeles City Department of Recreation and Parks. This cut in funding found the Recreation and Parks Department without sufficient funding to continue the trail maintenance program. In the years 1979-1980, however, we did not experience a significant increase in the mosquito population because it took that long for bulrush recruitment to fill in the trails previously cut in 1978. In 1981, we began to observe an increase in mosquito activity, even though the mosquitofish population remained high due to the increase in vegetation. In other words, the situation had reverted back to what had occurred prior to 1977. We increased our adulticiding and larviciding treatments to attempt to control the mosquitoes with essentially negative results. In 1982, the situation developed even further, causing an increase in mosquito service requests and light trap counts as shown in Table 1.

In 1983, the Department of Recreation and Parks sufficiently got their act together to cut trails again, but to a lesser degree than what was previously done, due to equipment breakdowns. However, this still opened the water up sufficiently to again allow the mosquitofish to forage and maintain the mosquito population below the nuisance threshold. That year, we only had one pesticide treatment.

In summary, the integrated pest management of this urban swamp essentially consisted of vegetation management to permit the biological control agent, *Gambusia affinis*, to forage and control the larval mosquito population. This interspersed with pesticide treatments of pyrethrin as an adulticide and GB 1111 as a larvicide when the mosquito population exceeded 0.50 female mosquitoes per trap night. Mosquito populations that averaged over 0.50 female mosquitoes per trap night seemed to exceed the nuisance threshold. At populations above 0.50 females per trap night, we received 10 or more service requests.

In conclusion, we submit that the combination of vegetation management and mosquitofish are able to keep the mosquito population in this urban swamp below the nuisance threshold and essentially reducing or eliminating the need for the use of pesticides while still providing a habitat suitable for migrating birds and other swamp wildlife.

THE USE OF OPERATIONAL COST RECORDS TO PROMOTE WATER MANAGEMENT

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A facet of our integrated control program is to use form letters to inform property owners about mosquito control costs on their property. This type of control is inexpensive yet effective and in our district is responsible for a large decrease in our operational expenditures. Eight years ago the County was divided into ten operational zones with a $13,000.00 aircraft budget. Next season, due to the reduction of mosquito source areas, we will have five operational zones. Last year's aircraft bill amounted to $888.00. In the last ten years multiple breeding sites on 1,543 parcels of property have been corrected. The great majority of these corrections are due to our letter writing program.

When the program was started during the spring of 1972, it was unsuccessful. The first letters were sent to a limited number of persons who had mosquito producing areas with high suppression costs. The letter explained the mosquito cycle, the time required for the mosquito to mature from egg to adult and requested their cooperation by managing the water in such a way that it would be gone in five days. In Jackson County, a land of bountiful water, nobody in the past had ever said anything about water management and to imply that millions of mosquitoes could come from an area in a pasture that dried up between irrigations was ridiculous, "Why everyone knows that mosquitoes come from ponds". The response to these letters was minimal. In two years it was evident that those who were going to cooperate had done so. The question was, "Do I try something else or stop the program?" The thought occurred to me, "Everybody relates to money", so I decided to try using the operational costs and the tax rates to motivate water management. The result of this approach was immediately noticed. However, there was a percentage of individuals who indicated that their high property taxes more than paid for the control activities on their property. To counteract this type of reasoning, we acquired a viewer and purchased microfiche from the Assessor's office which is used to determine vector control taxes on certain parcels of property. When control costs were greater than vector control taxes, the information was added to the letter, with the results again being increased cooperation.

One day while scanning a magazine called "Boardroom Reports", I read an article about junk mail which explained how a small percentage of responses would pay for all the junk mail and still show a profit. The thought occurred to me that perhaps the junk mail theory could be used to help reduce our costs by promoting mosquito control through water management. I decided to send letters to as many involved property owners as possible.

On parcels of land where control costs increased, the owner is notified and his aid in reducing our costs through water management is solicited. When costs are reduced such owners receive a letter thanking each for their cooperation and suggesting that he continue his efforts.

The result of this junk mail approach is positive, the outcome being additional corrections.

The compiling of field records into usable data, determining legal ownership and typing the many letters required the services of an office clerk.

Two years ago, we started to computerize our operations. The computer eliminated the full time office clerk and paid for itself in two years. I read in another article in "Boardroom Reports" where utility companies who sent computerized bills had a better response. My thoughts were "If utility companies obtain increased response from computerized letters, then a vector control district could obtain the same benefits." The bills were paid sooner and the number of unpaid bills decreased. With this thought in mind, we purchased superscript and computerized our letters. Last year the data were compiled, the letters typed and envelopes addressed at the rate of 150 a day. The first year's data indicated that a percentage of the property owners who received the computerized letters were more responsive. It will require at least two more years to determine if the trend is true and accurate.

There is one more item which I would like to bring to your attention. Most sales managers are aware that 80% of the sales are made on the fifth call or after. The same principal and persistence is in effect when selling mosquito control through water management. A certain amount of exposure is essential to the property owner before cooperation becomes apparent.

Last year, improved water management practices eliminated mosquito production on 11% of the irrigated agricultural properties which had breeding sites. We have had a 54% reduction in four years. At the present time, there are only 330 agricultural properties with mosquito sources on them.

CONTROLLING *AEDES SIERRENSIS* IN JACKSON COUNTY

A YEAR-ROUND PROGRAM

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INTRODUCTION.-*Aedes sierrensis* in Jackson County have become one of our most apparent and widespread mosquito problems to control and eliminate. The sources are varied, numerous and difficult to locate and treat.

In our observations of *Ae. sierrensis,* we collected and identified samples of adult females as early as May and as late as October. This represents a six month span in adult mosquito activity. Larval samples have been taken from various natural and man-made sources year-round. Jackson County is located in Southern Oregon, sharing a common border with Siskiyou County, California. It encompasses 2821 square miles and has a population of 132,700. In 1983, J.C.V.C.D. handled 1,268 calls, 982 of them being mosquito related and of those, 362 were *A. sierrensis*. In 1982, there 293 *Ae. sierrensis* calls and in 1981, only 40, which shows how drastic the increase of this mosquito has become and how our program for handling this problem needed to be evaluated.

SERVICE REQUESTS.-Our procedure for responding to such calls is as follows: A service request is taken over the phone and information is written down such as, when the person is being bitten, if they are bothered inside or outside and how long a time there has been a problem. This gives an indication of what type of mosquito the operator should be looking for when at the residence. The usual pattern for an *Ae. sierrensis* is: biting daytime, late afternoon, outside. This pattern varies slightly sometimes, and we get people being bitten in shaded areas around their house or garden at most any time of the day.

When the operator arrives at the address, an inspection is made around the house and an adult sample is taken, if available. If the sample proves to be *Ae. sierrensis,* then a notation is made on the service request card that a "spring check" is needed to locate the source. Sometimes the owner gets the sample which speeds up the process. We usually do not aerosol with our Leco ULV unless it is definitely determined to be mosquitoes. This policy of no mosquitoes - no spray, has caused a few negative remarks from citizens, but is necessary to protect both us and the taxpayer. If the operator cannot locate an adult sample, then the owner is requested to get one before any further action is taken. Throughout the summer, all *Ae. sierrensis* service requests are marked and at the end of the season sorted out for a more thorough inspection. *Ae. sierrensis* related service requests are taken from the first week of May until the last week of October. ULV aerosoling takes place from late May until mid-August.

Starting in September, after our regular season has ended, we go back to these service requests marked "spring check" and inspect each residence again. This inspection usually involves a 100 yard radius around the house and may include as many as 200 to 300 trees. The type of trees most often to cause problems are madrone and oak. If the house is located next to a grove of trees, then this is an operator's most likely area of inspection. Sometimes there may be just a single tree or stump in a person's yard that is causing the problem, but an inspection of the entire area is still done.

If trees are found to have cavities holding water, a sample of the water is taken with a glass syringe to check for the presence of larvae. A permanent map is drawn of the residence where a source is located, showing positions of trees with active cavities. These maps are numbered and added to our file of known treehole sources. This portion of the operation continues until early December.

Weather permitting, we start our treatment program in January with this file of over 550 maps. Each residence is visited by an operator and the sources are treated with either Dursban or Baytex. These are treated every year and marked on the back of each map how many trees were inspected, how many cavities, how many treated and with what material. This is a lengthy operation and time-wise brings us close to spring when the cycle repeats itself.

RAINFALL vs. SERVICE REQUESTS.-Reasons for the yearly increases in *Ae. sierrensis* calls might be explained by relating rainfall levels with *Ae. sierrensis* production, for example: In 1981, Jackson County received 10.43 inches of rain which is about 57% of the average rainfall for the area, 18 inches is the average. In 1982, we received 14.46 inches of rain, or 79.5% of the average. This represented a rainfall increase of approximately 4 inches, or 40% over the previous year. This might not seem like a very large increase, but in graphing out the daily precipitation levels, we discovered that in three separate months we received record amounts of rain. In February 1982, we received 3.64 inches which was the highest recorded measurement in that month for the past twelve years. Another observation was that the rainfall was not spread out evenly through the months, but usually fell all within a few days. This meant that we had received, on three or four separate occasions, large shots of rain within a few days, which had filled tree cavities and other sources that in previous years had been dry or had not held sufficient amounts of water to support a hatch of *Ae. sierrensis.* The finding of tree cavities throughout the late summer months still holding large amounts of water seemed to support this theory.

PROJECTIONS FOR THE FUTURE.-1984 would seem again to be a large *Ae. sierrensis* year, with almost 10 inches of rain above our average as of December 1983. With this knowledge of the effect of large amounts of rainfall in short periods of time in hand, we can expect by looking at our daily precipitation levels that 1984 could break our previous year's record of *Ae. sierrensis* service requests.

TREE CAVITY FILLING.-We have implemented a new program that will continue for the next few years that involves the filling in of all possible treehole sources with crushed granite. This is quite an undertaking considering we have well over 3,815 known *Ae. sierrensis* producing cavities to be filled and the only way to get material to these sources is by hand carrying it in plastic buckets.

We estimate that we will use 20 yards or 55,926 lbs. of crushed granite to fill these sources. Our tree cavity filling project so far this winter has been met with favorable comments from the public, even though people feel it is an overwhelming project to undertake.

Elimination of treehole sources seems the only sure way of eventually stopping the yearly increases in *Ae. sierrensis* service requests and keeping the amount of biting adults to an acceptable level.

BACILLUS THURINGIENSIS var *ISREALENSIS*.- The trial use of Bactimos Granular C in different areas of Jackson County involved 34 cavities with first, second and third larval instars of *Ae. sierrensis.* The cavities varied in size and amount of water and an average of 1.35 ounces of material per sources was used.

The results have not been completely satisfactory in most cavities. When sources were treated with BTI earlier in the winter, we have returned to find larvae still present, but in lesser amounts. Rainfall during this period was heavy and we feel that the material was diluted enough to allow a new hatch to survive.

CLIMBING SPURS.-The use of climbing spurs to reach tree cavities has been successful in some instances where the density of the wooded area made carrying ladders difficult.

Most tree cavities in madrone are basal or not much higher up on the trunk than 6 feet, but our oak tree sources have been found to have active cavities at 20 to 25 feet up.

In talking with residents on streets lined with large oaks, we have had some sources located by having them explain to us where they have observed birds taking baths higher up in the branches.

INACTIVE CAVITIES.-With treehole sources having been inspected for the past 5 to 6 years, we have noticed that some cavities treated with Dursban or Baytex seem to become inactive in 2 to 3 years. This may be due to the material tying up in the organic matter, causing residual carry-over into the next rainy season or perhaps the general profile of the tree cavity has been altered in such a way that it has become undesirable for oviposition.

MAN-MADE SOURCES.-Listed below are some artificial sources in which we have found *Ae. sierrensis* larvae present. All sources fill with rain water and are usually located in areas where leaves and other organic matter are present.

1. Tires
2. Plastic or metal buckets
3. Plastic 30 gallon trash barrels
4. Wooden barrels used for ornamental purposes
5. Tin cans and plastic jugs in trash piles
6. Old car bodies and parts
7. Rain gutters
8. Black plastic tarps over woodpiles
9. Bird baths
10. Small ornamental ponds
11. Uncovered boats
12. Discarded sinks and water troughs
13. Plant vases left outside

HAZARDS.-Some hazards encountered in treating treehole sources in Jackson County are as follows:

1. Ticks- In some areas tick infestation has been heavy and a quick check of the pant legs and jackets before returning to vehicles is necessary.

2. Poison Oak- Much of the area where madrone or oak groves are located are heavily overgrown with this plant, causing susceptible persons to move cautiously and probably making some basal tree cavities inaccessible.

3. Steep or Wet Hillsides- When treating a grove of trees located on either side of a steep ravine, rain dampened leaves and fallen branches make walking difficult, especially when carrying a solo backpack or other equipment. An unexpected trip downhill is not too unusual under these circumstances, but no major injuries have happened as of yet.

4. Animals- The treatment of some larger basal cavities in areas where neighborhood dogs or cats may use the water for drinking, has been brought to our attention by a few concerned citizens.

CONCLUSION.-It would seem that from the results of our past efforts to control *Ae. sierrensis,* we have been fighting an uphill battle. Though some sources become temporarily inactive, new sources turn up every year to add to our ever-growing permanent file.

In this situation, we are at the mercy of the elements and are faced with inaccessible source areas combined with limited time, manpower and money. We could then choose one of two options. Keep our program the way it is and use adulticiding every year as our primary deterrent, or eliminate the sources through a long-term treehole filling project. We have chosen the latter.

ROTATIONAL USE OF INSECTICIDES IN MOSQUITO CONTROL PROGRAMS

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ABSTRACT

The use of rotational applications of certain unrelated insecticides has been shown in the laboratory to be an effective means of delaying the development of resistance to organophosphates in *Culex quinquefasciatus*. This paper reports on the results of the initial phases of a study designed to determine whether such a program is effective under field conditions.

As all mosquito-control personnel are well aware, resistance to insecticides has become a significant obstacle to effective mosquito control. As a means of combating this problem, novel approaches to insecticide applications have been proposed, one of which is the use of unrelated compounds in a rotational scheme.

What is the basis for this approach? First, previous work in our laboratory has shown that resistance to certain insecticides declines when the treatments with that insecticide are withdrawn. This is believed to be due to the initial lower biotic fitness of certain insecticide-resistant populations, as has been demonstrated for a number of arthropods (Bhatia and Pradhan 1968, Brower 1974, Shaw and Lloyd 1969, Whitten et al. 1980). With *Culex quinquefasciatus* this decline is due to the demonstrated lower reproductive fitness of organophosphate-resistant individuals (Ferrari and Georghiou 1981). It would be impractical, of course, to withdraw selection pressure in the field by withdrawing all chemical control, so the use of unrelated insecticides in rotation has been proposed as a means to relax this pressure while maintaining continuous pest control. Laboratory studies with *Cx. quinquefasciatus* have shown that a decline in resistance to particular organophosphates does in fact occur when certain specific organophosphates are used in rotation (Georghiou et al. 1983). Thus, we do have laboratory studies confirming the feasibility of this approach to mosquito control. Very few studies have been done in the field, however, and those have shown mixed results. This may be due to the choice of inadequately distinct compounds for the rotational treatments: Rotational applications will not work unless the second compound allows for the complete relaxation of the selection pressure exerted by the initial compound, or unless resistance to these two compounds is negatively correlated (Georghiou 1965).

The choice for that second compound is of paramount importance. If cross resistance exists, resistance will be enhanced rather than delayed. So what requirements are there, then, for this second compound? It is imperative that the two compounds have distinctly different modes of action and detoxication pathways. If this is not the case, selection pressure may not be relaxed. Further, laboratory studies have suggested that specific rotation schemes must be tested individually in order to determine their effectiveness. It is a complex situation. Let us again emphasize that the two compounds must exhibit no correlation in their potential resistance mechanisms for rotational applications to be effective in delaying resistance development.

Taking these points into consideration, and with the cooperation of Frank Pelsue, Jack Hazelrigg and Jim Sides at the Southeast Mosquito Abatement District, we designed a study to determine whether the rotational use of two unrelated compounds could delay the development of resistance to an organophosphate in the field, as had been suggested by laboratory studies.

The insect monitored in our program was the southern house mosquito, *Culex quinquefasciatus*, and the compounds were chlorpyrifos (Dursban®), a relatively inexpensive organophosphate that is currently being used for mosquito control, and *Bacillus thuringiensis* var. *israelensis* (BTI). These two compounds have, as required, two distinctly different modes of action, and thus BTI allows for a period of relaxation of the chlorpyrifos selection pressure and vice versa. Resistance to BTI has been developed in the laboratory to only very low levels (Vazquez-Garcia 1983). The field area was in the southeast portion of Los Angeles County (Fig. 1). The area was divided into nine plots, each about 25 square miles. Individual plots received one of three treatments, as indicated (the alternation of chlorpyrifos and BTI described above, chlorpyrifos only, or BTI only), and each treatment scheme was carried out in three separated plots. Mosquito larvae and egg rafts were collected from the central square mile of each plot in order to minimize the effects of immigration from neighboring plots that had been under a different treatment schedule. All treatments and collections were carried out by the Abatement District's personnel.

Organophosphates had been used previously in the district, and as can be seen in Table 1, resistance to chlorpyrifos existed throughout the area at the beginning of our study. It is our hope that the further development of resistance to this organophosphate can be arrested or slowed by the proposed alternating applications. The mean resistance ratios for the plots under each treatment scheme did not vary significantly, so we can assume that changes observed over the course of this study are due to the imposed conditions.

What sorts of changes did occur? First,

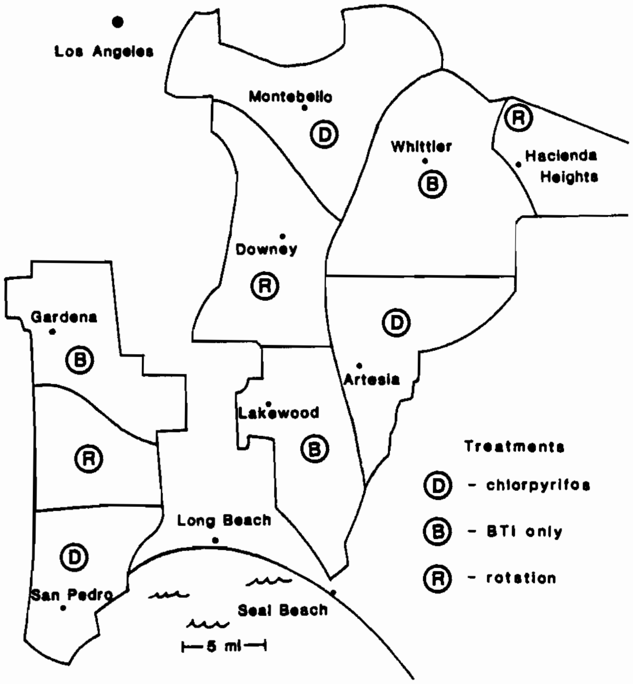

Figure 1.-Region under study (Southeast Mosquito Abatement District) and distribution of treatment programs.

Table 1.-Pre-experimental resistance ratios for chlorpyrifos throughout the area under study (LC_{50} level).

	$\bar{X}$	Range
Chlorpyrifos-treated plots	21	4-69
BTI-treated plots	18	4-41
Rotation plots	18	6-32

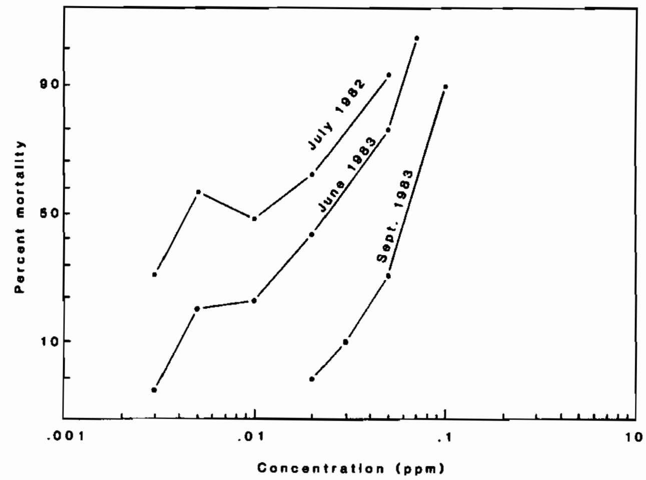

Figure 2.-Log dose-probit lines for chlorpyrifos in one of the chlorpyrifos-treated plots (San Pedro).

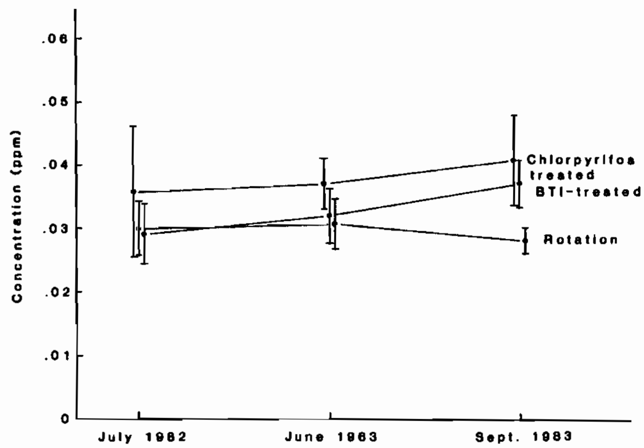

Figure 3.-Mean LC_{50} values for chlorpyrifos in *Culex quinquefasciatus* populations treated as indicated (bars designate standard error).

let's look at the results from just one of the plots that received only chlorpyrifos treatments (San Pedro plot, Fig. 2). Without a relaxation of selection pressure, we would expect resistance to chlorpyrifos to increase, and that is precisely what occurred in this plot, as reflected in the shift in the ld-p line: Higher concentrations of chlorpyrifos were required to kill a specific percentage of the population at the end of the second treatment season than were required to kill the same percentage of the population at the beginning of the study. There is also a concomittant decline of the percent of susceptible individuals in the population, as evidenced by the lowering or loss of the line's plateau. Aside from this plot, however, we saw no significant changes in the percent of susceptible individuals in the populations, and as almost all of our populations showed susceptible individuals accounting for less than 50% of our population, we used the LC_{50} and LC_{95} values as general indicators of changes in resistance levels.

At the LC_{50} level (Fig. 3), we do see the expected increase in resistance in the chlorpyrifos-treated plots, but there is a similar increase in the BTI-treated plots, where it was assumed that there was no exposure to chlorpyrifos. A Duncan's new multiple range test, however, indicated that the differences between the first and final values for these two treatment groups were not significant. For the rotational plot, on the other hand, we get a drop in the LC_{50} or resistance level, but again, this value is not significantly different from that observed in 1982.

At the LC_{95} level (Fig. 4), which is considered a more sensitive indicator of a population's resistance potential, the changes observed for the chlorpyrifos- and BTI-treated plots are not statistically significant. For the rotational

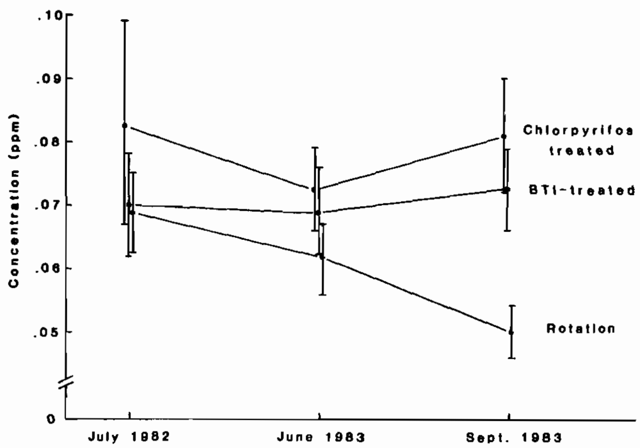

Figure 4.-Mean LC_{95} values for chlorpyrifos in *Culex quinquefasciatus* populations treated as indicated. (Bars designate standard error.)

group, however, there is a significant decline in the resistance level: The value obtained in September 1983 is significantly lower than the value obtained for these plots in July 1982 and from the values obtained in the other two treatment groups in September 1983. It would be very encouraging, indeed, if we could consider this very short-term information as indicating that the use of chlorpyrifos and BTI in rotation reduces the selection rate for chlorpyrifos resistance, but unfortunately, this phenomenon has not been observed in the laboratory with strains subjected to similar selection pressure. Further long-term studies are needed to determine whether these field data reflect a trend or a natural fluctuation. It is possible that other factors are contributing to this decline in resistance, such as refugia (untreated pockets) or the isolation of the sampled populations, the actual treatment levels at the collections sites, or other treatment and sampling variables, but our study was designed to minimize the effects of these variables on our results by the use of multiple collection sites, each assumed to be under similar environmental conditions. It should also be noted that although the mean resistance ratio for the rotationally treated plots was similar to those for the other plots, the maximum value was considerably lower. The treatment programs described above have been in effect for only two seasons, but with long-term research, both in the field and in the laboratory, we should be able to detect trends and to assess the efficacy of this rotational treatment. Such a program, if effective, may lead to more efficient and more economical procedures for mosquito control.

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TOXICITY OF THE IGR DIFLUBENZURON TO NEONATE, ADULT, AND GRAVID FEMALE *DAPHNIA MAGNA* STRAUS (CLADOCERA: DAPHNIIDAE) IN THE LABORATORY

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ABSTRACT

The toxic effects of the IGR diflubenzuron on neonate, adult, and gravid female cladoceran, *Daphnia magna* were studied in the laboratory. The neonates and adults were highly sensitive to low concentrations (<25 ppb) of a 25% WP formulation of diflubenzuron; neonates were 31X more sensitive than the adults. Exposure of gravid females to diflubenzuron (25% WP) at rates up to 5 ppb did not have any significant effects on the embryos enclosed in the brood pouch of *D. magna*.

INTRODUCTION.-In the past decade, insect growth regulators (IGRs) have received a great deal of attention as mosquito control agents. Of the urea type IGRs, diflubenzuron which inhibits chitin synthesis and cuticle deposition during metamorphosis of insects (Post and Vincent 1973) has been shown to exhibit a very high level of biological activity against a large number of mosquito species (Hsieh and Steelman 1974, Majori et al. 1981, Mulla and Darwazeh 1976, Mulla et al. 1975).

In mosquito larval habitats, a variety of organisms are equally exposed to various materials used for mosquito larval control. In several studies, diflubenzuron has been shown to have adverse effects on populations of aquatic nontarget invertebrates including species of Cladocera (Ali and Mulla 1978 a,b, Cunningham 1976, Julin and Sanders 1978, Miura and Takahashi 1974, 1975, Mulla et al. 1979). Among cladocerans, species of *Daphnia* are distributed worldwide and serve as an important link in the aquatic food chain. One species, *Daphnia magna* Straus is particularly important because it is common and abundant and cohabits with larvae of a variety of mosquito species. The present studies were, therefore, undertaken to elucidate the toxic effects of diflubenzuron on neonate, adult, and gravid female *D. magna* under laboratory conditions.

MATERIALS AND METHODS.-A parthenogenetic strain of *D. magna* was reared in the laboratory for over 30 generations under constant conditions. From this source, female *D. magna* were obtained and maintained in lots of 100 each, in separate glass dishes (30 X 30 X 15 cm), each dish containing 5 liters of tap water (pH 7.8, electric conductivity 719 µmhos/cm, total hardness 360 mg/liter, and alkalinity 304 mg/liter as $CaCO_3$). To obtain the test organisms of uniform size and age, gravid females were selected to start colonies in the dishes and were maintained for 1-2 days until the neonates were born. These neonates were transferred to similar glass dishes and were maintained in tap water for use in experiments. Five ml of a 1% suspension (in tap water) of the Brewer's yeast were added daily to each dish as food.

Experiment I: Acute toxicity (48 h) of diflubenzuron against 24-36 h old neonates and 7-8 days old adults was tested. For this experiment, neonates or adults in lots of 20 each were placed in 300 ml glass beakers. Each beaker contained 200 ml tap water to which 0.2 ml of the yeast suspension was added daily. A 25% wettable powder (WP) formulation of the IGR was employed. For treatments, 1% stock suspension (wt/vol) of the IGR was made in distilled water and the suspension was maintained with a magnetic stirrer to make the required serial dilutions and while making transfers of the IGR for treatments. Six different concentrations of diflubenzuron were added to 18 beakers containing neonates or adults; each concentration was replicated three times. Three beakers containing neonates or adults and receiving yeast served as controls. After 48 h of exposure under constant fluorescent light and 21± 1°C room temperature, mortality of neonates or adults was checked. A neonate or an adult was considered dead when its appendages ceased to move. The neonate or adult mortality at different concentrations of the IGR was corrected against mortality in the controls (Abbott 1925). The corrected mortality of each life stage was subjected to log-probit regression analysis to determine the LC_{50} levels. The experiment was repeated on three different occasions.

Experiment II: To study the effect of the IGR on gravid *D. magna*, 20 females (8-9 days old) were placed in each beaker containing 200 ml tap water and receiving 0.2 ml of 1% yeast suspension. In this experiment, 18 beakers were utilized and the gravid females were exposed to 0.05, 0.1, 1.0, and 5.0 ppb of the 25% WP of diflubenzuron. Each concentration was replicated three times and three untreated beakers served as control. The test was continued for seven days and the female mortality was noted on a daily basis. Also, the number of neonates appearing daily in each beaker was counted and the neonates were removed. The data were statistically analyzed by nonparametric methods because of the non-normal distribution of the data.

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Friedman's bivalent rank ANOVA (Friedman 1937) was used for multigroup comparison and Walsh's test (Walsh 1968) was used for a two group comparison. The experiment was conducted under constant fluorescent light and 21 ± 1°C room temperature.

RESULTS AND DISCUSSION.-Table 1 summarizes the LC_{50} levels (48 h) of neonate and adult *D. magna*. The neonate was 31 times more susceptible to the IGR than the adult. In general, both life stages of *D. magna* were highly sensitive to the relatively low concentrations (<25 ppb) of the 25% WP formulation of diflubenzuron used. Previously, Miura and Takahashi (1974) had reported a 50% mortality in a mixed population of water fleas within 48 h of treatment with a 25% WP of the IGR applied at a rate of 1.5 ppb. By contrast, Julin and Sanders (1978) using the 25% WP of diflubenzuron had reported an LC_{50} value (48 h) of 15 ppb of the IGR against first instar *D. magna*.

Table 1.-Toxicity of the IGR difulbenzuron[a] to neonate[b] and adult[c] *Daphnia magna*[d] in the laboratory.

Life stage	48 h lethal concentration (ppb) LC_{50}	C.I.[e]	Slope	r^2
Neonate	0.75	0.33 - 1.17	3.05	0.96
Adult	23.45	10.75 - 36.15	1.91	0.94

[a]25% WP formulation.

[b]24-36 hours old.

[c]7-8 days old.

[d]Under constant fluorescent light and 21 ± 1°C room temperature.

[e]95% confidence interval.

Table 2 summarizes the long-term effects of the IGR on gravid female *D. magna* exposed to five concentrations (0.05 to 5.0 ppb) of diflubenzuron (25% WP) for seven days. The table also shows the number of neonates taken daily from the treated and the control beakers. There was no significant (P=0.05) mortality of the females 24 h posttreatment at any of the test concentrations. The 0.05 and 0.1 ppb levels of exposure did not produce any significant mortality of the gravid females throughout the observation period. The higher concentrations (0.5, 1.0, and 5.0 ppb), however, were highly toxic and caused complete mortality of the females within 3-4 days of treatment; the extent of mortality was higher at the higher concentrations and in shorter time of exposure (Table 2).

The population trend of the neonates under different concentrations resembled the survival trend of the female parent. The neonates, like the gravid females, were not affected at 0.05 and 0.1 ppb levels of exposure; the numbers of neonates taken at the two exposure levels were comparable to the ones taken in the controls (Table 2). At higher concentrations of 0.5, 1.0, and 5.0 ppb, the daily population of neonates declined in proportion to the increasing mortality of the females. However, a comparison of the brood size of the surviving females (i.e., the number of neonates/surviving female) at different test concentrations of the IGR indicates no significant difference (χ^2 test; P=0.05) between the neonate populations taken in the treated and the control beakers. From this, it can be suggested that the formulation of diflubenzuron employed in this study, at exposure rates up to 5 ppb will not have any toxic effects on the embryos enclosed in the brood pouch of *D. magna*, although at rates lower than 5 ppb it proved toxic to the 24-36 h old neonates as shown in experiment I.

A number of previous field studies have shown that diflubenzuron (25% WP) applied at rates of 9 to 100 ppb for chironomid midge and mosquito control simultaneously reduced populations of Cladocera including daphnids (Ali and Mulla 1978b, Miura and Takahashi 1975, Mulla et al. 1975). The present laboratory studies confirm that *D. magna* is highly sensitive to the IGR and the various life stages of this daphnid would be severely affected even at lower rates than those employed for controlling chironomid midges and mosquitoes.

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Table 2.-Toxic and fecundity effects of the IGR diflubenzuron[a] on gravid female *Daphnia magna*[b] exposed to five concentrations of the IGR in the laboratory.

IGR concn. (ppb)	Gravid female (GF) or neonate (N)	Mean[c] no. live *D. magna* gravid females and neonate per beaker pre-, and posttreatment (days) Pretreatment	1	2	3	4	5	6	7	Mean[c] # neonate	Final % reduc. GF
0.05	GF	20.0	19.3 a	19.0 a	18.0 a	17.7 a	17.3 a	17.0 a	17.0 a		15
	N	(0.0)	(11.0)	(28.3)	(22.7)	(20.3)	(32.7)	(16.7)	(6.7)	19.8 a	
0.1	GF	20.0	19.3 a	18.7 a	18.3 a	18.3 a	18.3 a	17.3 a	17.3 a		13.5
	N	(0.0)	(15.7)	(24.3)	(23.7)	(30.7)	(34.7)	(20.0)	(8.3)	22.5 a	
0.5	GF	20.0	19.3 a	17.0 a	8.0 b	0.3 b	0.0 b	0.0 b	0.0 b		100
	N	(0.0)	(15.3)	(21.7)	(5.0)	(0.0)	(0.0)	(0.0)	(0.0)	6.0 b	
1.0	GF	20.0	20.0 a	18.0 a	2.7 c	0.0 b	0.0 b	0.0 b	0.0 b		100
	N	(0.0)	(15.3)	(0.3)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	2.2 b	
5.0	GF	20.0	20.0 a	15.3 b	0.7 c	0.0 b	0.0 b	0.0 b	0.0 b		100
	N	(0.0)	(15.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	2.1 b	
Control	GF	20.0	20.0 a	20.0 a	19.0 a	18.7 a	18.3 a	18.3 a	18.3 a		8.5
	N	(0.0)	(13.7)	(36.7)	(26.0)	(33.0)	(32.7)	(26.0)	(10.0)	25.4 a	

[a]25% WP formulation.

[b]8-9 days old; under constant fluorescent light and 21 ± 1°C room temperature.

[c]Means of gravid female in a column and the mean numbers of neonates in the column followed by the same letter are not significantly different at the 95% level of probability; Friedman's bivalent rank ANOVA tests and Walsh's test of nonparametric statistics. The mean numbers of neonates (separated by the aforementioned statistical tests) represent the mean of the total neonates taken per beaker during the seven days posttreatment period, while numbers in parentheses are the mean number of neonates per beaker taken daily.

IMPROVED EFFICACY OF ATTRACTANT-TOXICANT BAITS AGAINST *HIPPELATES* EYE GANTS[1]

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ABSTRACT

In controlling *Hippelates* eye gnats, the Coachella Valley MAD formulates a Synthetic Fly Attractant (SFA) mixture with a commercially available sugar toxicant bait. Female eye gnats are attracted by SFA, and a few seconds of feeding on the sugar toxicant results in their death. For ease of application as spot treatment, the two components are blended together and small quantities (5 to 10g) of the mixture are placed on the surface of damp ground.

Experimental results indicate that by sprinkling the sugar toxicant onto SFA mixture, rather than mixing the two together, efficiency of kill can be increased significantly. It was further determined that reduction in the amount of sugar toxicant by one half from the present level did not effect this increased efficiency. This technique, if adapted to operation programs, could result in substantial savings in cost of sugar toxicant bait for the control of *Hippelates* eye gnats.

INTRODUCTION.-*Hippelates* eye gnats are small, 2-3 mm long non-biting flies belonging to the family Chloropidae. They are highly attracted to man and domesticated animals, causing a great deal of annoyance and discomfort (Burgess 1951). When present in large numbers, they can be so pestiferous that they adversely effect any outdoor activity, be it work or play.

Implementation of control measures against these gnats in the Coachella Valley of southern California is the responsibility of the Coachella Valley Mosquito Abatement District. Current control technology employs the use of a synthetic fly attractant (SFA) mixture combined with a toxic sugar bait [Improved Golden Malrin® (IGM)] against the female gnats.

The SFA mixture is composed of trimethylamine hydrochloride, indole, ammonium sulfate and n-butyric acid blended with anchovy meal carrier (Mulla et al. 1976). This attractant mixture was developed from attractive chemicals isolated and identified from rotten chicken eggs by Hwang et al. (1975, 1976, 1976a).

The toxicant sugar portion of the mixture, Improved Golden Malrin® (Starbar, Dallas, Texas 75234) is a commercially available product. Its primary field use is for the control of house flies on farms and ranches. The toxic agent present in IGM is methomyl, a carbamate insecticide.

In actual practice, the two components of the formulation, SFA and IGM are mixed together in a ribbon blender in a 3:1 SFA to IGM ratio. This mixing makes for easy dispensing when applied in the field. In field application, a tablespoon of the mixture is dumped in a loose pile onto damp ground of a recently irrigated field or on ground wetted with water by the applicators. Moisture is needed to activate attractant chemicals as volatile attractant odors do not emanate when placed on dry substrate.

With escalating costs of the toxicant sugar bait formulation in the past few years, it became necessary to explore ways in which cost of sugar-toxicant bait could be reduced. The most successful alternative seemed to be a change in the method in which IGM and SFA mixture was presented to eye gnats. Rather than mixing them together (the practice now used), IGM and SFA could be applied separately. The method chosen was to sprinkle IGM on top of SFA placed on damp sand. Results of experiments using this procedure are presented here.

MATERIALS AND METHODS.-SFA composition was prepared in the laboratory as listed in Table 1. The sugar-toxicant bait IGM was used as it came from its manufacturer. All tests were conducted using 8 oz. dixie cups (No. 2168, American Can Co., USA) that contained damp Coachella

Table 1. Composition of Synthetic Fly Attractant[a/]

Chemical Ingredient	% by wt in formulation
Trimethylamine hydrochloride	2.5
Indole	0.25
Ammonium Sulfate	40.0
n-butyric acid	2.0
Anchovy meal	55.25

a/ For toxicant effect, Synthetic Fly Attractant was mixed with Improved Golden Malrin®, a sugar bait containing 1% methomyl, in a ratio of 1 part Improved Golden Malrin to 3 parts Synthetic Fly Attractant.

[1]These studies were supported in part by the Coachella Valley Mosquito Abatement District, Thermal, California.

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Valley Fine Sand and the various mixes to be tested. Cups were placed onto a rotary rod olfactometer (Mulla et al. 1973) in a field having a high density of eye gnats. The olfactometer revolves at 1/4 rpm. The experiments were run for 1-2 hours as the dead gnats accumulated in the cups. The cups were returned to the laboratory for fly counting under a dissecting microscope.

Statistical analysis on results of all tests were carried out by first transforming actual numbers to log (n+1). With the aide of a Compucorp Model 145E statistician computer, these figures were subjected to analysis of variance and Duncans Multiple Range Test to determine any significant differences (LeClerg 1957).

RESULTS AND DISCUSSION.-In the first test conducted, sprinkling of IGM on SFA was compared to mixing of IGM directly with SFA. The amount of SFA was kept constant while the amount of IGM was decreased (Table 2). In comparing the "sprinkling" and "mixing" methods, at all ratios tested, the "sprinkled" IGM significantly outperformed the "mixed" formulations. It was

Table 2. Effect of sprinkling IGM onto or mixing it into SFA and resulting toxicity to *Hippletes* eye gnats.

Formulation Ratio		g/cup		Mean no. Gnats/Cup[a/]	
SFA :	IGM	SFA	IGM	Sprinkled	Mixed
2	1	2	1.00	467 a	252 b
3	1	2	0.67	371 a	182 c
5	1	2	0.40	394 a	173 c
7	1	2	0.28	373 a	73 d

[a/] Mean based upon 5 reps. Means followed by same letters are not significantly different from each other at 5% level.

noted that regardless of the toxicant-attractant ratio, when IGM was "sprinkled", no significant differences were evident among the various mixtures. However, as the ratio of SFA to IGM increased (decreasing toxicant), significantly fewer gnats were found in the cups containing the "mixed" SFA:IGM formulations.

Further reduction in the amount of sugar-toxicant component was studied, using ratios of 9:1 and 12:1 (SFA:IGM) (Table 3). When IGM was sprinkled on top of SFA, ratios of 9:1 and 12:1 were as effective as the standard 3:1 ratio when mixed. Ratios of 3:1 and 6:1 with IGM sprinkled onto SFA yielded gnat kill that was significantly greater than the "mixed" 3:1 ratio.

When SFA-IGM formulation is applied in the field, its length of activity is a function of moisture in the ground surface. In the Coachella Valley, sufficient moisture could be available from 1-4 days after irrigation. To study this longevity of action, cups were "aged" in the laboratory and then field tested (Table 4, Test 1). In the

Table 3. Effect of mixing Improved Golden Malrin into or sprinkling onto Synthetic Fly Attractant

Formulation Ratio		g/cup		Formulation	Mean No.
SFA	IGM	SFA	IGM	Method	Gnats/Cup[a/]
3	1	4	1.33	Sprinkled	477 a
6	1	4	0.67		305 ab
9	1	4	0.44		210 bc
12	1	4	0.33		132 d
3	1	4	1.33	Mixed	132 cd

[a/] Mean based upon 6 reps. Means followed by same letters are not significantly different from each other at the 5% level.

first test, a 6:1 ratio mixture of "sprinkled" IGM was compared to the 3:1 "mixed" ratio. The "sprinkled" ratio (6:1) was significantly more effective than the "mixed" ratio (3:1) when fresh and at 3 days of aging. At 5 and 7 days of aging they appeared to be equal.

In the second test of this aging series (Table 4, Test 2) a 9:1 ratio mixture of "sprinkled" IGM was compared to the 3:1 "mixed" ratio. Through 0, 3 and 5 day periods, no significant differences between the two methods were evident. At 7 days, significantly fewer gnats were recovered from "sprinkled" cups than "mixed" cups. "Sprinkled" cups showed a significant loss in numbers at days 5 and 7 while "mixed" cups showed no significant loss through 7 days.

The reason for increased efficiency in the sprinkled method can be explained by the feeding activity of *Hippelates* eye gnats at the surface of the formulation. They do not dig down or rummage through the piles of SFA-IGM mixtures. Therefore, the more particles of IGM placed on the surface of the pile, the more apt the gnats are to receive lethal dosage. Ratios of 3:1 and 6:1 (SFA:IGM) "sprinkled" expose more IGM on the surface than the 3:1 ratio "mixed", while ratios of 9:1 and 12:1 "sprinkled" expose amounts of surface IGM that are comparable to the 3:1 "mixed" ratio.

In summary, we have demonstrated that sprinkling of toxicant bait increases the efficiency of gnat kill of the formulation over the present method of mixing attractants and sugar bait. The amount of IGM presently being used could be reduced by one half, without effecting the extent of this gnat kill. The amount of IGM used could even be reduced by two thirds, but results obtained would show no increase in efficiency. However, they would be comparable to results that are obtained now with 3:1 "mixed" ratio. Such reductions in the use of toxicant could result in substantial savings without loss of effectiveness.

Table 4.-Cup aging in laboratory and effect of sprinkling or mixing the IGM onto or into the SFA.

Test	Age (dys)	Mean no. gnats/cup for ratios[a/] 6:1 Ratio Sprinkled	9:1 Ratio Sprinkled	3:1 Ratio Mixed
1	0(fresh)	147 a	-	66 cd
	3	122 sb	-	67 cd
	5	85 abc	-	70 bcd
	7	70 cd	-	52 d
2	0(fresh)	-	278 a	235 ab
	3	-	283 a	267 a
	5	-	176 b	259 ab
	7	-	101 c	231 ab

[a/]Mean based upon 5 reps. Means followed by same letters are not significantly different from each other at the 5% level. Tests 1 and 2 analysed independently.

Further research is needed to develop a practical method of application under field conditions. As yet, no easy method of separate application of the attractant and sugar toxicant has evolved. Various methods are under consideration and will be explored this coming gnat season.

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MOSQUITO REPELLENTS FROM *ARTEMISIA*[1] PLANTS

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Artemisia species (Compositae: Anthemideae) is a group of aromatic plants with worldwide distribution. These and related plants produce some chemically interesting and biologically useful organic compounds. For instance, the well known anthelmintic, α-santonin, is obtained from the dried unexpanded flower heads of *Artemisia maritima* L. (Clemo et al. 1930). Insecticidal pyrethrins occur in the disk flowers of *Chrysanthemum cinerariaefolium* Vis. which also belongs to the tribe Anthemideae (mayweed Tribe).

In China, *Artemisia vulgaris* L. is used in traditional herb medicine. Old leaves of *A. vulgaris* are dried and made into a moxa (soft woolly mass) which is used as a cautery by being ignited on the skin. Young leaves of this plant can be added into rice cakes or dumplings for its fragrance.

It had come to our attention that *A. vulgaris* and related plants possessed insect-repellent activity (Prof. C.S. Tsi, private communication, 1982). Bundles of dry *A. vulgaris* are slowly burned to produce smoke which reportedly repels hematophagous insects. This has been a common practice for repelling biting insects particularly in rural areas in China for centuries. This practice is similar to the use of pyrethroid-containing mosquito incense sticks or mosquito coils in Asian countries.

In our preliminary studies (Hwang, Mulla and Axelrod, unpublished data), *A. vulgaris* was collected from the Ma-lian-wa Experimental station, Chinese Academy of Medical Sciences, Beijing, or purchased from local herb shops in Beijing. The herbs were air-dried and shipped to our laboratory. The dry herbs, possessing characteristic fragrance, were pulverized and extracted with a boiling mixture of benzene and methanol (3:1=V:V). The mixture was filtered to remove solid plant materials, and the filtrate was evaporated to dryness to give a green gum-like residue. The residue (10mg) was dissolved in acetone (2 ml), and the solution was applied to the skin of human arm in a circular area (3-cm dia.). The solvent was allowed to evaporate, and the portion of the skin covered with the *A. vulgaris* extract was exposed to female mosquitoes for a given period.

A plastic food container (11-cm dia., 7.5-cm height) with a circular screen window (4-cm dia) in the side and containing 20 female *Aedes aegypti* mosquitoes was used as a mosquito holding cage. In conducting bioassay tests for repellency, the screen window was pressed tightly against the arm treated with the *A. vulgaris* extract. The skin was exposed to mosquito landing and probing for 1 minute. Untreated skin (solvent only) was also exposed to mosquitoes as control. The test was replicated several times. The level of repellency was expressed in terms of percent repellency and calculated by the following formula:

$$\%\ \text{Repellency} = \frac{Nc - Nt}{Nc} \times 100$$

wherein Nc denotes the mean number of mosquitoes landing and probing on the control skin and Nt denotes that on the treated skin.

The mean numbers of mosquitoes landing on the treated and the control were 3 and 9, respectively, thus giving a repellency of 67%. The difference between the two means was significant at the 0.01 level. This preliminary test clearly showed that *A. vulgaris* contained repellent components which made attractive human arm 3 times less attractive. Our preliminary studies indicated that the herb *A. vulgaris* contains chemical compounds that possessed insect repellent activity. These findings prompted us to conduct further investigations on the nature of the repellent compounds produced in this plant.

In the isolation procedure, the pulverized *A. vulgaris* was steam distilled until no more volatile substances could be obtained. The distillate was extracted 3 times with benzene, and the combined benzene solutions were washed with water, dried over sodium sulfate, and evaporated to give the *Artemisia* essential oil.

The residue, obtained after steam distillation and mainly containing plant materials, was extracted with a mixture of benzene and methanol and the extract, after removal of the solvent, showed no repellency against *Ae. aegypti*.

The *Artemisia* essential oil, showing more than 90% repellency, was then chromatographed on a Florisil column and eluted with a series of solvents or mixtures of solvents with increasing polarity. Relatively larger quantities of eluates were obtained from hexane-eluted fractions which, upon bioassays tests, showed 80-95% repellency to biting mosquitoes.

The active fraction was analyzed by gas chromatography with a 0.25 mm x 60m capillary silica column packed with DB-5 (bonded SE-30). Identification of the components in the active

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fractions was made by comparing the retention index of a component with that of an authentic compound or by mass spectrometry. In the capillary gas chromatogram, 24 peaks were separated, and, among them, 13 peaks were identified. Compounds identified are camphene, β-pinene, myrcene, carvone, 1,8-cineole, α-thujone, linalool, β-thujone, camphor, isomenthone, borneol, terpinen-4-ol, and estragole. The active fraction was composed of 26% camphor, 22% borneol, 8% terpinen-4-ol, 8% estragole, 6% camphene, 6% β-pinene, 5% isomenthone, and 19% minor components.

These compounds identified from the essential oil of *A. vulgaris* were individually bioassayed for repellency against *Ae. aegypti* in the olfactometer described above. Each compound was dissolved in acetone or ethanol to make 10, 15, and 50 weight % solutions, and 0.1 ml of each solution was applied onto 6x6 cm area of the palm of a human subject resulting in obtaining dosages of 0.28, 0.42, and 1.39 mg/cm^2, respectively. Dimethyl phthalate and *N,N*-diethyl-*m*-toluamide (deet), two commonly used insect repellents, were used as standard for comparison.

As a result of bioassay tests, we found that several compounds showed significant repellency against *Ae. aegypti*. *dl*-Camphor, *d*-camphor, and terpinen-4-ol exhibited more than 90% repellency comparable to that of dimethyl phthalate. A mixture of α-and β-thujone and estragole possessed more than 80% repellency superior to that of deet. Linalool, (-)-camphor *l*-borneol, *dl*-isoborneol, *l*-methone, and isomenthone showed more than 70% repellency. β-Pinene, myrcene, α-terpinene, and 1,8-cineole showed some repellency only at higher dosages.

Except estragole which is *p*-allylanisole, all compounds isolated and identified from *A. vulgaris* and showing mosquito repellency are monoterpenes which are commonly present in various essential oils. Penfold and Morrison (1952) found that, of 40 Australian essential oils, the Huon pinewood (*Dacrydium franklini*) essential oil and the leaf oils of *Backhousia myrtifolia, Melaleuca bracteata*,and *Zieria smithii* were the most effective in repelling mosquitoes, March flies, and sand flies. Mayer (1952) reported that the essential oils of Eucalyptus and Caryophyllum repelled several species of mosquitoes. Although these essential oils were reportedly repellent to mosquitoes, none of the active compounds was chemically identified.

Terpinen-4-ol and 1,8-cineole were reported to possess repellency against mosquitoes. Thus, Lion Corporation (1982) in a Japanese patent disclosed that cyclic terpene alcohols, including terpinen-4-ol, were mosquito repellents. Nitto Electric Industrial Co., Ltd. (1981) disclosed that a composition containing cineole and one or more of 2,4-diethyltoluamide, triethylene glycol monoalkyl ethers, and triethylene glycol dialkyl ethers was a mosquito repellent for aerial and skin application. The compounds identified from *A. vulgaris* and showing repellency against *Ae. aegypti*, except the two compounds described above, are for the first time reported by us to exhibit mosquito repellency.

To develop these compounds into usable, long-lasting space or contact insect repellents, we are planning to conduct more detailed biological investigations and formulation studies.

ACKNOWLEDGEMENT.-The authors are indebted to Professor C.S. Tsi of the Research Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China, for supplying us with *A. vulgaris*.

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EFFECTS OF AN *ARTEMISIA CANA* EXTRACT ON THE DEVELOPMENT OF *CULEX QUINQUEFASCIATUS*

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ABSTRACT

The effects of four different plant extracts on larvae of *Culex quinquefasciatus* were determined. Using petroleum ether-methanol extracts of these plants, *Artemisia* extract caused the greatest mortality. When extracts were prepared using solvents with different dielectric constants, the methanolic fraction of *Elodea* and the petroleum ether fraction of *Artemisia* was found to be highly toxic.

These highly toxic plant extracts caused mortality and induced physical abnormalities in the surviving adults such as the inability to fly, the loss of leg parts or inability to leave the exuviae.

INTRODUCTION.-Mosquitoes often choose breeding sites dependent upon certain mosquito-plant relationships. Mosquitoes are attracted to certain vegetation, while some plants produce substances either repellent or toxic to mosquitoes (Bates 1949, Happold 1965). These various plant substances are potentially useful in mosquito control.

Field observations show the absence of immature stages of mosquitoes in ponds containing certain aquatic plants (Sherif and Hall 1984). Some species of blue-green algae inhibit mosquito reproduction in rice fields (Gerhardt 1954). Laboratory studies show that certain substances derived from algae are lethal to the larvae (Judd and Borden 1980).

Plant phenols and alkaloids (Levinson 1976) are known to be toxic to various insects, but the specific compounds that are inhibitory or lethal to mosquitoes are relatively unknown, especially in non-aquatic plants.

In this study we compare the effects of plant extracts on the survival of the immature stages of the mosquito.

MATERIALS AND METHODS.-*Culex quinquefasciatus* larvae were obtained from a laboratory-reared colony. Adults were fed a 10% glucose solution on cotton pads and larval stages were fed a (3:1) mixture of rat chow and Brewer's yeast.

The non-aquatic plant *Artemisia cana* (Tursh) was chosen because of its extensive medicinal use, especially in the Middle East, China and North Africa.

Four different plants: *Artemisia cana* (Pursh), *Elodea nuttallii* (Planch.), *Macrocystis pyrifera* (Linnaeus) C.A. Agardh, and *Spirogyra nitida* (Dillw.) were collected in southern California, washed several times with tap water, dried at 60° C, powdered and divided into two portions. The first portion (70 g) was refluxed for 8 h with absolute methanol and petroleum ether (3;1 vol./vol.). The second portion (70 g) was refluxed consecutively with three different solvents; absolute methanol, ethyl acetate and petroleum ether each for 8 h. The solvent was removed from the extracted material in a Buchler flush evaporator.

The extract samples were put into acetone solutions. To determine the maximum concentration of acetone which larvae and pupae can tolerate, larvae were exposed to various concentrations in water. Larvae and pupae were unaffected by concentrations up to 1% (vol./vol.). Thus acetone in concentrations not exceeding 1% was routinely used to prepare solutions containing the insoluble extracts. Solutions containing 6.5, 3.2, 1.3 and .65 mg of acetone soluble extract were prepared and added to 100 ml glass bowls. To each bowl, 20 first-stage mosquito larvae were added. There were four replicates of each concentration. Controls consisted of larvae grown in bowls containing 100 ml of distilled water with 1% acetone.

Mortality of each stage was determined every 1 or 2 days and dead organisms were removed from the bowls. In each case, adult emergence was determined by counting empty exuviae. Mosquitoes unable to completely leave the exuviae were included in the adults mortality counts.

Egg viability was determined by placing egg rafts in bowls containing the various extract treatments or else tap water and acetone as controls.

For further purification, plant extracts (100 μl) in acetone were applied to silica gel preparative plates and the plates were developed with methanol-petroleum ether (60-40). Visualization of the separated compounds was accomplished by placing the plate in a closed jar containing a few crystals of iodine. After visualization, spots were solubilized in acetone and bioassayed to determine their toxicity on the developing mosquito.

RESULTS AND DISCUSSION.-The inhibitory activity of methanol-petroleum ether extract of *Artemisia* and three other plants against larvae of *Culex quinquefasciatus* is shown in Figure 1. Among the four plants, the activity of the *Artemisia* extract was most toxic, with 97 percent mortality observed at a concentration of 60 ppm. The lowest mortality was caused by the *Spirogyra* extract, which was not expected to be highly toxic, and was chosen as a control in our experiment. Figure 2 shows that *Artemisia*

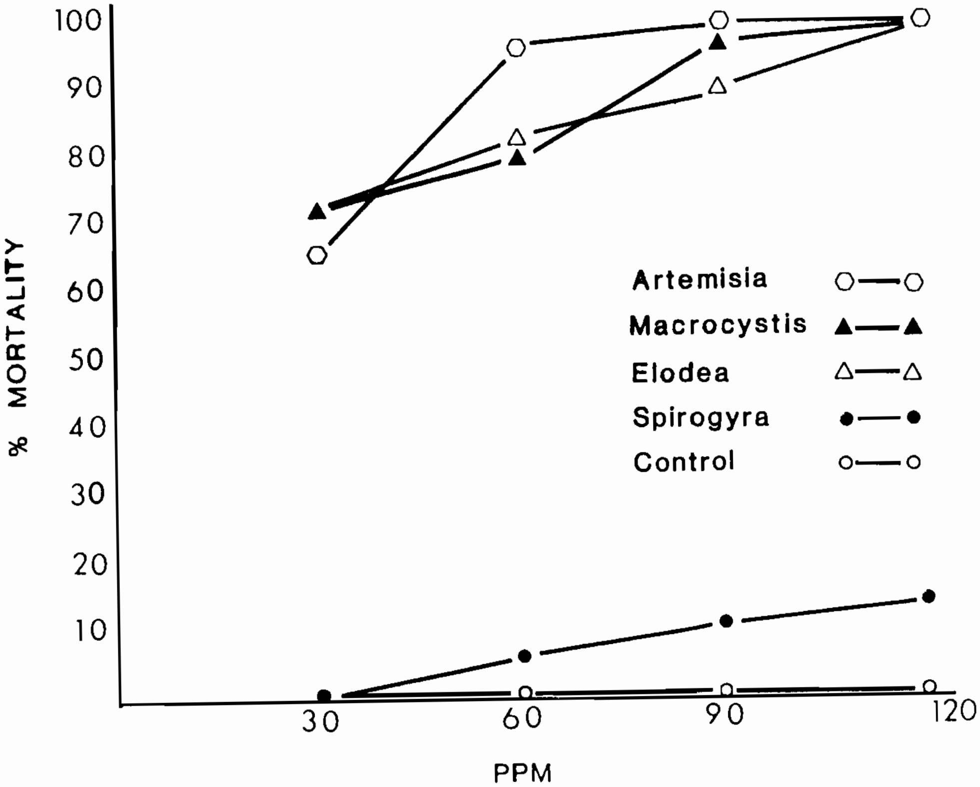

Figure 1. Effect of various concentrations of petroleum ether extract from four different plants on the development of *Culex quinquefasciatus* Say. Each point represents four replicates of 20 mosquitoes.

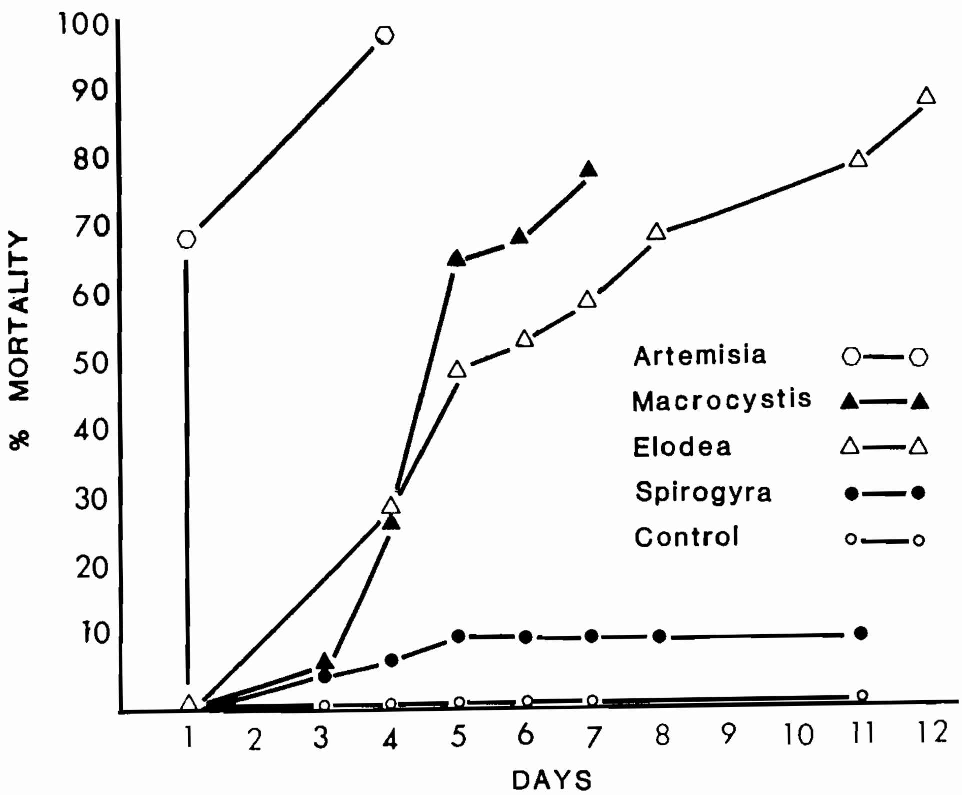

Figure 2. Effect of the petroleum ether extract (90 ppm) from four different plants on the development of the mosquito *Cx. quinquefasciatus*. Each point represent the mean percent mortality of four replicates of 20 mosquitoes.

Table 1. Evaluation of Different Slolvent Fractions from the plants: *Artemisia, Elodea* and *Macrocystis*.

Mean Percent Mortality in Larvae (L), Pupae (P) Adult (A) and Percent Emergence (E)

EXTRACT CONCENTRATION	6.5 mg/100 ml H_2				3.2 mg/100 ml H_2O			
	L	P	A	E	L	P	A	E
ARTEMISIA								
Petroleum Ether	100	0	0	0	100	0	0	0
Ethyl Acetate	5	10	10	75	20	5	0	75
Methanol	15	0	0	85	0	5	5	90
ELODEA								
Petroleum Ether	15	15	5	65	10	5	5	80
Ethyl Acetate	15	0	0	85	5	0	10	85
Methanol	100	0	0	0	100	0	0	0
MACROCYSTIS								
Petroleum Ether	90	0	0	10	10	10	0	80
Ethyl Acetate	10	10	10	70	0	5	5	90
Methanol	15	5	0	80	10	0	0	90
CONTROL	2	0	0	98	2	0	0	98

caused complete mortality after four days of exposing the mosquito larvae to the plant extract. Extracts of *Elodea* and *Macrocystis* not only produced lower mortality but also required prolonged exposure to be effective.

Data on the evaluation of the extract obtained with three different solvents, each with a different dielectric constant, is shown in Table 1. The data shows that the methanolic fraction of *Elodea* and the petroleum ether fraction of *Artemisia* caused the highest mortality. At low concentration (1.7 mg/100 ml) *Artemisia* was more effective than *Elodea* with a mortality of 60% compared to 30%. The highest mortality occurred during the larval stage, with few mosquito deaths occurring once the pupal stage was reached. Further tests are necessary to determine if mosquitoes reaching the pupal stage simply represent the resistant survivors from the larval exposure to the extract, or if the pupal stage itself is more resistant.

Unlike extracts prepared by the methanol-petroleum ether extraction, some extracts obtained with one of the three other solvents caused mortality in the adult stage as well as in the other stages of the mosquito development.

The toxicity produced by extracts of *Artemisia* using the three solvents is shown in Figure 3. Complete mortality was reached in three days by the petroleum ether fraction while a time of 8-11 days was required to cause a 25% mortality by the ethyl acetate fraction.

The effect of the low concentration of *Artemisia, Elodea,* and *Macrocystis* extracts on eggs caused an average delay of approximately 12 h in hatching as compared with the control. At high extract concentration the eggs did not hatch. Adult mosquitoes produced from treated eggs showed significant physical abnormalities. Such abnormalities include the inability to fly, loss of leg parts upon emergence and inability to completely leave the opened exuviae. The occurrence of these abnormalities was in the range of 3-4% of the emerging adults.

Separation by thin layer chromatography of the methanolic extract gave two spots with Rf values of .35 and .71. Upon bioassay, the spot with a Rf value of .71 was found more active, causing 72% mortality compared with 32% caused by the other spot.

The above study reveals that *Artemisia, Elodea* and *Macrocystis* possess biocidal activity against *Cx. quinquefasciatus*. Of the four plants tested, extracts from *Artemisia* were the most lethal.

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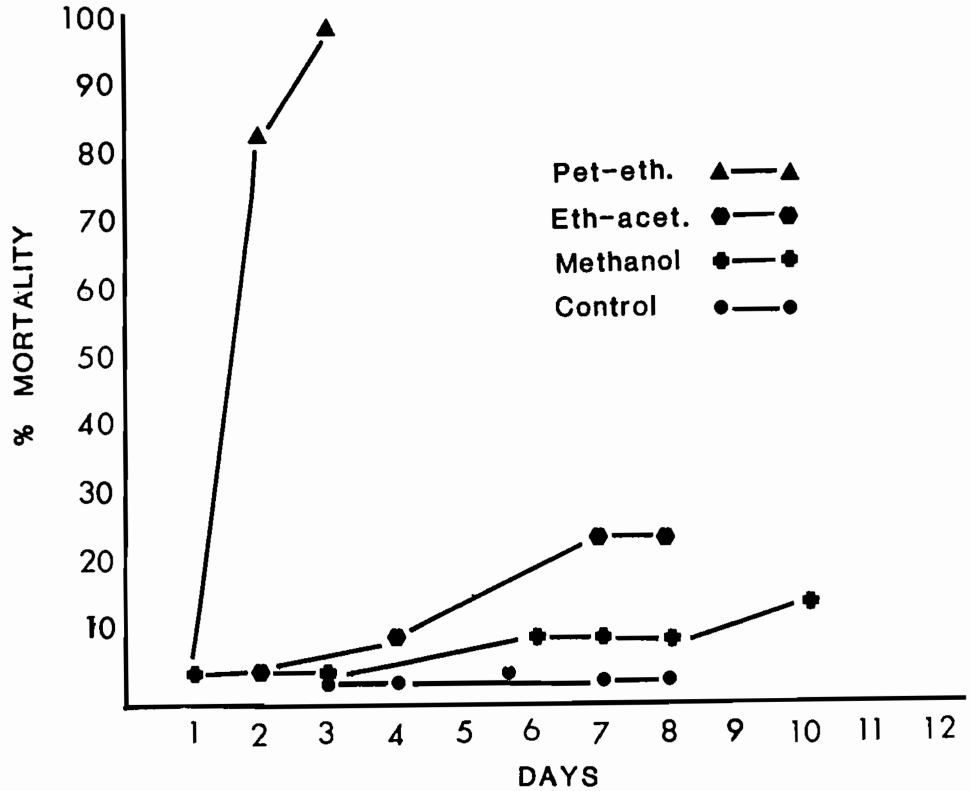

Figure 3. The effects of extracts of *Artemisia* prepared with different solvents. Each point represents the mean percent mortality of four replicates of 20 mosquitoes.

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FIELD TRIALS OF *BACILLUS THURINGIENSIS* H. 14 COMMERCIAL FORMULATIONS

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ABSTRACT

Several commercial formulations of *Bacillus thuringiensis* serotype H.14 were field tested by ground application methods in Fresno County, CA. Vectobac™ corncob granule was more effective against *Aedes nigromaculis* (Ludlow) larvae than against *Culex tarsalis* Coquilett; while the converse was true for Teknar™ corncob granule. Bactimos™ corncob granule showed good efficacy against both species, but a Bactimos™ corncob pellet was ineffective against *Cx. tarsalis*. All were applied at 10 kg/ha. Bactimos and Teknar granules were effective against *Culex* spp. in a milo field. Vectobac sand granule was ineffective until applied at 28 kg/ha. Vectobac aqueous suspension produced a 98% reduction of *Cx. tarsalis* at 0.5 kg/ha.

INTRODUCTION.-Testing of new formulations of *Bacillus thuringiensis* serotype H.14 (*B.t.* H.14) continues with attempts to find effective means for applying this agent against a wide range of species in diverse breeding sources, e.g., Garcia et al. 1983 and Mulligan and Schaefer 1983. Of the new formulations, several have been granules of various types. Because of the high competition among the manufacturers of *B.t.* H.14 for the mosquito control market, there has been a rush to market promising formulations before the full extent of their field utility is known. In this paper we discuss our field trials of several new commercial formulations of *B.t.* H.14.

MATERIALS AND METHODS.-The following commercial formulations of *B.t.* H.14 were evaluated in field tests; Abbott Laboratories Vectobac™ corncob granule (200 *Aedes aegypti* International Toxic Units per mg), Vectobac aqueous suspension (AS) (600 ITU/mg), Biochem Products Bactimos™ 5% (wt/wt) corncob grit granule (175 ITU/mg) and Bactimos 5% corncob pellet (400 ITU/mg) and Sandoz, Inc. Teknar™ corncob grit granule (260 *Ae. aegypti* Units per mg). Also a sand granule of Vectobac (100 ITU/mg) was prepared onsite; this was composed of 2.5% Vectobac technical powder (4000 ITU/mg), 2% GB-1356 oil (Witco Chemical Corp.) and 95.5% #16 Monterrey sand. Potency designations given in ITU/mg or AAU/mg were those determined by the respective producers; no verification assays were made by the authors.

Granules were applied by either whirling disc granule spreader or backpack power duster (Kioritz Corp., Tokyo, Japan). The AS formulation was suspended in 6 liters of water and applied by handcan sprayer.

Field trials of the commercial formulations were run against natural mosquito populations in Fresno County, CA. Formulation, rate (ITU or AAU/ha), area and species treated, date and application method are listed in Table 1. Immature mosquito populations were sampled by counting the numbers in each of 25-50 dips taken with a standard dipper. An adjacent, untreated control area for each trial was sampled likewise. Relative differences in populations were attributed to the treatments.

the numbers in each of 25-50 dips taken with a standard dipper. An adjacent, untreated control area for each trial was sampled likewise. Relative differences in populations were attributed to the treatments.

Since it is impractical to include all details recorded for each test, e.g., temperature, pH, wind, sky-cover, water depth, etc., this information will be on file and available to interested persons.

RESULTS AND DISCUSSION.-Results of the commercial formulation testing are shown in Table 1. Tests on 7-12 and 8-3-83 were conducted on an irrigated pasture against *Aedes nigromaculis* (Ludlow) larvae. All formulations were applied at a formulation rate of 10 kg/ha. When the two trials were averaged, the Bactimos granule and the Vectobac granule showed the greatest activities at 97 and 96% larval reductions respectively. These two formulations also had the lowest, relative, laboratory potencies as reported by the manufacturers. Bactimos pellet, with a potency doubled that of the former two formulations, averaged 92.5% reduction of larvae. Teknar granule, the lightest granule, produced an average of 89% reduction. However, against *Culex tarsalis* Coquillett, also at 10 kg/ha in an irrigated pasture, both Teknar and Bactimos granules produced excellent results, Vectobac granule did not perform as well and the Bactimos pellet failed (8-24-83).

Bactimos pellet and Vectobac granule were more active against *Ae. nigromaculis* than against *Cx. tarsalis*. Both formulations were large; the pellets were ca. 8 mm long and 3 mm diam and the granules averaged 5 mm. Conversely, the Teknar granule was small (ca. 1 mm) and was more effective against *Cx. tarsalis*. We speculate that *Ae. nigromaculis* larvae, being browse feeders, were more attracted to the larger-sized carrier particles and that the smaller-sized Teknar granule was more efficient in making the toxin available to *Cx. tarsalis* larvae. Hence, there appears to be a differential in activities of the formulations based upon carrier particle size.

The Bactimos granule also was small (ca. 2 mm) but effective against both *Ae. nigromaculis* and *Cx. tarsalis*, despite having the lowest formulated potency (175 ITU/mg). In contrast the

Table 1. Summary of applications of *Bacillus thuringiensis* H-14 formulations to mosquito breeding sources in Fresno County, CA, during 1983.

date	area (ha)	species	stadia	method of application	company formulation[1/]	rate (x 10^7 ITU/ha)	percent reduction
7-12	0.06	*Ae. nigro.*	3	whirling disc	Abb-corn G	200	99
					Bio-corn P	400	96
					Bio-corn G	175	97
					San-corn G	260[2/]	94
8-3	0.09	*Ae. nigro.*	2,3	whirling disc	Abb-corn G	200	93
					Abb-sand G	100	78
					Bio-corn P	400	89
					Bio-corn G	175	97
					San-corn G	260	84
8-24	0.07	*Cx. tar.*	1-4	whirling disc	Abb-corn G	200	82
	0.06				Bio-corn P	400	43
	0.05				Bio-corn G	175	96
	0.04				San-corn G	260	100
8-31	1.0	*Culex*[3/]	1-4	backpack blower	Bio-corn G	273	95
	0.5				San-corn G	455	93
9-1	0.5	*Culex*	1-4	backpack blower	Abb-sand G	185	67
9-6	0.02	*Cx. tar.*	1-4	handcan sprayer	Abb-AS	30	98
9-8	0.5	*Culex*	1-4	backpack blower	Abb-sand G	280	95

1/ Abb-Abbott Laboratories, Bio-Biochem Products, San-Sandoz, Inc.; G-granule, P-pellet, AS-aqueous suspension.

2/ The Sandoz designation is AAU/ha.

3/ Denotes a mixed population: *Cx. tarsalis*:*Cx. quinquefasciatus*, 2:1.

Bactimos pellet, with more than twice the formulated potency (400 ITU/mg), performed very poorly, especially against *Cx. tarsalis*. It appears that formulation potency is not as important as the physical characteristics of the formulation, in terms of field efficacy.

Bactimos and Teknar granules were applied by backpack blower to an irrigated milo field at 15.6 and 17.5 kg/ha, respectively (8-31-83). Both formulations provided good results against a mixed population of *Cx. tarsalis* and *Cx. quinquefasciatus* Say (Table 1); although, because of their light weight, their effective swath widths (14 and 12m) were half that of sand granules (24m). The Teknar granule was most subject to wind and had the most limited swath width.

All applications of the user-prepared, Vectobac sand granule were to permanent pasture sources. The sand granule, with a potency of 100 ITU/mg, was ineffective against *Ae. nigromaculis* at 10 kg/ha. It was also ineffective against *Culex* spp. at 18.5 kg/ha (9-1-83); although when applied at a very high rate of 28 kg/ha (9-8-83) good results were obtained against *Culex* spp.

Vectobac AS, applied at 0.5 kg/ha to a drainage ditch reduced *Cx. tarsalis* by 98%. This liquid formulation possessed good handling characteristics.

ACKNOWLEDGMENT.-The assistance and cooperation of David G. Farley and James R. Caton of the Fresno Mosquito Abatement District is gratefully acknowledged. This work was supported, in part, by a special California State appropriation for mosquito control research.

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EVALUATION OF BEECOMIST NOZZLES FOR AERIAL ADULTICIDING IN KERN COUNTY

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INTRODUCTION.-One of the biggest problems involved in the aerial application of insecticides against adult mosquitoes is the production of the correct spray particle size. Most conventional-type, atomization systems (hydraulic nozzles) produce a wide range of spray particles and much of the volume applied is lost by drift of fines (<10μ) and settling of the larger particles (>200μ). The loss of substantial proportions of the spray volume is not only cost inefficient but also results in the production of undesired insecticide residues.

A great deal of research has been conducted on the development of atomization systems which would produce a narrower range of spray particle sizes than the conventional systems commonly used; several manufacturers sell equipment designed for this purpose. One such device is the Beecomist nozzle; this device is a high-speed, rotary drum which displaces insecticide through its porous sides via centrifugal force. Each unit consists of a high-speed rotor, which turns at ca. 10,000 rpm, and a porous sleeve. Several sizes of sleeve porosity are available. These nozzles produce droplet diameters in a range of less than 100μ; in contrast, conventional spray particle diameters often range over 300μ. While conventional nozzles cost only a few dollars each, a Beecomist motor plus sleeve sells for ca. $550.

[1]Kern Mosquito Abatement District, 4705 Allen Road, Bakersfield, CA 93309

Aircraft equipped with Beecomist nozzles have been used for applying adulticides against mosquitoes in the southeastern U.S. and in Canada. An operational evaluation in California was conducted to assess their efficacy in Kern County.

MATERIALS AND METHODS.-A 1 lb. AI/gal. solution of Baygon in oil (Baygon 1 - MOS) was tested. Two Beecomist nozzles were mounted on a spray boom; each was located 7 feet outward from the aircraft fuselage and were oriented backward and 60° down from the boom (Figure 1). The discharge rate was calibrated by adjusting both pump pressure and orifice size of disks in flow-restrictors placed in the lines to the nozzles. For given sets of conditions, the actual discharge rates were measured following timed spray runs. Swath widths were determined by flying over lines of cages of mosquitoes placed at 10 or 20 ft. intervals over total spans up to 600 ft. Separate flights were made to evaluate results when flying into or with the wind as well as cross-wind. Droplet size was determined by microscopic measurement following collection of spray particles on teflon-coated slides (exposed in a rotator which turned at 4 revolutions/sec.). Temperatures were measured at 8 ft. and 30 ft., wind at 15 ft. and relative humidity at 4 ft.

RESULTS AND DISCUSSION.-A total of 17 separate spray trials were made, 10 with 20μ sleeves and 7 with 40μ sleeves. Attempts to align the flight paths into the wind, or downwind, led to highly variable results due to erratic crosswinds; this was especially true in the early

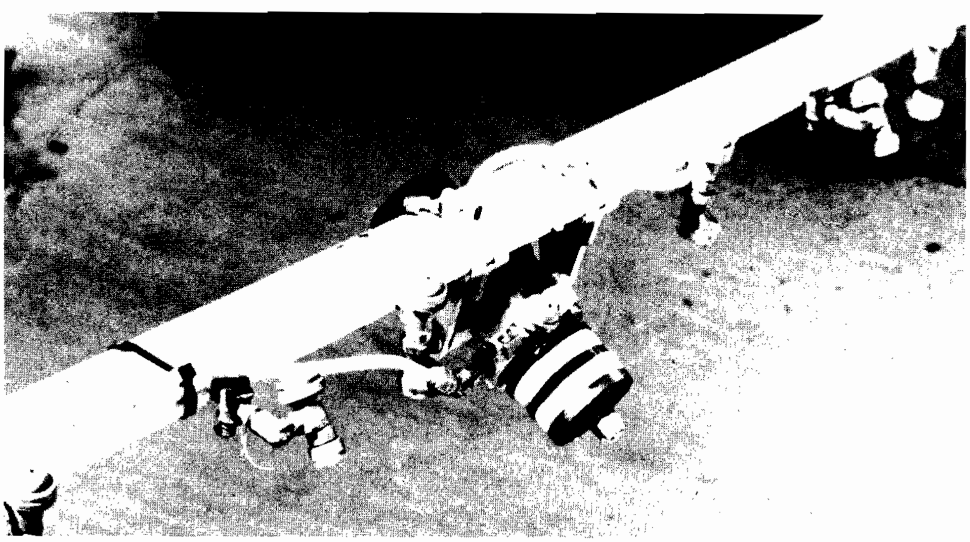

Figure 1. A Beecomist nozzle on spray boom mounted between the conventional hydraulic nozzles.

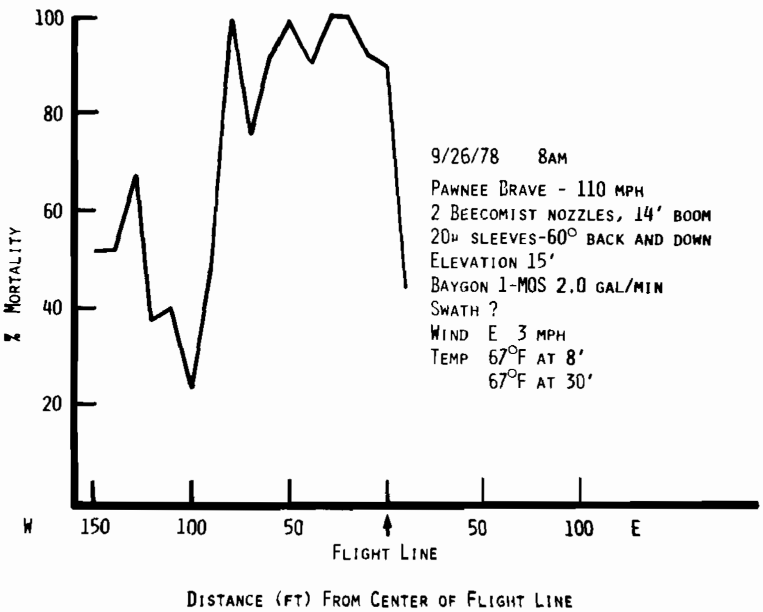

Figure 2. Typical results of variable winds on the swath following application of Baygon 1-MOS with Beecomist nozzles equipped with 20µ sleeves.

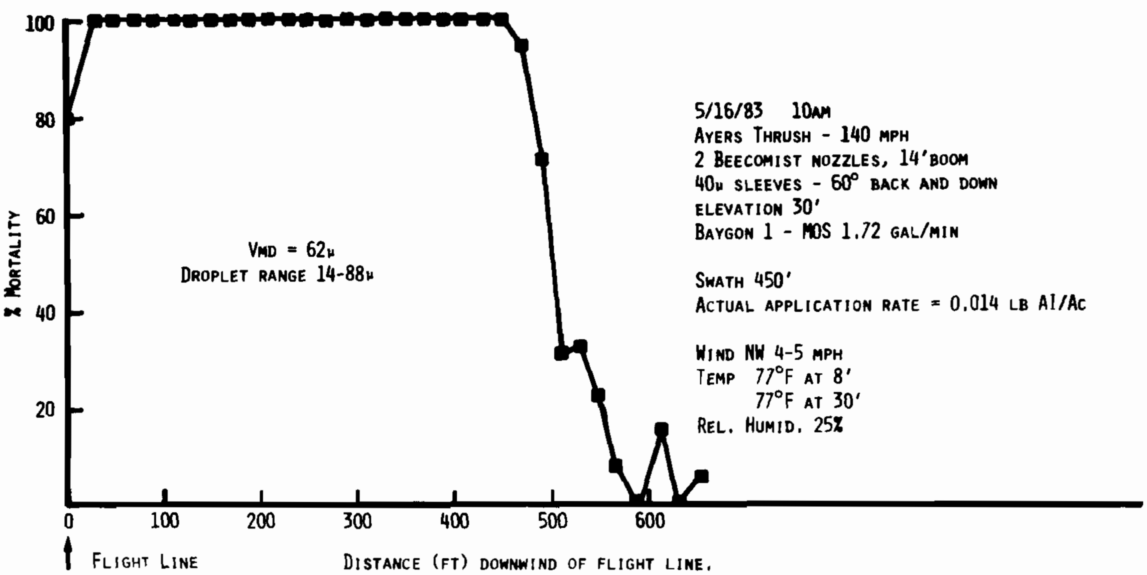

Figure 3. Drift application of Baygon 1-MOS using Beecomist nozzles equipped with 40µ sleeves.

morning hours. An example is shown in Figure 2 where the wind shifted 90° after initiation of the spray run. The small spray particles produced by the 20μ sleeves were so subject to crosswind effects that they were considered too fine for operational use. Also, it readily became apparent that, even using the 40μ sleeves, spraying would have to be conducted cross-wind. Thus, effective swath widths became dependent on drift applications.

Results with two 40μ sleeves showed that swath widths of 400 ft. (+) could be obtained even in the absence of inversions and at application rates far below those normally made (Figure 3).

While the Beecomist nozzles are quite expensive, they can achieve considerable savings of insecticide; again, effective swaths can only be expected through drift application. Under calm or very light and variable wind conditions, this application method was not operationally effective. The prevailing winds in the San Joaquin Valley are from the northwest or from the southeast; under these conditions, east-west flight lines allow for paralleling the section lines for pilot reference.

One big difference between Kern County and many other places where aerial adulticiding is practiced is the low humidity. This results in conditions of high particle evaporation and may largely account for our unsatisfactory experiences in using the 20μ sleeves.

ACKNOWLEDGMENTS.-These studies were supported by funds from a special California State appropriation for mosquito control research. The Baygon 1-MOS was donated by Mobay Corporation.

AIRCRAFT CLOSED MIX/LOADING SYSTEM FOR HANDLING WETTABLE POWDERS

Claude L. Watson

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Many Mosquito Abatement Districts use Baygon Wettable powders as an Adulticide for Mosquito control. The East Side Mosquito Abatement District has added another feature to its Closed Loading System for safety in handling wettable powders.

Regulations state that pilots who operate Pest Control Aircrafts shall not transfer, mix or load <u>liquid</u> Category One or Two pesticides containing organophosphates or carbamates, unless a closed system is used. Regulations do not require a closed system for Baygon powder even though it is a Category Two pesticide. Wettable powders are not considered as harmful as liquids. Therefore, the pilot can load his own aircraft without a closed system. A loader must be provided for the pilot if liquid Category One or Two is used, unless a closed system is used.

A few years ago, we had a pilot who claimed that he was poisoned from the Baygon Wettable Powder. The wettable powder was being dumped directly into the hopper of the aircraft. The pilot claimed that the dust would rise and collect around the hopper lid. When he would taxi out for take-off, the collected dust would blow back into his face. The breathing of the dust caused him to become nauseated. In a case of this nature the Closed Loading System would be the answer. The material is already mixed thoroughly when it is loaded into the plane.

Our current pilot is very pleased with the feature that we have added to the Closed Loading System for handling wettable powders. He feels that it is not only a much safer operation but it makes it much easier and faster to load.

Our new feature has been approved for wettable powders by the Stanislaus County Agriculture Department. The State of California Food and Agriculture Department has also inspected and approved the new feature. They found that the construction and design meet and exceed the minimum criteria. They stated that it was commendable that our system is employed for mixing, loading and transferring all materials for aerial applications and it reflects a sincere safety-oriented program.

After discussions with several insecticide specialists, I am convinced that in the near future there will be more and more demand for strenuous regulations on insecticides. This will call for more material to be handled through a Closed Loading System. Preparation now, would put you one step ahead of the game in a safety oriented program.

Table 2. Nematode infection rates of sentinel larvae in rice checks inoculated in 1983.

Inoculation Date[1]		Date of Sentinel Placement (1983)	Percent of Infected Sentinel Larvae[2]
6/3*		7/21	<1
6/10; 6/17	(N)	7/29	79
6/24	(N)	8/5	76
7/1	(S)	8/12	31
7/1	(N)	8/19	21
7/15	(1S)	8/26	81
7/15	(1N)	8/26	62
7/15	(2S)	8/26	86
7/15	(2N)	9/2	68
7/21	(S)	9/2	59
7/21	(N)	9/2	85

[1] S: south side of levee
N: north side of levee

* Field sprayed with Ordram® 15 minutes before postparasites were inoculated along this transect. This transect was sprayed at a rate of 100 g/100 m.

[2] Average of sentinel infection in 10 sentinel cages, each initially containing 20 second instar *Culex pipiens*.

work, the following recommendations for an operational protocol for use of *R. culicivorax* in California rice fields has been developed:

Inoculation rate

Ten to 50 grams of postparasites per 100 m transect with adjacent transects every 10 to 15 meters. The lower rate is probably sufficient for initiating and perhaps maintaining mosquito larval control for one season. The higher rate would increase overwintering capability. Inoculation rates assume close to a 1:1 ratio of male to female nematodes is used.

Inoculation Method

Apply nematodes in groups every one to two meters using a backpack sprayer or closed container that allows suspension of the nematodes in water. All sprayers tested to date have performed poorly due to the large size of the postparasites which gradually ruin or plug the pumping mechanism. Evaluation of alternate inoculation methods is necessary.

Monitoring

Periodic monitoring of inoculated sites is necessary to insure virulence of the laboratory-reared nematodes, to establish its persistence and to allow for adjustments in the inoculation protocol as needed.

Production

For a cost-effective program, an in vitro rearing method must be developed for *R. culicivorax*. At least five laboratories are currently attempting to develop an in vitro production method for this nematode.

Lagenidium giganteum. The choice of vegetable oils used to culture the asexual stage of *L. giganteum* was based on observations of their efficacy in the laboratory and on published studies (Domnas et al. 1982). The choice of linseed oil in both the maintenance medium and two of the media used for the field tests was based on the presence of high concentrations of esterified linolenic acid (C-18:3) in the oil (Hilditch and Williams 1964). Domnas et al. (1977, 1982) have reported that hemp seed extract was optimum for

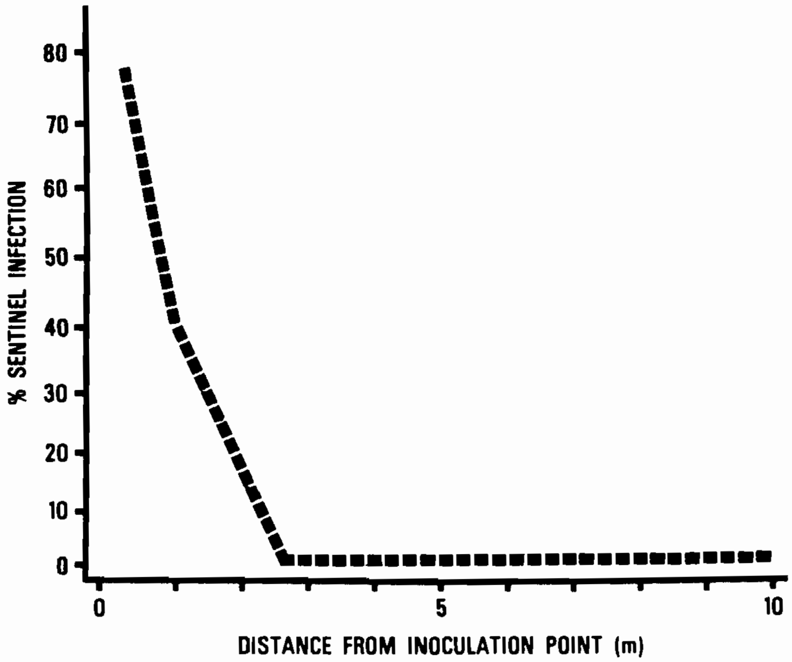

Figure 1. Distance migrated by *R. culicivorax* preparasites from the point of inoculation as monitored by sentinel larvae infection. Each point is an average of three separate trials, each of which employed 10 sentinel cages initially containing 20 second instar *Culex pipiens*, at 0, 1, 2.5, 5, and 10 meters from the point of nematode placement.

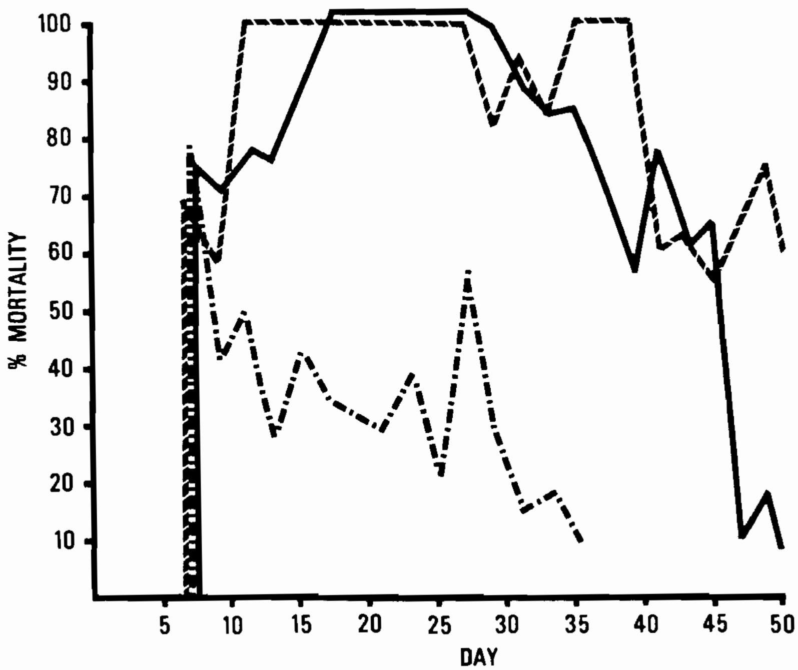

Figure 2. *Culex tarsalis* larval mortality due to *L. giganteum* oospore germination. Each point is the average of four pans containing one of the three dosages of oospores:

$3x10^4$:alternating dot/dashed line

$1.5x10^5$:solid line

$3.0x10^5$:dashed line

Table 1. Summary of Overwintering of *Romanomermis culicivorax* Between the 1982 and 1983 seasons.

Inoculation method/rate[1]	Inoculation Date (1982)	Date of Evaluation (1983)	Percent infection (no. of sentinels recovered)[2]
Spray/100	7/30	6/24	0 (166)
		7/1	29 (110)
Tubes/25	8/6	6/24	12 (97)
		7/1	32 (133)
Spray/25	8/6	6/24	1 (113)
		7/1	16 (160)
Tubes/50	8/13	7/1	5 (112)
		7/8	3 (100)
Spray/50	8/13	7/1	16 (114)
		7/15	0 (142)
Tubes/100	8/20	7/8	27 (137)
		7/15	5 (144)
Spray/100	8/27	7/8	14 (151)
		7/15	0 (109)
Spray/100	9/3	7/8	5 (126)
		7/15	0 (122)

[1] All overwintering studies performed in B. Lemanager's field at junction (NE corner) of Humphrey and South Butte Roads.

Spray: Inoculated using a compressed air backpack sprayer.
Tubes: Every 10 m the appropriate dose of postparasites was dumped into 1 m long plexiglass tubes (4 cm. diam.) placed into the benthos.

[2] For each transect, 10 sentinel cages placed 5 meters apart containing 20 second instar *Culex pipiens* each were used for monitoring infection.

seven days after rehydration of the oospores; motile spores, therefore, were released from germinating oospores ca. five to six days after rehydration, with actual initiation of the germination process (Kerwin and Washino 1983b) beginning 24 hours prior to spore release. At the lowest dosage investigated (3×10^4 oospores /1) larval infection peaked at day 7 of the test and persisted at rates around 50% for 20 days, after which activity quickly dropped (Figure 2). For the two higher oospore dosages, infection rates remained at 80% or greater for ca. 30 days, with some activity persisting until the test was terminated on day 51 (Figure 2).

DISCUSSION.-*Romanomermis culicivorax*. Results from the 1983 season confirm and extend those previously documented by this laboratory (Westerdahl et al. 1982, Westerdahl and Washino, unpublished data, Kerwin and Washino 1983a). A single application of *R. culicivorax* postparasites in a rice field will provide some degree of mosquito control throughout an entire season. Because of the low density of larvae in the inoculated sites, it is believed that most of the recorded infection is due to mating activity of the postparasites inoculated into the field rather than to recycling of the parasite in the indigenous population. The assumed continuous mating and/or egg hatching of the nematode is ideal for control of the multivoltine species associated with rice culture in the Central Valley.

Based on the last several years of field uation of infection rate. Sentinel larvae were used to evaluate overwintering of nematodes inoculated in 1982, establishment of the nematode in sites inoculated in 1983, or distance from the point of inoculation that larval infection occurred. For the migration evaluations, 10 sentinel cages were placed at each of the following distances from the inoculation point: 0 m, 1 m, 2.5 m, 5 m and 10 m.

Lagenidium giganteum **field tests.** An in vitro culture of *L. giganteum* was maintained on solid media consisting of (g/1 deionized water): 1.25 glucose; 1.25 Difco peptone; 1.25 Ardamine pH (autolyzed yeast extract, Desmo Chemical Corporation, Elmsford, NY); 2.0 corn oil; 1.0 Grumbacher linseed oil; 0.5 mM Ca^{2+}; and 20 Difco agar. Weekly transfers of the fungus were made using zoospore suspensions.

For the field trials the fungus was cultured on a growth medium identical to the maintenance medium described above but without the peptone and agar, or on this basal medium with the corn and linseed oils replaced by 3.0 g/1 wheat germ oil or 2.0 g/1 wheat germ oil plus 1.0 g/1 linseed oil. Cultures were grown in one liter of medium in 2500 ml Fernbach flasks for six days on a rotary shaker at 110 rpm. Each flask was inoculated with 20 ml zoospore suspension. After six days of growth, the initial liter of each medium was stored at 15°C in an incubator for one week while the second liter of each was grown.

Inoculum was sprayed using a compressed air backpack sprayer at a rate of 0.5 and 1.5 liters of medium per 10 m^2 in a rice field at the Colusa Wildlife Refuge maintained for migrating birds. Three sentinel cages per 10m^2 plot containing 10 second instar *An. freeborni* and 20 second instar *Cx. tarsalis* larvae each were placed in the test plots immediately following inoculation and two weeks post-inoculation. *An. freeborni* larvae were not available for the second sentinel evaluation. After three days in the field, larvae were collected and returned to the laboratory to monitor percent infection.

Lagenidium giganteum **oospore evaluations.** *L. giganteum* oospores were grown in liquid shake culture on a variety of media containing yeast extract, glucose, cholesterol, triolein or trilinolein, lecithin, and millimolar amounts of calcium and magnesium. Spores were collected on filter paper and stored at room temperature in petri dishes for a minimum of two months before initiation of germination. Oospores were rehydrated in a small volume of water and counted using a hemocytometer. Germination/pathogenicity evaluations were performed in an incubator at 30± 2° C in small enamel-lined pans each containing one liter of deionized water. Four replicates at three concentrations (3×10^4, 1.5×10^5, and 3.0×10^5 oospores/liter) were initiated. Twenty late third or early fourth instar laboratory-reared *Cx. tarsalis* larvae were added to each pan and a small amount of ground fish meal placed in each. As they pupated, the immatures were removed from the pans and held for two additional days in small plastic cups to monitor for infection. An equal number of larvae was added back to each pan. Seven days after rehydration, when the first sign of *L. giganteum* infection was evident, all larvae and pupae were removed from the pans and placed in small plastic cups for two additional days. From that point until termination of the experiment six weeks later, all larvae were removed from the infection pans every two days and replaced with 20 late instar *Cx. tarsalis* larvae per pan. Dead larvae were immediately examined under a dissecting scope to determine whether they were infected with the fungus. Living larvae were held for two additional days to check for latent infection. Larvae were removed every two days to insure that all larval infections were due to oospore germination rather than zoospore release from previously infected larvae.

RESULTS.-*Romanomermis culicivorax* **field trials.** The nematode successfully over-wintered along eight different lines of inoculation in four separate rice checks in which postparasites had been applied in 1982 (Table 1). Rates of infection varied from 0 to 32% with great variation in nematode activity documented from week to week along the same transect. There was no obvious correlation between the different application rates (25, 50 or 100 g postparasites per 100 m) or methods (spray vs. tubes) and nematode activity in these fields.

Migration of preparasites away from the point of inoculation upon hatching was minimal (Figure 1). In three separate evaluations nearly 80% of sentinel larvae were infected along the inoculation transect but nematodes were present in only 2% of larvae placed 2.5 m from the transect. Infection at very low levels did occur as far as 10 m from the point where postparasites were originally placed in the rice field (Figure 1).

Application of *R. culicivorax* postparasites resulted in high levels of infection among sentinel larvae (21 to 86%) placed along inoculated transects (Table 2). An inadvertent test of postparasite tolerance to the herbicide Ordram occurred when shortly after beginning nematode dissemination on June 3 a plane began spraying the field with this chemical. We ceased operations until spraying ceased and resumed inoculation ca. 15 minutes after the Ordram application was completed. The very low level of nematode activity along this transect (Table 2) suggests that this compound had an adverse effect on the postparasites.

Lagenidium giganteum **field trials.** At the inoculum rates used, the asexual stage of *L. giganteum* successfully infected *An. freeborni* and *Cx. tarsalis* on a rice field habitat (Table 3). With the exception of the 0.5 liter inoculation using the basal growth medium supplemented with 3 g/1 wheat germ oil, infection rates were comparable in all plots. This study confirmed that *An. freeborni* larvae were less susceptible than *Cx. tarsalis* to the asexual stage of *L. giganteum* (Fetter-Lasko and Washino 1983, Westerdahl and Washino, unpublished data).

Lagenidium giganteum **oospore evaluations.** Larval mortality over the course of the monitoring period due to oospore germination is summarized in Figure 2. The first infections were observed

EFFICACY OF *ROMANOMERMIS CULICIVORAX* AND *LAGENIDIUM GIGANTEUM* FOR MOSQUITO CONTROL: STRATEGIES FOR USE OF BIOLOGICAL CONTROL AGENTS IN RICE FIELDS OF THE CENTRAL VALLEY OF CALIFORNIA

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INTRODUCTION.-Successful implementation of biological control programs on an operational level requires knowledge of environmental, behavioral and other host-mediated factors which affect the interaction of the host and pathogen (Pinnock and Brand, 1981). Efforts to introduce the merthimid nematode *Romanomermis culicivorax* and the oomycetous fungus *Lagenidium giganteum* as alternatives to the chemical control programs currently used to control mosquitoes in the Central Valley have included studies on factors affecting the establishment and virulence of these agents in rice field habitats (Westerdahl et al. 1982, Fetter-Lasko and Washino 1983). Among the factors that have been investigated are water temperature and chemistry (Brown and Platzer 1977, Westerdahl et al. 1982, Fetter-Lasko and Washino 1983), presence and abundance of nontarget aquatic invertebrates (Westerdahl and Washino; Kerwin and Washino, unpublished data), herbicide and pesticide applications and soil type (Westerdahl and Washino, unpublished data).

Perhaps the most important factor to be considered in attempting biological control of mosquitoes in Central Valley rice fields are the spatial and temporal distribution of the larvae. Mosquito density in rice fields varies significantly from field to field (Collins and Washino 1979; Palchick and Washino 1979), with a small percentage of the fields apparently producing a large proportion of the adult mosquitoes. When the low field density is considered with the multivoltine nature of *Anopheles freeborni* and *Culex tarsalis*, the two major pest species associated with rice fields, a major concern of any biological larval control strategy is how to introduce the control agent such that a moderate level of pathogen activity is sustained throughout the four to five month mosquito breeding season.

Most field studies using *R. culicivorax* have involved direct application of the preparasitic stage of the nematode (reviewed by Poinar, 1979; Peterson, 1982) which penetrates the cuticle of susceptible mosquito larvae and develops in ca. six days into the postparasitic stage. Use of preparasites allows an assessment of control potential within one week of applications, but persistence depends upon a sufficiently dense indigenous larval population at the time of application to insure completion of its life cycle.

Lagenidium giganteum field evaluations have involved application of the asexual stage of the fungus (Jaronski 1982, Washino 1982, Fetter-Lasko and Washino 1983, Jaronski and Axtell 1983, Kerwin and Washino 1983a). Use of the ephemeral zoospore stage is subject to the same host density constraints as described for *R. culicivorax,* although recycling of the fungus has been documented following application of zoospores in several environments (Fetter-Lasko and Washino 1983, Jaronski and Axtell 1983). Only recently with the development of an in vitro method for production of *L. giganteum* oospores has it has been possible to evaluate the sexual stage of the fungus for mosquito control (Kerwin and Washino 1983b).

Postparasite applications of *R. culicivorax* have proven to exert some degree of control of larval populations in California rice fields throughout the breeding season (Westerdahl and Washino 1982, Kerwin and Washino 1983a). Further evaluation of the efficacy of this nematode is included in this report along with field testing of the asexual zoospore stage and laboratory evaluation of *L. giganteum* oospore germination. These results are discussed in relation to strategies for the introduction of biological control agents into rice fields for mosquito control. Advantages of postparasite versus preparasite applications of *R. culicivorax* and use of the sexual rather than the asexual stage of *L. giganteum* are summarized.

MATERIALS AND METHODS.-*Romanomermis culicivorax* **field applications.** *R. culicivorax* postparasites were reared at the Sutter Yuba Mosquito Abatement District according to established protocol (Critchfield et al. 1982). Weekly from June 3 through September 9, 400 g of postparasites were inoculated ca. 2.5 meters from rice field levees at a rate of 50 g per 100 m linear transect. Initially, postparasites were disseminated using compressed air backpack sprayers; however, due to the relatively large size of the postparasites, the pumping mechanism of the sprayers invariably became inoperable within several weeks. From early July until termination of the inoculations, nematodes were dispensed in water suspensions from backpack sprayers from which the pumping apparatus was removed. The spray tanks were constantly shaken and aliquots of the nematodes dumped every 2.5 m through the exit port to which the spray hose and nozzle were previously attached. All fields inoculated with *R. culicivorax* were located near the town of Sutter, CA.

Beginning June 24 sentinel larvae were placed weekly in rice fields to monitor preparasite activity. Sentinel cages containing 20 second instar *Culex pipiens* larvae were placed 5 meters apart, with 10 cages placed along a given transect. Larvae remained in the field for three days and were brought back to the laboratory for eval-

TECHNIQUES OF COLD FOGGING FOR ADULT MOSQUITOES

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Don Womeldorf presented a talk at the American Mosquito Control Association in 1983. The title was "The California Experience with ULV Aerosols - An Annotated Bibliography". Don lists in chronological order papers published in the Proceedings of the California Mosquito and Vector Control Association, dealing with the development, efficacy and safety of ULV aerosols in California mosquito control. He has condensed these papers and people who are interested in cold foggers (as we seem to call them), should take time to read these papers. In preparing this paper, I was reminded of all the work and time that a group of us have put into the development of machines, chemicals, dosage rates, techniques in general, and last but not least, weather. You can have the best machine and the best chemical, but if the weather is not right for good spraying, you might as well forget it.

Many of you sitting out there already know what I'm about to say. What you need for good spraying weather is an inversion - simply - cool on the bottom, warm on the top. In the past we have talked marine air. Norm Akesson and his people used the term 'sea breeze'. I feel that the marine air coming into the valley from the bay area, creates a south east breeze that has a cooling effect on our north valley. Gene Kauffman says he doesn't need marine air as he gets an inversion every night. He is getting a cool air flow from somewhere, maybe from the hills to the east. I see this condition in Willow which is 35 miles north of Colusa and on the west side of the valley about 5 miles from the foothills. There are times when Colusa has a lapse condition (warm bottom-cold top) as did Willows in the early evening. After the evening progressed, the air movement from west to east from the hills produced an inversion. Colusa still did not have an inversion. Sometimes the inversion in Willows may not last long, but if we can have 1½ to 2 hours, we can treat the cities.

There are conditions during the day which we use as information for the evening. One of the main sources of information has been the barometer. Over the last few years, we have kept information on barometer reading and "spray or no spray" nights. If the barometer is 29.9 to 30 plus, we have a good indication of a "no go" night. It it's under 29.9, it will look better. Another indicator we use is to call the Weather Bureau in Sacramento for the wind velocity predicted for air coming from the bay area into the valley. If winds are predicted at 15 mph or less, then we probably will not receive any cooling. Fifteen to 20 mph is the gray area. With no cooling air, we can expect a hot, quiet night, and no night work. We prefer wind velocity from ½ - 5 mph, with a steady direction.

Now comes 'instrumentation'. Sutter-Yuba has one of the better operational set-ups in our area (Slide I). The tower which is about 50 ft. in height, has three temperature sensors as shown (Slide II and III) leading down into recording devices (Slide IV and V). The towers at Colusa and Willows consist of two sensors leading into meters in the offices. The sensors are about 9 ft. and 33 ft. (3M and 10M). The reason for the height of the sensors, I don't know. Sometime way back, Norm Akesson said that it was the distance, and we did it.

Now, the "Stability Ratio". There is a chart which you can use that will take the weather measurement data and transpose it into a number that will determine a 'go or no go' night (Slide VI). The left side is the temperature difference and the bottom is wind velocity. Follow the curve to a number and the higher the number, the better the spray night.

I have used my Colusa and Glenn Districts as examples of how we have learned to determine weather in regard to spray nights. The point I want to emphasize is this: Get into your area day and night and learn the indications that the weather gives you on whether it is a 'go or no go'. Sometimes out there, there is an inversion.

Last but not least, there is a District to the north of me that has a way of determining spray nights. The manager runs out of the office and grabs a handful of dirt and throws it into the air. If it lays down and the drift is to his liking, then they spray. This method is called the "Tehama Modified Method". Thank you, Mel Oldham for your scientific contribution.

Table 3. Infection Rates of *Lagenidium giganteum* in *Anopheles freeborni* and *Culex tarsalis* Larvae in a California Rice Field

Medium[1]	Inoculation Rate (Liters/10m^2)	Monitoring[2] Period	Percent Infection[3] *Anopheles freeborni*	*Culex tarsalis*
Wheat germ	0.5	A	57	67
		B	--	24
Wheat germ	1.5	A	83	100
		B	--	15
Wheat germ/ linseed	0.5	A	79	100
		B	--	5
Wheat germ/ linseed	1.5	A	76	100
		B	--	0
Corn/linseed	0.5	A	78	100
		B	--	7
Corn/linseed	1.5	A	86	100
		B	--	11

[1] Basal growth medium of (g/L deionized water): 1.25 glucose, 1.25 peptone, 1.25 Ardamine pH, 0.075 $CaCl_2 \cdot 2H_2O$, supplemented with 3.0 g/L wheat germ oil; 2.0 g/L wheat germ oil plus 1.0 g linseed oil; or 2.0 g/L corn oil plus 1.0 g/L linseed oil.

[2] A: Sentinel larvae placed in field immediately following fungal inoculation.
B: Sentinel larvae placed two weeks post-inoculation.

[3] *A. freeborni* larvae were not available for the second (B) monitoring period.

zoosporogenesis by *L. giganteum.* It has been assumed that the type and concentration of sterols in hemp seeds were responsible for its utility despite the inability to stimulate zoosporogenesis using a comparable mixture of purified sterols (Domnas et al. 1977). It is possible that the highly unsaturated nature of the constituent fatty acids of hemp seeds plays an important role in the induction of zoosporogenesis. Because zoosporogenesis involves a series of discrete fusion events (Olson et al. 1981) any compound affecting cleavage and fusion behavior will probably also affect zoospore formation. The nature of membrane fatty acids, especially the degree of unsaturation, has a marked effect on fusion-related processes (Cullis and deKruijff 1978). This class of compounds, therefore, merits its evaluation in future studies on the molecular biology of zoosporogenesis by oomycetous fungi.

Using the experimental protocol described, in vitro-cultured *L. giganteum* oospores germinated in the laboratory over a several month period following activation by successive dehydration and rehydration. This nonsynchronous germination is ideal for mosquito control in Central Valley rice fields where multiple generations occur throughout the growing season. Germination of the spores is likely to be even less synchronous under field conditions and a single application of oospores would probably exert some control on larval populations from late May when fields are first flooded until late September or early October when fields are drained.

With additional infective asexual zoospores released from mosquito larvae which are infected by germinating oospores *L. giganteum* has the potential to maintain itself at low levels during the breeding season in the absence of a suitable host, yet rapidly increase its activity to epizootic levels when larval populations are high. Larger scale tests are planned for the next two seasons to further evaluate the efficacy of *L. giganteum* for field control of mosquitoes.

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LABORATORY STUDIES ON THE EFFECTS OF *LAGENIDIUM GIGANTEUM* COUCH ON *PSOROPHORA COLUMBIAE* (DYAR & KNAB) AND *ANOPHELES QUADRIMACULATUS* SAY IN TEXAS[1]

Thierry M. Work[2] and Jimmy K. Olson[3]

Culex quinquefasciatus Say, known to be susceptible to *Lagenidium giganteum* Couch, was used as a control organism in a laboratory study to determine if the fungus was pathogenic to second/third instar larval stages of the mosquitoes *Psorophora columbiae* (Dyar & Knab) and *Anopheles quadrimaculatus* Say. This study also attempted to find the concentrations of *L. giganteum* needed to give optimum mortality for the three mosquito species. To expose the mosquito larvae to the fungus, sections of sporulating agar plate cultures of *L. giganteum* were homogenized in one liter of deionized water. These fungus water mixtures were then sprayed into pans containing second/third instar larvae of the three mosquito species.

Ps. columbiae and *An. quadrimaculatus* were found to be susceptible to infections by *L. giganteum*. This was demonstrated by observing fungal sporangia within larval cadavers. The observation of exit tubes, and the formation of progeny zoospores from sporangia within *An. quadrimaculatus* larvae indicated that *L. giganteum* is able to propagate in this mosquito species. Mortality induced by the fungus on all three species of mosquitoes was erratic; therefore, the concentrations of fungus needed to give optimum mortality for the three species of mosquitoes was not determined.

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A LABORATORY STUDY OF CRUSTACEAN PREDATION ON MOSQUITO LARVAE

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ABSTRACT

Crustacean predation on *Culex tarsalis* larvae were conducted in the laboratory using Ostracoda, Branchiopoda and Copepoda; only subclass Copepoda can prey on early stages of larvae when they are provided as food sources, a single copepod (*Cyclops vernalis* Fisher) consumed a mean of 1.83 first stage larvae in a 48 hour period with a range of 0.86 to 2.8.

Subclass Ostracoda (*Cypris subglobosa* Sowerby) and Branchiopoda (*Simocephalus* spp.) failed to prey on healthy mosquito larvae, but they may compete with mosquito larvae for available food.

Crustaceans are the most abundant invertebrate in Fresno westside rice fields (Miura et al. 1981). Among them, subclass Ostracoda predominated, followed by Branchiopoda (Cladocerans) and Copepoda.

It is also known that some crustaceans prey on mosquito larvae (Jenkins 1964), e.g., in the laboratory, one tadpole shrimp *Triops longicaudatus* (Le Conte) (*Apus lucasanus* Packard) consumed 308 2nd instar mosquito larvae in 5 days and another devoured 475 2nd instar larvae in 4 days (Mail 1934), and a single copepod [*Mesocyclops obsoletus* (Koch)] destroyed 15 to 20 instar mosquito larvae in a 24 hour period (Bonnet and Mukaida 1957).

This study was designed to answer these questions: 1. Can crustaceans (ostracods, cladocerans, and copepods) prey on *Culex tarsalis* Coquillett larvae? 2. If so, how many and which stages of larvae can they prey on for a limited time period?

MATERIALS AND METHODS.-The rate of crustacean predation was studied for various predators and prey densities in the laboratory. The crustaceans tested as predators were Ostracoda (*Cypris subglobosa* Sowerby), Branchiopoda (*Simocephalus* sp.), Copepoda (*Cyclops vernalis* Fisher). All were collected from rice fields, located about 10 km. SW of Dos Palos, California. They were sorted and conditioned to laboratory conditions for 2 days prior to testing. The crustaceans were loaded (numbers as per tables 1 and 2) into 12 oz. styrofoam cups containing ca. 300 ml. of tap water held at 21-25°C. Laboratory colonized *Culex tarsalis* larvae were used as prey organisms. One to two egg rafts were added to each cup and the rafts were examined every day for hatch. After all eggs were hatched, the rafts were carefully taken out of the cups and then total number of hatched, unhatched and damaged eggs were counted and tabulated. The rate of predation was determined at 48 hour posthatch by counting live larvae. The number of larvae dying from causes other than predation was calculated by establishing a control unit for each test. Tests were repeated three times for Ostracoda and Branchiopoda (Cladocerans) and seven times for Copepoda.

RESULTS AND DISCUSSION.-The data obtained from copepod predation studies is shown in Table 1. Individual *Cyclops vernalis* consumed an average of 1.83 first instar *Cx. tarsalis* larvae in a 48 hour period. The copepods were observed to attack and injure healthy larvae. Injured and weakened larvae were then clasped by one or more copepods and the body contents sucked out; subsequently, the dead bodies were discarded (Fig. 1,A). Attacking techniques were "hit and run" or "nibbling", usually by a single copepod on a single prey. However, on several occasions we have observed simultaneous group attacks by two or more copepods. Preferred attack points were membraneous areas in the posterior end of the thorax and the 8th abdominal segment, however some copepods attacked the conjunctives between abdominal segments (Fig. 1,B).

Copepods may play an important role in supressing immature (at least early instar) mosquito population densities in rice fields because the former are so abundant (Fig. 2) and distributed fairly uniformly in rice paddies (Fig. 3). In addition, their group attacking and ravenous behavior may enhance this predation efficiency.

Table 2 shows the results obtained from cladoceran and ostracod predation studies. Neither cladocerans nor ostracods appeared to prey on healthy first instar larvae of *Cx. tarsalis* when only larvae were provided as a food source. They may, however, compete with mosquito larvae for available food.

Ostracods, according to Pennak (1953), are omnivorous scavengers; their foods consist mostly of bacteria, molds, algae, and detritus. Some larger ostracods can feed on dead animals. Our study indicates that they cannot prey on healthy mosquito larvae, but on several occasions we have observed them feeding on injured, but still alive, larvae in the test cups (Fig. 1,C).

In summary, of the three subclasses of crustaceans tested, only Copepoda successfully preyed on early stages of *Cx. tarsalis* larvae. Subclass Ostracoda and Branchiopoda appeared to be unable to prey on healthy larvae, but Ostracoda consumed injured live larvae. Copepoda may have an important role in suppression of mosquito population densities in rice fields because of their behavior, abundance and popu-

Table 1. A Copepod predation of *Culex tarsalis* in the laboratory.

Test	No.raft	No.hatched	No.larva[a/]	No.copepod	%Survival	%Reduction[b/]	Reduction/ copepod
				Control			
1	2(333)	330	312	0	94.5	5.5	0
2	2(225)	223	196	0	87.9	12.1	0
3	1(166)	166	162	0	97.6	2.4	0
4	1(108)	103	98	0	95.1	4.9	0
5	2(235)	230	194	0	84.3	15.7	0
				Test			
1	2(254)	249	80	76	32.1	65.0	2.13
2	2(346)	337	49	102	14.0	84.8	2.80
3	1(145)	145	32	128	22.1	76.0	0.86
4	1(159)	147	108	12	73.5	20.0	2.42
5	1(185)	180	82	74	45.6	50.4	1.23
6	1(153)	148	24	49	16.2	82.4	2.49
7	1(86)	84	33	41	39.3	42.8	0.88

[a/] number of larvae at 48 hrs post hatch.

[b/] adjusted by the Abbotts formula.

Table 2. A crustacean (ostracods and cladocerans) predation of *Culex tarsalis* in the laboratory.

Test	No.raft	No.hatched	No.larva[a/]	No.crustacean	%Survived	%Reduction[b/]	Reduction/ organism
				Control			
1	2(333)	330	312	0	94.5	5.5	0
2	2(225)	223	196	0	87.9	12.1	0
3	1(166)	166	162	0	97.6	2.4	0
				Ostracod			
1	2(275)	268	257	10	89.9	3.64	0
2	2(236)	227	223	10	98.2	0	0
3	1(121)	113	111	10	98.2	0	0
				Cladoceran			
1	2(295)	276	258	68	93.5	0	0
2	2(226)	117	109	33	93.2	0	0
3	1(114)	105	98	48	93.3	0	0

[a/] number of larvae at 48 hrs post hatch.

[b/] adjusted by the Abbott's formula.

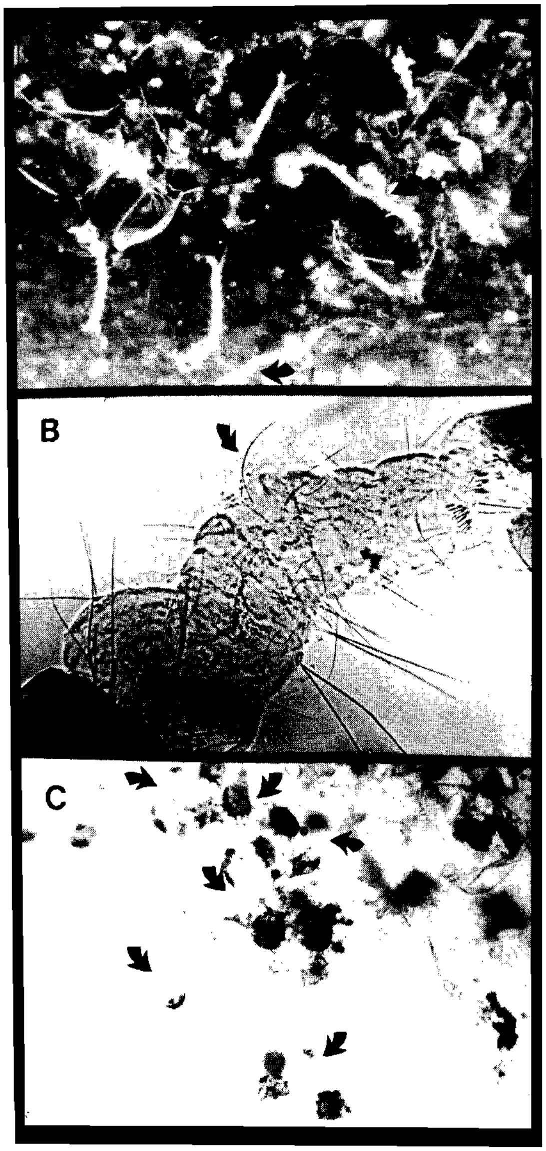

Figure 1. Crustacean predation on mosquito larvae.

A. Dead mosquito larvae (arrows) on the bottom of a copepod cup.

B. A micrograph of freshly injured mosquito larva showing attacking points (arrow).

C. Mosquito head capsules (arrows) on the bottom of a cup containing copepods and ostracods.

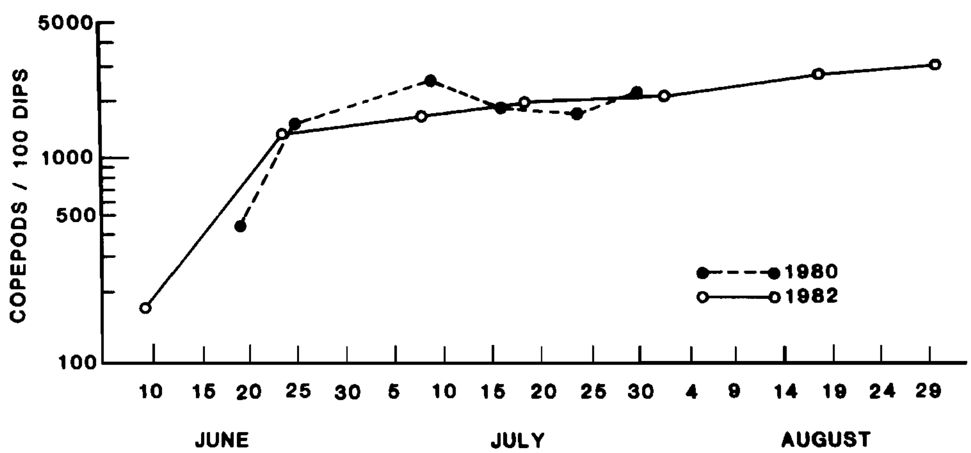

Figure 2. Seasonal population densities of copepods in Fresno Westside ricefields.

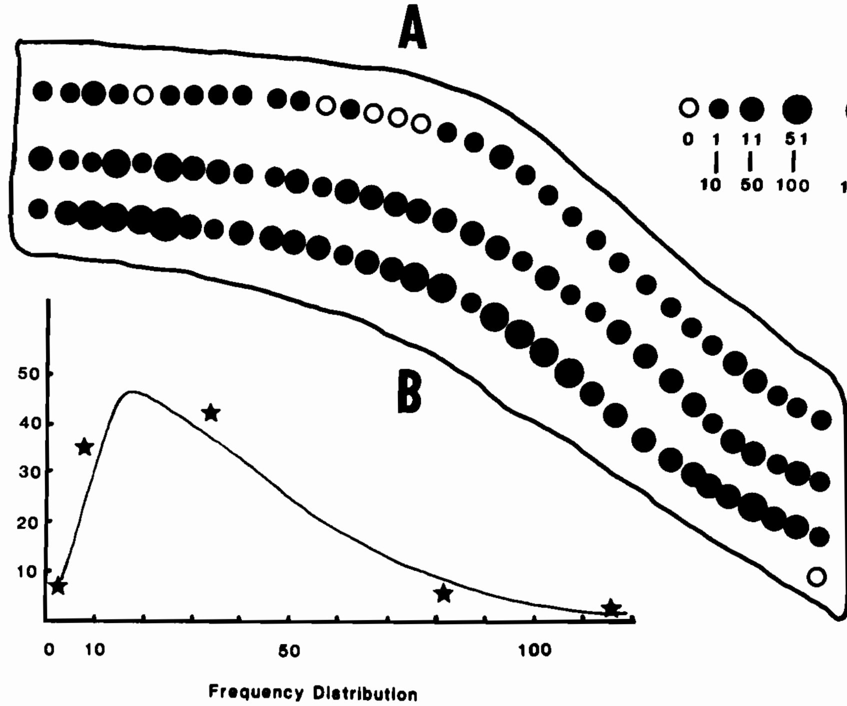

Figure 3. Distribution of copepods in Fresno Westside rice fields.
A. Spatial distribution in a paddy (No. copepod/dip).
B. Frequency distribution. X-axis - no. copepod/dip, Y-axis - frequency of each collection (modified from Miura et al. 1981).

lation density which grows as the season progresses.

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INITIAL OBSERVATIONS OF *ROMANOMERMIS CULICIVORAX* AS A BIOLOGICAL CONTROL AGENT

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INTRODUCTION.- Sutter-Yuba Mosquito Abatement District has had the objective of rearing the mermithid nematode *Romanomermis culicivorax* on an operational level since 1980. This year that objective was realized. A total of 7,545 g post-parasites and approximately 40 million pre-parasites were applied throughout the district. One zone in the district was given priority for nematode availability, monitoring and evaluation. Biological control using nematodes was used as much as possible. This paper will deal with the work done in this geographical zone.

The Sutter-Yuba Mosquito Abatement District, located in the Sacramento Valley, is comprised of 660 square miles and is principally agricultural. The primary crop is rice, 138,000 acres in 1982, which is the largest single producer of mosquitoes. The district must also deal with extensive drainage systems and seepage associated with agriculture.

MATERIALS AND METHODS.-Sites for nematode utilization were selected that had been or were active sources of mosquito production. Sites with *Gambusia affinis* present were not used. After nematode application, no other methods of control were used. Site selection leaned toward varied soil types, degree of canopy, and vegetation.

Nematodes were introduced as both pre-parasites and post-parasites (Figure 1). Pre-parasites were introduced where larvae were present at greater than 1 per dip at the initial inspection. However, where larvae were present at less than 1 per dip pre-treatment, post-parasites were used. Pre-parasites were applied at a rate of 1000 per m² and post-parasites at a rate of .40 g per m. There are approximately 2,500 post-parasites per g. A certain degree of discretion was used at each particular site in determining the planting rate. An irrigation canal for example, where the larval habitat at the banks is separated by a stream of swiftly moving water was planted on each side as if two separate sources. Where the actual larval habitat had a margin exceeding 2.5 m in width, the planting rate was increased to .80 g per m. The planting of pre-parasites required a determination of actual larval habitat in m².

Post-parasites were transported to the field in plastic vials with 1 g of nematodes and approximately 10 ml of water. Pre-parasites were transported in 3 gallon plastic hand cans with added water for a more uniform application. Applications were made manually. Pre-parasites were sprayed evenly over the target area. Post-parasites were deposited 1 g each 2.5 m. At the time of application, a site description was made and soil and water temperatures recorded.

Post-parasite sites were monitored for infection at the 3rd, 4th and 5th week post-treatment and then at various times later during the season, where time permitted. Pre-parasite sites were monitored initially at 3 to 7 days post-treatment and then the 4th week, and subsequently when time permitted. This sequence of sampling attempted to coincide with active pre-parasites and possible larval infection, based on the lifecycle of the nematode. At each sampling,

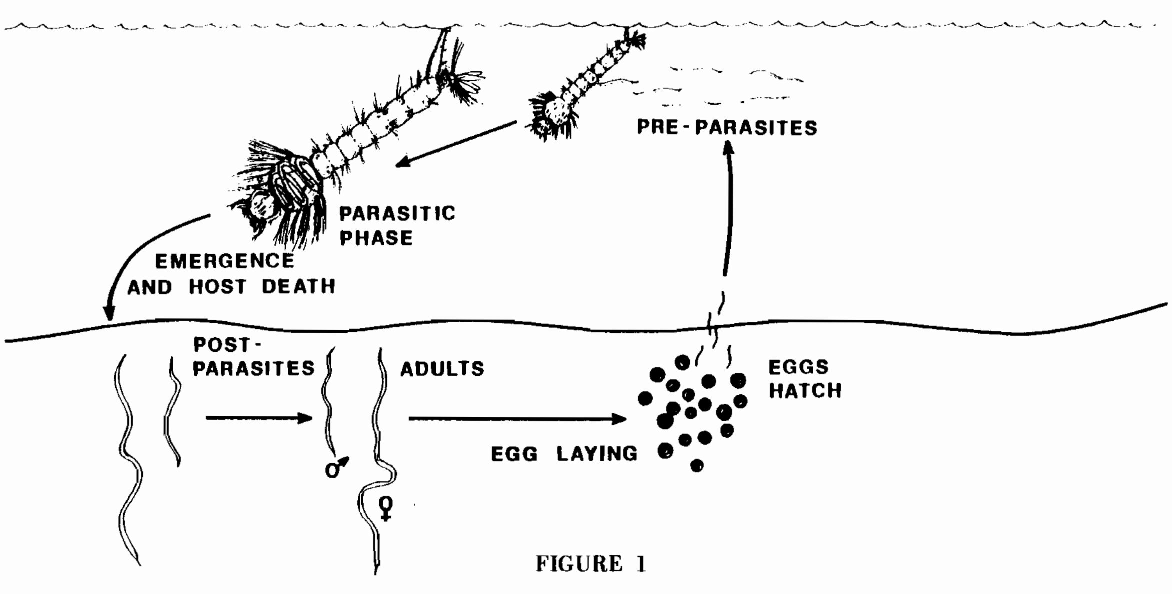


FIGURE 1

soil and water temperatures were taken. A standardized method was used to obtain temperature, insuring precision between sites. Larvae recovered were lab reared to 4th instar, then dissected to determine infection.

Where no larvae could be recovered post-treatment, sentinel cages were used. Cages contained 20 lab reared 2nd instar *Culex pipiens* larvae and were placed for 48 hours and then retrieved. After retrieval, the larvae were reared to 4th instar and then dissected to determine infection. The sentinel cage consisted of a plastic container 15 cm. deep with a diameter of 20 cm. The bottom, lid and 4 large side panels were removed and replaced with very fine mesh screen. Three large fishing floats were attached to the side to ensure flotation.

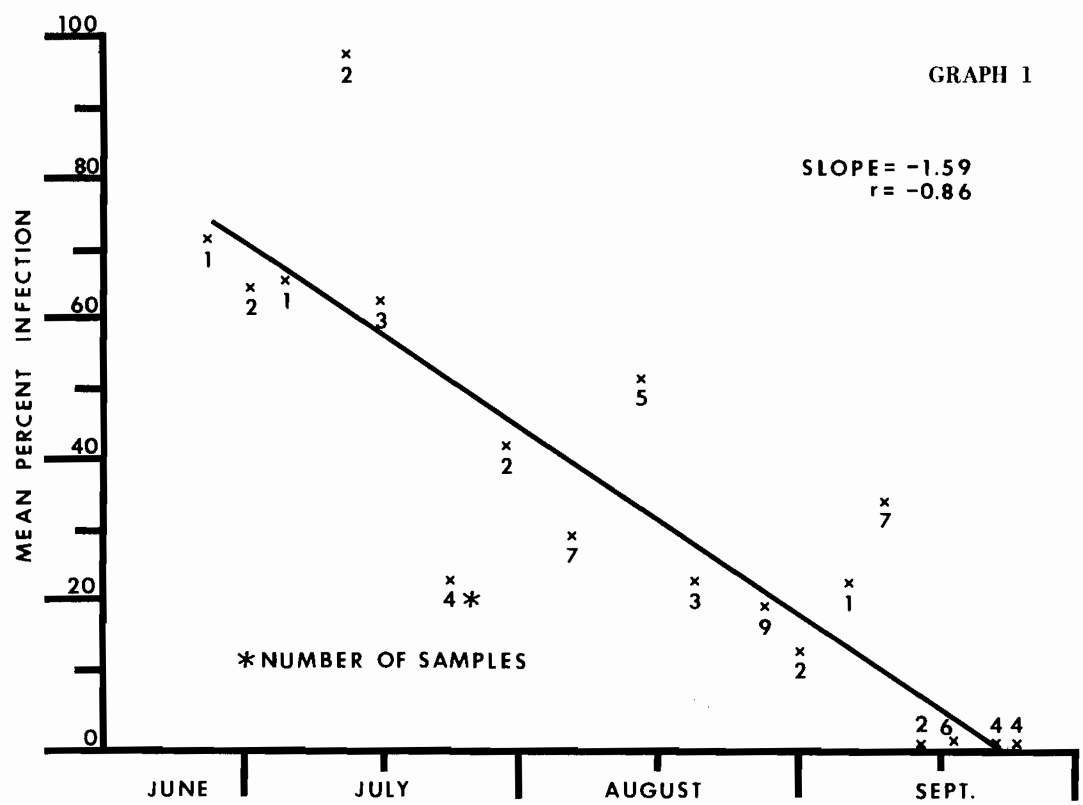
RESULTS AND DISCUSSION.-A total of 27 sites were planted with nematodes. Of these sites, 22 were sampled for infected larvae. In 65 instances, larvae (wild and sentinel) were recovered from sites to determine infection. The mean infection of sites sampled was 27.85 ± 9.09 at the .05 confidence level. Of the 22 sites sampled, 13 showed infection at some point during sampling, or 59.0% of those sampled.

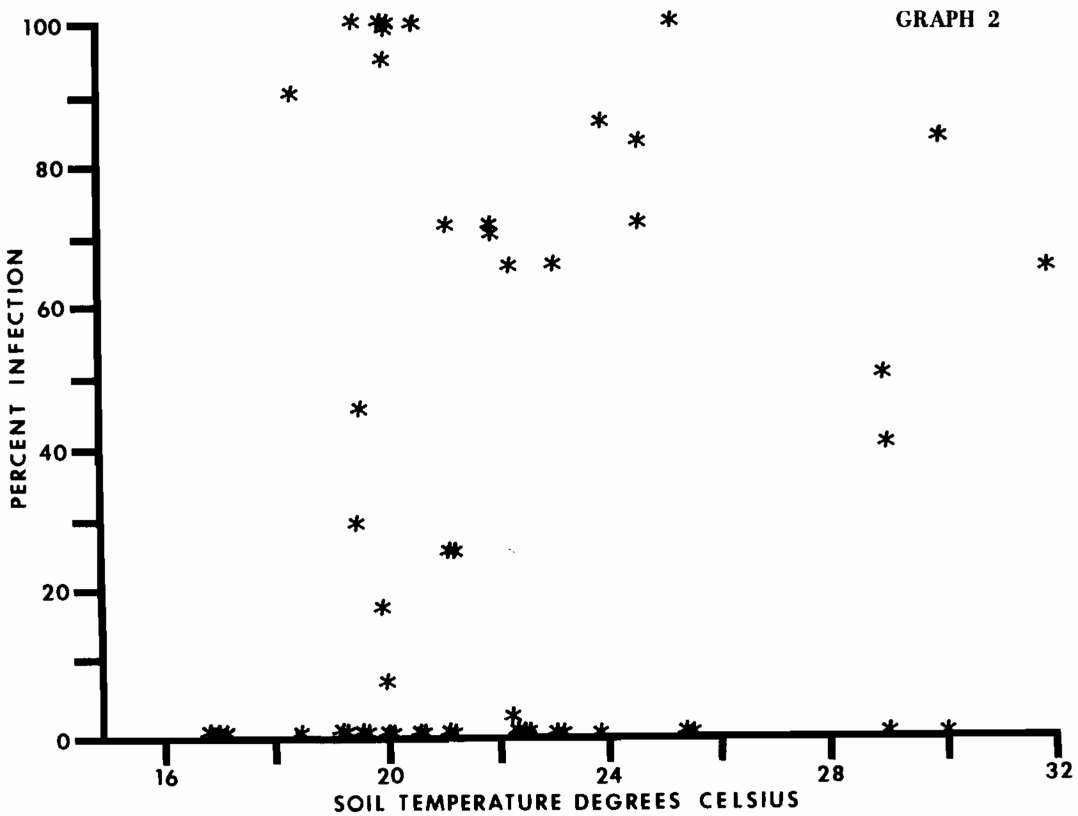
Several environmental variables were examined. The first of these was seasonality. Mean percent infection was graphed in relationship to the time period extending from June to September (Graph 1). The number next to each point represents the number of sites sampled. The regression line shows a definite trend toward decreasing infection for the months of June to September. Operationally, the pattern demonstrated by this data may dictate planting nematodes up to, but no later than, the beginning of September. Several questions are raised. Will the post-parasites planted during September produce pre-parasites and possible infection the following spring? Also, how early in the spring will diapause cease and parasitism resume? We will attempt to answer both of these questions next season.

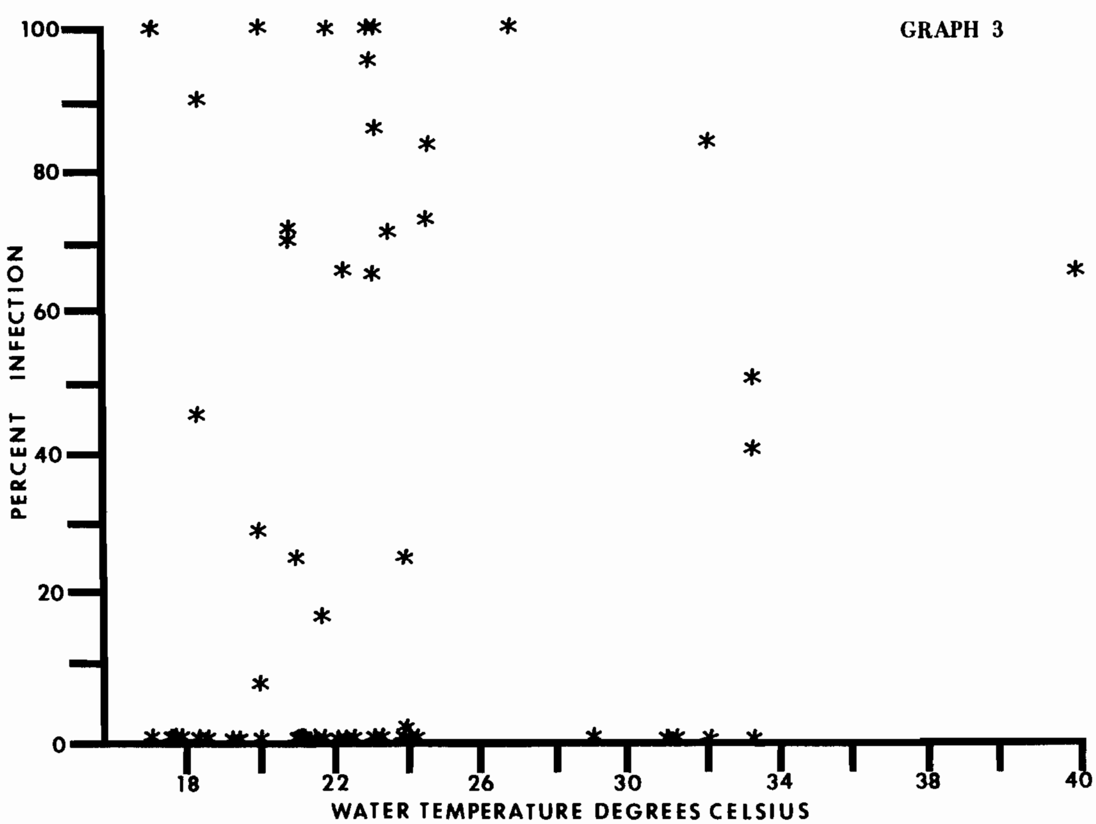
The second variable we looked at was soil temperature at the time of sampling. Percent infection was graphed in relationship to soil temperature at time of sampling (Graph 2). There appears to be no significant relationship here, given the range in soil temperature between 16.5 and 32.0°C.

The third variable examined was water temperature at the time of sampling. Percent infection was graphed in relationship to water temperature at the time of sampling (Graph 3). Here again, there appears to be no relationship given the range in water temperature between 17.0 and 40.0°C. These water temperature results really came as no surprise. Predominately, our water temperatures fall into the ranges that the literature suggests as being optimal for the infection of *Culex pipiens* (Brown, Platzer 1977).

Soil types did not seem to influence infection rates. The percent infection was very even between the three predominant soil types in our test zone - Placentia Hildreth, Stockton, and Landlow series.




GRAPH 2
PERCENT INFECTION
0
20
40
60
80
100
16
20
24
28
32
SOIL TEMPERATURE DEGREES CELSIUS


GRAPH 3
PERCENT INFECTION
0
20
40
60
80
100
18
22
26
30
34
38
40
WATER TEMPERATURE DEGREES CELSIUS

The last variable considered was canopy. There were diverse degrees of canopy in our test zone, with no degree of canopy showing a greater infection rate over another.

A perplexing situation arose using the sentinel cages. There was a high mortality of the 2nd instar larvae during the 48 hour period that the cages were left at the sites. On several occasions, 100% mortality occurred. Two possible reasons for this phenomenon are suggested. One suggests that heavily parasitized sentinel larvae are killed immediately and decay in the field (University of California, Davis; personal communication). Secondly, flatworms may be preying upon the sentinel larvae. The latter hypothesis seems unlikely after several nocturnal and diurnal investigations of sentinel cages revealed no flatworms or dismembered larvae. Since sentinel cages were only used when wild larvae could not be found, we feel that multiple parasitism or water quality are probable factors. This problem will receive more testing next season.

Initial evaluation of the post-parasite application rate of .40 g per m in relationship to the rate of .10 g per m, which was used in all other zones of Sutter-Yuba MAD shows that the rate of .40 g per m yields much higher infectivity. We will continue to use this higher rate next season.

Next season, only sites in the experimental zone with larvae present will be planted. We feel that larvae available for parasitism immediately by pre-parasites are very important for the establishment and cycling of nematodes.

The operational use of nematodes and their initial evaluation have answered some questions and also created several new ones. We feel that more use and testing is warranted to determine the efficacy of the nematode as a biological control agent in selected habitats of Sutter-Yuba Mosquito Abatement District.

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BIOLOGICAL MOSQUITO CONTROL: BROADER RECOGNITION OF PAST SUCCESSES AND FUTURE RESEARCH EMPHASES

E. F. Legner[1] and R. D. Sjogren[2]

ABSTRACT

Natural enemy systems that persist in the environment increase their effectiveness through field reproduction, and may coevolve with their hosts to minimize the development of resistance to pesticides. Some biotic insecticides are commercially available for short-term direct mosquito control. An extended view of biological control likelihood should differentiate geographic areas, habitat characteristics and human annoyance tolerance levels. Intensified studies of certain invertebrates, fungi and bacteria are desirable.

The accepted definition of biological control from a medical viewpoint recently stated by the 6th World Health Organization Expert Committee on Vector Biology is, "The control of pests, including the vectors of human disease, by direct or indirect use of natural control agents with or without their metabolites." Implied in this definition is the presence of some living natural biotic agent. The first effective effort to organize information on biological control of medically important pests was made by Jenkins (1964). Following accelerated research emphasis in the 1960's and 1970's, new reviews have dealt with various aspects of mosquito biological control (Bay 1973, 1974; Bay et al. 1976; Chapman 1974; Garcia & Dahlsten 1980; Hertlein et al. 1980; Laird 1971, 1980; Legner et al. 1974; Service 1983; World Health Organization 1973, 1982).

Although considerable research has shown that natural enemies can effectively reduce mosquito numbers, a controversy still exists about the practical value of biological control (Service 1983). As is typical of biological control generally, control of mosquito larvae and pupae is usually not as immediate as field workers customarily experience with insecticides. In many cases, however, mosquito reductions at differing levels have been continuous, and in a few cases permanent. Careful field studies of semi-permanent habitats also have shown the value of resident natural enemies in maintaining the mosquito larval densities at levels which do not require cultural or insecticidal control measures. Care must be exercised to preserve natural predator complexes without disrupting significant interacting predator/mosquito components by inappropriate insecticide usage. Recognition of such areas can greatly reduce the cost of mosquito abatement, but careful studies over several years and seasons are usually required (Case & Washino 1979; Collins & Washino 1978; Glenn & Chapman 1978; Hauser et al. 1976, 1977; Washino 1981). Where field reproduction of natural enemy complexes occurs, the theoretical possibility of coevolution with their mosquito hosts exists, which could minimize development of host resistance, resulting in greater predator/prey stability at lower mosquito larval population densities.

Sometimes periodic additions of natural enemies to chronic mosquito breeding areas are effective in reducing breeding to levels acceptable by local residents. This strategy has gained the most attention by researchers who are invariably led to such problem areas. Various organisms have given practical control under favorable environmental conditions with this approach (Ali & Mulla 1983; Axtell et al. 1982; Chapman 1974; Garcia & DesRochers 1979; Garcia et al. 1980; George et al. 1983; Goldberg & Margalit 1977; Jaronski & Axtell 1983; Legner 1977, 1978a, 1979; Legner & Yu 1975; Legner et al. 1975b; Levy & Miller 1977a, 1977b, 1978; Miura et al. 1980; Sjogren & Legner 1974; Sweeney et al. 1983; Schaefer & Kirnowardoyo 1983; Yu & Legner 1976; Yu et al. 1974a, 1974b, 1975). However, to date mass production techniques are limited (Legner 1978b; Legner & Medved 1974b; Legner & Tsai 1978; Legner et al. 1976; Lenhoff & Brown 1970; Medved & Legner 1974; Nelson 1979; Tsai & Legner 1977); and only parasitic nematodes, *Bacillus thuringiensis* Serotype H-14 and *Bacillus sphaericus* Neide are available for widespread use (Schaefer & Kirnowardoyo 1983; Service 1983). When recycling of these organisms does occur, it is usually significant only during the year of application, with winter carryover not being high enough to evoke satisfactory mosquito reductions during the early part of the season of the following year.

Natural enemies may occasionally be added to mosquito breeding habitats where they persist, dependably recycling over time. The minnows, *Gambusia* spp. and *Poecilia reticulata* Peters are most commonly used in this manner (Bay et al. 1976; Gall et al. 1980; Hoy & Reed 1970, 1971; Hoy et al. 1971, 1972; Mulligan et al. 1983). The

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use of native fishes also appears promising (Legner & Medved 1974a; Legner et al. 1975a; Walters & Legner 1980). Three species of cichlids, *Sarotherodon mossambica* (Peters), *S. hornorum* Trewazas and *Tilapia zillii* (Gervais), have become permanently established in ca. 2,000 hectares of *Culex tarsalis* Coquillett breeding habitat in the irrigation system of southeastern California. In these areas, mosquitoes are controlled by the dual action of the fish feeding on protective aquatic vegetation which created favorable breeding habitat, and by direct predation of mosquito eggs, larvae and pupae (Legner 1978a, 1983; Legner & Fisher 1980; Legner & Medved 1973a, 1973b; Legner & Murray 1981; Legner & Pelsue 1983; Legner et al. 1975c). This example of permanent biological mosquito control is dependent on favorable water temperatures that allow persistence of the subtropical cichlids, and a continuous supply of irrigation water to canals and drainage ditches. It is probably applicable only to areas where similar sophisticated water management can guarantee a permanent water supply.

Another case of permanent biological mosquito control is in the paved river drainage of southwestern California where two species of cichlid fish, *S. hornorum* and *S. mossambica*, are established at high population densities. Aquatic vegetation can accumulate there around debris in the form of boards and tires, providing protective niches for *Culex tarsalis*. Starving populations of the cichlids (Legner & Pelsue 1980, 1983) constantly forage at these sites, eliminating both the aquatic vegetation and the mosquito breeding there. However, the principal food sustaining these fish is chironomid larvae, which allows them to build up annually to the large numbers necessary for effective aquatic weed control (Legner & Medved 1973b, Legner et al. 1983). Persistence of these subtropical species in winter is apparently dependent on an artificial source of warm water supplied by a power generating plant adjacent to one of the paved channels.

Practical Considerations.--The relative stability of aquatic habitat is a principal determinant in the successful utilization of biological control agents for mosquitoes. To achieve predator-prey equilibrium at a level which will prevent disease transmission and/or sustain mosquito pest populations below the human annoyance tolerance level, cost considerations usually dictate that the control agent must reproduce after inoculation to achieve satisfactory control levels. For this reason, the permanent and semi-permanent water genera *Culex, Culiseta* and *Anopheles* have received more attention in biological control than have *Aedes* or *Psorophora*. In the sporatic, intermittant water habitat of the latter, brood development can occur in 3.5 to 7 days requiring costly inundative releases of predatory agents or applications of mosquito pathogens which serve as biological insecticides. The highest levels of control have been achieved under low to moderate larval population densities. Massive synchronous brood development by *Aedes* or *Psorophora* may at times exceed the controlling capacity of economically feasible inoculation levels. In such cases, concurrent inoculations of compatible chemical control agents can be used (eg. juvenile hormones).

In stable aquatic habitats, *Gambusia affinis-affinis* (Baird & Girard) continues to be the primary biological control agent used in early season inoculative releases. Adverse side effects of this species causing phytoplanktonic blooms (Hurlbert 1975, Hurlbert & Mulla 1981, Hurlbert et al. 1972) have never been reported outside of aquarium environment (Walters & Legner 1980). A number of effective insect and noninsect invertebrate biological control agents of mosquitoes are also known (Chapman 1976). The most promising agents in permanent water habitat appear to be hydra, freshwater flatworms and nematodes for which mass culture procedures have been developed, albeit in need of improvement. For sporadic floodwater habitats the most promising and potentially economical biological insecticide is the bacterial pathogen *Bacillus thuringiensis* var. *israelensis* de Barjac.

As for environmental alterations to enhance the success of biological control agents, empirical observations indicate the need for water and nutrient level manipulations under some conditions to achieve a balanced invertebrate fauna to support necessary biological control agents, in the absence of mosquito larvae. Due to great physical and chemical diversity among habitats, environmental manipulation will probably continue to play a secondary role to that of recognition of habitats in which conditions favor the maintenance of control-producing populations of natural enemies. Economic considerations and conflicting land use patterns limit the number of aquatic habitats amenable to achieving dependable biological control.

Specific Biological and Operational Considerations.--A biological mosquito control agent must be dependable over a wide range of environmental conditions, particularly when operations personnel are required to recognize or predict when and where an agent will work effectively and when and where it will not. Unpredictability of control is a frustrating and difficult complicating factor for field personnel, and one which control administrators strive to reduce to the greatest extent possible. The high level of training and time (ie. monitoring) required for field personnel to utilize biological control agents is expensive. However, both are essential to an accurate recognition of the varying environmental conditions including seasonal shifts in water quality, temperature and water permanence, which may result in control failures. To this end, there is a need for operational districts to support funding to conduct the necessary studies. A high degree of confidence is also necessary in the introduction of a mass cultured organism (or any control, for that matter), to insure with reasonable certainty that known levels of control will be achieved for the duration necessary.

Operationally, unless large acreages are contiguous and permit ready access for treatment and evaluation, a control district finds it difficult to utilize a biological control agent. If the agent can survive and exert control only in select and dispersed locations, its use will be economically impractical.

Logistically, the time spent in travel to a site to determine its suitability for a certain biological control agent, then return for treatment and subsequent follow-up is too time consuming for a large number of small sites. Conventional methods of sweeping through an area, controlling all mosquito breeding in sites with insecticides or oils at scheduled intervals, is a more cost effective approach. Implementing a number of different kinds of control methods within an area at the same time, each with its specific requirements, is more complex to supervise. Intermittent water breeding sites (ie., where the site floods, dries and refloods during the year) hold water at erratic intervals, which frustrates the utilization of biological control organisms. Conscientious effort put forth is voided when erratic weather or human activities alter site stability. Reflooding results in mosquito return but little if any predator carryover.

A biological control program must have the total support of the program administrator to enable it to reach it highest level of effectiveness (ie., support from the top down through the ranks, including employees that frequently are skeptical and resist complex technology). Combined with the natural reluctance of governing boards to appropriate funds for anything but the "sure thing," adds up to a high risk venture for an administrator to support a large mass culture program for areas less than ca. 4,000 hectares of stable, semi-permanent water. For large acreages, such as rice, cost benefits are more favorable.

With tight money situations, managers look closely at the cost per hectare per year required to achieve effective control, choosing the cheapest environmentally compatible control method possible. If a biological control agent is most feasible for large acreages, a manager is not likely to put forth the effort necessary to use the control technique in small sites, particularly if the cost of training employees and repeated visits to the sites to determine if control is still in effect are high, and there is much likelihood that less than a uniform 95% overall control will be achieved.

Thus, the primary obstacle to a wider application of biological mosquito control appears to be resistance among abatement agencies to adopt new technologies because of the aforementioned reasons. The laboratory production, storage and field transfer of biological control organisms requires a high degree of sophistication, which is usually unavailable at the operations level. Meanwhile, there are few commercial sources for most organisms in numbers required for mosquito control. A major thrust to an expanded reliance on biological controls will develop when highly trained personnel are employed at the operational level and/or environmentally acceptable chemical controls become unavailable.

Future Considerations.--Biological mosquito control must be considered according to geographic area and habitat. There must be a recognition of those systems in which biological control works (eg., irrigation system in the lower Colorado Desert, duck club ponds, rice fields, etc.). More intensive studies of those systems are needed to learn why biological control works, so that correct decisions can be made for other areas, and certainly to preclude its attempted use where it is not possible (eg. subtropical cichlids in colder areas or in semi-permanent water). New insights are also needed into what acceptable mosquito production levels are relative to breeding site locations and adjacent human population, which would allow less than 100% control with biologicals to be operationally feasible. The current goal of 100% control for all breeding sites which drives most programs, needs to be reevaluated through development of population models which could weigh the variables of adult emergence, distance from breeding sites, adjacent production acreage, daily survivorship rates, biting behavior, etc. for treatment threshold decisions.

An intensive study of certain invertebrates, especially flatworms, is needed. The wider appraisal and application of *Dugesia tigrina* Girard and *D. dorotocephala* (Woodworth) may be expected to produce startling results; and the discovery of new strains with resistance to environmental contaminants and insecticides may be possible. Flatworms of the genus *Mesostoma* are especially potent mosquito destroyers, and mass production of at least one species is needed to test inoculation effectiveness. Various species of freshwater hydra, fungi in the genera *Lagenidium* and *Culicinomyces,* and predatory Hemiptera in the genera *Notonecta, Buenoa* and *Plea,* are candidates with promising biological control potential.

There is also a need to examine and categorize those ecosystems in which biological control effectiveness is limited. Such areas include the tundra, wilderness snow melt, wilderness swamps and most pastures and intermittant rainwater depressions. As further research provides a fuller understanding of the factors associated with different resulting levels of control that can be achieved with biological agents, it will increase confidence in the predictability of their use and enhance their cost-effective integration into operational mosquito control programs.

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A PROGRESS REPORT ON A METHOD OF REARING *GAMBUSIA AFFINIS* ON AN OPERATIONAL SCALE

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ABSTRACT

The strategy for finding a method of rearing 3,000 pounds of *Gambusia affinis* per year is presented. A general description of the hatchery facilities that have been constructed is given along with a brief discussion of the results to date.

INTRODUCTION.-The use of *Gambusia affinis*, the mosquitofish, for mosquito control has been hampered by an inadequate and unreliable supply of these fish. Pond culture efforts have encountered many hurdles, with mortality during the winter being one of the most important problems. This project was initiated to study methods of mass-production and rearing, utilizing close control of the fish and economies of scale.

The specific goal of our project is to develop a hatchery method that can produce 3,000 pounds of *Gambusia affinis* per year at a cost of $7.50 per pound including cost of labor and facilities. The strategy by which we plan to reach that goal has four elements: 1) fry production and grow-out throughout the year, 2) operation in a greenhouse with supplemental heat, 3) use of a closed system with biological filters to maintain water quality, 4) minimizing the load on the biological filters by carefully metered feeding.

MATERIALS AND METHODS.-We have constructed our rearing tanks in a 20x50 foot fiberglass greenhouse located in the corporation yard of the Contra Costa Mosquito Abatement District. Supplemental heat is provided by a propane heater capable of delivering up to 80,000 BTU's per hour. Each of the 12 rearing tanks is 8x8 feet x 16 inches deep, the walls are of 0.5 inch plywood with 2x4 inch wooden reinforcements along the top edges. Two sheets of plastic lining (20 mil vinyl under 6 mil polyethylene) hold the water and filter materials. The upper sheet protects the lower from abrasion by the gravel and sand in the filter bed while preventing the fungicide in the lower sheet from reaching the water in the system.

Water is circulated through the filterbed by the air-lift method whereby air is bubbled at the bottom of a column of water in a 3 inch diameter lift-pipe to decrease the specific gravity of the mixture in the column. The light (air-water) mixture moves upward and spills out the top of the lift-pipe. Water enters the bottoms of the lift-pipes through a grid of eight horizontal slotted 1.5 inch diameter pipes across the bottom of the tank after initially passing down through the filterbed. A high volume, low pressure supply of air comes from a blower powered by a 1.5 hp. electric motor. Air is delivered through a 3 inch diameter pipe to each tank. Two 1.5 inch diameter manifolds distribute air along the ends of each tank and into 0.5 inch diameter air lines which are inserted in each of 16 lift-pipes. All pipes are PVC plastic which has the advantages of being inexpensive, low in toxicity, and easily plumbed.

The biological filters currently in use have a bottom layer of 2 inches of pea gravel, which covers and separates the grid of horizontal pipes, and a 5 inch layer of No. 3 aquarium sand. Water depth of about 13 inches has been maintained to maximize submergence of the air line, which in turn maximizes the rate of water circulation.

An automatic feeder control system has been designed and constructed as an integral part of this project. The system and its rationale are described elsewhere (Hoy 1984). If one assumes a growth rate of 7 percent per day, which is less than one third the maximum demonstrated growth rate (Wurtsbaugh and Cech 1983), and a maximum loading of less than one half the theoretical maximum for the biological filter, 12 tanks operating throughout the year should produce approximately 3,285 pounds of fish per year. This system also can be used to control the auxillary heating of the greenhouse and to record air and water temperatures.

RESULTS AND DISCUSSION.-Using biological filters with 2 inches of gravel and 5 inches of sand at least 6 pounds of fish per tank can be supported. Furthermore, fish born in the system have reached sexual maturity in 60 days. Fish in the system were gravid through December, although only limited numbers were available.

To reach our goal of a system that can produce 3,000 pounds of fish per year, the depth of the biological filter will probably need to be increased to the maximum depth, i.e., 12 inches. Furthermore, the circulation through the filter may need to be higher than the rate we are currently using. Therefore, to date our goal seems to be attainable.

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Figure 1. Plywood fish tanks. Note sheet plastic liners uncovered grid of pipes in the bottom of the tank, and reinforcement along the tops of the tanks on the right.

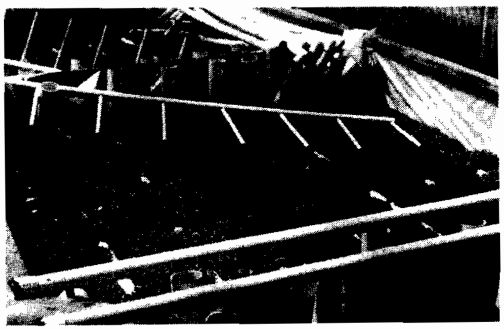

Figure 2. Exploded view of the air-lift manifold, showing the horizontal 1.5 inch manifold with eight 0.5 inch air lines.

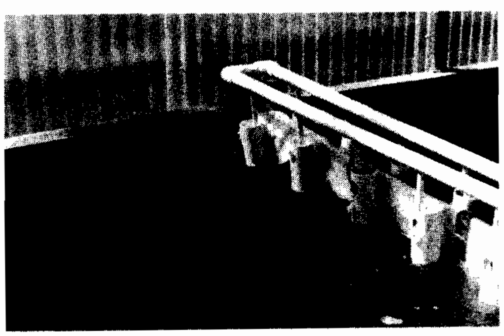

Figure 3. Assembled manifold and lift-pipes.

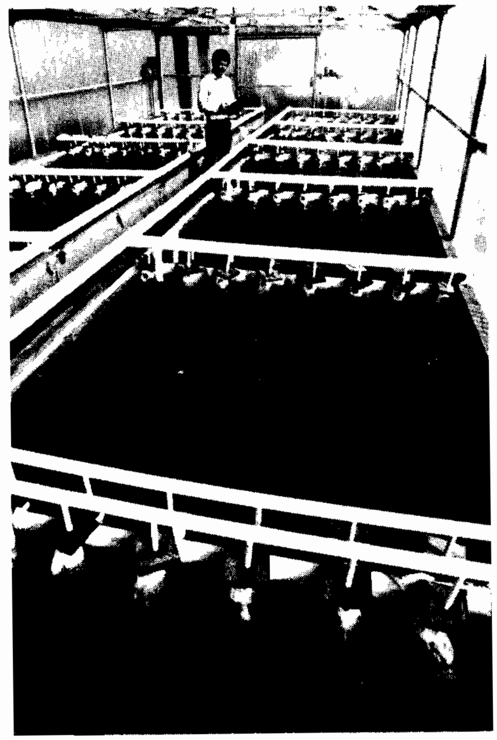

Figure 4. Completed system. Note 3 inch manifold running the length of the system and wooden splash guards covering each lift-pipe.

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IMPLICATIONS OF LABORATORY MOSQUITOFISH EXPERIMENTS FOR POPULATION DEVELOPMENT IN RICE FIELDS

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ABSTRACT

Development of mosquitofish *(Gambusia affinis)* populations in rice fields is variable and not well understood. To closely observe the effects of plant cover on reproduction, cannibalism, and resulting population development, populations were grown in aquaria with different levels of artificial cover. In all treatments, parturition occurred in an initial burst during the first few weeks after stocking, followed by 7 to 8 weeks with no reproduction and a second period of parturition from both stocked females and their offspring. Recruitment (survival of fry) varied among treatments. No young survived without plant cover, while substantial population growth was seen in all treatments with moderate to high plant cover. These observations provide possible explanations of population behavior observed in rice fields, and may have some implications for mass culture.

INTRODUCTION.-Research into use of mosquitofish, *Gambusia affinis* (Baird and Girard), for control of mosquitoes in California rice fields has shown that there is considerable variability in both fish population growth and resulting mosquito control. Observations of fish populations (e.g., Norland and Bowman 1976, Reed and Bryant 1974, 1975, Farley and Younce 1978) have shown considerable variation, ranging from fields in which populations never develop, to those in which populations increase more or less steadily from the start of the season. Observations of the efficacy of *Gambusia* for mosquito control have also shown mixed results (Hoy and Reed 1971, Hoy et al. 1971, 1972).

The experiments discussed here are part of continuing research designed to provide the information necessary to formulate a model of mosquitofish utilization in California rice fields. This formulation requires an understanding of two processes: (1) population growth after stocking, and (2) the mosquito control that results from a fish population of a certain size and age structure. These processes depend on a variety of factors, such as growth and reproductive capacity of the stocked fish, plant cover, stocking density, field temperature, pesticides, presence of predators, and density of various food items (including mosquito larvae). Both of these processes have been investigated experimentally over the past several years (Botsford et al. 1984, Cech et al. 1980, Linden and Cech 1983, Wurtsbaugh and Cech 1983). Our focus here is on the first process, because a description of the stock of fish present at a certain time is required before the predatory capacity of those fish can be described.

In field observations to date, we have seen a variety of mosquitofish population responses after initial stocking. We have collected and analyzed population data over the course of two seasons in rice fields in the UC Davis Experimental Rice Facility and in commercial fields in the San Joaquin Valley near Fresno (Botsford et al. 1984). In our Davis field in 1981 (Fig. 1a), we observed little population increase for a few weeks after stocking, followed by a sudden burst due to synchronous reproduction, a period of stable population size in mid-season, and finally a second burst of reproduction at the end of the season. In four paddies in the same location in 1982 (Fig. 1b), there was a burst of reproduction immediately after stocking followed by a sharp decline, a period of relatively low populations in mid-season, and (in three out of four paddies) a second burst at the end of the season.

To describe this variability in population behavior, we view *Gambusia* population development in rice fields in terms of four recognizable phases:

- Phase 0: initial period of little change in numbers.
- Phase 1: initial pulse of reproduction.
- Phase 2: period of increase or decrease in population.
- Phase 3: second pulse of growth (not always seen).

There are two primary areas of variability which can strongly affect population development and level of mosquito control. First, the timing of the initial reproductive pulse is unpredictable; second, the direction of change during phase 2 is variable, ranging from a sharp population decline to a slow increase. Both of these sources of variability are clearly related to the balance between reproduction and mortality of mosquitofish, and could be causally related to a number of factors (such as cannibalism, other predation, food availability, stress and disease). The experiment described here was designed to determine the potential effect of varying levels of plant cover on population development, especially effects on reproduction and cannibalism.

METHODS.-To closely observe reproduction and mortality, we stocked eight 75 liter aquaria

Figure 1(a)

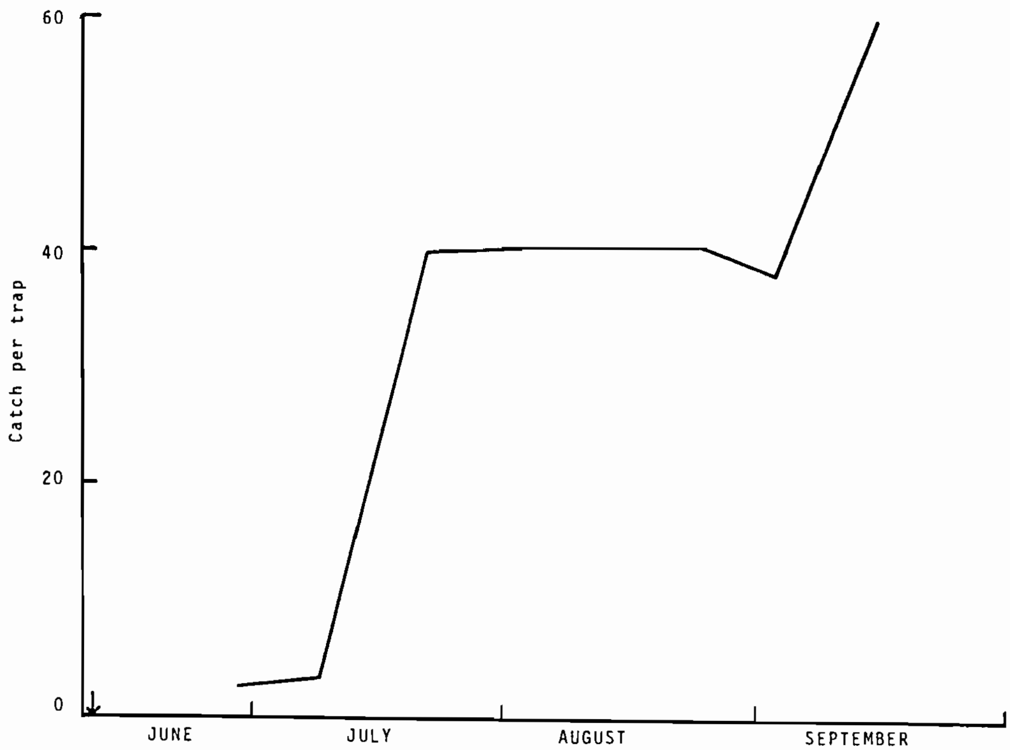

Figure 1(b)

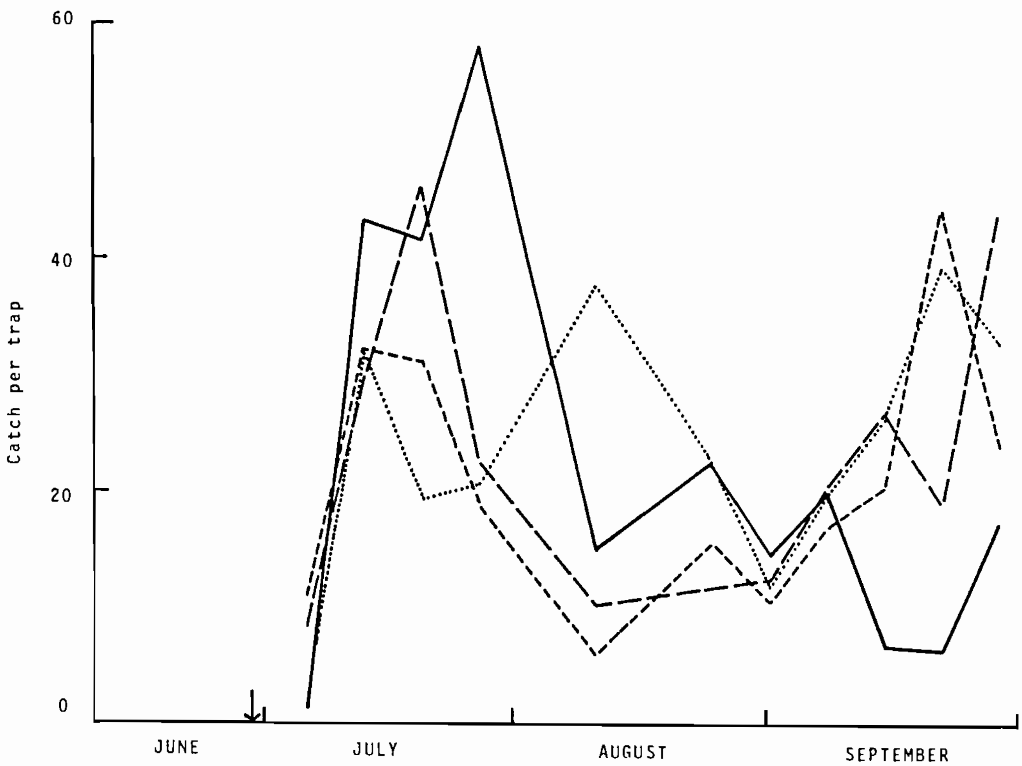

Figure 1. Relative abundance of mosquitofish in experimental rice paddies in Davis: (a) a single paddy in 1981, (b) four paddies in 1982. Arrow on bottom axis indicates stocking date.

with adult fish (four females and two males in each) collected from a local creek. All tanks were maintained at 26-27°C, at a neutral photoperiod of 12 hours light and 12 hours dark. The eight tanks were divided randomly into four pairs, each pair receiving a different treatment. Fish were maintained on a diet of Tetramin® Staple Food for tropical fish, with supplemental feeding of live small invertebrates. The treatments differed in level of artificial vegetation (plastic 'rice plants' and plastic floating weeds) as follows: (A) no vegetation, (B) moderate density of vegetation, (C) high density, and (D) variable density--starting with no cover and increasing in stages to high density. Tanks were observed daily to track female reproduction and fry mortality. A complete census of the populations was made weekly, in which the length of each fish present was recorded. The experiment was started in June 1983, and continued for 20 weeks.

RESULTS.-Reproduction was similar in all treatments, starting with noticeable parturition in the first few weeks, followed by a period of essentially no parturition for seven to eight weeks, then scattered parturition by the stocked females, and finally some parturition by early offspring during the final two weeks (Fig. 2).

In spite of similar reproduction in all the tanks, there were notable differences in population development (Fig. 3). In the no cover treatment (A), there was an overall decline in population, and no fry survived more than 5 days. In all treatments with cover, there was an overall increase in population, with no significant differences among the three treatments. In general, we see a pattern similar to the 1982 Davis fields (Fig. 1b). In the lab, the early increase was clearly due to synchronous reproduction, followed by a decline due to cannibalism. The subsequent level period was due to low reproduction with cannibalism removing all fry produced. Finally, there was a period of reproduction, with some fry surviving to adulthood.

DISCUSSION.-These results suggest possible mechanisms contributing to variability in mosquitofish population development in rice fields. There are several ways in which the mechanisms observed in the laboratory experiments could contribute to the two variable phases of development discussed above. First, the duration of phase 0 (timing of the initial population increase) may be related to availability of plant cover early in the

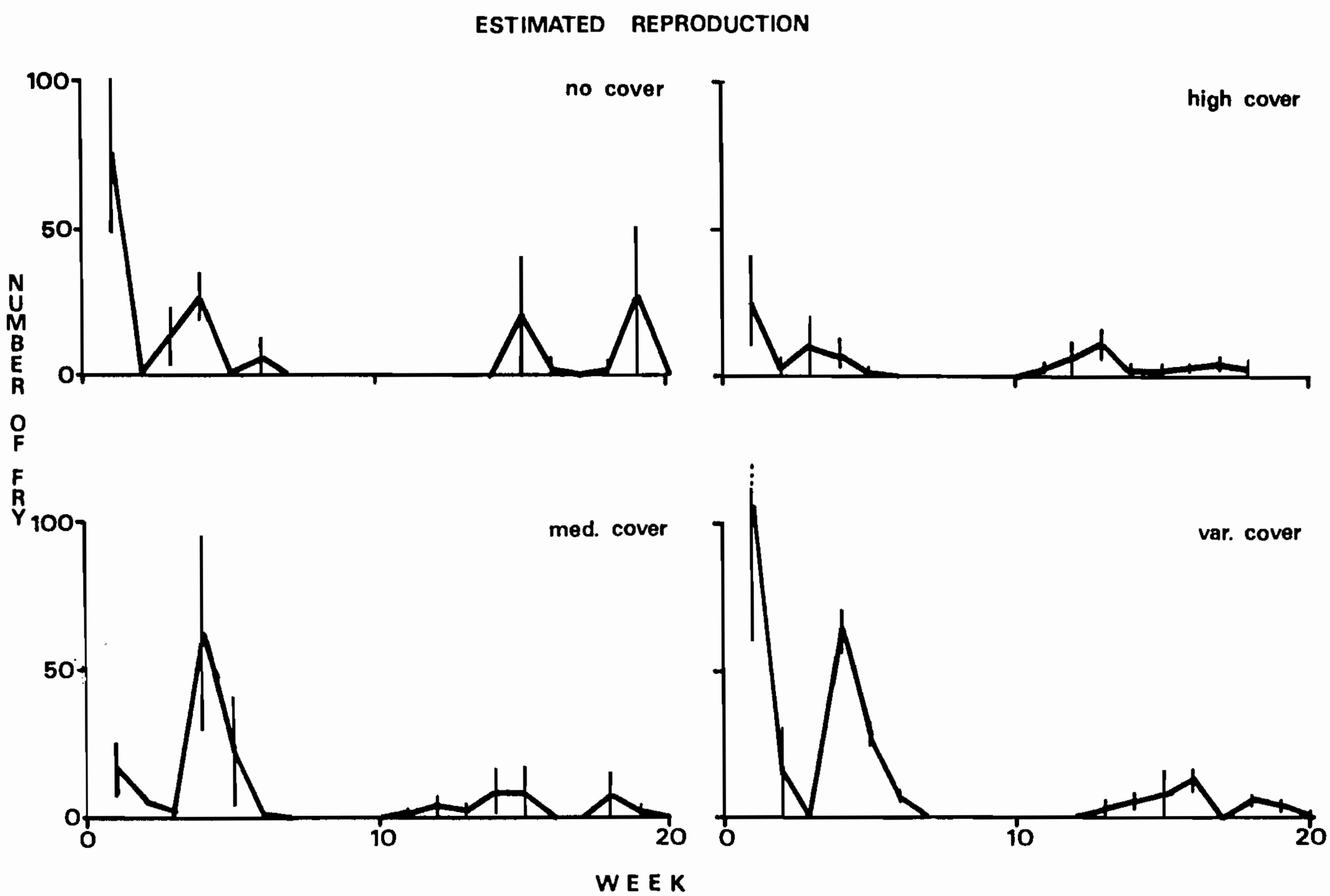

Figure 2. Observed reproduction in four experimental treatments. Curves connect means of two replicates; vertical bars connect the two observations.

rice season. Our experimental results indicate that the level of cannibalism of fry by adult *Gambusia* depends on plant cover. Plant cover could influence other types of predation as well. Thus, we might expect population development early in the season to be suppressed until the rice has grown to provide adequate cover, either through cannibalism of whatever reproduction occurs, or through selective repression of reproduction under high-risk conditions. This result corresponds with the observation of Farley and Younce (1977) that early stocking of fish does not lead to higher final population numbers.

Second, variation in the direction of change during phase 2 (middle phase) depends on the balance between reproduction and mortality. Population behavior during this phase could be affected by the occurrence of a pause in reproduction such as was observed in our laboratory populations, in combination with cannibalism and other predation. This pause is, however, unexpected on the basis of previous laboratory observations of mosquitofish reproduction, which indicate that individually reared mature females reproduce at regular two to three week intervals (B. Vondracek, unpublished data). Additionally, a comparison of recruitment in our 1981 and 1982 Davis fields showed no detectable difference (Botsford et al. 1984). No direct data are available regarding timing of reproduction in rice fields, although Maglio and Rosen (1969) observed a similar lack of mid-season recruitment in a pond in New York. We plan to collect more detailed data on field reproduction by direct observation of female reproductive condition throughout a rice season.

The similarity of population development in our laboratory experiment with that in the 1982 Davis fields is striking, but this does not imply that the mechanisms observed in the laboratory are the same as those controlling field populations. The laboratory populations were at much higher densities than those typical of rice fields. Also, environmental conditions in the laboratory, especially light and temperature regimes, were not the same as in the field. These conditions may influence *Gambusia* reproduction.

Effective use of *Gambusia* for mosquito control in rice fields has two limitations: (1) cost and availability of stock fish, and (2) the performance of fish once stocked. The first is primarily an aquaculture problem, and has received much attention recently. However, the second continues to be a major problem. We do not know why *Gambusia* are effective in some fields but not in others. Better understanding of the factors that

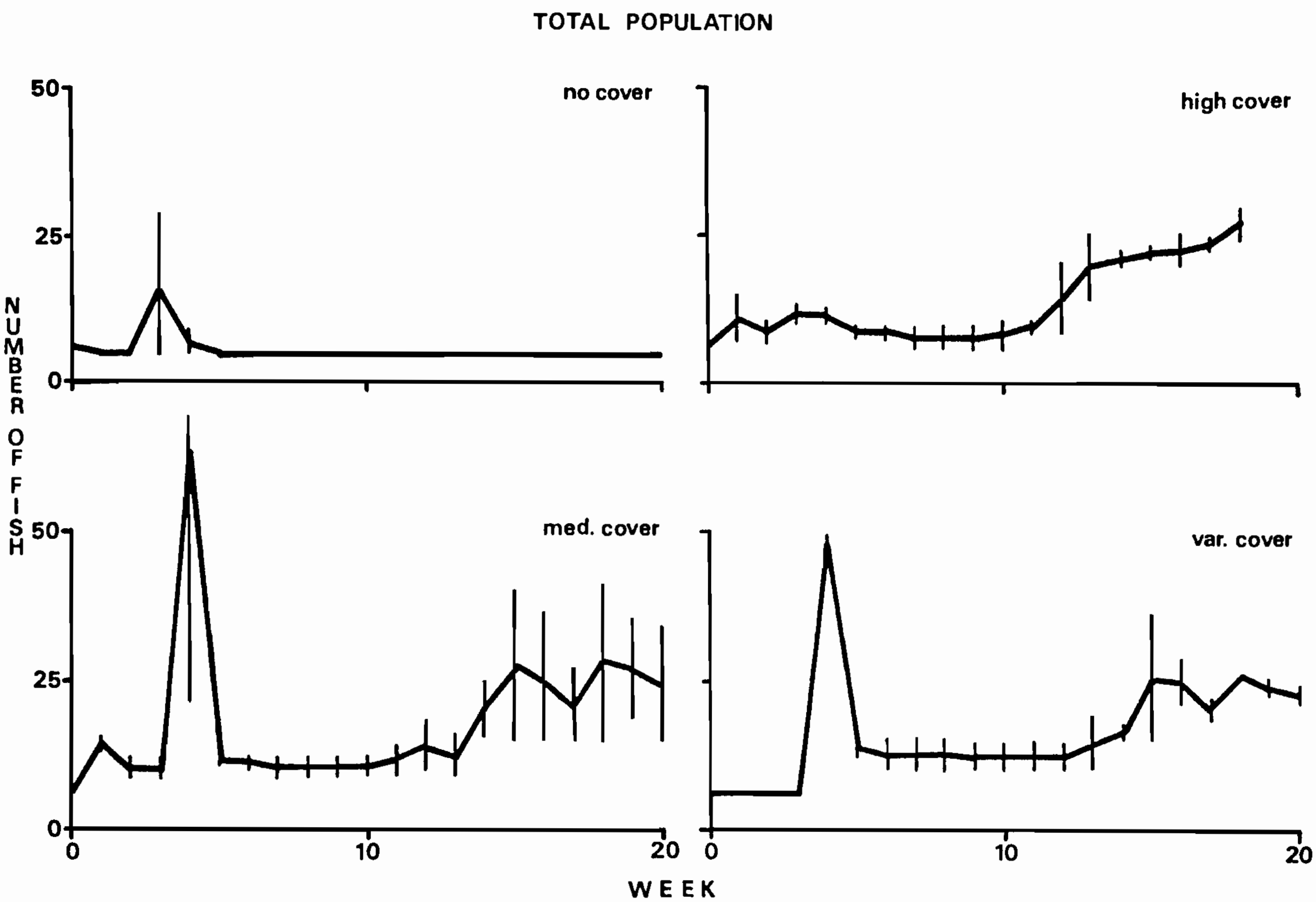

Figure 3. Total population in four experimental treatments. Curves and vertical bars as in Fig. 2.

influence *Gambusia* reproduction and mortality during critical phases of population development is necessary for cost-effective mosquito control. Better understanding of intra-population interactions may also suggest directions for improvements in mass culture techniques.

ACKNOWLEDGEMENT.-This research was supported in part by a grant from the University of California Mosquito Research Program.

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PROGRESS IN THE AQUACULTURAL OVERWINTERING OF MOSQUITOFISH: POND CONFIGURATION AND SOLAR GREENHOUSE EXPERIMENTATION

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INTRODUCTION.-One of the major obstacles limiting the culture and use of mosquitofish, *Gambusia affinis* (Baird and Girard) in California's Sacramento Valley has been the inability to consistently provide an overwintering habitat conducive to the survival of large populations of this valuable biocontrol agent. Over the last decade, overwintering ponds stocked each fall with nearly or newly mature mosquitofish have been harvested each following spring with extremely variable results. Spring fish yields have ranged from less than 10% of the initial fall stocking to as much as 90% of the biomass that had been stocked the previous fall. Obviously, many variables adversely affect fish growth and survival; we have long endeavored to identify these negative factors and design overwintering ponds that would hopefully minimize these unfortunate losses. One of the design variables in overwintering ponds that has received some attention has been that of pond depth.

Previous observations and experimentation (Coykendall 1977, Johnson and Gieke 1977) have supported the hypothesis that deeper ponds provide more stable temperature environments than do shallower ponds; but our operational tests have been unable to conclusively demonstrate the validity of this reasoning. Consequently, a small-scale experiment to test this hypothesis was conducted to examine actual temperature regimes, changes in biomass, condition, gender-related survival, and effects upon the reproductive readiness of fish maintained in simulated ponds representing three different pond depth configurations.

Experiments with stocks of mosquitofish overwintered in geothermally-heated waters of southern Oregon (Cheyne 1981), have demonstrated that excellent growth, reproduction and survival rates can be achieved when outdoor ponds receive adequate supplemental heating. With this in mind, a small electrical heating system was subsequently installed in an extra pond of the pond depth study to provide information which could be contrasted with collected data from the unheated ponds comprising this experiment. This was also performed to help determine the benefits of a higher temperature regimen for mosquitofish overwintered in this locale.

With encouraging results from ongoing cooperative research in geothermal aquaculture, coupled with observations made during the course of this pond depth/pond heating experiment, the decision was made to construct a small solar greenhouse to assess its water heating potential for both rearing and overwintering mosquitofish. In this second study temperatures were monitored in two stocked breeding tanks - one of which was installed inside the solar greenhouse, the other outside to provide ambient data for comparison.

MATERIALS AND METHODS: **Pond depth Study**.-Ten rectangular ponds were excavated in the fall of 1982. Each measured approximately 3.2 m by 0.7 m. Three depth configurations were selected (0.9, 1.5 and 2.1 m), with three ponds representing each depth. Commencing on January 7, 1983, an extra pond of 1.5 m depth was heated by means of a separate electrical heating system supplied with water by a submersible pump situated in the pond. Temperatures in this heated pond averaged 23.7± 1C over the remainder of the experimental period. All ponds were lined with black polyethylene film and filled and maintained at the test depth with wellwater. On November 18, 1982, each of the ten ponds received 136 g (corresponds to 550 lbs per acre) of mature mosquitofish of mixed gender (70.6% ♀♀: 29.4% ♂♂). Temperatures in ponds representing the different experimental treatments were monitored by means of two remote probe thermographs.

Fish were fed Tetramin Basic Flakes® on weekdays at a level corresponding to 3% of their combined weight as stocked. This ration size was usually slightly in excess of what would be consumed in any 24-hour period. Fourth instar larvae of the mosquito, *Culex pipiens* were supplementally fed on Fridays to provide feed on the weekends. As expected, the fish in the heated pond consumed far more feed than those fish stocked in the unheated ponds and the amount initially fed (3%) was not nearly enough; hence the ration size was gradually increased to a level slightly exceeding the daily consumption rate (8%).

Individual fish used in this study were sampled and examined to make calculations and assessments of growth, condition and reproductive readiness. Fifty fish of each sex, randomly selected from the fish population used to stock this experiment, were weighed and measured at the inception of the study; likewise, similar samplings were conducted for the fish from all ponds at the termination of this experiment on April 13-14, 1983.

Data collected were statistically analyzed through the use of pond and treatment means, t-tests and simple correlation. The coefficient of condition or condition factor (K) was calculated through the use of the formula: $K=10^5W \div L^3$, where W represented individual fish weight in grams and L its total length in millimeters (Carlander 1969). The condition factor is a measure of robustness of a fish; high K values usually denote fatter or healthier fish while low values usually are indicative of fish in poorer condition. However, sexual dimorphism in poeciliid fishes usually prevents valid comparisons between male and female.

Solar Greenhouse Project.-Solar greenhouses differ from standard greenhouses in that solar designs incorporate several unique features which enable them to more effectively capture and store thermal energy that is gained during sunny periods. At night this stored energy is slowly reradiated throughout the greenhouse. Overall, significantly higher temperature regimes can be maintained over a 24-hour period than would be possible in a standard greenhouse without the added expense of supplemental heating systems.

The small ($46m^3$) geodesic structure constructed for this study incorporated the following passive techniques to maximize thermal energy capture and storage efficiency. First, double-film glazing was affixed to all surfaces of the greenhouse. Second, the northern greenhouse walls were further insulated by inserting styrofoam packaging material between the glazing films. These two procedures served to minimize rapid heat loss at night which commonly occur in ordinary single-glazed greenhouses. Third, black plastic drums filled with water were stacked inside the greenhouse in front of its styrofoam-insulated walls. These drums were used to capture and store thermal energy for delayed reradiation at night and cooler periods.

On March 9, 1983, 100 unconditioned female mosquitofish were collected, divided into two equal lots and stocked into two shallow (15 cm) plastic tanks, each having a volume of approximately 265 liters. One of these tanks had been located 33 cm above ground level, parallel to the south wall inside the greenhouse; while the other was similarly oriented but was fully recessed into the soil approximately 5 m east of the greenhouse.

The primary goals of this preliminary experimentation were to monitor and evaluate water temperatures inside and outside the solar greenhouse. Secondarily, female fish were stocked into the tanks to observe their behavior and to determine if a somewhat elevated temperature regime in the greenhouse would stimulate earlier parturition than would be obtained under ambient conditions outside this structure.

To prevent cannibalism of newborn fish by the adult females, a hardware cloth reef enclosing bundled plastic bird netting was set into both tanks. Periodic dipping and observations were used to determine parturition dates. Remote probe thermographs were used to record water temperatures, which were then converted to mean daily values for all evaluations and comparisons. Temperatures were monitored until January 15, 1984; however, parturition data wasn't collected after July 1, 1983 due to the excessive heat attained inside the greenhouse.

RESULTS AND DISCUSSION: **Pond Depth Study.**-Water temperature data, summarized in Figure 1 and Table 1, demonstrated two fairly pronounced trends among ponds representing the three depth variables. For the first three months of the study where pond temperatures were continually dropping, the deepest (2.1 m) ponds were always warmer than the shallowest (0.9 m). Conversely, as spring approached and the ponds became warmer, the shallowest ponds warmed most rapidly and to the greatest extent. Significant differences also occurred in the magnitude of daily temperature variation among the three depth

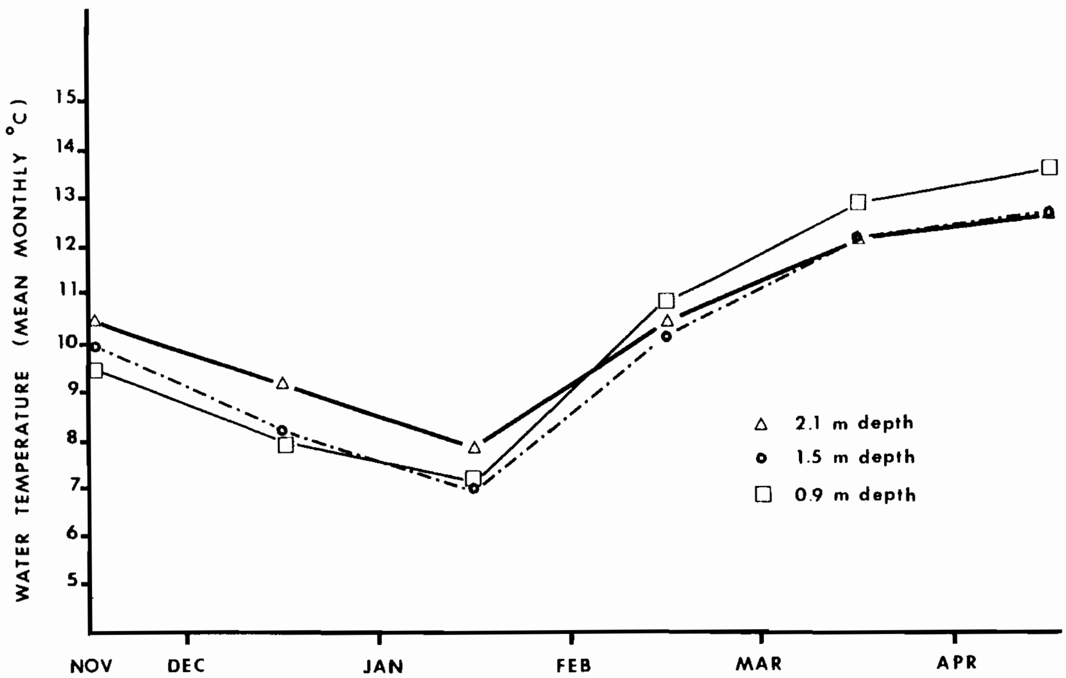

Figure 1. Monthly mean water temperatures for each of the three pond depths tested. Each treatment comprised of three ponds maintained at the same depth.

Table 1. SUMMARY OF MEAN WATER TEMPERATURES (C) AND POND VOLUMES (m^3).

MONTH/ YEAR	0.9 m DEPTH	1.5 m DEPTH	2.1 m DEPTH	HEATED POND
NOV. 82	9.5	10.1	10.5	-
DEC. 82	8.0	8.2	9.2	-
JAN. 83	7.2	7.0	7.8	20.7
FEB. 83	10.8	10.1	10.5	25.1
MAR. 83	12.9	12.2	12.2	25.0
APR. 83	13.6	12.6	12.7	22.8
MEAN DAILY VARIATION, C	1.4	1.2	1.0	1.0
MEAN POND VOLUME, m^3	2.0	3.3	4.7	3.3

Table 2. THE EFFECTS OF POND DEPTH & SUPPLEMENTAL HEATING ON MOSQUITOFISH BIOMASS, CONDITION, GENDER-RELATED SURVIVAL & REPRODUCTIVE READINESS (TREATMENT MEANS).

POND NO.	POND DEPTH (m)	BIOMASS RCVR'D (g)	NO. FISH RCVR'D	PERCENT ♂	PERCENT ♀	BIOMASS ♂ (g)	BIOMASS ♀ (g)	CONDITION FACTOR (K) ♂	CONDITION FACTOR (K) ♀	NO. ♀ GRAVID	% ♀ GRAVID
								RANDOM SAMPLING (n=50)			
1,2,3	0.9	128.2	341	29.2	70.8	18.7	109.5	0.952	1.152	25	16.7
4,5,6	1.5	133.1	308	27.5	72.5	16.6	116.5	0.956	1.265	67	44.7
7 * 9	2.1	132.1	322	32.2	67.8	21.8	110.3	1.009	1.267	48	48.0
10	HEATED	411.0	364	33.5	66.5	33.5	377.5	1.011	1.357	49	98.0

* Pond No. 8 data excluded.

treatments, in that as water depth increased, the less the mean daily fluctuation (r= -0.999, $P < 0.05$). This appears to be simply a demonstration of the greater temperature stability afforded by deeper, thus greater pond volumes.

Data summarized by treatment in Table 2 indicated that the mean biomass of the mosquitofish recovered from ponds representing the intermediate depth (1.5 m) was slightly, but not significantly greater (133.1 g/pond) than that harvested from the shallowest pond treatment (128.2 g/pond). Unfortunately, the biomass data collected from the deepest ponds were of somewhat limited value, as the fish from one pond in this series (#8) uncharacteristically experienced over 50% mortality due to unknown causes. The fish biomass collected at the end of the study from the single heated pond (411 g) was three times that initially stocked. Of that biomass, all but 1.7 g represented originally-stocked fish. This remaining biomass represented 20 newborn fish which were first observed on March 20, 1983.

The mean number of fish recovered from all depth treatments didn't vary significantly; thus obvious trends weren't observed which could favor one depth over another in terms of survival through the 1982-83 winter here.

Final sex ratios were also difficult to interpret; none were radically different from the initial sex ratio observed in those fish selected for this study (29.4% ♂♂: 70.6% ♀♀). The proportion of males was slightly greater in recoveries from the heated pond (33.5%) and the deepest pond series (32.2%) than from the shallowest series of ponds (29.2%) or the intermediate depth ponds (27.5%). Male mosquitofish are typically less hardy than their female counterparts, especially in their cold tolerance limits. Apparently, in this experiment they were simply not stressed enough so that final sex rations were appreciably altered in favor of the females.

Mean biomass values for both sexes recovered from all ponds, except those from the heated pond, were not significantly different from each other. However, the biomass of the females collected from the heated pond (377.5 g) was over three times the mean biomass for unheated ponds (112.3 g - combined treatments).

Random sampling data offered more conclusive interpretations than the foregoing enumeration and biomass information. The females recovered from the heated pond were in much better condition (K=1.357) than those from the unheated ponds (K=1.223, combined treatment means); but this difference wasn't as pronounced in comparisons of male fish. Perceptible trends, even though not statistically significant, were observed in the mean K values obtained for both sexes in the three treatments, as condition factors appeared to be positively correlated with increasing depth.

Finally, reproductive readiness, as measured by the absence or presence of viable eggs in the female fish, provided a very significant trend statistically correlated ($r=0.816$, $P < 0.01$) with pond depth.

Comparing unheated ponds, the mean percentage of gravid females increased with increases in pond depth. In the shallowest ponds only 16.7% of the females were gravid; this measurement rose to 44.7% in the fish from the intermediate depth treatment. The deepest ponds produced a female population in which 48% of those examined were gravid. In addition, as pond depths increased so did fecundity, egg size and viability. Females sampled from the heated pond were essentially all gravid (98%) and most eggs removed and examined were in an advanced state of development. Embryonic tissue was very commonly observed developing within these eggs.

Solar Greenhouse Project.-As expected, water temperatures monitored in breeding tanks, both inside and outside the greenhouse for the duration of the study, were significantly different (t-test, $P < 0.01$) . Overall, the greenhouse yielded water temperatures for the study period that averaged 5.9C higher than that of the ambient control. The mean temperature for greenhouse water was 21.9C; whereas the ambient water was 16.0C. Figure 2 depicts the mean temperature data for both treatment and control for this ten-month preliminary study. The greenhouse water, in addition to being warmer, also had a much greater daily temperature variation than did the ambient water. For the study period the mean daily variation was 10.4C for the greenhouse water; while it was only 5.1C for the ambient water. This may prove advantageous in the summer months as fluctuating temperature environments have been hypothesized as being more beneficial for the growth and reproduction of mosquitofish than are those having relatively stable or constant temperatures (Hubbs 1971). Protracted periods of inclement weather that obscures the sun and is normally experienced each winter (November-March) in the Sacramento Valley, are typified by low elevation cloudiness with frequent rain and cooler conditions coupled with ground-

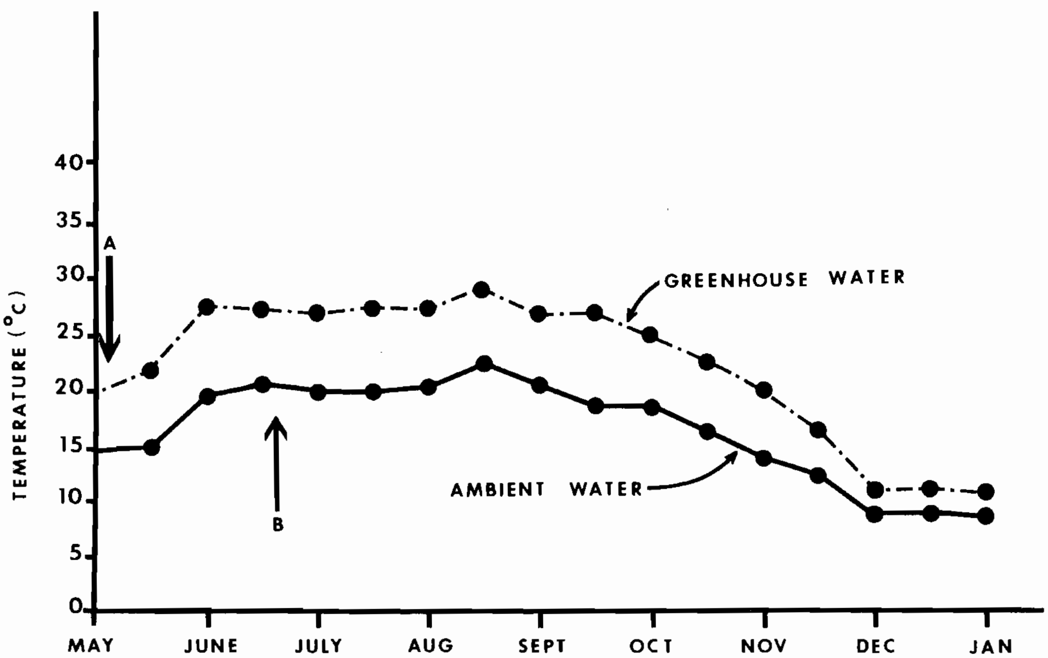

Figure 2. Mean water temperatures recorded daily and summarized bimonthly. Dates of first parturition by female mosquitofish indicated by arrows: A = greenhouse fish: B = fish held under ambient conditions.

level fog. During these periods the heat gain in the greenhouse water was substantially less than what it had been during the summer months.

The unconditioned stocks of female fish stocked into both environments were far more active in the warmer greenhouse water and as a result consumed much more feed. In terms of the onset of reproductive activities, the greenhouse females delivered their initial brood of young on May 3rd; while ambient control females had their first broods of young on June 19th - a difference of 47 days. From these observations, it can be seen that this solar greenhouse provided a warmer, more variable temperature environment which likely promoted much earlier parturition. In the last 15 days of May, uncontrolled greenhouse water temperatures climbed beyond the lethal limits of the originally-stocked females in the greenhouse, so data relating to brood intervals couldn't be collected. Woven shade material was then placed over the greenhouse tanks and later over the greenhouse itself to limit excessive solar insolation which would have otherwise developed. Even with this shading the normal high ambient daytime air temperatures of the late spring and summer combined with an inadequate venting system resulted in water temperatures that would still often exceed 35C. Adult fish exposed to this fluctuating high temperature regimen often succumbed; however, newborn fish appeared to be far more tolerant and survived these high temperature excursions.

CONCLUSIONS AND RECOMMENDATIONS: **Pond Depth Study**.-The results of this experiment furnished some evidence, but didn't conclusively demonstrate the superiority of deeper pond designs over shallower ones in this region. Noticeably higher mortality or losses in biomass weren't found to be significantly correlated with increases in pond water depth. Slight differences in condition factor among both sexes may indicate that these deeper ponds demanded a little less from the fish metabolically. With regard to reproductive readiness, the results were far more revealing; as pond depth positively corresponded with enhancement in the development of stored eggs in overwintered female mosquitofish.

The small ponds designed and excavated for this study didn't simulate operational ponds as closely as they should have - they were simply too deep with respect to their individual surface area. Tall shrubbery adjacent to the pond site also sheltered the ponds somewhat from prevailing southerly winds. Consequently, wind current influences commonly experienced in the District operational ponds were unfortunately minimized in these simulated ponds. Also, undesirable shading effects from bordering walnut trees and shrubs likely inhibited insolation levels somewhat. The application of artificial heat to one of the ponds produced impressive results. Fish thrived in this environment; sizable gains in biomass, condition and reproductive readiness were very evident. Obviously, it was possible to aid overwintered mosquitofish by thermally enhancing their environment.

Solar Greenhouse Project.-The results of this project promote some optimism in the value of "free" solar energy to help provide a thermally-enhanced aquatic habitat for mosquitofish. This fish survives nicely in a warmer winter environment and with supplementally-heated water there is also the potential for growth and reproduction during times of the year that are often life threatening to fish populations kept under ambient conditions.

It would be impractical and uneconomical to attempt to propagate massive numbers of fish in solar greenhouses; but it would be desirable to be able to produce numerous young fish to use to restock ponds and other habitats when ambient conditions are too harsh to permit overwinter survival. Of even greater potential benefit is the goal of raising mosquitofish through the winter to an age that would, when stocked in the spring, reproduce substantially sooner because of prior greenhouse conditioning than would stocks carried through the winter in outdoor ponds.

As a consequence of these encouraging preliminary results, this use of solar energy is being expanded through the District's current construction of a much larger solar greenhouse, which will hopefully make much more efficient use of this energy source.

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CONTINUING STUDIES OF *GAMBUSIA AFFINIS*, *LEPOMIS CYANELLUS* AND THEIR INTERACTIVE EFFECTS ON MOSQUITO ABUNDANCE IN EXPERIMENTAL RICE PLOTS

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ABSTRACT

Studies evaluating the effects of the mosquitofish *(Gambusia affinis)*, the green sunfish *(Lepomis cyanellus)* and their interactions on mosquito abundance and community structure were continued (see Blaustein and Washino 1983. Proc. Calif. Mosq. and Vector Control Assoc. 51:42) in 1983. Our results indicate that *L. cyanellus* may provide control for *Culex tarsalis* but appear to reduce the effectiveness of *G. affinis* to control *Anopheles freeborni*.

This study was conducted in replicate plots constructed by forming (0.75 m^2) rings with sheet aluminum and forcing them into the rice substrate. Twenty of these plots were randomly assigned to 4 treatments: (1) Control - no fish, (2) *G. affinis*, (3) *L. cyanellus* and (4) Both fish spp. Treatments containing *L. cyanellus* received 5 (0.5 g) immatures. Faunal abundance within these plots was monitored through the course of the rice field season by dipping and by minnow traps.

Analyses of variance (repeated measures design) were conducted on abundances of *Cx. tarsalis*, *An. freeborni*, Ephemeroptera, Zygoptera and *G. affinis*. *Cx. tarsalis* abundances were significantly reduced in all of the fish treatments as compared to the control. *An. freeborni* populations were significantly reduced only in the *G. affinis* treatment; no differences were found in *An. freeborni* abundance between the control and the treatments containing *L. cyanellus*. Ephemeroptera abundance was significantly reduced in the *L. cyanellus* and mixed- spp. treatments but not in the *G. affinis* treatment. No treatment effects could be demonstrated for Zygoptera abundance. *G. affinis* populations were drastically reduced in the presence of *L. cyanellus*. The results suggest that *L. cyanellus* may be detrimental to *An. freeborni* control since this fish does not significantly reduce the populations of this mosquito but may reduce populations of *G. affinis*.

PRELIMINARY STUDIES OF THE INTERACTIVE EFFECTS OF SOUTHERN NAIAD AND THE MOSQUITOFISH ON RICE FIELD FAUNA

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ABSTRACT

We have initiated a study to determine how *Gambusia affinis*, southern naiad (a submergent aquatic plant - Najas spp.) and their interaction affect mosquito abundance and community structure. Our preliminary results indicate that both *G. affinis* and southern naiad have individual treatment effects as well as interactive effects on rice field fauna.

This experiment was conducted in 20 (0.75m^2) rings constructed from sheet aluminum which were forced into the rice field substrate. These rings were randomly assigned to 4 treatments: (1) No *Gambusia*, no naiad, (2) *Gambusia*, no naiad, (3) No *Gambusia*, plus naiad, (4) *Gambusia*, plus naiad. *Gambusia* treatments received 1 gravid *G. affinis;* southern naiad was planted in the appropriate rings and weeded from others. Fauna within these rings were monitored through the course of the rice field season by dipping and by minnow traps.

Analyses of variance (repeated measures design) were conducted on population sizes of *Culex tarsalis*, *Anopheles freeborni*, Ephemeroptera, Zygoptera and *G. affinis*. *G. affinis* depressed *An. freeborni* and *Cx. tarsalis* populations while southern naiad did not affect abundances of these mosquitoes. No treatment effects were demonstrated for Ephemeroptera. Plots with naiad had significantly more Zygoptera than plots without the vegetation, if *Gambusia* were absent. Plots with naiad also supported higher populations of *G. affinis*, suggesting that the vegetation provided refuge for fish fry against larger conspecifics and/or provided more abundant prey. Southern naiad did not generally achieve high densities in this study; more dramatic effects may be demonstrated with greater densities of this vegetation.

PANEL: ECOSYSTEM SIMULATION AND MOSQUITO CONTROL IN A FRESHWATER MARSH: THE COYOTE HILLS MARSH MODEL

INTRODUCTION

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The following eight papers all deal with some aspect of the ecological interactions in a freshwater marsh at the Coyote Hills Regional Park in Fremont, California. These papers have much more in common than their study site. These papers are presented together here, as they were in Long Beach, because they represent a unified systems approach to mosquito control studies. These studies are a continuation of calibration studies (Collins et al. 1983 and Schooley 1983) in support of an ecosystem simulation model designed for use by the Alameda County Mosquito Abatement District (Schooley et al. 1982).

A detailed description of the site and the major biotic components of the aquatic system can be found in the references given above. In summary, this marsh is rapidly being surrounded by urban development and is also a source of anopheline mosquitoes. The areas producing the anopheline mosquitoes are those which develop extensive growths of the pondweed *Potamogeton pectinatus*. An ecosystems model was developed in order to aid in the prediction of mosquito production in mid-summer based on spring conditions in the marsh. A computer based model was necessary because of the complexity of the biological interactions involving pondweed, mosquito larvae and their predators, and the impact of management options such as altering marsh depth, stocking with fish or spraying with insecticides. Field studies began in 1982 to calibrate the model (Collins et al. 1983 and Schooley 1983).

The eight studies reported here continue and expand the calibration begun last season. In addition, the work this season included both laboratory and field experimentation to test implications and assumptions of the model. The modelling effort was not done solely to make predictions of the system but also to aid in our conceptualization of the system and to help us generate hypotheses concerning the structure and function of the system. These hypotheses can later be tested in the field and laboratory and will ideally increase our understanding of other aquatic systems as well as the Coyote Hills marsh. Three different research groups were assembled and coordinated by Fred Roberts of the Alameda County MAD to continue the calibration and experimental program (Roberts et al. 1983). The first group from the Division of Entomology and Parasitology at UC Berkeley examined the seasonal patterns of the pondweed and mosquitoes, the seasonality of the dominant invertebrate predators and their prey and the influence of trails made in the pondweed by waterfowl on the survival and age structure of the anopheline population. Their results are given in the first three papers of the panel. The second research group was from the Department of Biological Sciences at California State University, Hayward and they examined allelochemical interactions between pondweeds and mosquito larvae, and details of the fish community including seasonal patterns of abundance, prey selection in the field, and experimentation on the influence of pondweed on predation rates. Their results are presented in the next four papers. The final paper of the panel discusses the abundance of adult mosquitoes in the Coyote Hills marsh area and is the result of collaboration of the Division of Biological Control at UC Berkeley and the Alameda County MAD.

As you will see in the following reports, just as many new questions have been posed as were answered by last seasons research. During 1984, the three research groups will continue their fruitful collaboration in the areas of sensitivity analysis, field experimentation and adult life histories.

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SEASONAL PATTERNS OF PONDWEED STANDING CROP AND *ANOPHELES OCCIDENTALIS* DENSITIES IN COYOTE HILLS MARSH

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ABSTRACT

Extensive areas of the water surface of Coyote Hills Marsh are occluded by dense mats of sago pondweed, *Potamogeton pectinatus*. The resulting plant-water-air interface serves as habitat for larvae of the mosquito *Anopheles occidentalis*. Biweekly samples of pondweed standing crop taken throughout 1983 showed that sprouting from tubers and seeds occurred in March and April and that plant surfacing occurred in early June. The intertwining canopy of leaves and stems that developed at the water surface fully occluded the surface from July through mid-October. *Anopheles* larvae occurred in the marsh only when this canopy was present. However, the decline in larval abundance in August suggests that the mosquitoes were unable to sustain their summer production despite the continued availability of appropriate habitat.

INTRODUCTION.-The Coyote Hills Marsh Model is a computer-based simulation model designed to integrate the objectives of both mosquito control and wildlife management (Roberts et al. 1983). This paper presents the results of a time-series analysis of the two main components of the model, the mosquito *Anopheles occidentalis* and its pondweed refuge, *Potamogeton pectinatus*.

STUDY SITE.-Coyote Hills Marsh (Alameda County, California) is located along the southwestern margin of San Francisco Bay. Although historically a tidal salt marsh, it is now a diked floodwater catch-basin and therefore contains fresh water for much of the year (Collins et al. 1983). The shallow margins of the main pond support cattails (*Typha* spp.) and bulrushes (*Scirpus* spp.). From late spring through early autumn, the deeper (ca. 1 m), interior section of the pond supports a dense surface mat of *Potamogeton*, which in turn provides habitat for *An. occidentalis*.

MATERIALS AND METHODS.-We chose dry-weight standing crop as the best measurement of *Potamogeton* phenology. Standing crop also serves as the best quantifiable correlate of the water surface area that is occluded by pondweed. From January through May 1983, prior to surfacing, the pondweed was sampled biweekly using an Ekman grab sampler (area = 0.023 m^2). Because the Ekman takes a bottom sample as well as the attached material above it, these early season samples allowed us to analyze the relative contribution of overwintering seeds and tubers to the new year's crop.

Once pondweed reaches the surface, the leaves and stems quickly become intertwined and form a dense surface mat. If sampled from above, the mat will sink for several meters around and thus disrupt animals both at the surface and through the water column. To avoid such disruption, we developed a pull-up sampler to use from the pond bottom upward to the surface. The sampler consists of a 2-m PVC pole with a 28-cm circular ring (area = 0.062 m^2) attached to the lower end and at right angles to the pole. The ring is 15 cm from the pole and supports a conical, 90-μm mesh bag.

The samplers were implanted by cutting a T-shaped notch in the intertwined canopy of pondweed. The stems were cut at their base with a scythe and allowed to float up. The sampler was then slipped under the vegetation, ring first, and implanted into the pond bottom. We implanted all 15 samplers prior to pulling the first one, thereby allowing at least an hour for recovery of the habitat from disruption. The samplers were retrieved by pulling quickly upward until the ring was just out of the water, which allowed water to drain through the net. By cutting pondweed around the ring, the amount of pondweed per unit area was obtained. Samples were dried at 70°C for one week to determine dry-weight standing crop. The pull-up sampler also provided us with a quantitative measurement of invertebrates, including mosquito larvae, from the water surface, the pondweed canopy, and the water column below.

Sampling began in March 1983 and will continue through April 1984. Fifteen samples have been taken biweekly (spring, summer, autumn) or monthly (winter) from a site in which depth, temperature, and wind and sun effects are uniform. Laboratory procedures for separating invertebrates from the pondweed are described by Lamberti and Resh (1984). Mosquito samples were taken concurrently with each pull-up sample using a standard one-pint dipper. Three dips were taken within one meter of each pull-up sample and combined.

RESULTS.-Growth of *Potamogeton* in Coyote Hills Marsh began in early March at the southern reaches of the marsh and in mid-April at our study site in the northern end (Fig. 1). The year's first shoots sprout from overwintering

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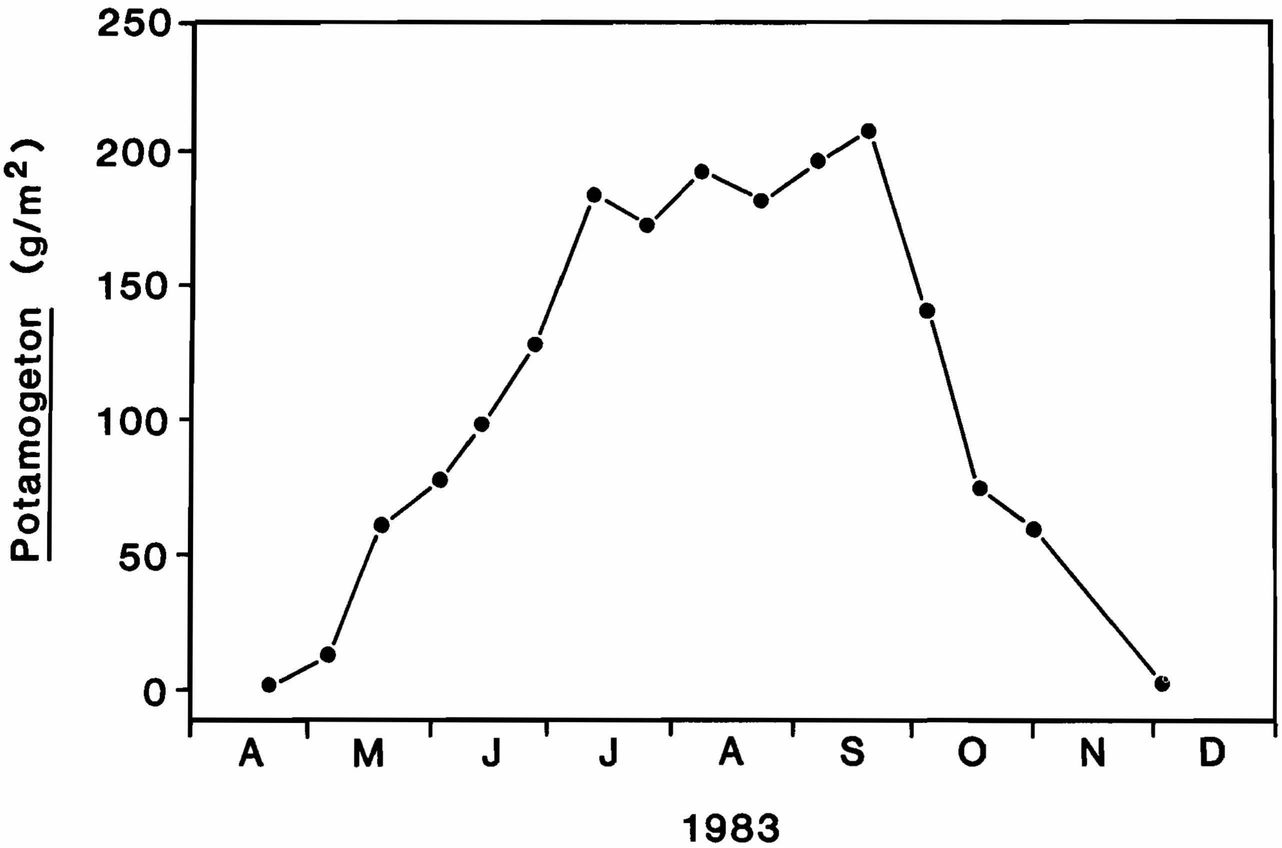

Figure 1. Seasonal pattern of *Potamogeton pectinatus* standing crop in Coyote Hills Marsh. The pondweed surfaced in early June, formed a complete canopy by early July, and persisted until mid-October.

tubers and seeds. Contents of the Ekman grab samples showed that there were about 10 times as many seeds (ca. 300/m^2) as tubers (30/m^2) in most areas of the marsh. However, laboratory studies indicated that all tubers sprouted and successfully produced new plants, whereas less than 4% of the seeds sprouted. Therefore, at least 60-70% of the new year's crop is probably derived from tubers. The distribution of seeds, tubers, and early season main stems throughout the marsh was aggregated, with coefficients of variation of 60, 68, and 87%, respectively. This patchiness was due in part to differences in pond depth. Although past construction activities have tended to give the ponds of Coyote Hills Marsh a relatively uniform depth, early season plant biomass transects across a 20-m wide channel, which ranged in depth from 86 to 120 cm, indicated a significant negative correlation between dry-weight biomass and depth ($r = -0.61$, $p = 0.001$).

The time at which *Potamogeton* first reached the water surface was also variable in different parts of the marsh. Surfacing occurred about mid-May in the southern reaches of the marsh, but not until early June at our study site (Fig. 1); standing crop in early June was approximately 75 g/m^2. By early July, pondweed reached approximately 150 g/m^2, and the water surface was fully occluded. Standing crop leveled off in mid-July, remaining between 170 and 210 g/m^2 until 19 September, and then declined steadily through the autumn. The increasing load of senescing leaves and stems caused the canopy to sink below the surface in mid-October and remain there until the entire canopy decayed in mid-December. Therefore, only from July through September did pondweed fully occlude the marsh surface and provide maximal habitat for *Anopheles* mosquitoes.

In our main-Pond study site, pull-up samples showed that *Anopheles occidentalis* first appeared in mid-June (Fig. 2), coincident with pondweed surfacing. Mosquito densities, then, appeared to rise in conjunction with *Potamogeton* biomass. However, the apparent link between *Potamogeton* surfacing and *An. occidentalis* appearance is misleading. *An. occidentalis* first appeared throughout the marsh in mid-June regardless of spatial differences in the timing of *Potamogeton* surfacing. In fact, Crandell Creek, which is a nearby fresh-water inlet to the marsh, was fully occluded with pondweed at least one month prior to the appearance of *An. occidentalis.* Thus, the timing

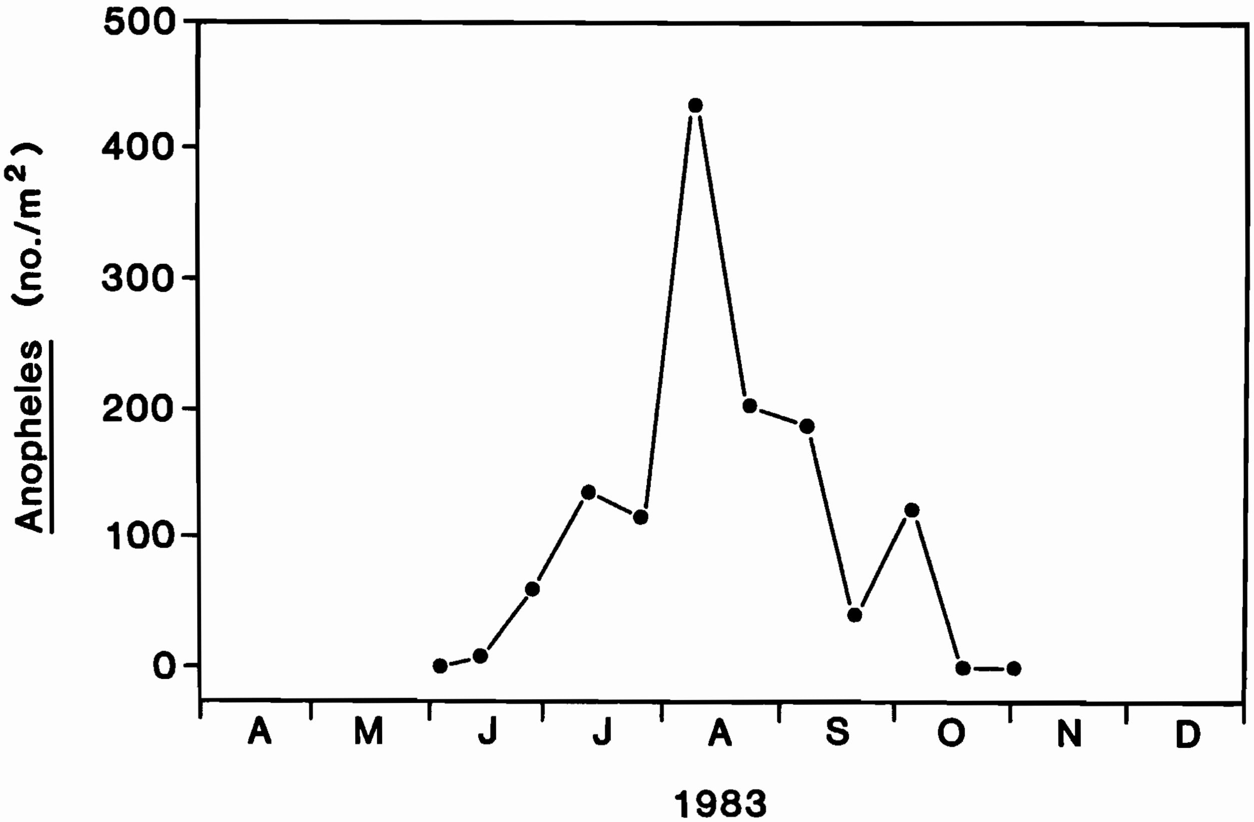

Figure 2. Seasonal pattern of *Anopheles occidentalis* densities in Coyote Hills Marsh as estimated from pull-up samples.

of the first brood of *An. occidentalis* appears to be related to either a delay in first reproduction (e.g. due to cool temperatures) or slow immigration from remote sites, rather than to the phenology of *Potamogeton* and the availability of refuge.

An. occidentalis larvae reached a peak abundance of $435/m^2$ in early August, but numbers then declined sharply (Fig. 2). Larvae were absent by mid-October, which coincided with the decline of pondweed below 75 g/m^2. At this point the plants began to senesce and lose buoyancy, which caused the mat to drop below the surface and thus minimize the availability of refuge for mosquitoes.

Concurrent dip sampling allowed us to establish the relationship between the absolute *An. occidentalis* population measure provided by the pull-up samples and the relative measure provided by dip samples. Linear regression analysis indicated that there was a strong, positive correlation ($r = 0.65$; $p = 0.001$) between the two measures. Both methods agreed on the timing of larval appearance, but the peak estimated by dipping (40 larvae/3 dips) occurred about 3 weeks later than the peak estimated by pull-up samples (Fig. 3 compared to Fig. 2).

Age-structure analyses provided no evidence for the progression of well defined broods through the summer. In fact, from mid-July until late October, relative abundances of the four larval instars remained fairly constant, as shown by the coefficients of variation for each instar, which during this period averaged less than 30%. Such consistency in age structure suggests that despite the large fluctuations in larval density, there was continuous recruitment through much of the summer.

DISCUSSION.-The close relationship between the presence of *An. occidentalis* and the intertwining surface mat of pondweed agrees with past studies of anopheline mosquitoes (Hall 1972). Hess and Hall (1943) and Rozeboom and Hess (1944) found a strong, positive correlation between *Anopheles* abundance and the amount of air-water-plant intersection. In a narrow-leaf, floating plant such as *Potamogeton pectinatus*, the intersection line per unit surface area is maximized, which provides optimal habitat for *Anopheles* larvae.

Despite the synchrony between appearance and disappearance of *An. occidentalis* larvae and the pondweed canopy, mosquito densities did not closely track pondweed biomass. For example, although pondweed biomass remained fairly constant from July through September, mosquito density peaked in August and then declined sharply. We, and other groups working on this project, are presently analyzing several potential factors that may cause the discrepancy in the patterns

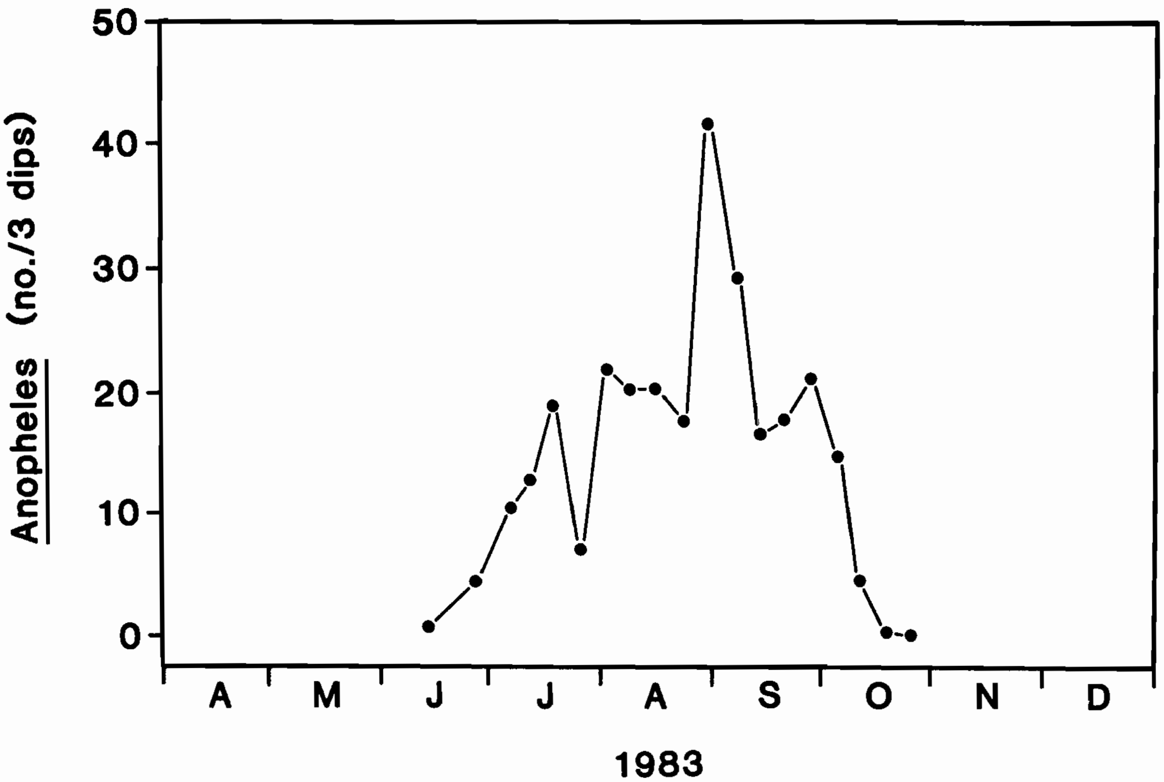

Figure 3. Seasonal pattern of *Anopheles occidentalis* abundance in Coyote Hills Marsh as estimated from standard one-pint dip samples.

observed for pondweed and mosquitoes, such as: (1) predation by fish and invertebrates, particularly damselflies; (2) availability of alternative prey for marsh fish; (3) mortality from *Potamogeton* toxins; and (4) temperature and diapause-induced emigration in *An. occidentalis*. Analysis of such covariates in this system will substantially refine the accuracy of the Coyote Hills Marsh Model, and thus improve the criteria by which marsh management decisions are made.

ACKNOWLEDGMENTS.-This study is the result of a cooperative effort among personnel of the University of California at Berkeley, California State University at Hayward, the East Bay Regional Park District, and the Alameda County Mosquito Abatement District. Support for this research was provided by University of California Mosquito Research Funds and the Alameda County Mosquito Abatement District.

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SEASONAL PATTERNS OF INVERTEBRATE PREDATORS AND PREY IN COYOTE HILLS MARSH

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ABSTRACT

Seasonal fluctuations in the densities of invertebrate predators of *Anopheles* mosquitoes and alternative prey for mosquitofish *(Gambusia affinis)* were examined in 1983 to calibrate the Coyote Hills Marsh Model. Population dynamics of most marsh invertebrates were closely related to the dynamics of sago pondweed (*Potamogeton pectinatus*); such relationships can be expressed by linear equations, which are also suitable for incorporation into the model. The most abundant alternative prey items for marsh fish were chironomid midge larvae and microcrustaceans, especially cladocerans and copepods. Damselfly naiads accounted for most invertebrate predation on *Anopheles* larvae; in August 1983 they consumed over 30% of early-instar mosquitoes in Coyote Hills Marsh.

INTRODUCTION.-The seasonal patterns of the invertebrate predators of *Anopheles* mosquitoes and the alternative invertebrate prey for mosquitofish, *Gambusia affinis*, are important components of the Coyote Hills Marsh system in Alameda County, California. A computer-based simulation model, the Coyote Hills Marsh Model, has been designed to integrate control methods for anopheline mosquitoes in Coyote Hills Marsh, with the eventual goal of applying this model as a management tool in a diverse array of wetland habitats (Roberts et al. 1983, Schooley 1984). However, realistic predictions of anopheline dynamics with this model depend on accurate representation of the dynamics of invertebrate predators and prey in the marsh (Collins et al. 1983). This report summarizes the results of 1983 field studies designed to determine: (1) the seasonal abundances of invertebrate predators of mosquitoes and alternative prey for mosquitofish, (2) the relationship of these organisms to the primary mosquito refuge in the marsh (sago pondweed, *Potamogeton pectinatus*), and (3) the extent of invertebrate predation on *Anopheles* (mostly *An. occidentalis)* populations.

MATERIALS AND METHODS.-Field sampling of pondweed and the associated invertebrate fauna in Coyote Hills Marsh was conducted from March 1983 through April 1984. Sampling methods are described in detail by Balling and Resh (1984). In the laboratory, individual pondweed samples were repeatedly flushed with water and the dislodged macroinvertebrates were collected in a 90-μm mesh net. All macroinvertebrates, except for Chironomidae, were then identified and enumerated; microcrustacea were subsampled prior to enumeration.

Chironomid midge larvae (Diptera), which occupy tube-cases on the pondweed stems and leaves, could not be dislodged using the standard washing technique. Instead, separate samples of pondweed were collected from the marsh and treated with a mild (5%) hydrochloric acid solution that caused the midge larvae to evacuate their cases; midge larvae were then enumerated.

RESULTS AND DISCUSSION.-The phenology of pondweed highly influences the dynamics of the invertebrate populations in Coyote Hills Marsh. The seasonal patterns of pondweed and mosquito populations have been described by Balling and Resh (1984). The following sections describe the invertebrate fauna of Coyote Hills Marsh and its relationship to pondweed biomass and mosquito dynamics.

The aquatic invertebrate fauna of the marsh is taxonomically diverse; the Insecta are represented by 9 orders, 23 families, 39 genera, and at least 43 different species. In addition, there are 10 species of non-insectan invertebrates that commonly occur in the marsh. The number of invertebrate species collected on a particular sampling date peaked at 25-30 species in early summer and then declined through the autumn.

The density of macroinvertebrates, exclusive of Chironomidae, increased rapidly from June to July, stabilized between 1500-2000/m^2 from August to September, and then declined in October. A high proportion of the seasonal variation in macroinvertebrate density can be explained by changes in pondweed biomass (Table 1).

Alternative Prey. The most abundant macroinvertebrates in the marsh are chironomid midge larvae, which are also important alternative prey for mosquitofish and stickleback (*Gasterosteus aculeatus*) in the marsh (Fleming and Schooley 1984). The Chironomidae, which are mostly represented by *Paralauterborniella subcincta* but include at least six species, build and occupy tube-cases on the submerged pondweed; relatively few midges are present in the anoxic sediments of the marsh. Population dynamics of chironomids closely followed the seasonal pattern of pondweed biomass; midge densities peaked at more than 100,000/m^2 during midsummer, which is over 50 times the peak density of all other macroinvertebrates combined. Similar to the pattern for total number of macroinvertebrates, the density of chironomids can be accurately predicted using a linear equation based on pondweed biomass (Table 1). These equations will be incorporated as components in the Coyote Hills Marsh Model.

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Table 1. Linear regression equations describing the relationship between biomass of *Potamogeton* and densities of selected macroinvertebrate taxa in Coyote Hills Marsh.

x (g/m^2)	y (no./m^2)	regression equation (y = a + bx)	R^2
Potamogeton	macroinvertebrates	y = -268.5 + 9.2x	0.82
Potamogeton	Chironomidae	y = -9520 + 488x	0.85
Potamogeton	Zygoptera	y = -143.5 + 2.6x	0.48

Table 2. Consumption of first (I) and second (II) instar *Anopheles occidentalis* by damselfly naiads in relation to the total number of *An. occidentalis present in the marsh during 1983.*

	11 June	8 Aug.	6 Sept.
I + II *Anopheles*/m^2	45	95	85
I + II *Anopheles* in Zygoptera foreguts/m^2	3	42	7
Total *Anopheles*/m^2	48	137	92
% *Anopheles* ingested	6.3	30.7	7.6

Microcrustacea in the marsh are largely represented by cladocerans and copepods. These organisms move freely in the water column where they feed on plankton. Thus, unlike macroinvertebrates, the microcrustacea are not epiphytic and their dynamics are not directly linked to pondweed phenology. Both Cladocera and Copepoda showed abundance peaks in the early summer and early autumn. The highest densities were about 65,000/m^2 for Cladocera (autumn) and 75,000/m^2 for Copepoda (summer and autumn). Because of their high densities and year-round presence in the marsh, microcrustacea serve as important alternative prey items for mosquitofish (Fleming and Schooley 1984).

Invertebrate predators. Invertebrate predators may be seasonally important agents in the regulation of mosquito populations in the marsh. This predator guild includes hydrophilid and dytiscid beetles (eight species combined; densities about 100/m^2) and *Notonecta*(20/m^2). However, the most numerous invertebrate predators in the marsh are damselfly naiads (Odonata: Zygoptera), which are represented by three species: *Ischnura cervula, Enallagma carunculatum,* and *Enallagma civile.* The dynamics of damselflies generally followed the seasonal cycle of pondweed; damselfly densities peaked in late summer at about 1300/m^2. The relationship between *Potamogeton* and Zygoptera (three species combined) can be described by a linear equation that explains about one-half of the variation in damselfly densities (Table 1).

Damselfly densities peaked shortly after *Anopheles* in Coyote Hills Marsh, which suggests that predation by damselflies may have been partially responsible for the decline in the mosquito population in midsummer (Balling and Resh 1984). We examined the foregut contents of damselfly naiads collected in June, August, and September 1983. The foreguts of those damselflies held mostly chironomid larvae and cladocerans. However, the foreguts of later-instar naiads held first- and second-instar *Anopheles* larvae (Table 2). Damselflies consumed only a small percentage (6-8%) of the mosquito population in June and September, but mosquito densities were low during those periods (Balling and Resh 1984). However, in August when mosquito densities were high, damselflies consumed over 30% of the early-instar larvae present in the marsh.

The importance of damselfly predation in regulating *Anopheles* populations will depend on the consumption rate of these organisms compared to the recruitment rate of mosquitoes into the habitat. The relationship between these factors will be examined during 1984 using field and laboratory experiments. The results of these studies will be integrated into the simulation model to provide a realistic estimate of invertebrate predation in Coyote Hills Marsh, and ultimately, to increase the accuracy and applicability of this approach to mosquito management.

ACKNOWLEDGMENTS.-This study is the result of a cooperative effort among personnel of the University of California at Berkeley, California State University at Hayward, the East Bay Regional Park District, and the Alameda County Mosquito Abatement District. Support for this research was provided by University of California Mosquito Research Funds and the Alameda County Mosquito Abatement District.

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DO WATERFOWL AFFECT MOSQUITOES IN COYOTE HILLS MARSH?

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ABSTRACT

Results of field studies conducted at the Coyote Hills Marsh, Alameda County, California, indicate that foraging by waterfowl on sago pondweed, *Potamogeton pectinatus*, can prevent the formation of a dense, floating plant canopy, and also can accelerate the rate of canopy decay. Consequently, the density of *Anopheles* larvae is reduced, possibly because of predation by fish or decreased availability of oviposition sites. Such waterfowl activity also causes the population age structure of *Anopheles* to vary within a habitat. The value of waterfowl for mosquito control may depend upon the timing of their migration, relative to both peak densities of mosquito larvae and pondweed surface cover.

INTRODUCTION.- Waterfowl that graze on vegetation while at the water surface (i.e., dabbling ducks) have been shown to affect the distribution, density, and relative abundance of aquatic plants in many shallow lakes and marshes (e.g., Boorman and Fuller 1981; Danell and Sjorberg 1982; Krull 1970). At the Coyote Hills Marsh (Coyote Hills Regional Park, Alameda County, California), dabbling ducks feed intensively on sago pondweed, *Potamogeton pectinatus*, which in some areas of the marsh can form a dense floating plant canopy. Since this canopy of pondweed functions as a refuge for mosquito larvae from predation by fish, we hypothesized that disruption of the canopy by waterfowl might indirectly affect mosquito population dynamics. This paper presents the results of the first studies that were designed to examine this hypothesis.

METHODS.-**Study Area.** The Coyote Hills Marsh was created from highly disturbed salt marsh remnants to improve local flood control and provide wildlife habitat, and to benefit environmental education programs. The floral mosaic at the marsh reflects a history of varied landuse patterns (Collins et al. 1983), and a complex habitat plan. Zones of emergent cattails and bulrushes separate the main marsh basins from a surrounding flood plain that supports halophytes as well as freshwater and upland plant species. The important mosquito species that are associated with the pondweed at the marsh are *Anopheles freeborni* and *An. occidentalis*. Seasonal patterns of pondweed and epiphytic invertebrate species that in combination probably comprise the main diet for dabbling waterfowl at the Coyote Hills Marsh are presented by Balling and Resh (1984) and Lamberti and Resh (1984).

Sampling Design. We monitored populations of *Anopheles* larvae and described changes in pondweed standing crop for areas of the marsh where waterfowl were active, and for adjacent areas from which waterfowl were physically excluded. Three waterfowl exclosures were constructed during early August 1983 in a portion of the marsh where dabbling ducks congregated to feed and rest. Each exclosure consisted of a square frame of small-diameter plastic pipe that supported 2.5 - 5.0 cm mesh nylon netting and enclosed 10 m^2 of marsh surface. The open top and bottom and the large mesh size allowed invasion into the exclosures by vertebrate and invertebrate mosquito predators, including adult and immature Odonata (dragonflies and damselflies), mosquitofish, and aerial-feeding insectivorous birds (e.g., swallows and flycatchers).

The immediate vicinity of the exclosures was selected as the control area. In addition, samples taken within the exclosures and control area were compared to other samples collected in a nearby portion of the marsh where waterfowl were uncommon. This latter area is henceforth referred to as the sheltered site. For a description of the methods used to sample *Anopheles* larvae and pondweed at this latter site see Balling and Resh (1984).

Within each exclosure and the control area 16 dip samples of *Anopheles* larvae were taken in a gridded pattern each week, beginning in mid-August and ending in mid-October. The number of individuals of each age class (i.e., instars 1-4 and pupae) was recorded for each sample, and all samples were returned to their places of collection.

The number of waterfowl that remained within a 2 ha area around the exclosures during our sampling efforts was determined by direct count. These counts indicated the abundance of dabbling ducks that foraged in the study area, and therefore provide a measure of waterfowl activity that might affect the availability of mosquito refuge.

Visual estimates of percent surface cover were made regularly for each exclosure and the control area. At the end of the study, a complete inventory was made of the total standing crop of pondweed in each exclosure. The pondweed was collected with a rake, dried at about 75°C for 2 weeks, and weighed.

RESULTS.-We consistently observed that 70-100 waterfowl were present in the marsh on each sampling date (Table 1). The majority of these waterfowl were surface-feeders (e.g., mallards, gadwalls, pintails), but included some species that dive to forage if sufficient food is unavailable at the surface (e.g., American coots).

Foraging by waterfowl rapidly reduced the plant cover in the control area. Percent surface cover declined more gradually within the exclosures (Table 2), and also at the sheltered site.

Table 1. Total number of dabbling waterfowl that were present in the marsh during mosquito sampling.

Date	Count
Aug. 16	100
Aug. 22	86
Aug. 29	89
Sept. 6	90
Sept. 12	81
Sept. 19	71
Sept. 26	87

We expected that after waterfowl were excluded from an area, the percent cover and pondweed standing crop would increase; however, this anticipated response only occurred in one of the three exclosures. The largest amount of pondweed occurred at the sheltered site, and the least amount occurred in the control area.

Beginning in early September, the density of *Anopheles* larvae decreased throughout the marsh. However, mosquito densities were always higher at the sheltered site than within the exclosures, and were always lowest in the control area (Table 3). Patterns of change in mosquito density varied among the three waterfowl exclosures (Figure 1). In two exclosures, where percent cover decreased throughout the study, mosquito densities also tended to decrease. In the single exlosure where percent cover increased there was a corresponding increase in the density of mosquito larvae. After the last week of September, no mosquito larvae were found in any exclosure.

The population age structure for *Anopheles* was relatively constant at the sheltered site, and second through fourth instars were numerically dominant. A similar age structure was observed for larvae in the exclosures on certain dates, but during the last week in August, recruitment of first instars increased substantially (Figure 2) in the one exclosure where percent cover had also increased. Subsequent effects of this recruitment on age structure could be detected for the cohort over time (Figure 2). In the control area, age structure appeared highly variable, probably because of the very low densities.

During the first two weeks of the study, larval populations within the control area, exclosures, and sheltered site were similar in age structure; however, by early September all recruitment in the control area had apparently ceased (Figure 2). Recruitment continued in at least one exclosure until late September, and continued at the sheltered site for about one month longer.

DISCUSSION.-The foraging behavior of dabbling waterfowl at the Coyote Hills Marsh resulted in widespread disruption of the pondweed surface canopy. An obvious characteristic of intensive foraging was the appearance of white pondweed stems intertwined with partially submerged, shredded pondweed leaves. Stem-cutting apparently accelerated the senescence of the canopy, and consequently its subsidence or its removal by wave action. This is why only traces of pondweed were found in the control area at the end of the study.

Since the waterfowl exclosures were not constructed until late summer, they encompassed pondweed that had already been disrupted by foraging waterfowl. The effect of this foraging was apparently too extensive in two of the three exclosures for the pondweed to recover and form

Table 2. Percent plant cover by date for each waterfowl exclosure, and total pondweed standing crop contained within each exclosure at the end of the study.

Exclosure	Percent Cover							Ending Standing Crop (kg)
	August			September				
	16	22	29	6	12	19	26	
A	75	60	60	50	35	20	15	1.79
B	75	60	60	60	50	50	40	3.26
C	80	70	80	90	85	80	85	4.75

Table 3. Density of *Anopheles* larvae by sampling date for the waterfowl exclosures, the control area, and the sheltered site. Data were combined for all three exclosures. Since original data for the sheltered site were based on 3 dips per sample, the variance among individual dips was not available.

Date	Density of *Anopheles* Larvae		
	Exclosures	Control Area	Sheltered Site
	$\bar{x}$ (sd) / dip	$\bar{x}$ (sd) / dip	$\bar{x}$ / dip
Aug. 16	6.0 (5.5)	2.0 (2.1)	20.7
22	3.9 (3.5)	2.2 (2.4)	17.6
29	4.1 (1.8)	1.1 (1.2)	41.5
Sept. 6	3.9 (3.5)	1.4 (2.6)	29.1
12	2.9 (2.8)	0.4 (0.6)	26.6
19	2.4 (3.2)	0.1 (0.3)	17.5
26	0.1 (0.5)	0.0 (0.0)	21.0

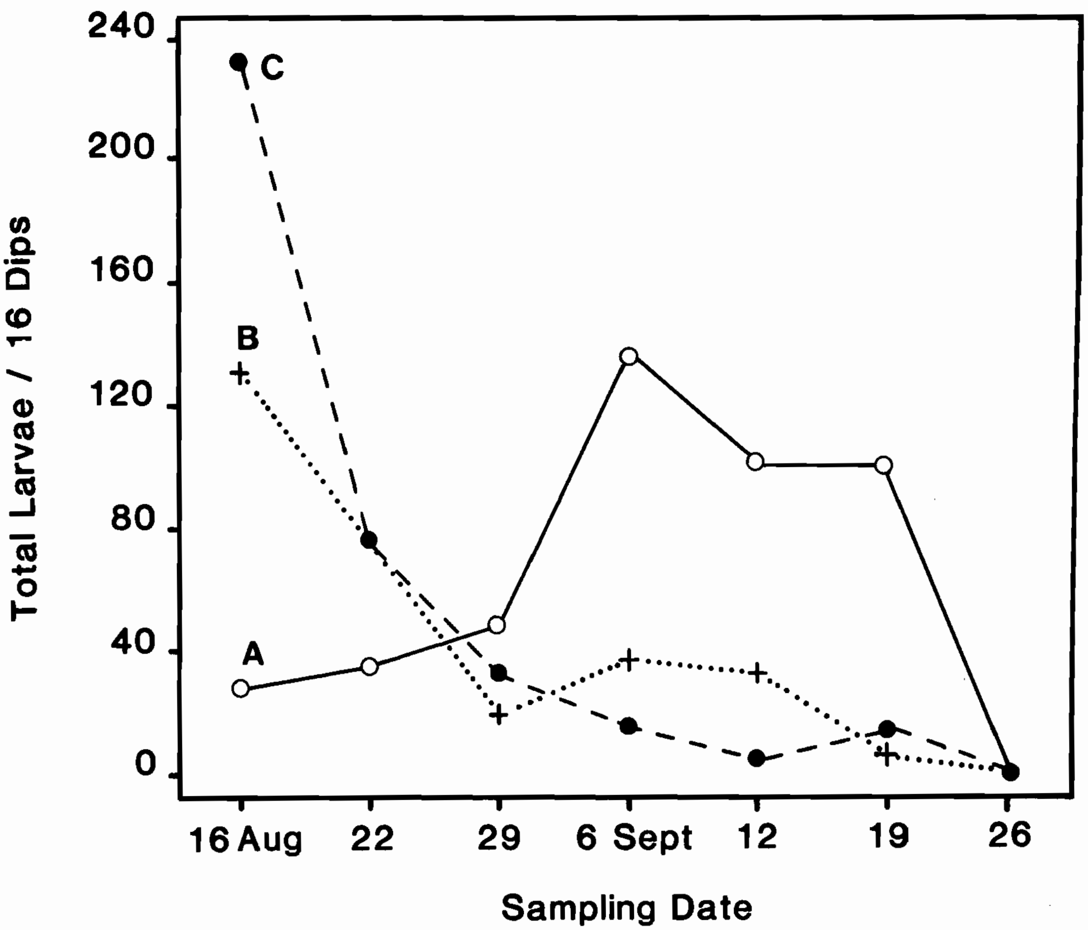

Figure 1. Total number of mosquito larvae collected in each exclosure on each sampling date.

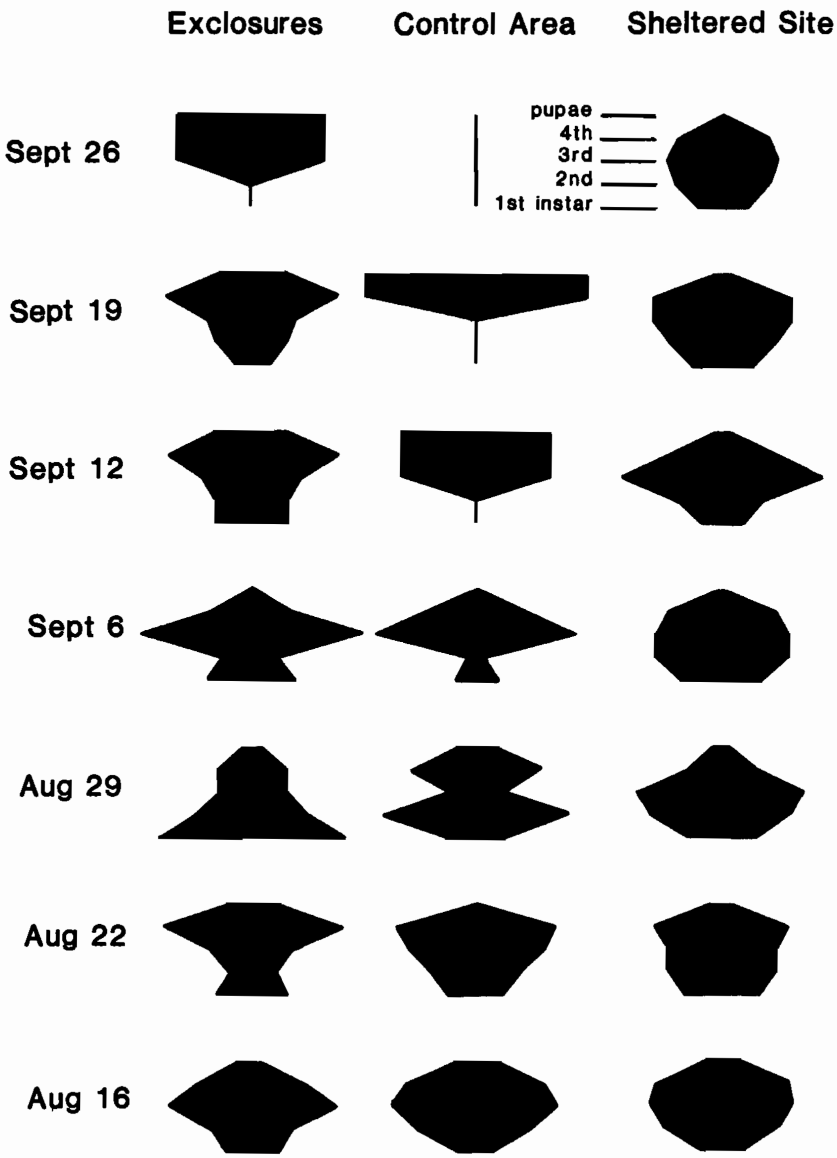

Figure 2. Population age structure by sampling date for the waterfowl exclosures, control area, and sheltered site. Data were combined for the three exclosures. Note that an increase in recruitment is apparent as a numerical dominance of first-instars in the exclosures on August 29. As these recruited larvae mature (i.e., during later sampling dates), dominance shifts to later instars.

a healthy surface mat. Instead, the pondweed in these two exclosures continued to deteriorate throughout the study. This suggests that dabbling waterfowl can prevent canopy formation, and that continuous foraging is not required to accelerate canopy senescence.

The relatively high rate of mosquito recruitment that we observed for one exclosure may represent the importance of pondweed surface cover for mosquito oviposition and survival. If the pondweed canopy enhances oviposition, then the exclosure with the most pondweed cover would receive the most recruitment. Since the pondweed canopy serves as a refuge from fish predation, and since mosquitofish at the Coyote Hills Marsh apparently prefer early-instar *Anopheles* (Fleming and Schooley 1984), an increase in pondweed could cause an increase in the relative abundance of younger larvae.

At the Coyote Hills Marsh, the timing of migration of dabbling waterfowl can influence their value for mosquito control. Densities of *Anopheles* larvae tend to be highest during August, and pondweed standing crop is largest from July through September (Balling and Resh 1984). However, the autumn immigration of waterfowl does not usually begin until October, and the spring emigration preceeds both the formation of a pondweed canopy and the appearance of *Anopheles*. Therefore, to increase the value of waterfowl in mosquito management, efforts might be made to increase the number of waterfowl that are summer residents.

In this regard, differences between the effects of residential and migrating waterfowl on the pondweed canopy should be examined. For example, we have observed that summertime residents create pathways through the pondweed between the marsh margin, where the waterfowl nest, and the foraging grounds at the marsh interior. We have also noted that *Gambusia* can use these pathways to invade the mosquito refuge (Collins et al. 1983). This suggests that the beneficial effects of foraging by dabbling waterfowl may be complemented by other waterfowl activities. Field research is now being conducted to measure how populations of *Anopheles* larvae respond to waterfowl feeding activity that occurs early in the pondweed growth period, and to determine the relative importance of waterfowl pathways for enhancing natural mosquito control. The results of these studies will be useful in the design and management of ecologically diverse marshes that have a reduced potential to support mosquitoes.

ACKNOWLEDGMENTS.- This study is the result of a cooperative effort among personnel of the University of California at Berkeley, California State University at Hayward, the East Bay Regional Park District, and the Alameda County Mosquito Abatement District. Support for this research was provided by University of California Mosquito Research Funds and the Alameda County Mosquito Abatement District.

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DISTRIBUTION AND ABUNDANCE OF TWO MARSH FISH: THE MOSQUITOFISH *(GAMBUSIA AFFINIS)* AND THE THREESPINE STICKLEBACK *(GASTEROSTEUS ACULEATUS)*

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ABSTRACT

In support of an ecosystem simulation model designed for vector control, the distribution and abundance of two significant mosquito predators were studied. They are the mosquitofish (*Gambusia affinis*) and the threespine stickleback (*Gasterosteus aculeatus*). The summer distributions and abundance of each within the Coyote Hills freshwater marsh indicates that the mosquitofish is in relatively low numbers, less than 1 per m^2, in July and increases to 18.6 per m^2 by September. Conversely, the stickleback goes from a high of 27 per m^2 in July to 9.17 per m^2 in September. The vertical distributions of the two species within the dense pondweed were also analyzed. All of the mosquitofish collected were in surface traps while the sticklebacks were confined to mid-water and a few bottom water traps. Finally, a simple method of estimating the density of mosquitofish from a catch per unit effort method is presented. The utility of this population estimate is guarded because of the low correlation between the population density and the catch per unit effort.

INTRODUCTION.-In the operational use of the ecosystem simulation described by Schooley et al. (1982) a variety of biological inputs are required. Many of these inputs are routine measurements of water depth, dominant vegetation, water temperature and density of mosquito larvae (Roberts 1982 and Roberts et al. 1983). One measurement which is not routine for the Alameda County Mosquito Abatement District is the density of fish in general or even the mosquitofish (*Gambusia affinis*). The current simulation model is designed to produce simulations under a wide variety of initial conditions including fish density. One of the key features of the application of the model is that a vector control biologist could survey existing conditions in the marsh, use this as input to the model and make predictions of future conditions for that season and to test various control options such as spraying insecticides or stocking fish.

The study reported here is designed to support the current modelling effort and is broken into three projects. The first is a continuation of the calibration of fish distribution and seasonal abundance in the marsh, begun last season (Schooley 1983). The second is the examination, on a fine scale, of the vertical distribution of mosquitofish and the threespine stickleback (*Gasterosteus aculeatus*). The objectives of this second project were to examine how the density of pondweed alters the distribution of two abundant mosquito larvae predators and determine the proper configuration for minnow traps in estimating fish abundance. The final project reported here is the testing of minnow traps for making quantitative estimates of mosquitofish abundance. This project is patterned after the work done in rice fields (Miura et al. 1982) where mark and recapture population estimates are used in generating a regression of catch per unit effort against mosquitofish density. This will allow the vector control biologists in the future to trap mosquitofish, calculate an average catch per unit effort and then use the regression equation to predict mosquitofish density without the problems of yearly mark and recapture or unit area sampling techniques.

METHODS AND MATERIALS.-__Quantitative Sampling.__ Quantitative fish samples were taken using a hoop net. The hoop net consisted of a 3.8 cm diameter copper pipe formed into a 111 cm diameter hoop which was filled with sand through a fitting in the pipe. The copper hoop was attached by 12 velcro tabs to a rip-stop nylon base with two draw strings sewn into it. This allowed detachment and closure of a cylindrical nylon screen net, of the same diameter, in a purse net fashion. Styrofoam flotation was sewn into the top of the net. This net design was the most practical method available for sampling fish in the dense floating vegetation of the marsh. The hoop net was tossed about 3 meters from a boat, into undisturbed water, sinking immediately. Pondweed *(Potamogeton pectinatus)* was then removed from the center of the net by hand and the bottom of the net drawn closed. Fish were preserved in 10% formalin and later counted, weighed, measured and sexed, in the case of mosquitofish. Samples were taken every three weeks from July through October at four areas in the northern basin of the marsh and two areas in the southern portion of the marsh. At each hoop net location, measurements were taken of the water depth, air temperature, water temperature (surface and bottom), distances to the nearshore, nextshore, boardwalks and open water. Open water is defined as an area of at least one square meter with no vegetation at the water surface. Distances were measured with a double image coincidence hand held range finder. In addition, two pondweed samples were taken adjacent to each hoop sample with a 0.065 m^2 pull-up sampler.

__Vertical Distributions.__ For this microhabitat study, three Gee's minnow traps, lined with galvanized window screen, were placed one meter apart in an equilateral triangle, one on the surface, one at midwater and one on the bottom. Traps were individually suspended from P.V.C.

poles and depth settings were easily adjusted for each trap location by changes in the length of the suspension line. Settings were conducted from a kayak so not to disturb vegetation and bottom mud. Traps were checked after 24 hours for 1-3 consecutive days with 4 trials in August and 8 trials in October. Fish were identified and counted in the field and then released.

Mark and Recapture. Mosquitofish were collected from the freshwater marsh at Coyote Hills for marking and later release. The captured fish were put directly into a 20 gallon holding tank, treated with 2 ppm $KMnO_4$ and 500 ppm NaCl and held for at least 18 hours to allow for acclimation to holding conditions, before marking. Marking procedures followed Vondracek et al. (1980) and Stewart and Miura (1982), with a few modifications. In brief, mosquitofish were marked by spraying them with a fine powdered flourescent pigment under high pressure (175 psi) for 10 seconds. Bottled nitrogen was the compressed air source. Fish were then rinsed immediately with clean water and returned to the treated holding tank. Sprayed fish were held for no less than 4 days, to observe mortality. Marked fish were then separated, counted and released into an enclosure erected in the marsh.

Three 3.65 m by 3.65 m plastic enclosures were constructed in the main marsh. Care was taken not to disturb the vegetation nor scatter the fish from the enclosure area. Boards and styrofoam were used for edge flotation to hold up the enclosure; this flotation system withstood pressure from sitting waterfowl. Rocks secured the plastic to the bottom. Marked fish released into the enclosure were allowed to acclimate for 24 hours before two Gee's minnow traps, lined with galvinized window screen, were set within the enclosure. For each trial, the traps were set at the surface, approximately 2 meters apart, and checked at 24 hour intervals for two consecutive days. Several trials were run in July, August, and October. The standard depth, temperature and pondweed measurements described earlier were taken after each trapping.

Recaptured fish were checked for markings using an observation box equipped with a black light and a small aquarium, as in Stewart and Miura (1982). Marked and unmarked fish were counted and recorded. An estimate of the population density was calculated using the Lincoln Index (Southwood 1978). A regression was then run using the average number of fish per trap (catch per unit effort) as the independent vari-

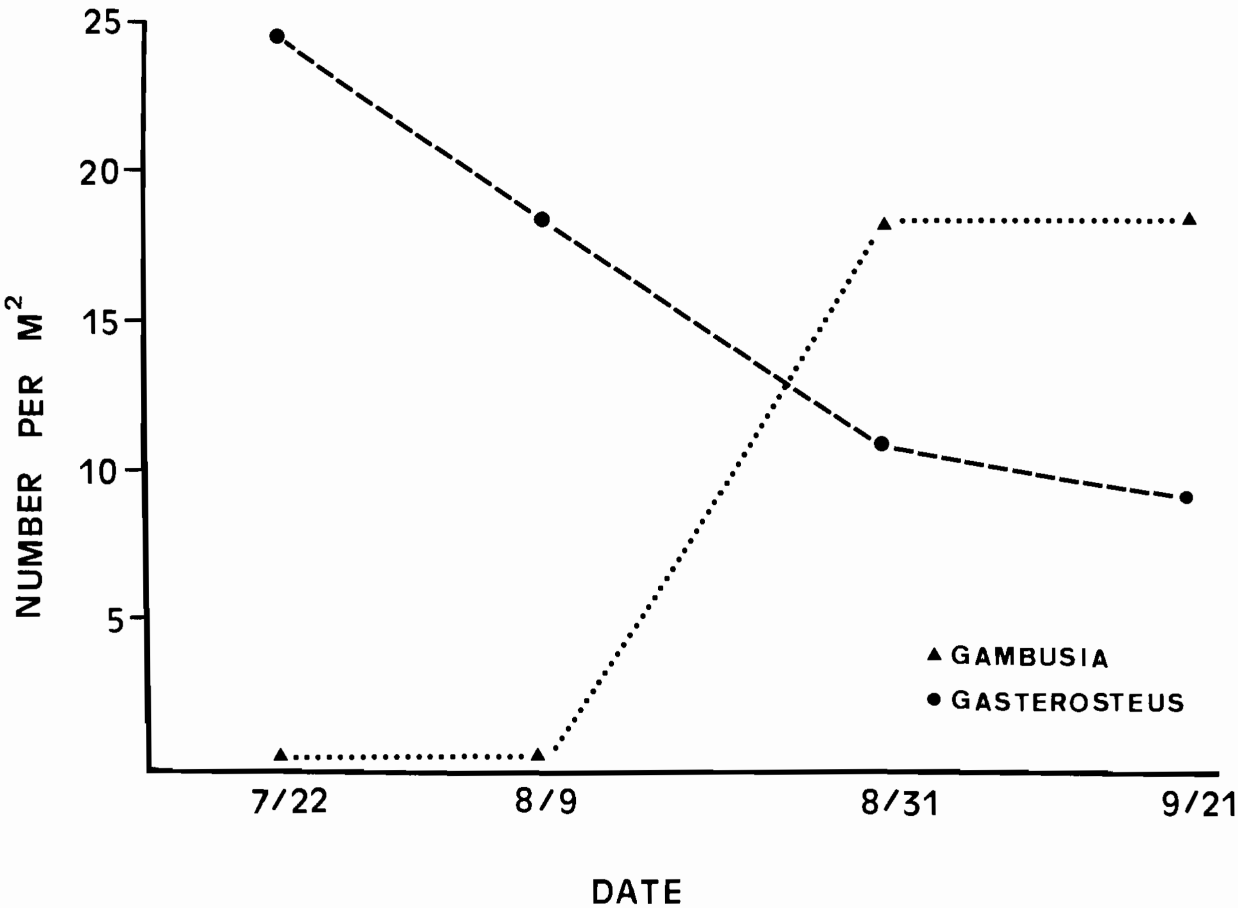

Figure 1. Summer abundances of the mosquitofish (*Gambusia affinis*) and the stickleback (*Gasterosteus aculeatus*) collected by hoop nets.

able and population density as the dependent variable, similar to the index developed by Miura et al.(1982).

RESULTS AND DISCUSSION.-The seasonal patterns of fish abundance show a remarkable shift occurring in mid-August (Figure 1). The mosquitofish show an explosive growth which is not uncommon for this species while the stickleback shows a consistant decrease in abundance from an early summer high. It is interesting to note that, although the shift in relative abundance of the two fish is dramatic, the total of the two only increases from approximately 25 to 27 ind/m^2. In comparison, Miura et al. (1982) report the growth of mosquitofish indigenous to the rice fields near Fresno, California, reached densities of 2.5 ind/m^2 by the end of August during 1980, while *Potamogeton* covered areas of the Coyote Hills marsh supported densities of 18.2 ind/m^2 in August of 1983. In rice fields stocked with approximately 0.16 ind/m^2 in May, total mosquitofish densities reached approximately 17 ind/m^2 by the end of August 1980 (Miura et al. 1980). The Coyote Hills marsh is, therefore, supporting as many mosquitofish as rice fields where they are stocked as a biological control for mosquitoes.

The density of fish during the summer of 1983 was 27 percent lower than summer of 1982 (Table 1). The majority of this decline was due to the much lower density of the Sacramento Blackfish (*Orthodon microlepidotus)*. The two most abundant fish, the mosquitofish and the stickleback, decreased in 1983, but not significantly. We currently have no explanation for the dramatic decrease in the Sacramento blackfish population, nor is it clear how this decrease affects the rest of the community.

In the vertical distribution sampling there were no surprises concerning the distribution of mosquitofish (Moyle 1976). One hundred percent of the fish taken in summer and fall were in surface traps. The stickleback, however, showed a more complex vertical distribution pattern (Figure 2). In July over 70 percent of the fish were trapped at mid-water in the lower edge of the dense *Potamogeton* canopy. In November as the pondweed was rapidly breaking up and sinking to the bottom, the sticklebacks were more equitably distributed than in July, but were still uncommon at the surface. Also of interest is the fact that two fish which were rare in July, sculpin and sunfish, were more abundant in November and were very evenly distributed within the water column (Figure 3). Neither of the latter two species were significant predators on mosquito larvae (Fleming unpublished) but did prey on both mosquitofish and stickleback. The results for the mosquitofish support our earlier assumption that mid-water minnow traps are not needed in censusing mosquitofish populations in mark and recapture studies.

In the mark and recapture study done here, marking mortalities were kept at less than 10% by selection of larger fish for marking. We found in preliminary markings, that smaller individuals (<35mm) and males suffered the highest marking mortalities, these are similar to the results of Stewart and Miura (1982). We, however, worked with smaller sample sizes and could size select our fish prior to spraying to reduce mortality.

The linear regression equation developed gave a rather poor fit (Figure 4). The intended application of this equation was for the estimation of mosquitofish densities in spring when densities are typically very low in systems like the Coyote Hills marsh. The application of this technique in rice fields gave a reasonably good fit to catch data which exceeded an average of 15 fish per trap. Only 25% of our trappings reached this level. At 15 fish per trap the estimated population densities were between 3.7 and 5.9 fish per meter square in the rice fields (Miura et al. 1982). Coyote Hills marsh densities greater than

Table 1. Mean densities of fish during the period August to October, 1982 and 1983.

SPECIES	Density No/m^2 1982	1983
Gambusia affinis (mosquitofish)	12.8	12.5
Gasterosteus aculeatus (threespine stickleback)	16.8	14.8
Orthodon microlepidotus (Sacramento blackfish)	8.4	0.4
Cottus sp. (Sculpin)	2.7	2.8
Cyprinus carpio (carp)	1.1	0.2
Lepomis microlophus (Redear sunfish)	0.4	0.2
Total	42.2	30.9

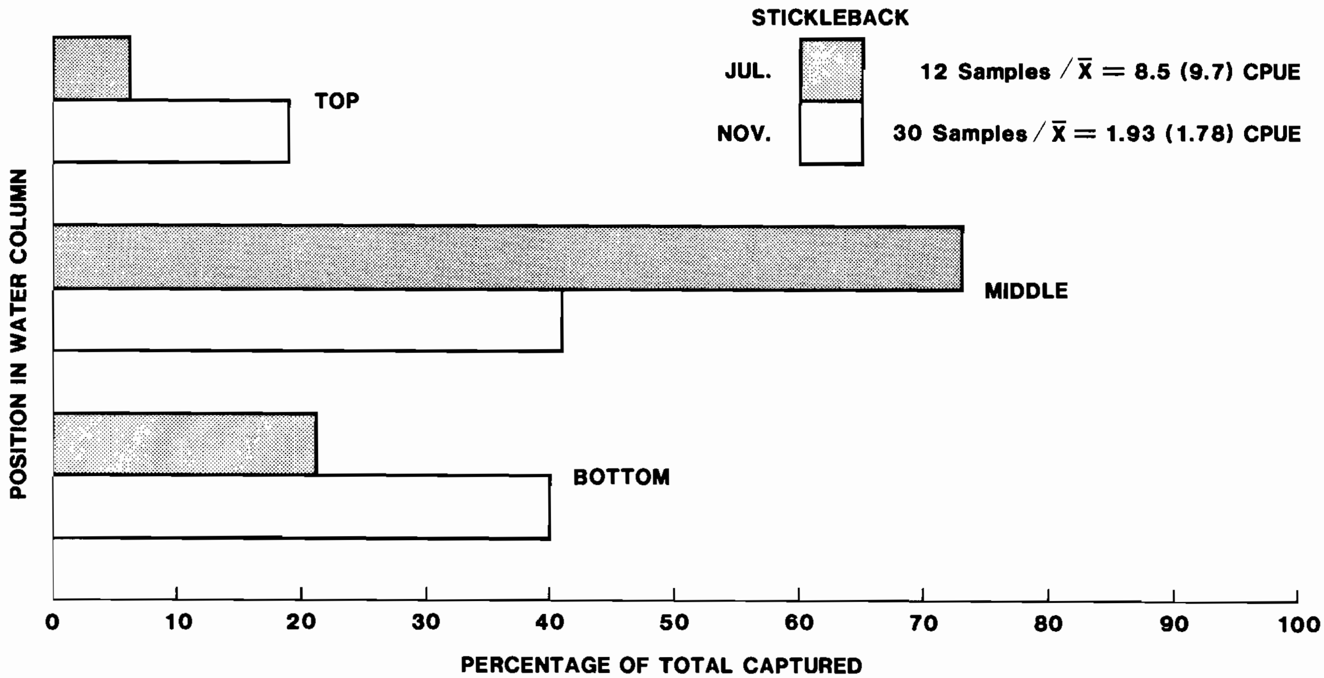

Figure 2. A seasonal comparison of the relative catches of the stickleback in minnow traps at different positions in the water column. ($\bar{x}$ = mean catch per trap or catch per unit effort (standard deviation))

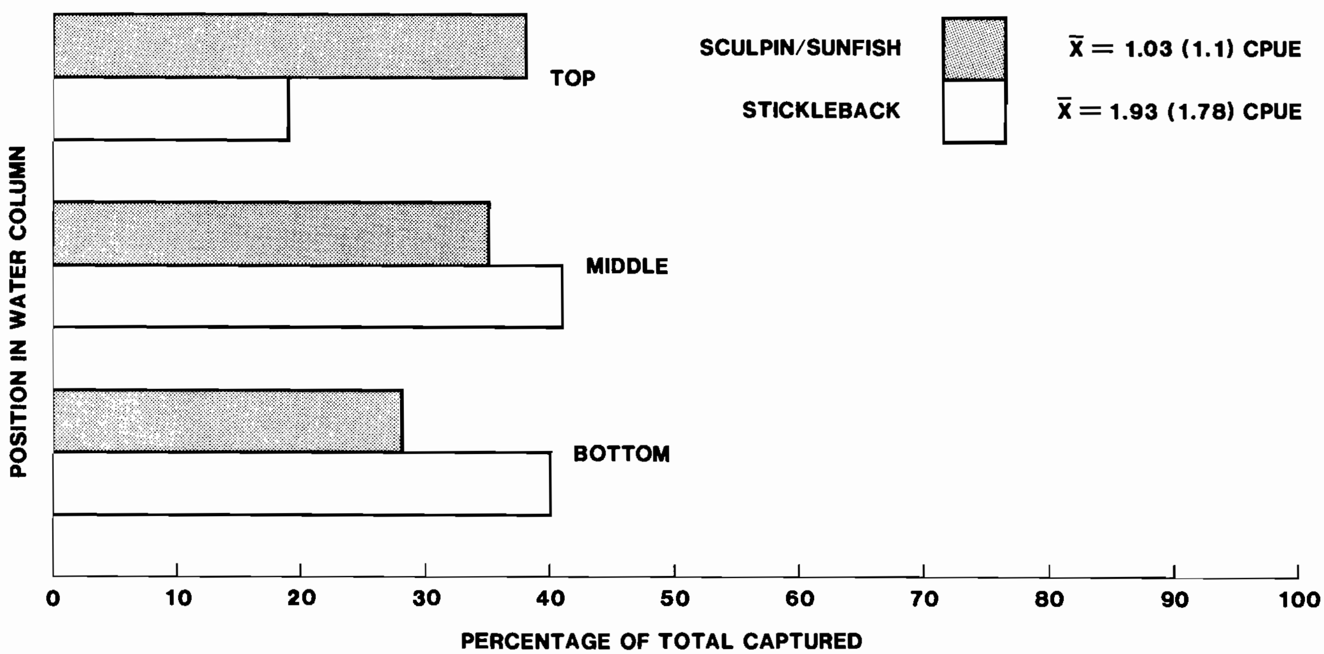

Figure 3. A comparison of the relative catches in minnow traps at different positions in the water column of stickleback verses sculpin and sunfish combined during November. ($\bar{x}$ = mean catch per unit effort (standard deviation))

3.0 fish per meter square were not reached until mid-August. In addition to the normal high variance expected when estimating small populations using mark and recapture techniques, two portions of the design also need to be considered; the size of the enclosures used (13.4 m^2) and the patchiness of pondweed within each enclosure. We believe the "edge effect" was significant in altering fish distributions and that larger enclosures would offset some of these problems. Larger enclosures with an increase in traps per enclosure, would also reduce some of the variablility produced by significant differences in pondweed biomass over a few meters distance. These differences in pondweed biomass produce obvious shifts in the abundance of several invertebrate prey species (Collins et al. 1983, Balling and Resh 1984, and Lamberti and Resh 1984) which can lead to differences in mosquitofish production. In summary, the use of the catch per unit trapping methods are clearly one of the easiest methods available to us for estimating mosquitofish abundance. However, due to the high variance at low densities it will probably give more accurate estimates of fish densities later in the season than it will in the spring.

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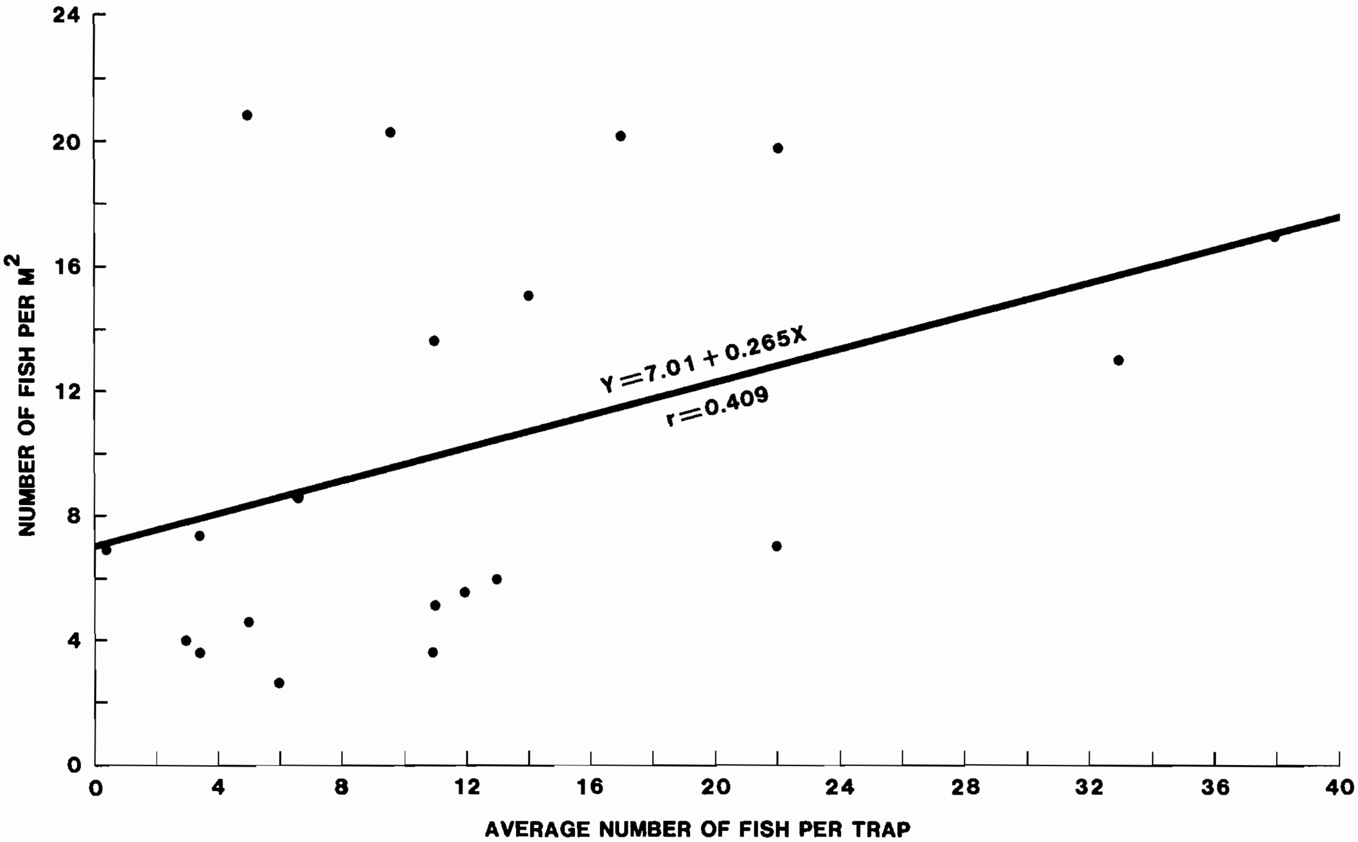

Figure 4. Mosquitofish density as a function of average catch per unit effort from the mark and recapture study.

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FORAGING PATTERNS AND PREY SELECTION BY MARSH FISH

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ABSTRACT

Prey selection and feeding electivity in the mosquitofish (*Gambusia affinis*) and the threespine stickleback (*Gasterosteus aculeatus*) were examined during the summer of 1983, in the Coyote Hills Freshwater Marsh, Fremont, California. One of our objectives was to study the effect the fish had on mosquito populations.

In both species, intraspecific prey selection changed over the summer. These changes, however, had little effect on the interspecific prey selection differences, which were dramatic. The mosquitofish diet was composed of a pleuston (i.e. adult diptera and gerrids) and nekton portions. Averaged over the summer, the nekton portion of the mosquitofish diet consisted of: 43.44% cladocera; 27.66% copepods; 13.31% chironomids; 5.08% culicid larvae; 1.13% corixid adults; and 9.38% others. Stickleback, which fed only on nekton prey, had a diet of: 48.24% chironomid larvae; 35.26% cladocerans; 15.66% copepods; .33% ostracods; .12% culicid larvae; and .39% others.

Electivity values for the mosquitofish indicate significant selection for mosquito larvae. Selection for the mosquito larvae increased during the month of August. This increase in electivity values corresponded with increases in the pondweed (*Potamogeton pectinatus*), in the density of mosquito larvae and in the percentage of the mosquitofish diets made up by pleuston.

Stickleback had insignificant electivity values for the mosquito larvae. Mosquito larvae were therefore taken in proportion to their abundances. In August, larval and pupal chironomids made up 85.66% of the stickleback diet. By September the numbers of chironomids found in the guts declined to 10.82% of the total intake. Correspondingly the number of cladocerans increased. Of particular interest was the change in the electivity values of these prey. The electivity value for chironomids changed from zero to significantly negative; for cladocerans the electivity value went from zero to significantly positive. These changes correspond to a dramatic decrease in the chironomid population. The fact that the electivity values have changed are consistent with a switching behavior between the two prey items.

EFFECTS OF PONDWEED DENSITY ON PREDATOR EFFICIENCY: *GAMBUSIA AFFINIS* AND *GASTEROSTEUS ACULEATUS* AS PREDATORS ON *ANOPHELES OCCIDENTALIS* LARVAE

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ABSTRACT

Previous work in the Coyote Hills Freshwater Marsh had shown a negative correlation between the presence of pondweed, *Potamogeton pectinatus,* and the mosquitofish *Gambusia affinis*. One hypothesis to explain this distribution pattern was that the pondweed was negatively affecting the mosquitofish's ability to locate and capture surface prey.

Our experiments were designed to test implications of this hypothesis, and to cross compare pondweed effects on *Gambusia affinis* with *Gaterosteus aculeatus,* the threespine stickleback, another very common fish in this system. The experiments reported here used early instars of *Anopheles occidentalis* as the surface prey.

Six runs were conducted with each fish species. A run entailed placing three fish and fifty anopheline larvae into one of six brown plastic tubs, each containing a different amount of pondweed. Pondweed densities corresponded to 0, 10, 25, 50, and 100% maximum densities found in the marsh. A run lasted 24 hours, at the end of which the fish were removed and the surviving larvae counted. Significant results from our testing show a linear relationship between increasing pondweed density and decreasing predator efficiency. Both fish were adversely affected by the pondweed with the stickleback showing the greater effect. At all levels of pondweed density the stickleback had more trouble capturing anopheline larvae than did mosquitofish.

The implications these results have on the Coyote Hill Marsh Models are: (1) that the assumption of a linear interference by pondweed on mosquitofish predation of mosquito larvae is reasonable and (2) that the two most abundant vertebrate predators on mosquito larvae are affected differentially by the presence of pondweed.

TOXICITY OF *MYRIOPHYLLUM* AND *POTAMOGETON* TO MOSQUITO LARVAE

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ABSTRACT

Extracts of the common pond weeds *Myriophyllum spicatum* and *Potamogeton pectinatus* were individually assayed against first and second instar larvae of field collected *Anopheles occidentalis* and laboratory reared *Culex pipiens.* When larvae were exposed to increasing organic extract concentrations of 3.2 mg/100 ml H_2O, 6.4 mg/100 ml H_2O, 9.58 mg/100 ml H_2O and 12.75 mg/100 ml H_2O, various levels of cumulative mortality to pupation were observed.

An. occidentalis larvae collected at Coyote Hills East Bay Regional Park, Fremont, Ca., a fresh water marsh dominated by *Potamogeton*, were assayed against *Potamogeton* extract collected at Coyote Hills. Duncan's Multiple Range Test (P=0.01) showed larval mortality at all concentrations of extract to be significant. Larvae of *An. occidentalis* from Coyote Hills were assayed against *M. spicatum* collected from quarry ponds in Livermore, Ca. Cumulative mortality at all concentrations was significant and cumulative mortality in the pond water control group, one of two controls, the other being distilled water with appropriate solvents, also showed significant mortality. *Culex pipiens* showed resistance at all concentrations of both extracts except at 9.58 mg/100 ml H_2O. While the two highest concentrations were not significantly different from each other, the highest was not significantly different from the control.

An. occidentalis larvae collected at Livermore, Ca., showed a differential susceptability toward the larvacidal activity of *Potamogeton* from Coyote Hills. Assays at 6.4 mg/100 ml H_2O were within four percent of the mortality shown by Coyote Hills larvae, but assays at 3.2 mg/100 ml H_2O were significantly lower than for mosquitoes from the Coyote Hills, but were significantly different from the controls. Perhaps the most interesting and important finding in this work is that Coyote Hills *An. occidentalis* exhibit a susceptability to toxins present in pond water samples from *M. spicatum* dominated ponds. There is a strong similarity between mortalities observed in pond water and those observed in extract concentrations of 6.4 mg/100 ml H_2O. This finding suggests that further work should be directed toward determining whether there is release and accumulation of toxins during an annual cycle, and whether the natural accumulation of hydrophyte toxins during an annual cycle, and whether there is a natural accumulation of hydrophyte toxins is sufficient to reduce mosquito populations.

PRELIMINARY INVESTIGATIONS ON ADULT *ANOPHELES OCCIDENTALIS* ACTIVITY AT COYOTE HILLS MARSH[1]

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ABSTRACT

Studies on the activity of adult *Anopheles occidentalis* in the Coyote Hills Marsh area are reported. Adults reached peak numbers in August and declined thereafter and were not found after mid-October. There was a positive correlation between the numbers of larvae surveyed in the marsh and the numbers of adults recovered in red boxes. Preliminary parity studies indicate that the majority of females undergo a single gonotrophic cycle.

INTRODUCTION.-This paper summarizes studies on adult *Anopheles occidentalis* populations in the Coyote Hills Marsh. This work is being conducted as part of a large research effort whose objectives are to develop a comprehensive model of the anopheline interactions in the marsh ecosystem (Roberts et al. 1983). The information reported here was from studies started in the late spring of 1983 and is therefore only preliminary. The major objectives of this phase of the project is to describe the population dynamics of adult anophelines produced in the marsh system.

MATERIALS AND METHODS.-Adult *Anopheles occidentalis* activities were monitored with New Jersey light traps and with red boxes (dimensions 1 cubic foot) at stations set up in the vicinity of the marsh. The light traps were located in suburban areas two miles due east and three miles northeast of the marsh. They were operated on a weekly basis from mid-April through the first week in November. These traps have been operated at these sites for several years before the start of this study. One of the major purposes for these traps was to monitor *Anopheles* encroachment into residental areas.

Four areas relatively close to the marsh were chosen as sites for red box collection stations. A set of three boxes were hung about three feet from the ground in willow trees bordering the southern edge of the marsh at intervals of about 25 meters. Another set of three were positioned in a similar manner in willows along the eastern perimeter of the park, a distance of about 400 meters from the marsh. The final groups were placed on the ground on sloping rocky terrain. One group of four boxes were placed under low shrubs (*Baccharis* sp.) about 100 to 150 meters from the marsh while the remaining five boxes were placed behind the Park Headquarters under California laurel (*Umbellularia californica*) approximately 200 meters from the marsh. Mosquitoes were collected from the boxes with a modified portable vacuum sweeper either once or twice weekly from mid-July to November and then counted and identified in the laboratory. Adult females were examined for the presence of blood and developing eggs. A total of 136 females were dissected to determine parity status. Larval populations in the marsh were assessed by taking 10 dips once a week and averaging the results.

RESULTS AND DISCUSSION.-*Anopheles occidentalis* were only found in light trap collections on two occasions during 1983. A single male was collected in mid-June from the trap two miles east of the marsh and a single female during the last week in August in the trap located three miles northeast. This would suggest than *An. occidentalis* rarely move distances of two miles or more. However, an intermediate light trap station to be located approximately one mile from the marsh and another adjacent to it planned for this season may give us a better understanding of the pattern of movement for this species.

The use of red boxes proved satisfactory for monitoring *An. occidentalis* at the marsh site. Figure (1) shows the relationship between larval densities and the recovery of adults from red boxes. There was a positive correlation between the numbers of both sexes found in the boxes and the number of larvae surveyed in the marsh. The four small peaks observed for males suggests that at least four generations occurred from July to October; however, this cannot be supported statistically. Boxes positioned on the sloping terrain to the south of the marsh contained the greatest number of mosquitoes. A total of 1,109 adults in about an equal sex ratio were retrieved between July and mid-October. Four boxes on the south slope accounted for over 75% of the adult mosquitoes recovered.

Table (1) shows the results of ovariole dissections. Unfortunately, these analyses were started late in the season and only 136 females were examined. These preliminary results indi-

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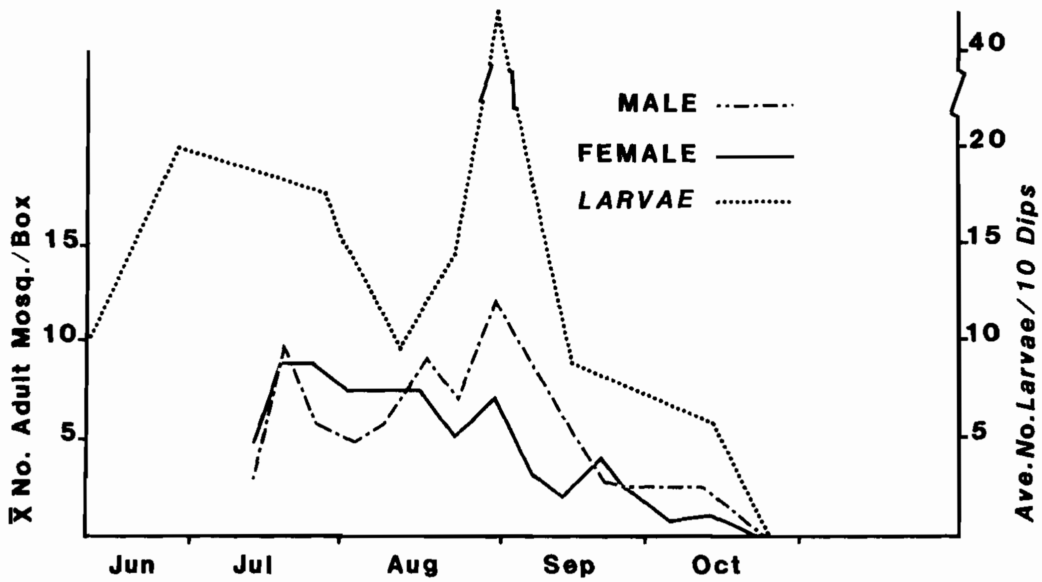

Figure 1. Comparison of adult and larval collections of *An. occidentalis* in Coyote Hills Marsh, Alameda County, 1983.

Table 1. Gonotrophic cycles of *An. occidentalis* Coyote Hills Marsh, Alameda County, Calif. 1983.

	Aug	Sept	Oct	Fat Body Develop.
Nulliparous	24	4	6	0
Parous-1	22	30	2	-
-2	0	1	0	-
-3	0	0	0	-
Gravid	24	22	1	-
Total	70	57	9	0
% Parous	66%	93%	33%	-

cate, however, that the majority of females undergo only a single gonotrophic cycle indicating that the lifespan of adults is probably not long. Much more data is required, however, before any conclusive statements regarding gonotrophic patterns and physiological age can be made for these populations of *Anopheles*.

CONCLUSION.-These preliminary studies indicate that *Anopheles occidentalis* was the only anopheline utilizing the marsh in 1983. Several generations are produced during the summer with peak populations occurring in August. Larvae and adults were not observed after October. Examination of a relatively few females indicate that they undergo a single gonotrophic cycle during their life near the marsh. Further research is necessary to verify the preliminary information presented in this paper.

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FACTORS AFFECTING MOSQUITO LARVAL ABUNDANCE IN NORTHERN CALIFORNIA RICE FIELDS

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INTRODUCTION.-Rice fields in the Sacramento Valley are major breeding sites of *Culex tarsalis* and *Anopheles freeborni* in northern California. A variety of studies have noted that the density of mosquito larvae varies significantly from field to field (Case and Washino, 1979). It has yet to be shown what factors might be important for enhancing oviposition or larval survival or both.

Potential factors can be grouped into three categories--biological, chemical and physical. Biological factors include the presence of predators and the interaction of the mosquito larvae with other organisms in the rice field ecosystem. Chemical factors include variations in ion concentrations in the water and the timing and application rates of herbicides and pesticides. Physical factors include water source, rice height, water depth, water temperature and field preparation.

MATERIALS AND METHODS.-Fields in south Sutter County were monitored once per week from June 20 until September 19 or when the water was turned off. Each field was dipped by 3 people spaced 20 meters apart, taking 3 dips with a one pint mosquito dipper in each spot. Dips were repeated for 20 stops at 5 meter intervals on a transect perpendicular to the access road and parallel to the levee. Mosquito larvae per 3 dip interval were recorded as to species and number per instar. Samples were then put through 200 micron mesh screens, placed in ½ gallon buckets and transported back to the lab in a styrofoam cooler. Estimates of zooplankton populations and abundance of other fauna were recorded within 24 hours.

At the time of collection, the water temperature (including maximum and minimum for the week), rice height above water and water depth were recorded. Water samples for chemical analysis (pH, Ca, Mg, Na, Cl, SO_4, NO_3, PO_4 and B) were taken every other week and analyzed by Land, Air and Water Resources Soil Extension, UC Davis. On alternate weeks, invertebrate predators were collected and immediately frozen in dry ice, to be tested by EIA for presence of mosquito larvae in gut contents. This work is in cooperation with Dr. C. H. Tempelis, UC Berkeley.

Table 1. *Culex* collections in fields with and without *Mesostoma*.

Sampling period: flatworms	Sampling period: *Culex*	Fields with flatworms[a]	Fields without flatworms[a]
7/4	7/4	10.7 ± 17.1 (6)	31.9 ± 45.6 (14)
	7/11	5.2 ± 8.0 (6)	29.4 ± 36.1 (14)
	7/18	9.8 ± 20.8 (5)	62.3 ± 90.8 (11)
7/11	7/11	22.5 ± 49.4 (6)	21.9 ± 24.0 (14)
	7/18	79.3 ± 127 (4)	34.8 ± 59.8 (12)
	7/25	42.3 ± 70.1 (6)	26.3 ± 30.9 (12)
	8/1	26.8 ± 47.7 (6)	28.3 ± 41.3 (12)
7/18	7/18	21.7 ± 23.7 (3)	51.5 ± 86.8 (13)
	7/25	27.0 ± 38.4 (3)	37.1 ± 50.9 (13)
	8/1	11.7 ± 20.2 (3)	26.1 ± 38.1 (13)

[a] Mean number of larvae per 180 dips ± S.D. (fields sampled).

Growers were asked to submit pesticide and herbicide application records which are still being collected. The information obtained from them will be compared to fluctuations in the mosquito populations over the season.

RESULTS.-Flatworms have been suggested as being important predators of *Culex tarsalis* larvae in the rice fields (Case and Washino, 1979) and *Mesostoma* nr. *lingua* seems to be the predominant flatworm present in the rice fields. Therefore, fields were evaluated as to whether there was a difference in the number of *Culex* larvae collected in the presence or absence of *Mesostoma*. In addition to looking at the number of *Culex* for the same time period as when the fields were determined to be ± flatworms, it was also of interest to investigate a possible delayed predation effect (Table 1). For the three time periods evaluated, there was no significant difference in the number of *Culex* collected between fields with and without flatworms. This was true for 4 weeks after the +/- flatworm determination.

Further discussion in this paper will be limited to physical factors. Continuing an evaluation of the hypothesis of Collins and Washino (1979) that new fields were supposed to support higher populations of *Culex tarsalis* larvae, data were compared for new fields (first year planted in

Table 2. Larval collections in new (first year to be planted in rice) and old (greater than 1 year planted in rice) fields.

Collection	*Culex tarsalis*		*Anopheles freeborni*	
week	New[a]	Old[a]	New[a]	Old[a]
6/20	1.0 ± .82 (7)	6.8 ± 12.0 (6)	1 ± .37 (7)	.67 ± 1.63 (6)
6/28	13.6 ± 15.1 (9)	14.1 ± 18.8 (9)	.67 ± .71 (9)	1.3 ± 2.1 (9)
7/4	16.5 ± 17.7 (10)	34.6 ± 53.7 (10)	2.2 ± 2.6 (10)	3.6 ± 7.9 (10)
7/11	28.4 ± 36.04 (10)	15.8 ± 28.3 (10)	12.5 ± 13.6 (10)	8.5 ± 16.1 (10)
7/18	63.1 ± 90.6 (8)	28.6 ± 67.1 (8)	*53.5 ± 36.9 (8)	12.2 ± 28.2 (8)
7/25	*50 ± 57.7 (8)	17.6 ± 29.4 (10)	30 ± 34 (9)	18.4 ± 19.4 (10)
8/1	37.8 ± 42.3 (9)	16 ± 38.6 (10)	*128. ±114.8 (9)	16.5 ± 16.4 (10)
8/8	25.7 ± 36.1 (9)	6 ± 8.9 (10)	239.7 ±300.6 (9)	69.3 ± 66.8 (10)
8/15	17.9 ± 45.3 (10)	1.2 ± 3.5 (10)	168.7 ±222.4 (10)	80.1 ± 49.3 (10)
8/22	5.1 ± 12.7 (10)	3.36 ± 6.74 (11)	145.7 ±179.3 (10)	57.45 ± 27.2 (11)
8/29	6.3 ± 19.6 (10)	.3 ± .7 (10)	156 ±155.4 (10)	62.2 ± 41.7 (10)
9/5	4.25 ± 11.2 (8)	3 ± 8.5 (8)	*229.7 ±170.0 (8)	87.25 ± 75.4 (8)

[a] Mean number of larvae per 180 dips ± standard deviation (fields sampled).

* Significant difference between mosquito populations from new and old fields at the .05 level using 2 tail student t-test.

rice) and old fields (more than one year in rice). New fields (Table 2) had a significantly greater number of *Culex* larvae during only one sampling period (7/25). *Anopheles* populations were significantly larger in new fields during the 7/18, 8/1 and 9/5 sampling periods. It is interesting to compare this with 1982 when there was no significant difference for either *Culex* or *Anopheles* collections between new and old fields.

Another theory of Collins and Washino (1979) was that well-water irrigated fields would yield more *Culex* larvae than surface or ditch-water irrigated fields. From the 1983 data there was no difference in *Culex* collections from the different water sources. The *Anopheles* collections, however, were greater in the well water fields for three sampling periods. This contrasts with 1982 when *Anopheles* larval densities were greater in well water fields July to mid-August, but lower in late August to early September.

Increased laser leveling of rice fields has raised the question of how this will affect mosquito populations. This year there was no difference between regular fields and laser fields for either *Culex* or *Anopheles*. In 1982 there were more *Culex* in laser planed fields.

Neither rice height nor minimum water temperature seemed to influence the number of larvae collected. Greater numbers of *Anopheles* were collected in fields with lower maximum water temperature (<27°C) (Fig. 1); greater numbers of *Culex* were collected in fields with higher maximum temperature (>39°C) (Fig. 2). Fields with deeper water (>20 cm) had more *Anopheles* than shallower fields (Fig. 3). Water depth did not seem to be associated with *Culex* abundance.

There were a few fields that stood out as being unusual in the high numbers of *Culex* collected, especially late in the season. High numbers of *Culex* were collected from these fields in late August through early September. In other fields, *Culex* peaked in mid-July and leveled off to low numbers for the remainder of the season. These were organic fields (no herbicides or pesticides) and were drained and dry for three weeks in mid-season (7/18-8/1).

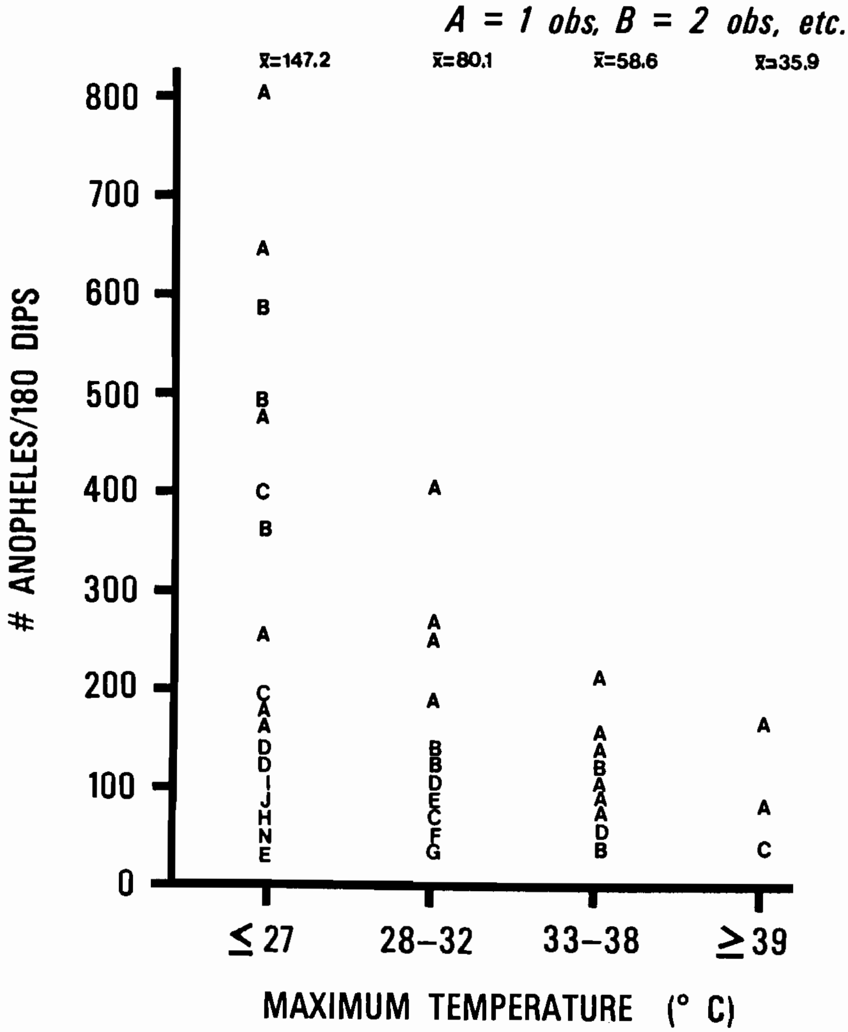

Figure 1. Association of *Anopheles freeborni* larval collections and maximum water temperature.

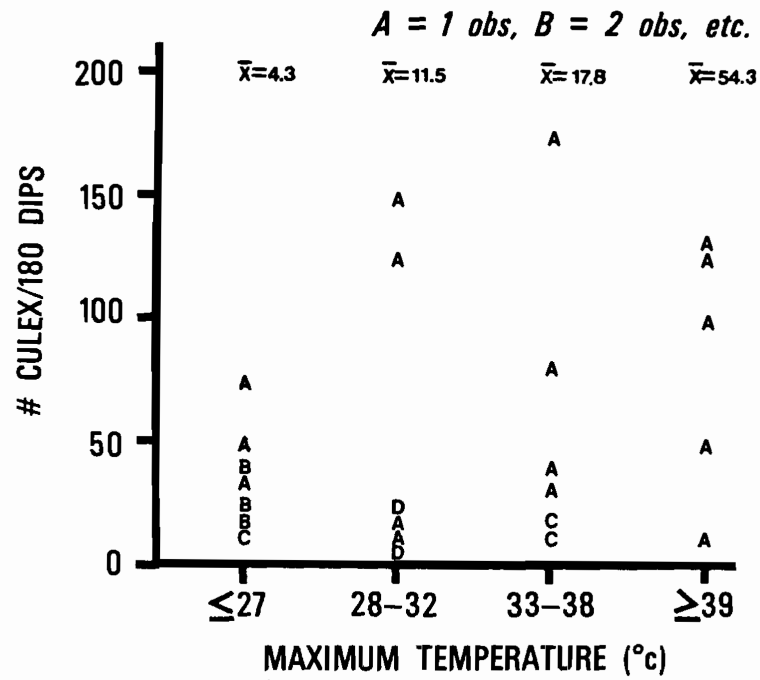

Figure 2. Association of *Culex tarsalis* larval collections and maximum water temperature.

They had a high chloride ion concentration (mean 2.06 me/1) compared to other fields (mean 0.39 me/1). The calcium level was also higher than for other fields. It would be interesting to further evaluate these fields for other factors that might be influencing mosquito larval abundance.

DISCUSSION.-The lack of continuity from 1982 to 1983 in population trends related to field age, water source and laser leveling limits the usefulness of these factors for predicting larval abundance. The association between temperature and larval abundance warrants further investigation including laboratory studies attempting to discern the effect of temperature on oviposition and larval survival. The relationship between water depth and *Anopheles* abundance may be a reflection of the temperature relationship, as deeper fields would be expected to have less temperature fluctuation and hence lower maximum temperature.

ACKNOWLEDGMENTS.-We are grateful for the assistance of G. Kauffman at Sutter Yuba MAD. These studies were supported in part by Special Mosquito Augmentation Fund, University of California, and Research Grant RF-83-543, U.S. Department of Agriculture, Science and Education.

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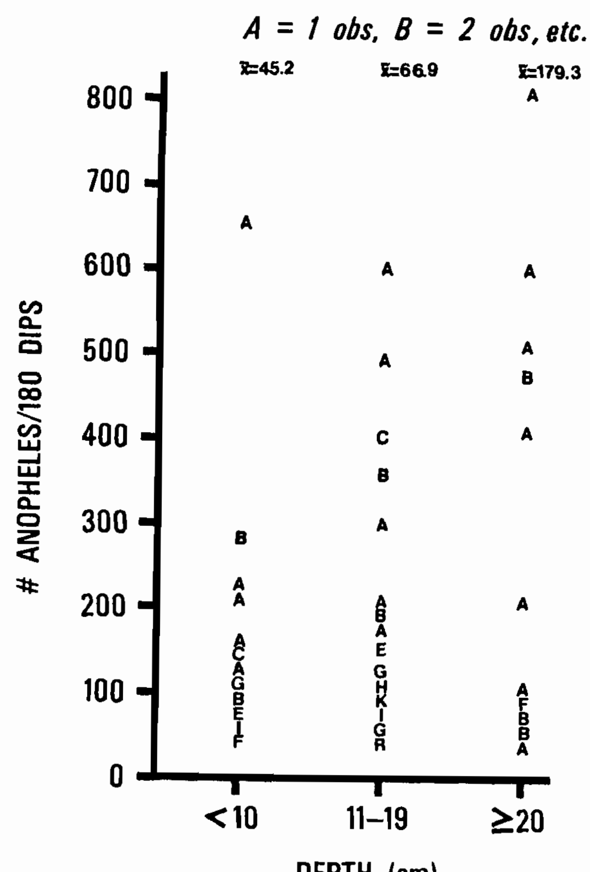

Figure 3. Association of *Anopheles freeborni* larval collections and water depth.

AGE STRUCTURE AND SURVIVORSHIP OF *CULEX TARSALIS* IN CENTRAL CALIFORNIA RICEFIELDS

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ABSTRACT

Sampling of immature populations of *Culex tarsalis* in rice fields of Fresno and Kings Counties was carried out to determine age structure and stage-specific survivorship estimates. Counts of each stage were converted to absolute density estimates and divided by the time duration of that stage and plotted against time in days to development. A modified k factor analysis was performed on selected data for comparison of different time periods and field situations.

INTRODUCTION.-In mosquito control programs it is important to know what counts of immature stages will represent in terms of expected adult production. Many factors affect immature survival and result in corresponding adult emergence. This study was conducted to determine survivorship rates of immature stages of *Culex tarsalis* in some rice fields of central California.

The life table approach to population study allows one to summarize survival and mortality rates. A preliminary step in life table construction is the determination of age distribution. Once the age distribution is coupled with stage duration, stage specific survival estimates can be made. Varley and Gradwell (1970) emphasize that, for this approach, it is best to use continuous and intensive sampling techniques in a single habitat rather than sampling different populations in similar habitats over several years. Our approach was to sample two different fields intensively and continuously to gain information on population dynamics.

MATERIALS AND METHODS.-The immature populations of *Culex tarsalis* were sampled from two different rice fields in central California. In 1982 a large field (90 ha) was sampled in Kings County and in 1983 a smaller field (8 ha) in western Fresno County. The pint dipper (473 ml capacity) was used for sampling larvae and pupae. Sampling in Kings County took place from August 23 to September 21. Some samples were counted in the field while others were concentrated and taken to the laboratory for counting. Four transects were made across the field with either 50 or 100 samples per transect over the sampling period. The field in Fresno County was sampled from July 13 to August 24. Sampling was almost daily until no more immatures were found. A set number of 60 samples was taken in a section of the field where presampling had indicated a concentration of *Cx. tarsalis* immatures. All samples from 1983 were concentrated and taken to the laboratory for counting. In the lab, the samples were sorted and identified to stage under a microscope.

The immature sampling data was analyzed for time-specific survivorship using the methods of Service (1976). Stage duration was developed from data collected in 1982. Survivorship curves were constructed with methods described in Service (1976) and Southwood (1978). Estimates of absolute population densities were made from conversions of mean dipper counts according to Stewart and Schaefer (1983). A modified k factor analysis was performed on selected data following Varley and Gradwell (1960) and Southwood et al. (1972).

RESULTS AND DISCUSSION.-Figure 1 shows the mean dipper counts of *Cx. tarsalis* from 1982 sampling. There were four mosquito control treatments applied during the season on this field. The first two were with Bti followed with one of parathion and a final treatment with Bti on August 20. After a population reduction due to the second Bti treatment and later parathion treatment, we thought we might be able to follow development time in the field. The third treatment with Bti gave a good starting point for a new generation of immature stages. The bracketed segment A in Figure 1 shows the period of time over which data from collected samples shown in Table 1 was taken. Table 1 shows the results of sampling and was the basis of stage duration estimation. Days of stage duration were estimated to be: 2.0 for I, 1.5 for II, 1.9 for III, 3.2 for IV and 1.9 for pupae. These results are consistent with those reported by Bailey and Gieke (1968) and Rosay (1972).

Figure 2 shows a survivorship curve using data from 1983 sampling. The curve results from a plotting of the number of each stage (in meter square values) divided by the appropriate stage duration against age in days of the immature stage. A smooth curve was fitted through the midpoints of the stage histograms. In Figure 3, a similar curve is shown for Kings County data. It can be seen in these curves that the histograms representing I stage larvae is smaller than those of II stage larvae. This seems to represent an error in sampling yet the 95% confidence interval for I stage larvae (as shown in Figure 2) is relatively wide. Also, *Cx. tarsalis* is a species with overlapping generations and asynchronous egg laying. Eggs are laid in rafts which can lead to a clumped distribution pattern and higher sampling variance.

The mean dipper counts over the sampling season in 1983 are shown in Figure 4. There were no control measures applied to this field for mosquitoes other than early season planting of

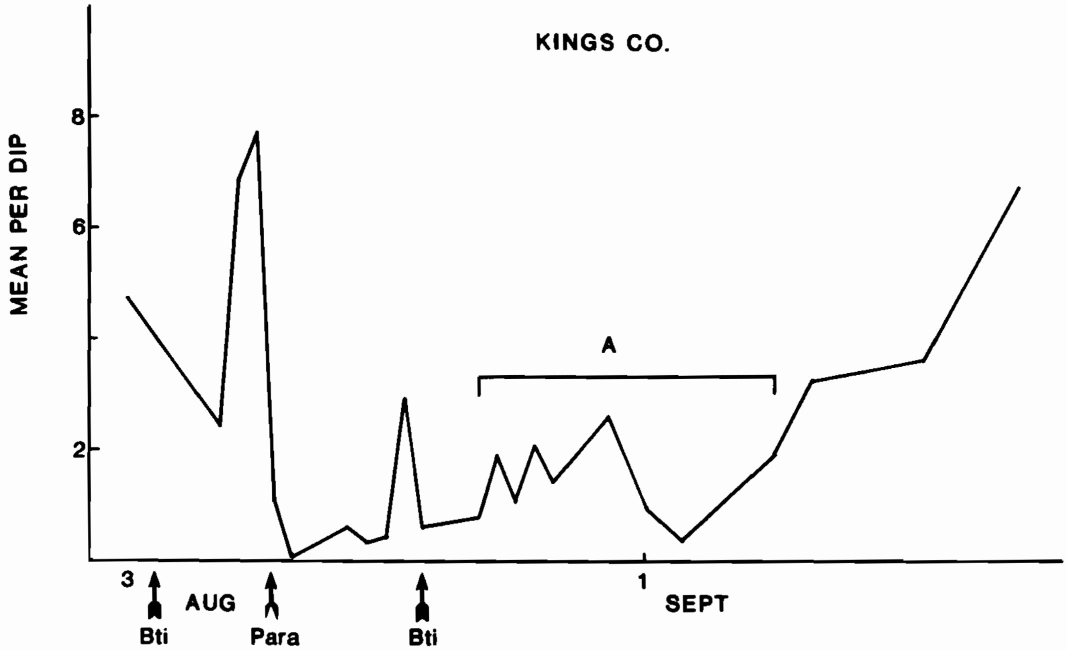

Figure 1. Mean dipper counts of *Culex tarsalis* from 1982 sampling. Counts are combined totals of all immature stages. The bracketed area shows the period of most intensive sampling.

Table 1. *Culex tarsalis* dipper samples from 1982 in a Kings County rice field. The counts are expressed as percent of total for each day. The diagonal line approximately represents the development time of one immature generation.

Date		Stage I	Stage II	Stage III	Stage IV	Stage P
August	20	Treatment with Bti				
	21					
	22					
	23	49.1	50.0	0.9	0	0
	24					
	25	39.5	38.4	22.1	0	0
	26	48.6	18.5	16.4	9.6	0
	27	37.4	12.2	33.9	16.5	0
	28	5.2	32.8	37.9	24.1	0
	29					
	30	19.4	18.5	16.9	34.7	10.5
	31	11.7	10.8	12.6	53.2	11.7
September	1	33.3	2.9	0	39.1	24.6
	2	3.6	0	10.7	75.0	10.7
	3	11.1	8.3	2.8	33.3	44.4
	4	2.3	22.7	2.3	54.5	18.2

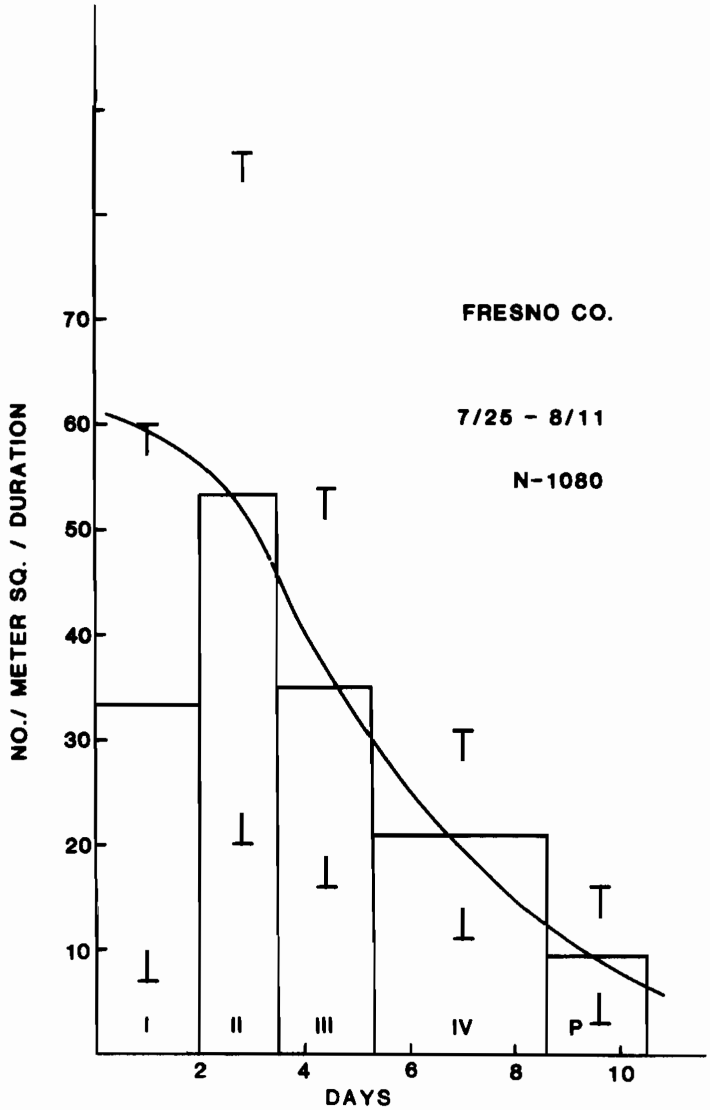

Figure 2. Age distribution and survivorship curve for the immature stages of *Culex tarsalis*. 95% confidence limits of the mean total frequencies are indicated for each stage.

mosquitofish. The population of immature mosquitoes was sampled until dipper counts reached zero in mid August. The bracketed areas shown in Figure 4 represent times when the population was either at its peak or into a decline. These periods were used for k factor analysis of the figures from survivorship estimates.

Table 2 represents calculated k values for 1982 and 1983 data. In this table, values for I and II stage larvae were combined to ensure positive values for k analysis. From July 14 to July 31, 1983, when the population was peaking, the K value is higher than for the period of July 25 to August 11 when it was declining. Approximately equal time spans are compared to cover one generation and keep uniformity. The decline in mortality was not great but shows that the population decline was probably related to factors other than larval mortality. The overall K value for the 1982 sampling in Kings County is lower than that for the Fresno County field. The field in Kings County had a higher overall field population of *Cx. tarsalis* but also a smaller predator complex.

The development of survivorship estimates and mortality comparisons can be important in the description of population dynamics of *C. tarsalis* in rice fields. Larval densities in and of themselves do not realistically describe potential adult populations or mortality trends.

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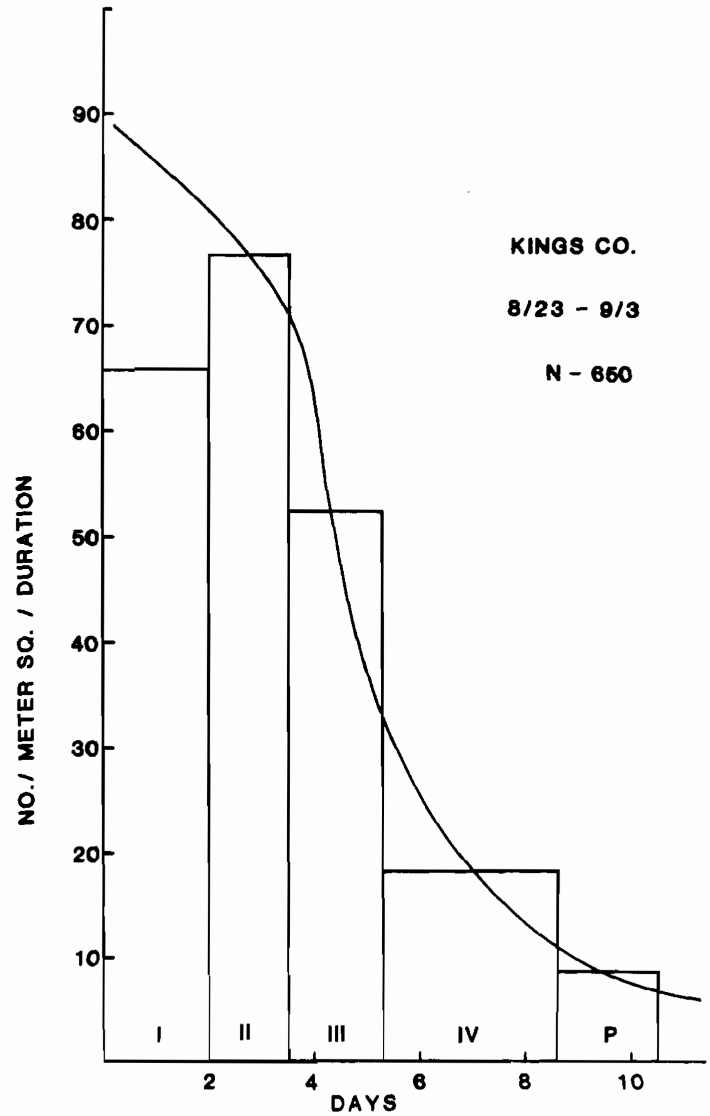

Figure 3. Age distribution and survivorship curve for the immature stages of *Culex tarsalis.*

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Table 2. K values of *Culex tarsalis* from rice fields in Fresno and Kings Counties.

	Stage	No/m²/duration	Log	k
		Fresno County Field		
July 14 to July 31	I & II	161.43	2.2080	0.5298
	III	47.66	1.6782	0.3144
	IV	23.11	1.3638	0.4596
	P	8.02	0.9042	K=1.3038
July 25 to August 11	I & II	86.20	1.9355	0.3909
	III	35.04	1.5446	0.2240
	IV	20.92	1.3206	0.3493
	P	9.36	0.9713	K=0.9642
		Kings County Field		
Aug 23 to Sept 21	I & II	142.50	2.1538	0.4351
	III	52.33	1.7187	0.4560
	IV	18.31	1.2627	0.3143
	P	8.88	0.9484	K=1.2054

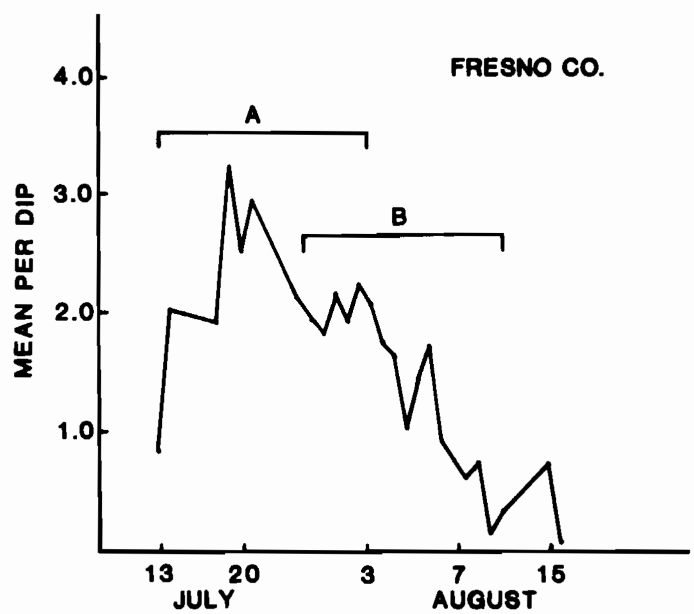

Figure 4. Mean dipper counts of *Culex tarsalis* from 1983 sampling. Counts are combined totals of all immature stages. The bracketed areas indicate periods of greatest significance.

PREDICTING *CULEX TARSALIS* ABUNDANCE IN KERN COUNTY

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The question has been asked at encephalitis virus surveillance meetings and workshops: how can we combine climatological data with historical abundance patterns to predict the size of *Culex tarsalis* populations? Time series analyses (Hacker et al. 1973), stochastic probability models (Moon 1976) and thermal summation models (Toscano et al. 1979, Ring and Harris 1983) have been used in recent efforts to forecast insect abundance. The present analysis utilized step-wise multiple regression techniques and was based on records from New Jersey light traps operated by the Kern Mosquito Abatement District at 23-25 rural sites in the years 1978 through 1983.

The mean seasonal abundance patterns for these 6 years fell into two fairly distinct categories: 1979, 1980 and 1981, when the peak abundance of *Cx. tarsalis* came in September, and 1978, 1982 and 1983 when populations were high in June, then fell off or stayed at about the same level and went up again with a second peak in the fall (Figure 1). 1978 and 1983 followed very wet winters, but 1982 was an average rainfall year in Bakersfield (Table 1). Even when the July through December rain for the previous year was added, the rank order of years did not change. So the populations must be influenced by a combination of rain and something else, perhaps temperature.

Monthly indices (female mosquitoes per trap night) were calculated for *Cx. tarsalis* and *Culiseta inornata* for each trap. The actual trap indices, which have a very skewed distribution, were converted to natural logarithms (LCTAR and LCINOR). This stabilized their variance so that standard regression procedures could be used. Rainfall and temperature data were from the U.S. Weather Station at the Bakersfield Airport. The

Table 1. Cumulative rainfall, January 1 - June 30, recorded at Bakersfield, California.

Year	Inches of rain
1978	8.79
1983	6.89
1980	5.83
1979	5.18
1982	4.99
1981	4.60

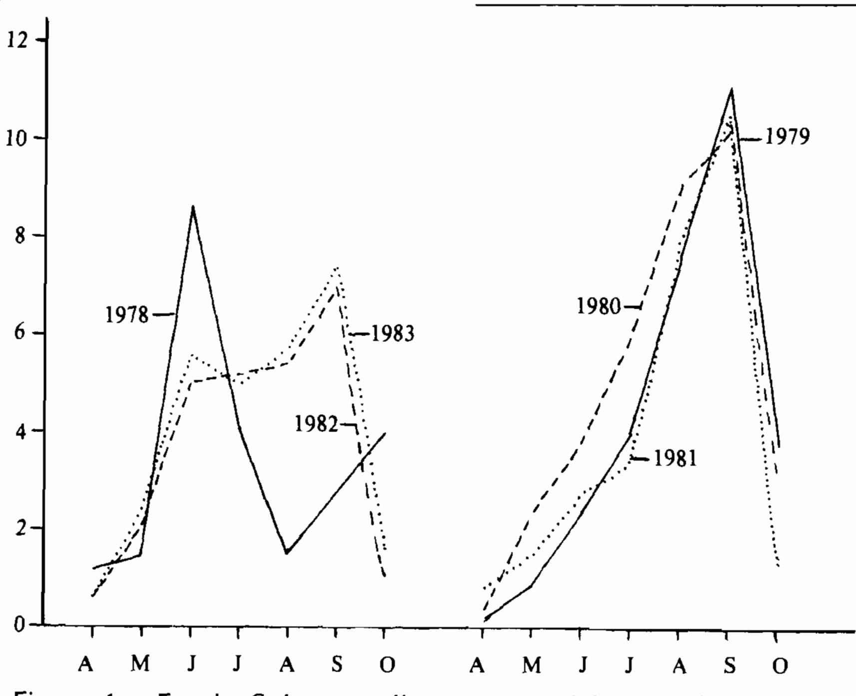

Figure 1. Female *Culex tarsalis* per trap night, rural New Jersey light traps, Kern Mosquito Abatement District.

meteorological variables included rain in inches (RAIN), cumulative rain since January 1 (CRAIN), mean temperature in °F (TEMP), number of degree-days above 65°F (DDAY), and cumulative degree-days since January 1 (CDDAY).

Preliminary analyses determined that moon phase, cloud cover and barometric pressure had no significant influence on trap catches. Day length was highly correlated with abundance, but covaried significantly with temperature and was not included.

The next step was to develop a series of equations which would attempt to predict *Cx. tarsalis*, the dependent variable, for a given month as a function of the values of all 7 variables in a previous month. The statistic that is used to indicate how well a regression model fits the data is R^2, which tells what proportion of the total variation in the dependent variable, *Cx. tarsalis* abundance in our case, is explained by the model. The stepwise multiple regression procedure selects in order of importance those independent variables that make significant contributions to the value of R^2. If none of the independent variables has a high enough correlation with the dependent variable, none will be chosen and no equation will be developed.

This happened when an attempt was made to predict April abundance using only the climatological data for March. March temperature and cumulative rainfall had the highest correlation with *Cx. tarsalis* abundance for April, so an equation was developed using only those two independent variables:

$$LCTAR_{APR} = -1.58 + 0.033(TEMP)_{MAR} + 0.024(CRAIN)_{MAR}.$$

R^2 was only 0.035, which is very poor; it says the model only explained $3\frac{1}{2}\%$ of the variation in *Cx. tarsalis* abundance in April.

The stepwise regression procedure worked for the succeeding months. Attempts to predict May or June abundance with March data were unsatisfactory. But for a May prediction based on April data, 5 of the 7 variables were used in the equation, and the R^2 was 0.58:

$$LCTAR_{MAY} = 2.11 + 0.78(LCTAR)_{APR} + 0.30(LCINOR)_{APR} - 0.24(CRAIN)_{APR} - 0.0087(CDDAY)_{APR} + 0.37(RAIN)_{APR}.$$

The closer we get to the month we're trying to predict, the better model we end up with, which certainly makes sense:

$$LCTAR_{JUN} = 1.07 + 0.71(LCTAR)_{APR} + 0.42(LCINOR)_{APR};\ R^2 = 0.37.$$

$$LCTAR_{JUN} = -1.30 + 0.90(LCTAR)_{MAY} + 0.23(CRAIN)_{MAY} + 0.0022(CDDAY)_{MAY};$$

$R^2 = 0.53.$

Culiseta inornata abundance was a significant variable in equations for May and June using April data, but was not selected for any subsequent months, probably because this species is rarely collected in midsummer.

At this point, it would seem that trying to predict abundance before the end of April is a waste of time. It also becomes obvious that the best way to estimate next month's population size is to look at the current month's *Cx. tarsalis* indices. This confirms empirically what was shown theoretically in the model developed by a former Berkeley doctoral student, T. E. Moon (1976).

The consistency of the models for June (above), July and August was encouraging, and illustrated the combined influence of rain and temperature:

$$LCTAR_{JUL} = 2.17 + 0.82(LCTAR)_{JUN} - 0.20(CRAIN)_{JUN} - 0.00082(CDDAY)_{JUN};$$

$R^2 = 0.63.$

$$LCTAR_{AUG} = 3.36 + 0.76(LCTAR)_{JUL} - 0.31(CRAIN)_{JUL} - 0.00065(CDDAY)_{JUL};$$

$R^2 = 0.65.$

For each of these three months, the variables selected for the model were the previous month's *Cx. tarsalis* abundance, cumulative rainfall and cumulative degree-days. But the signs, plus or minus, of the coefficients associated with rain and temperature were *not* consistent. For the June equation, they were both positive, indicating that a lot of rain and warm weather will increase the June *Cx. tarsalis* levels. For July and August they were both negative, which says midsummer populations should be lower in wet, warm years. It also could mean that if populations are high in June, control efforts are intensified and the summer populations are reduced.

In any case, by September the only thing that mattered was how many mosquitoes there were in August. And the equation for September had the highest R^2 value of all, 0.72:

$$LCTAR_{SEP} = 0.53 + 0.86(LCTAR)_{AUG}.$$

The equation for October indicated fall populations would be decreased if significant amounts of rain fell in September; R^2 was 0.51:

$$LCTAR_{OCT} = -1.97 + 0.56(LCTAR)_{SEP} + 0.34(CRAIN)_{SEP} - 0.54(RAIN)_{SEP}.$$

(Note: these equations were developed for a specific set of rural Kern County traps, and should not be expected to work for traps in a different location.)

The model was tested by using the equations to predict monthly *Cx. tarsalis* indices for both a late-peak year, 1980, and an early-peak one, 1982. In each case, the estimated abundance was very close to the actual observed data, which is precisely what one would expect since these two years represent one-third of the total data used to develop the model. The bottom line, of course, is how accurately will the model predict 1984 abundance?

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GENETIC IMPROVEMENT AND INBREEDING EFFECTS IN CULTURE OF BENEFICIAL ARTHROPODS

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ABSTRACT

Improvements in reproductive potential of beneficial arthropods and a minimization of inbreeding in culture may be possible using simple techniques. However considerations of population genetics are complex to the point of discouraging practical breakthroughs. This lack of understanding should not impede implementation of technology that may lead to improved biological control.

Genetic studies of beneficial arthropods reveal great complexities, which cause hesitation to experiment with improvement at the applied level. However, researchers overlook probable field examples of genetic improvement, and there is a tendency to devote an inordinate amount of time viewing limited genetic diversity revealed by electrophoretic analysis.

Genetic considerations are important to biological control by suggesting improved strategies for the acquisition of new natural enemies from abroad, in their breeding for introduction programs, and in their mass release for direct control (Levins 1969; Lucas 1969; Mackauer 1976, 1980; Myers & Sabath 1980; Remington 1968; Whitten 1970).

According to past records, 80% of the natural enemies introduced into a new environment for biological control in the United States have failed (Clausen 1956); 42% of the Canadian projects failed between 1929 and 1955 (Turnbull & Chant 1961); and 90% failed worldwide (Turnbull 1967). There is an apparent modern trend toward more failures (Hall & Ehler 1979, Ehler & Andres 1983).

Many of these failures involved natural enemies that were poorly adapted to the host species against which they were introduced, or were handled inefficiently, released in inadequate numbers and under improper circumstances. Adverse environmental conditions at the time of liberation are cited as principal causes for failure. These include unavailable hosts or host plants, scarcity of food, water or shelter, severe competition with analogous organisms, adverse toxic effects of chemicals; adverse cultural practices; diapause problems (Legner 1979b); and host-parasite asynchrony. Researchers are often able to minimize most of these obstacles or at least recognize their presence so that establishment trials may be repeated during favorable intervals; but the genetic make-up of the colonized natural enemies is usually unknown (Hoy 1976; Mackauer 1972, 1976; Messenger & van den Bosch 1971; Myers & Sabath 1980; Roush 1979).

Biological control traditionally involves the permanent transfer of natural enemies from one geographic area to another. These natural enemies, or colonizers, after leaving their source population, are faced with problems not encountered previously. While searching in the new environment, they invariably confront situations completely different from those prevailing at their points of origin. Due to the comparatively small numbers of individuals in the colonizing flock, with respect to the point of origin, intraspecific competition is relaxed, enabling less fit genotypes to survive, reproduce and interact. Thus, a high genetic variability develops which may eventually give rise to a new genotype. Such a process was apparently recorded in England by Ford & Ford (1930) with the colonial checker-spot butterfly, *Euphydryas aurinia* (Rottenburg). Furthermore, if the colonizer originates from a marginal population, it may more successfully exploit its genetic opportunity because of a smaller genetic load and a possible lower inversion heterozygosity (Force 1967, Remington 1968).

A group of colonizers is an atypical isolate of the source population. In the new area to which it is introduced, it no longer is subjected to the diluting effect of gene flow from the main body of the population. A change in gene frequencies occurs which is called the Founder Principle. However, relaxation from intraspecific competition may be accompanied by an increased interspecific competition to which the colonizer may not be preadapted; and new environmental conditions may make such competition impossible. When the environmental resistance is in the form of already established natural enemies, the introduction of superior species may be difficult or impossible (Ehler & Andres 1983).

When a colonizer encounters related species in the new environment, the outcome of such encounters depends on the perfection of the prezygotic barriers to hybridization and upon the relative fitness of the hybrid and the parental species (Remington 1968).

Callan (1969) offered a list of three major genetic groups of entomophagous colonizers, (1) colonizers with built-in success which become rapidly established; (2) colonizers with delayed success; and (3) colonizers which are predestined to fail. Remington (1968) and Lucas (1969) presented opposite views of the genetic make-up of populations and what should be sought in biological control. However, Myers & Sabath (1980) suggest that Lucas failed to distinguish between probable marginal and central populations.

The Field Population at its Origin.-The size of the population at its origin determines its genetic variability due to mutation (including chromosomal abberations), outcrossing (the larger the population the higher the outcrossing rate), and genetic drift, which endangers very small populations by the loss of genes. The original population may be either continuous and numerous, continuous and rare, subdivided into semi-isolated segments or demes, and subdivided into wholly isolated demes (Remington 1968).

The central portion of the source population dwells in the ecologically optimal region of the species, while marginal portions are found near the ecological boundaries of the species. Data for central and marginal insect populations comes principally from *Drosophila* studies conducted in the Western Hemisphere by Carson (1955, 1959, 1965), Dobshansky (1956), Prakash (1973), and Townsend (1952); and for insects and vertebrates, Nevo (1978).

Compared to marginal populations, central populations are subjected to greater heteroselection, they are larger and outbreeding, they have increased concealed genetic variability and carry a higher genetic load. They may show increased inversion heterozygosity and, therefore, may not be as evolutionarily plastic. Central populations are adapted to the average environment.

Marginal populations may be subjected to greater homoselection and are small and inbreeding. They have less alleles per locus and increased homozygosity in *Drosophila*. There is a lesser genetic load, but they may be endangered by genetic drift. They may show less heterozyzosity for inversions and, therefore, higher evolutionary plasticity. Marginal populations are adapted to narrower niches. Remington (1968) and Messenger & van den Bosch (1971) discussed in greater detail the characteristics of these two major types of populations. Nevo (1978) showed electrophoretic data which suggest opposite allelic characteristics from those described by Remington (1968), from a study of both vertebrates and invertebrates. Myers & Sabath (1980) concluded that generalizations about central and marginal populations are not valid and cannot be used as a basis for decisions on where to collect biological control agents. But this conclusion seems to weigh the electrophoretic data more heavily than the cytological evidence from *Drosophila*. The fact that most of the alleles marked in electrophoresis are probably neutral (99% as believed by many) casts doubt on conclusions referring to "expressed" hetero- or homozygosity. Futuyma (1979) revealed further limitations of electrophoretic data, and especially emphasized the possible role of regulator genes, which cannot be assessed biochemically.

Cultured parasitic Hymenoptera show low levels of enzyme polymorphism (Crozier 1971, 1977; Kawooya 1983; Metcalf et al. 1975), suggesting that most introductions that involved culturing probably were also deficient in allelic variability. Thus, such cultures may have conformed closely to the description of the marginal population given by Remington (1968).

Natural Enemy Introductions.-The existence of races in natural enemies is widely known; but in foreign exploration genetic variability is usually not clearly expressed. Thus, the stress has been to introduce natural enemies from varied areas whenever possible to grain greater genetic heterogeneity (Whitten 1970).

When natural enemies are first introduced to new environments, the pace of genetic drift is accelerated and quick evolution anticipated. The introduced organism by virtue of both having been "sampled" from the original population and then passed through the bottleneck of culture, theoretically contains but a small fraction of the original gene pool. Many of the lost alleles may have been essential for fitness, and a marked trend for greater homozygosity exists (Legner 1979a, Unruh et al. 1983).

Most natural enemy introduction attempts fall naturally into two or three phases. The first phase involves an initial search for natural enemies where little is known about what species exist or their potential for biological control (Zwölfer et al. 1976). Restricted financial support usually dictates a less than thorough sample of the indigenous area. The second phase is taken after repeated search has turned up a few natural enemy species, but initial colonization has failed. Additional information is available since the first attempt. Continued searching is carried out, or previously discovered species are tried again in the quest for greater genetic diversity. A final phase may be entered by researchers cognizant of the importance of genetic make-up, which involves the acquisition of seasonal and geographic strains of an initially colonized species. New species are often discovered in this process whose activity may be confined to certain seasons. However, the numerous steps outlined by Mackauer (1980) to assure genetic stability in laboratory stocks can rarely be taken.

Improving Fitness of Natural Enemies.-Fitness of a given enemy to a target environment may be improved generally in two ways (1) by artificial selection, in which a stock of the enemy is created by selection in the laboratory to enable it to cope with some limiting environmental factor such as temperature or insecticide treatment (Hoy 1976, 1979); and (2) by increasing genetic diversity through hybridization or by colonization of a greater number of individuals from the source population. A more plastic or diverse stock is created which, after colonization, will have an increased chance for improvement through natural selection. A third possibility, genetic engineering, has not yet been attempted in biological control.

Examples of artificial selection are few, and its practicality in continued improved biological control is doubtful. Difficulties include a lack of knowledge concerning the genetic basis for inheritance of the desired characteristics. There is also usually little information on the amount of genetic diversity on which to base selection. The possibility of unintentional co-selection for detrimental qualities is always present (Ashley et al. 1973).

Nevertheless, there have been numerous attempts to select adaptive features for beneficial organisms. One of the most common efforts involves the improvement of climatic tolerance. Wilkes (1947) attempted this with *Dahlbominus fuscipennis* (Zetterstedt); and DeBach & Hagen (1964) and White et al. (1970) reported on work with *Aphytis lingnanensis* Compere.

Improvements in the sex ratio to favor females were sought by Wilkes (1947) with *Dahlbominus fuscipennis* and Simmonds (1947) with *Aenoplex carpocapsae* (Cushman). Host-finding ability was improved in *Trichogramma minutum* Riley by Urquijo (1951); and a change of host preference was induced in *Horogenes molestae* (Uchida) by Allen (1954, 1958), and *Chrysopa carnea* Stephens by Meyer & Meyer (1946).

Resistance to DDT was produced in *Macrocentrus ancylivorous* Rohwer by Pielou & Glasser (1952); while in predatory mites resistance was produced to phosphate insecticides (Croft 1970, Croft & Brown 1975), to permethrin (Hoy & Knop 1981, Hoy et al. 1982), and to sulfur (Hoy & Standow 1982).

Interspecific crosses between two species of *Spalangia,* parasites of synanthropic flies, in Australia yielded a hybrid in the field with improved fecundity and longevity (Handschin 1932, 1934).

Intraspecific crosses (crosses between strains) have resulted in improved host preference behavior in the tachinid *Paratheresia claripalpis* (Box 1956), in increased laboratory productivity in the braconid *Apanteles melanoscelus* (Ratzeburg) (Hoy 1975) and in improved fecundity and longevity with *Spalangia* and *Muscidifurax* parasites of synanthropic flies (Legner 1972). In the latter case, the reproductive potential was utilized, which gave a strong measure of fitness probably influenced by many polymorphic genes. True fitness in the field, of course, is also influenced by other behavioral traits such as habitat selection (see Hoy 1976). However, the process of intraspecific hybridization and heterosis is probably natural, causing the hybrids to be more vigorous and better able to withstand environmental resistance, and to extend their range in all niches a population has mastered (Carson 1959). The selection of appropriate strains for intraspecific crosses is critical, as detrimental outcomes due to negative heterosis (hybrid dysgenesis) may occur (Croft 1970, Mahr & McMurtry 1979, Legner 1972). Hoy (1976) and Whitten & Foster (1975) discuss genetic improvement further.

Experimental field demonstrations of natural enemy improvement through heterosis apparently exist. The mite predator, *Phytoseiulus persimilis* Athias-Henriot, which was initially established in California from a culture obtained in Chile, improved its effectiveness following the subsequent introduction of another strain from Italy (McMurtry et al. 1978). A triple hybrid of *Apanteles melanoscelus* gave good inundative release effects, although the final degree of host parasitism was not higher than that rendered by nonhybrids (Hoy 1975). However, field establishment might not have been successful with either of the single strains available at the time.

Fitness of parasitic insects can also be improved physiologically without any apparent genetic change, as evidenced by experimental cold storage treatments (Legner 1976).

Prolonged Culture.-The problem in culture is to judge whether the stock material is genetically changed as time goes on. Some commercial insectaries in California have maintained sustained cultures of beneficial arthropods for over 45 years without knowingly changing their stock or its effectiveness. In many cases a beneficial species becomes established from cultures started with very few founders (Mackauer 1972, Simmonds 1964); and DeBach (1965) found no correlation between the number of individuals liberated and the probability of establishment.

Studies of three parasitic species, *Muscidifurax raptor* Girault & Sanders, *M. zaraptor* Kogan & Legner (Legner 1979a), and *Aphidius ervi* Haliday (Unruh et al. 1983), show that cultures are indeed changed genetically with time. In the former two species, cultures maintained for over 100 generations (25 days allowed for one generation) were compared to those gathered from the field just one or three generations earlier. An examination of their reproductive potentials indicated an immediate loss of wild alleles during the first couple of generations in culture. However, considerable heterogeneity (and presumably heterozygosity) was retained in culture over the 100 generations (Legner 1979a). Declines in allozyme variability in laboratory populations of *A. ervi* (Unruh et al. 1983) support the initial loss of heterozygosity in cultures of arrhenotokous Hymenoptera.

There is no clear agreement, however, on how to retain heterozygosity. Unruh et al. (1983) believed that the only way to prevent genetic drift in laboratory culture is to keep population sizes large. Wright (1951) recommended subdividing the population into several smaller subpopulations (stepping stones) among which gene flow may occur. A compromise suggested by studies with *Muscidifurax* species (Legner 1979a) might be considered as follows:

Initial acquisitions of field cultures could be converted to a series of inbred lines, maintained without gene flow among them to guarantee their separate characteristics and the retention of a greater number of alleles with respect to all lines cultured. This is possible with some hymenopterous species because genetic decay is uncommon or unknown (Crozier 1970, Legner 1979a). The number of individuals in each line could be held relatively low with the heterozygosity among lines retained by having a large number of such separate lines. The more lines initially established from individuals acquired in the field, the greater the genetic variability present among all of them. Since gene flow is eliminated, there would be a reduced tendency for certain genotypes to dominate as in a single large culture. The total number of individuals of a species thus cultured might not be much greater than that recommended by advocates of the large populations; the greatest increase in labor being that associated with tending separate units.

The technique would have to be modified for Hymenoptera possessing the Whiting single locus, multiple allele scheme of sex determination (Crozier 1971, Whiting 1943). Also, variability in the stock of inbred lines would probably not reconstitute the original sampled population (Wright 1980). Admittedly, duplicating the structure of the original population is impossible. However, the inbred isolated line approach would offer a further step in the direction of increasing heterozygosity. Not employing the technique guarantees losing heterozygosity. For example, in the *Muscidifurax* study (Legner 1979a), contrary to expectations, traits for both high and low reproductive potential were lost in prolonged culture. Such traits, along with other unknown attributes of fitness, such as high searching capacity, might have been preserved had original genomes been isolated. Thus, although Unruh et al. (1983) believed that inbred lines do not presently represent a practical alternative for maintaining genetic variability in biological control programs, it seems that they may be an expedient way to retain greater heterozygosity than is now usually being retained.

Finally, is there a need for heterozygosity in our imported biological control organisms in the first place? Introductions from marginal homozygous populations may yield organisms with the capacity for rapid change in the new environment (Remington 1968). Because, as mentioned earlier, conditions at the place of introduction always differ to some degree from the place of origin, the colonizer invariably is faced with differences which may require it to modify its genotype in order to be maximally successful. Thus, organisms with greater homozygosity may be better candidates for introduction because they have a better capacity for evolving into new superiorly adapted types (Remington 1968). In biological control which aims at reducing pest densities, this has important implications. Liberations of the previously described inbred lines in different geographic portions of the introduction area offers a means for testing this hypothesis. Some support for its validity is the evidence of many successful biological control introductions having obviously involved highly inbred, homozygous lines of natural enemies (Mackauer 1972).

The foregoing briefly illustrates the complexities involved in genetic considerations of natural enemy introduction, which leaves some researchers perplexed when considering practical solutions. This was again made obvious in a recent statement by Unruh et al. (1983) that, "Genetic drift, as well as inbreeding and selection occurring in founder colonies, transport, quarantine and culture of natural enemies, will deter us from reaching our goals until we grasp the nature of variation within and among populations." However, achievements in the improvement of fitness are common to entomologists and plant scientists as well (Hoy 1976). Since laboratory techniques for creating apparently better adapted strains are available, and field demonstrations are known (Hoy 1976, 1982a, 1982b; McMurtry et al. 1978; White et al. 1970), there is no reason why we cannot proceed with other planned attempts.

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A TIME SEGREGATED SAMPLING DEVICE FOR DETERMINING NIGHTLY HOST-SEEKING PATTERNS OF FEMALE MOSQUITOES[1]

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ABSTRACT

Nightly host-seeking patterns of *Culex tarsalis*, *Culex quinquefasciatus* and *Culiseta inornata* were investigated in a mixed agricultural habitat in the southern San Joaquin Valley, Kern County, California, USA. Host-seeking patterns were determined by hourly collections using a 110 V AC time segregated sampler, or TSS, that featured a CDC miniature light trap fan, regulated flow of a bottled CO_2 attractant, variable timing modes, and live-trapping of large collections of adult mosquitoes.

Most female *Cx. tarsalis* and *Cx. quinquefasciatus* were collected between 2000-2400 h, with peak host-seeking activity 1-2 h after sunset. Female *Cs. inornata* exhibited a distinctly bimodal pattern in October with most activity occurring ca. 1 h after sunset and ca. 1 h prior to sunrise.

INTRODUCTION.-Determination of the nocturnal host-seeking or biting rhythms exhibited by both vector and nuisance mosquito species indicates when disease transmission is most likely to occur and when adulticides can be most effectively applied. Nocturnal biting patterns of *Culex tarsalis* Coquillett were described briefly by Nelson and Spadoni (1972) in the Sacramento Valley of California; however, the biting patterns of *Cx. tarsalis*, *Culex quinquefasciatus* Say and *Culiseta inornata* (Williston) have not been investigated in the San Joaquin Valley of California.

Studies were initiated in Kern County, California, during the summer of 1983 to examine the nightly host-seeking patterns of *Cx. tarsalis*, *Cx. quinquefasciatus* and *Cs. inornata* in a mixed agricultural habitat. Since western equine encephalomyelitis (WEE) virus has been prevalent in Kern Co. in recent years, collections were made with a CO_2 attractant rather than using human bait. Females collected at CO_2 bait have been shown to be at the host-seeking stage of ovarian development (Reisen et al. 1983). Hourly collections were made with a 110 V AC, time segregated sampler (TSS) developed at the Arbovirus Field Station. Unlike similar time segregated devices developed by Standfast (1965) and Mitchell (1982), the TSS live-traps adult female mosquitoes and provides sufficient space for large hourly collections.

The present paper includes a brief description of our time segregated sampler, operational data comparing TSS sampling efficiency with that of a CDC trap baited with dry ice, a brief synopsis of the nocturnal host-seeking patterns exhibited by *Cx. tarsalis* and *Cx. quinquefasciatus*, and preliminary data on the nocturnal host-seeking pattern of *Cx. inornata*.

MATERIALS AND METHODS.-**Description of TSS**: The components of the TSS consist of a wooden frame stand (S), aluminum ramp (R), trolley (T) and counterweight system (W), a 110 V AC timer grouping (M) and solenoid, and a dual regulated CO_2 delivery system (C) (Figure 1). Pieces of the stand, ramp and counterweight support mast are modular so that the sampler can be disassembled for transport to the field. When fully assembled, the TSS measures 3' wide x 3' high (end) x 6' long x 6.5' high (height including counterweight mast).

Stand and Ramp: The stand is constructed from standard grade lumber (2" x 4" and 2" x 2"-ramp supports) and the ramp from aluminum angle equal leg (various sizes), "U" channel, and kick plate (Figure 1A and B). Six 3 1/2" dia. holes, equally spaced in each of two 3' x 10" kick plates, provide the opening and attachment sites for the collection cartons. Collection cartons are secured to the underside of the ramp by 4" x 3" clothes dryer reducer wrapped with foam rubber weather stripping (Figure 2).

Trolley: The aluminum trolley is supported by 4 screen door rollers and accommodates the 6V DC CDC miniature light trap (Hausherr's Machine Works, Toms River, NJ), 110 V AC Dormeyer® 4 x 240 solenoid (Dormeyer Electric Co., 3418 N. Milwaukee Ave., Chicago, IL), 2 CF6 V8 Carefree® gel cell batteries (Eagle-Picher Industries, Inc., Seneca, MO) connected in parallel to operate the fan motor at sufficient RPM's for 16 h, and plastic CO_2 dispersal funnel (Figures 1C and D). Hourly collections were accomplished by moving the trolley down the ramp at 60 min. intervals. The CDC trap is positioned directly over the opening to each collection carton by a system of carefully placed window locks secured

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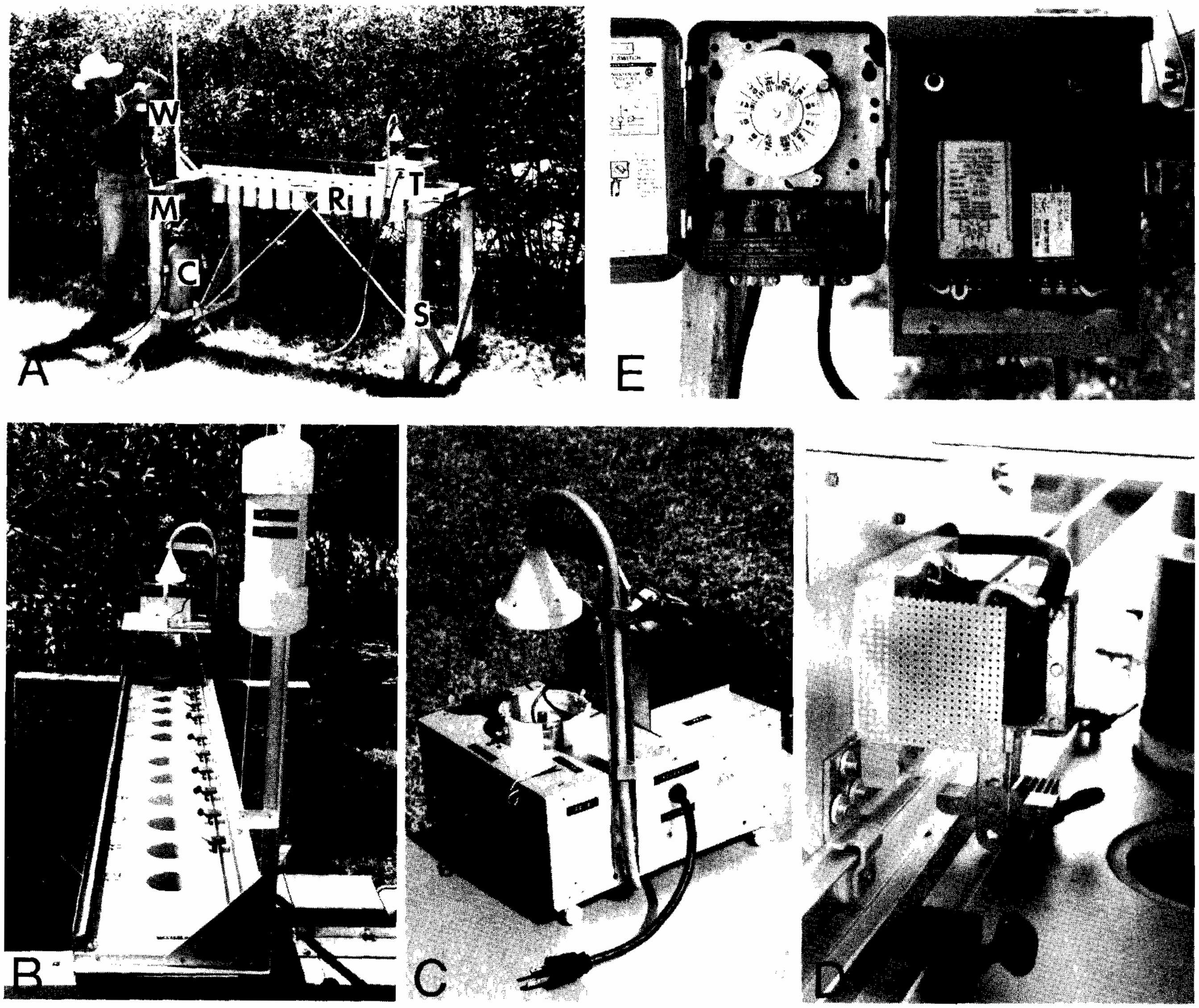

Figure 1. A. Assembled TSS showing the wooden stand (S), trolley (T), ramp (R), counterweight (W), timer grouping (M), and CO_2 cylinder (C). B. End view of TSS. C. Trolley. D. Solenoid and E. Timer.

to the "U" channel (Figures 1B and D). When released by the solenoid, the counterweight pulls the trolley down the ramp until it is stopped when the "T" arm of the solenoid contacts the front edge of the next window lock.

Counterweight system: Movement of the trolley is initiated by a counterweight that pulls in line with the solenoid arm to minimize lateral torque when the trolley is stopped (Figure 1B). The counterweight (PVC pipe and caps) is elevated by an aluminum mast and a pulley system to maintain alignment and to prevent the weight from contacting the ground.

Collection cartons: Collection cartons are fabricated from 1 qt., 3 3/8" dia. Fonda® No. 109 paper cans (Saxon Industries, Inc., Union, NJ). The cone insert (Figure 2 SC) is made from fiberglass window screening and has a 1/4" dia. opening at the tip. Mosquitoes are forced through the opening in the cone by the CDC trap fan without apparent injury. Design and placement of the cone prevented the escape of trapped mosquitoes in all-night laboratory evaluations with known numbers of mosquitoes.

Timer grouping: Movement of the trolley is controlled by a Dayton® No. 2E021A 24 h single throw 110 V AC timer and adjustable Agastat® No. SCR72A1BA solid state, repeat cycle timer (2-60

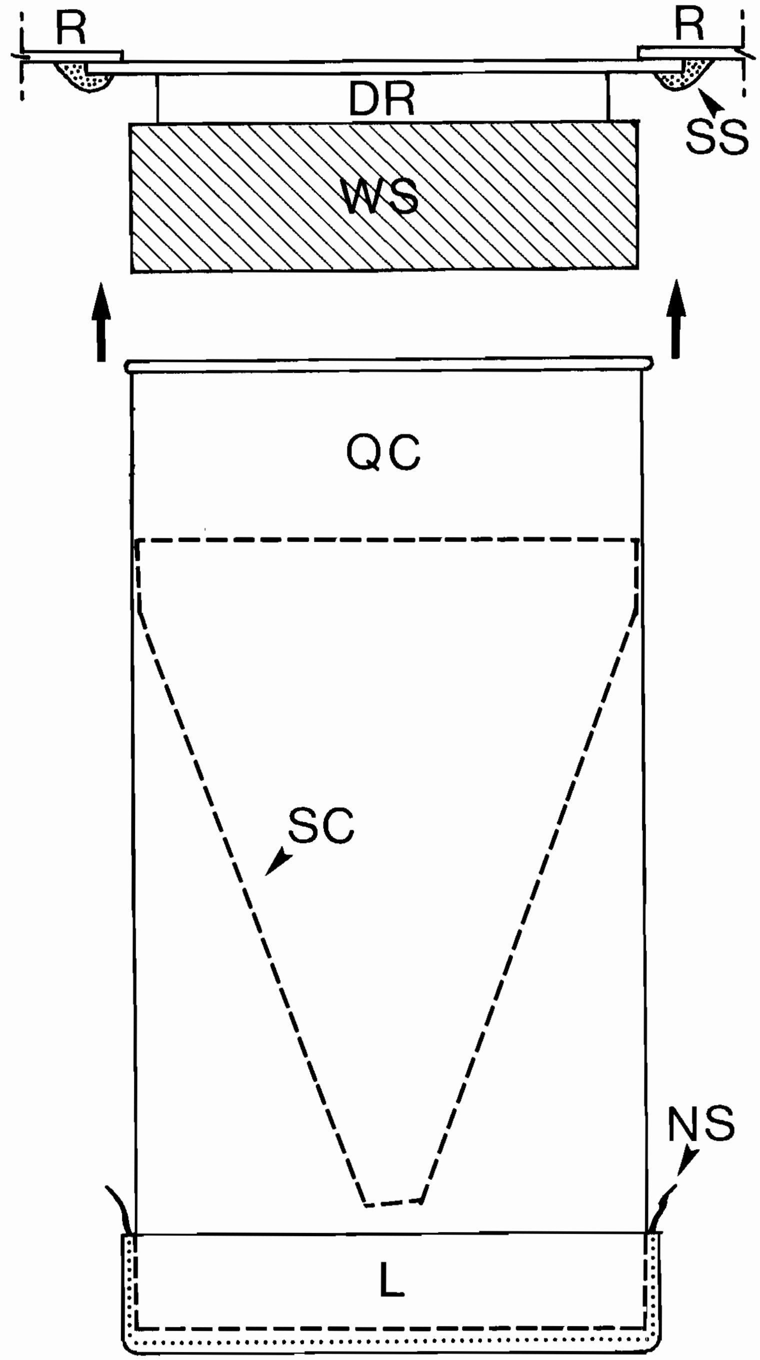

Figure 2. Attachment of the 1 quart collection cartons (QC) to the ramp (R). Indicated are the 4" x 3" clothers dryer reducer (DR), 1" wide foam weather stripping (WS), silicon seal (SS) for cementing the dryer reducer to the ramp, fiberglass window screen cone (SC), nylon screeing (NS) and quart carton lid (L).

min. time off, 0.5 - 15 sec. time on. Amerace Corp., Union, NJ) (Figure 1E). The repeat cycle timer is energized only during the "on" cycle of the 24 h timer, i.e., 1900-0700 h. A time delay of 0.5 secs. is used to close and open the solenoid, and release the trolley (CDC trap) which is then stopped over the next collection carton. An optional in line relay was installed to protect the Agastat® timer against an electrical overload.

CO_2 **delivery system**: An adjustable flowmeter (0-2 liters/minute) was used to control the release of CO_2 through the plastic diffusion funnel at 1 liter/min. A dual gauge regulator maintains a constant CO_2 pressure (10 psi) to the flowmeter from a 20 lb. (1000 psi) capacity CO_2 tank.

Field Evaluations: The sampling efficiency of the TSS was compared with that of a CDC miniature light trap (Sudia and Chamberlain 1962) baited with ca. 2.0 kg of dry ice (CO_2T) at a mixed agricultural habitat (John Dale Ranch, or JD) located ca. 10 km W of Arvin, Kern County, California, USA. The TSS was operated from 1900-0700 h at weekly intervals concurrent with 4 CO_2T's spaced at 60 m intervals in an E-W transect along a mature stand of Fremont cottonwood (*Populus Fremontii* Wats). The TSS was placed midway between 2 CO_2T's at the west end of the transect.

CO_2T and TSS collections were processed the day following the night of trap operation. Mosquitoes collected during each hour were combined per trap night for statistical comparison with CO_2T collections. Mean collection size (females/trap night) and the similarity of seasonal changes in the relative abundance of *Cx. tarsalis* and *Cx. quinquefasciatus* sampled by the TSS and CO_2T's were evaluated by paired t-test and time series correlations ($N = 50$), respectively (Sokal and Rohlf 1969).

RESULTS AND DISCUSSION.-Overall, the TSS collected significantly more host-seeking female *Cx. tarsalis,* but significantly fewer host-seeking female *Cx. quinquefasciatus* than the CO_2T's (Table 1). Changes in relative abundance of female *Cx. tarsalis* were detected similarly by both sampling methods. Marked differences in the efficiency of the TSS and CO_2LT's to sample female *Cx. quinquefasciatus* were observed in late September and October and may have been related to the release rate of the CO_2 attractant. Reeves (1953) demonstrated that nightly catches of female *Cx. quinquefasciatus* were increased as the CO_2 release rate was decreased from 2.5 liters/min. to 0.025 liters/min. Apparently, the 1 liter/min. flow rate of CO_2 supplied to the attractant funnel of the TSS was too concentrated for sampling *Cx. quinquefasciatus,* but was well suited for attracting female *Cx. tarsalis.*

Nocturnal host-seeking patterns determined by the TSS for female *Cx. tarsalis, Cx. quinquefasciatus,* and *Cs. inornata* are illustrated in Figure 3. Hourly collection data are presented as the percent of the total number of females collected per trap night. Peak activity of *Cx. tarsalis* and *Cx. quinquefasciatus* occurred 1-2 h after sunset and decreased thereafter. Most females (>56%) of both species were collected between sunset and midnight. Female *Cx. quinquefasciatus* remained relatively more active after midnight than female *Cx. tarsalis*, but activity decreased more abruptly 2-3 h prior to sunrise. The nightly host-seeking pattern exhibited by female *Cs. inornata* sampled in October-November was distinctly bimodal, with peaks 1 h after sunset and 1 h before sunrise. An unexplained smaller peak occurred from 2300-2400 h.

Table 1. Comparison of the mean numbers collected per trap night of female *Culex tarsalis* and *Culex quinquefasciatus* sampled by the TSS and CO_2 traps at John Dale Ranch, 1983.

	Trap Abundance Indices	
Collection Method	$\bar{x}$±S.E/Trap Night (n = 50)	Correlation (n = 50)
Culex tarsalis		
TSS[1]	205.1 ± 32.9***[3]	r = 0.92***[4]
CO_2T[2]	167.3 ± 21.7	
Cx. quinquefasciatus		
TSS	19.4 ± 2.8	r = 0.57***
CO_2T	32.2 ± 4.5***	

[1]Time-segregated sampler, CO_2 gas released at ca. 1.0 liters/min.

[2]Mean of 4 CO_2 traps (CDC miniature light trap baited with ca. 2 kg of dry ice and operated without light) at John Dale Ranch.

[3]Mean number of females collected per trap night significantly greater in paired t-test, (df = 48) *** $P < 0.001$.

[4]Time series correlation between TSS and CO_2T samples (df = 48) *** $P < 0.001$.

The nocturnal patterns of human biting activity determined by Nelson and Spadoni (1972) for female *Cx. tarsalis* in the Sacramento Valley agreed well with the patterns resolved at JD. The similarity in the host-seeking patterns of two geographically separate populations of *Cx. tarsalis* indicated a possible uniformity in the nocturnal activity of this species in the Central Valley of California.

Based upon the host-seeking activity data presented herein, adulticiding to control *Cx. tarsalis* and *Cx. quinquefasciatus* should com-

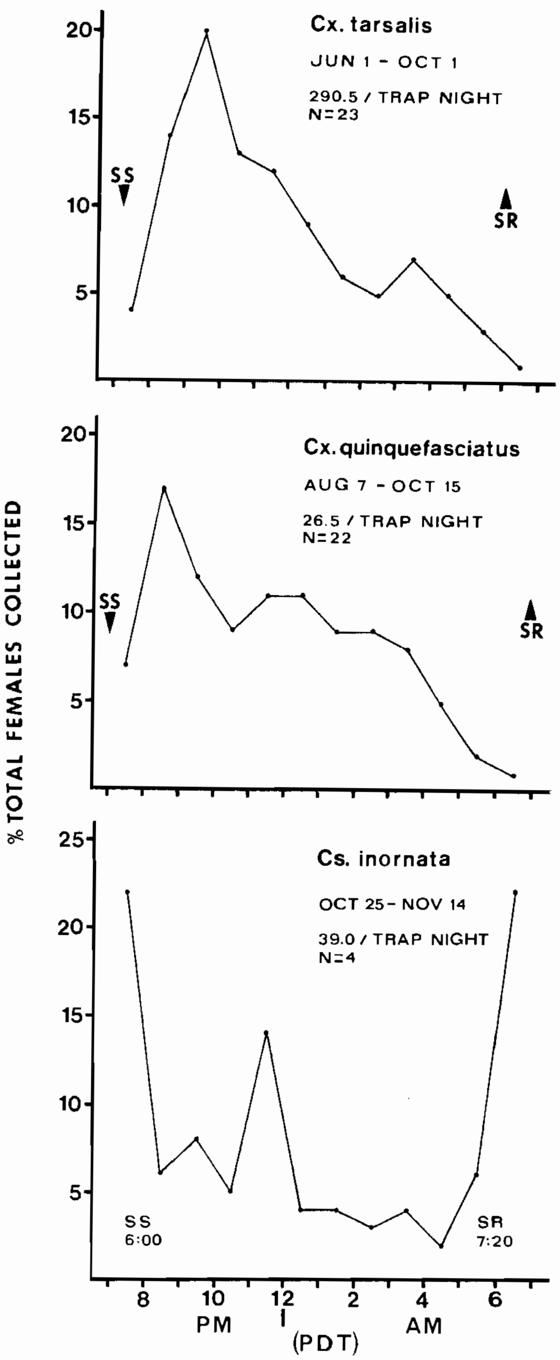

Figure 3. The nocturnal host-seeking patterns of *Culex tarsalis*, *Culex quinquefasciatus* and *Culiseta inornata*. N = number of nights the TSS was operated for determining species specific host-seeking patterns. Time scale of Pacific Daylight Time (PDT). Arrows indicate the mean time of sunrise (SR) and sunset (SS) for the sampling period.

mence shortly after sunset and continue from 3-4 h thereafter. Applications of adulticides during the second half of the night or early morning would be less effective in contacting host-seeking females. Epidemiologically, the greatest risk for arboviral transmission by *Cx. tarsalis* would most likely exist during the 3-4 h period after sunset. Studies are planned to resolve that portion of the night when parous and potentially infective females are host-seeking.

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WATER HYACINTH AQUACULTURE AS A POTENTIAL MOSQUITO SOURCE[1]

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Water hyacinth (*Eichornia crassipes*) introduced as an ornamental, was first recorded in California near Clarksburg in 1904. In the last decade this prolific weed invader has been actively promoted as a potentially inexpensive mechanism for the treatment of wastewater. Aquaculture systems have provided effective treatment of wastewater in three ways: 1) as bio-filters, supporting large and diverse populations of bacteria which break down organic materials, 2) in the absorption of nutrients and 3) in sedimentation of wastewater solids. Hyacinths appear to be able to reduce BOD, nitrogen and suspended solids. These systems have been promoted partially on the assumption that they would require little management and would not generate environmental problems. However, by the end of 1980 several mosquito abatement districts responded to citizen complaints of mosquito problems associated with water hyacinth sewage treatment. The final outcome was removal of the hyacinths at considerable costs to the sewage treatment plants.

In view of the interest in aquaculture systems and the associated mosquito problems, a two-year program monitoring mosquito production in an experimental wastewater-water hyacinth aquaculture project was completed at the Roseville, California sewage treatment facility. Larval and adult mosquito populations, mosquitofish (*Gambusia affinis*), water temperatures, water quality, and general pond conditions were monitored throughout two mosquito seasons.

The investigators concluded that:

1. Intolerable levels of *Culex peus, Cx. pipiens* and *Cx. tarsalis* are produced in treatment facilities using water hyacinth aquaculture. Water hyacinths are not recommended for treatment of primary sewage.
2. It may be possible to reduce mosquito production to acceptable levels by intensive pond management and by maintaining high densities of mosquitofish.
3. Aeration of ponds is advantageous.
4. Winter dieback of hyacinths will contribute to accelerated eutrophication, anaerobic conditions and fish kills.
5. Ponds must be designed to enhance fish predation, plant harvesting, pond drainage and total removal of the plants.

Promotion of water hyacinth in the treatment of sewage wastewater is continuing, with a major multi-million dollar pilot project in San Diego, an experimental facility in Stockton and proposals for more in Monterey County. In order to minimize mosquito production, mosquito control agencies must monitor and work closely with facilities using or contemplating the use of water hyacinths in wastewater management.

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[1]Abstracted from "Survey of mosquito production associated with water hyacinths," 1983, 46 pp., a report completed in cooperation with the State Water Resources Control Board and the consulting firm of Dewante and Stowell.

ADEDINE FLIGHT DISPERSAL STUDIES IN AN ALPINE HABITAT

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ABSTRACT

To evaluate Giemsa for marking adult mosquitoes by treating the larvae as presently being done in New Jersey studies by workers at Rutgers University, three separate laboratory experiments and one field trial were conducted utilizing Giemsa concentrations of 15 to 20 mg/ml. Results were inconsistent and pending further work in the future, subsequent mark-release-recapture trials in California and Oregon were conducted using the more conventional fluorescent powders to mark adults. Studies in California were preliminary to the main study in Oregon where a total of 3,054 mosquitoes were marked and released; sixty-three were recaptured within 72 hours (i.e., 2.1%); 18 were *Aedes hexodontus* and 45 were *Aedes communis*. The results of these trials demonstrated the ability of snow pool mosquitoes to fly at least 2 km distance from their breeding site to seek blood meals. This matter will have to be taken into account in defining the magnitude of a control program necessary to sufficiently suppress the biting mosquito population associated with recreational sites in alpine areas.

A MICROCOMPUTER-BASED SYSTEM FOR FEED CONTROL, TEMPERATURE CONTROL AND TEMPERATURE RECORDING IN A *GAMBUSIA* HATCHERY

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ABSTRACT

An automatic feeding system for a *Gambusia* hatchery facilitates delivery of small amounts of feed at many times throughout the day, thereby allowing steady consumption and minimizing waste feed that places an unproductive load on a hatchery's biological filters. Furthermore, automatic feeding reduces labor costs and scheduling problems.

The automatic feeder is simply a hopper over a shuttle-like feed release that is activated by a small solenoid and a counterspring.

Signals that activate the solenoid are generated, according to user specified times and amounts, by a 6502 type microcomputer. Relay devices isolate the 5 volt microcomputer electronics from the 110 volt solenoids. As many as 6 relays may be activated, each with different specified amounts of feed, at up to 24 feeding times per day. Up to 4 solenoids may receive signals from a single relay, therefore this system can coincidentally control up to 24 feeding devices. The feeding system has been expanded to sense, control and record air and water temperatures.

The electronic components of the system are an Apple-compatible microcomputer, a TV monitor, a disk drive, an input-output board, a clock and a temperature board with sensors. The easily modified program is written in BASIC and is available upon written request.

IMPACT OF *GAMBUSIA AFFINIS* WINTERING AT MENDOTA WILDLIFE AREA[1]

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ABSTRACT

Mosquitofish, *Gambusia affinis* (Baird and Girard), were stocked in a flooded wetlands field in the Mendota Wildlife Area during the fall of 1982 to determine: 1) overwintering success, 2) impact on aquatic invertebrate populations and 3) efficacy against mosquitoes. During spring draining 14.4 kg of mosquitofish, 11% of the stocked fish biomass, were recovered. Ten invertebrate taxa were collected in significantly greater ($P \leq 0.05$) numbers in the stocked field and nine taxa were collected in greater numbers in a similar, unstocked field. There was no difference between fields in the mean numbers of taxa collected by any sampling method, area sampler, dipper or minnow trap. *Culex tarsalis* Coquillett larval population in the stocked field was apparently reduced by 72% seven days after stocking.

INTRODUCTION.-The mosquitofish, *Gambusia affinis* (Baird and Girard), is an important component in riceland mosquito control programs in California. Each spring, with the advent of the rice and mosquito-growing season, many mosquito abatement districts stock newly flooded rice fields with mosquitofish. Most fish are obtained through active fish culture systems. In the fall with the draining of the rice fields, a small percentage of the fish are recaptured to restock or supplement the culture system; however, the vast majority of *G. affinis* are carried off in canals to an unknown fate and are lost of ricelands as a mosquito control agent.

Concurrent to the fall draining of ricelands, wintering waterfowl refuges flood their wetlands to attract and support migratory waterfowl through the winter. Often, water is held in these wetland fields until spring draining, which coincides with spring flooding of ricelands. Our study was developed to determine whether these synchronous regimes can be combined to produce a passive means for wintering mosquitofish to stock ricelands.

Recent studies by wildlife management personnel show that invertebrates, especially chironomid midge immatures, are very important in the diet of wintering migratory waterfowl in the Central Valley of California (Beam and Gruenhagen 1980 and Euliss 1984). Thus, when dealing with waterfowl habitats, it is necessary to ascertain the effect of any outside management implementation on the aquatic invertebrate component.

Our study was designed to determine: 1) the overwintering success, 2) impact on aquatic invertebrate populations and 3) efficacy against mosquitoes of *G. affinis* at the Mendota Wildlife Area.

MATERIALS AND METHODS.-The study sites were two, flooded, wetland fields within the Mendota Wildlife Area. Both fields were managed for and predominately composed of barnyardgrass (watergrass), *Echinochloa crusgalli*, and irrigated with water from the Fresno slough of the San Joaquin River. The fields were located 2.5 km apart and had the same soil type, Tachi clay. One field (13 ha) was stocked at a rate of 10 kg unsorted *G. affinis*/ha with fish collected from ricefield drainage ditches by seining (4.8 mm opening seine). The other field (10.4 ha) was left unstocked.

Aquatic invertebrates and mosquitofish populations were sampled from mid-September 1982 through April 1983 by three methods: 0.045 m^2 area sampler, standard mosquito dipper and modified minnow trap. Area sampling methods, including processing, counting and identification of organisms, generally followed those described by Takahashi et al. (1982); however, instead of box-shaped sampling units, a single-unit, cylindrical (24 cm diam.) device was used. Water containing organisms and benthos was removed and condensed through a 1.0 mm opening screen, washed and placed in alcohol. Twelve samples were collected from each field on nine dates during the study.

A 400 ml (6 x 10 cm diam.), long-handled dipper was used to collect six samples along a transect in each field. Each sample was composed of 15 dips, 6 liters of surface water, condensed through a 250μ opening screen and placed in alcohol. There was a total of nine sampling dates.

Minnow traps were 40 x 22 cm diam. and the interiors were lined with 1.5 mm window screen. Ten of these traps were placed in each field, collected after 24 h and the contents of each trap were put in separate jars with alcohol. There were six trapping dates during the study.

All samples were transported to the laboratory where the organisms were identified and counted. The data was analyzed by analysis of variance (ANOVA) on a Hewlett-Packard 9830A calculator.

RESULTS.- Twenty-five taxonomic groups were sampled in numbers sufficient for statistical analysis by two-way ANOVA. Where a taxon was

[1]California Department of Fish and Game, located in western Fresno County, California.

sampled by more than one method, data from the method which yielded the most numbers was used. In every case there was a significant difference ($P \leq 0.05$) between sampling dates. Overall means, generated through the entire study period, of 19 aquatic invertebrate taxa were found to be significantly different between the stocked and unstocked fields. These means and the sampling method utilized are listed in Table 1.

Ten invertebrate taxa were collected in significantly greater numbers in the field stocked with mosquitofish. These taxa were Cladocera, Eucopepoda, Ephemeroptera, Corixidae, Notonectidae, *Laccophilus, Thermonectus, Tropisternus,* Ephydridae and Oligochaeta. Mosquitofish were also more numerous in the stocked field.

In the unstocked field the following taxa had significantly greater populations: Podocopa, Zygoptera, Anisoptera, *Agabus, Berosus, Enochrus,* Ceratopogonidae, Syrphidae and Chironomidae collected by area sampler. There were no significant differences between fields in the means of *Hygrotus, Hydrophilus,* Culicidae, Tabanidae and Chironomidae sampled by dipper.

As a measure of diversity, the mean numbers of taxa collected by field and sampling method were statistically analyzed with paired t-test. The mean numbers of total invertebrate organisms

Table 1. Overall means and statistical analysis (ANOVA) of invertebrate organisms sampled from an unstocked wetlands field and a field stocked with 10 kg of *Gambusia affinis*/ha in the Mendota Wildlife Area (fall 1982 through spring 1983).

taxa	sampling method 1/	mean no. organisms 2/ unstocked	stocked
Arthropoda			
Crustacea			
Cladocera	D	807	1345*
Eucopepoda	D	95	232*
Podocopa	D	32*	4.5
Insecta			
Ephemeroptera	A	4.6	7.8*
Odonata			
Zygoptera	A	5.4*	2.8
Anisoptera	D	2.1*	0.74
Hemiptera			
Corixidae	T	4.5	16*
Notonectidae	T	0.45	2.7*
Coleoptera			
Laccophilus	T	0.12	1.2*
Hygrotus	A	0.093	0.11
Agabus	T	0.77*	0.37
Thermonectus	T	0.40	1.7*
Berosus	A	4.4*	2.9
Hydrophilus	T	0.017	0.17
Tropisternus	T	0.73	1.9*
Enochrus	A	0.13*	0.019
Diptera			
Culicidae	D	3.9	2.4
Chironomidae	D	117	135
Chironomidae	A	151*	77
Ceratopogonidae	A	7.1*	0.33
Tabanidae	A	0.23	0.27
Syrphidae	A	2.5*	0.63
Ephydridae	D	0.63	2.3*
Annelida			
Oligochaeta	A	17	52*
Chordata			
Osteichthyes			
Gambusia	T	0.13	8.9*

1/ D = dip samples, per 6 liters; A = area samples, per 0.045 m^2; T = trap samples, per trap.

2/ Asterisk * denotes significantly greater mean ($P \leq 0.05$) than that in other field.

Table 2. Statistical comparison (pared t-test) of mean numbers of taxa and organisms collected by 3 sampling methods from stocked (10 kg *G. affinis*/ha) and unstocked wetlands fields.

Sampling Method	Taxa		Organisms	
	Unstocked	Stocked	Unstocked	Stocked
Dip	13.5	11.9	1081	1735
Area	14.9	12.9	207	153
Trap	8.3	9.0	9.5	26.6*

*Mean was significantly greater ($P \leq 0.05$) than that in unstocked field.

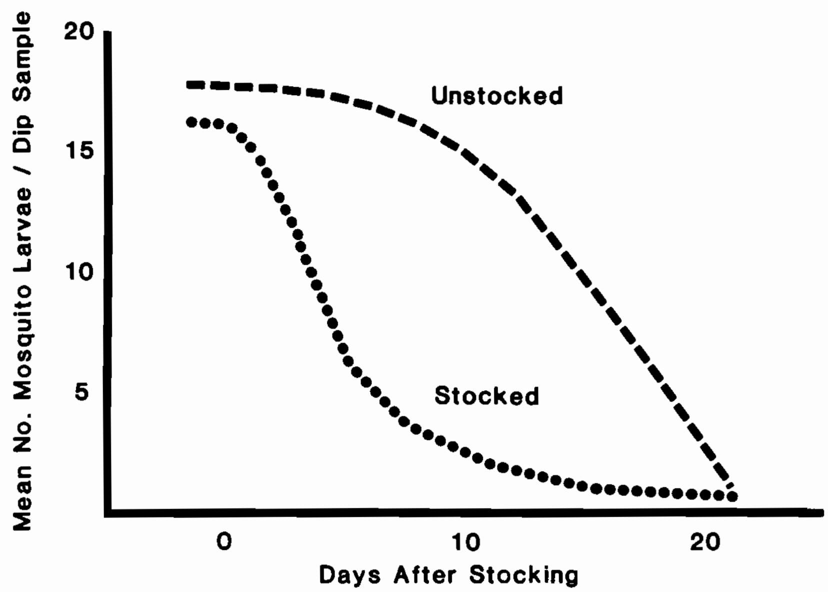

Figure 1. Comparison of *Culex tarsalis* immature populations in a wetlands field stocked with 10 kg *Gambusia affinis*/ha and an unstocked field (per 6 liters surface water).

collected were likewise analyzed. Results are shown in Table 2. Although the unstocked field average 1-2 more taxa per collection date than the stocked field by dip and area sampling methods, the means were not significantly different ($P>0.05$). Significantly more organisms on an average were collected in the stocked field by trap than in the unstocked field; however, mean numbers of organisms collected by other methods showed no significant difference between fields.

While ANOVA showed no significant difference between fields in numbers of mosquitoes collected through the entire study; there was an apparent reduction of 72% below the unstocked field seven days after stocking (Fig. 1). Virtually no mosquitoes were collected from either field after 21 days post-stocking. *Culex tarsalis* Coquillett was the predominant species.

Draining of the stocked field was initiated 6/1/83. Collection of mosquitofish in a screened outflow enclosure and by limited seining yielded 14.4 kg of *G. affinis*. This partial recovery of the available mosquitofish biomass represented 11% of the stocked biomass.

DISCUSSION.-Mosquitofish, transplanted from ricefields in the fall, successfully overwintered in retrievable numbers in a flooded wetlands field on the Mendota Wildlife Area. While the physical layout of the field prevented complete drainage and full recovery of *G. affinis*, 14.4 kg of mosquitofish were collected. It was estimated that this figure represented between 10-33% of the actual *G. affinis* biomass present at the time of draining. This estimated biomass, 43-144 kg, is a substantial amount with respect to the initial stocked biomass, 130 kg. Improvement of the drainage system within the wildlife area could greatly facilitate recovery of *G. affinis*.

Based on both the mean numbers of organisms by taxa collected and the measure of diversity between the two fields, there seems to be no overall detrimental effect upon the faunal constituents from wintering *G. affinis*. Major differences in the numbers of certain taxa between fields were evident, however, no consistent trend was apparent. Such differences between fields as were found might reasonably occur without any effect of treatment.

It is significant that cladoceran and copepod populations were greater in the stocked field. Several studies have indicated mosquitofish prey heavily on crustaceans (Ahmed et al. 1970, Farley and Younce 1977 and Miura et al. 1979); although Farley (1980) found that crustaceans were not selected in proportion to their abundance. Further, these studies showed chironomid immatures to be highly selected by mosquitofish; however, Bay and Anderson (1966) considered the impact of this predation upon the midge population to be negligible. While our study showed no difference in chironomid populations between fields by dip sampling, there was an apparent 50% reduction of larger chironomids in the stocked field by area sampling. The effect of wintering *G. affinis* on chironomids should be further studied in view of the importance of midges to waterfowl feeding ecology.

That mosquitofish produced minimal impact on invertebrate populations may be a result of reduced activity. Wurtsbaugh and Cech (1983) found temperature to be important in movement, food-consumption and growth of juvenile *G. affinis*. At lower water temperatures (10 and 15°C) swimming speed and activity were greatly diminished, with concomitant reduced food-consumption and growth rates. Mean daily air temperature through our study was 11.1°C (November 1982 - April 1983) and 8.1°C for December - January. The activity and food-consumption rates of the wintering fish were probably very low throughout most of our study.

In conclusion, the waterfowl refuge habitat is promising as a wintering preserve for *G. affinis* from ricelands and a source for subsequent restocking. Of added benefit is possible reduction of mosquitoes in the refuge habitat.

ACKNOWLEDGMENT.-The assistance and cooperation of personnel of the California Department of Fish and Game (Mendota), especially R. Huddleston and J. Beam, and the Fresno Westside Mosquito Abatement District is gratefully acknowledged. This work was supported, in part, by a special California State appropriation for mosquito control research.

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TOWARDS AN INTEGRATED MOSQUITO CONTROL STRATEGY FOR GRAY LODGE WILDLIFE REFUGE WITH EMPHASIS ON THE FLOODWATER SPECIES: *AEDES MELANIMON* AND *AE. NIGROMACULIS*[1]

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ABSTRACT

Studies related to the development of an integrated control program for mosquitoes produced on Gray Lodge Wildlife Refuge are reported. The principal species occurring on the refuge are *Aedes melanimon, Ae. nigromaculis, Culex tarsalis,* and *Anopheles freeborni*. Light trap monitoring in conjunction with field flooding patterns and larval survey indicate that the aedine mosquitoes are largely produced on the refuge while *Cx. tarsalis* and *An. freeborni* have important breeding sources outside as well. Light trap analysis indicated that the most abundant species were *An. freeborni* and *Ae. nigromaculis* in 1982 and *Ae. melanimon* and *Cx. tarsalis* in 1983. Speculation is given for these differences. Summer flooding of fields for millet production formed suitable oviposition sites for female *Aedes* spp. Larvae are frequently found in association with bermuda grass and populations requiring control action are generally restricted to a few fields. Treatment with granular formulations of *Bacillus thuringiensis* var. *israelensis* appears practical and is recommended for control of the *Aedes* spp.

INTRODUCTION.-A research program to develop a mosquito control strategy for Gray Lodge Wildlife Refuge was initiated in 1977 by several governmental agencies. The proposed plan was comprehensive and included studies ranging from the effect of managerial practices on mosquito production to the assessment of the success of any control action attempted (Lusk, 1979). Intensive field investigations were begun in 1978 and continued through 1979 by Hanna (1982) who studied the ecology of *Aedes melanimon* and *Ae. nigromaculis* and examined several methods for their control. Hanna conducted tests with the mosquitofish, *Gambusia affinis*, and the insecticide Chlorpyrifos against the *Aedes* on the refuge and found that neither tactic provided more than marginal control. Consequently, further research was necessary in order to develop a satisfactory program for mosquito suppression. In 1981 results of preliminary field trials with the microbial pathogen *Bacillus thuringiensis* var. *israelensis* serotype H14 (BTI) were encouraging. Investigations with BTI along with studies on the ecology and biology of mosquitoes on the refuge were then continued through 1983 by the present authors.

The studies reported in this paper primarily highlight work conducted in 1982 and 1983. Although research has been conducted at the refuge over the last six years, major changes in flooding practices after 1981 make comparisons with earlier studies impossible to analyze except in general terms. As in most field studies of this nature more questions were raised than answered. However, it is believed that enough information was generated to offer a preliminary strategy for the control of the floodwater *Aedes* spp. produced on the refuge.

STUDY AREA.-The Gray Lodge Wildlife Refuge is almost entirely in Butte County and is located approximately five miles west of Gridley just north of the Sutter Buttes. In consists of about 3,200 hectares of which the majority is flooded at least once during the year as waterfowl habitat (for a more detailed description see Lusk (1979), Conte and Hanna (1980), Rosenfield et al. 1981), and Hanna (1982)). During the first three years of the project, approximately 400 hectares were flooded in May and June and approximately 1,600 hectares during September and October (Hanna 1982). During 1981 major changes in the flooding practices were initiated, and included a marked reduction in the spring flooding to about 250 hectares and a major increase in the fall flooding to 2,500 hectares (Rosenfield et al. 1981). This general pattern has continued through 1983 although some fields that were flooded in the spring of 1981 were not flooded in the spring of 1982 and 1983, and in 1983 the "spring" flooding was not begun until the first part of July.

MATERIALS AND METHODS.-Adult mosquito activity was monitored by New Jersey light traps set at three locations on the north, east and southwestern borders of the refuge (Figure 1).

[1]This study was funded in part from special funds for Mosquito Research in California as well as the following cooperating agencies: Sutter-Yuba Mosquito Abatement District, California Department of Health Services, United States Fish and Wildlife Service and the California Department of Fish and Game.

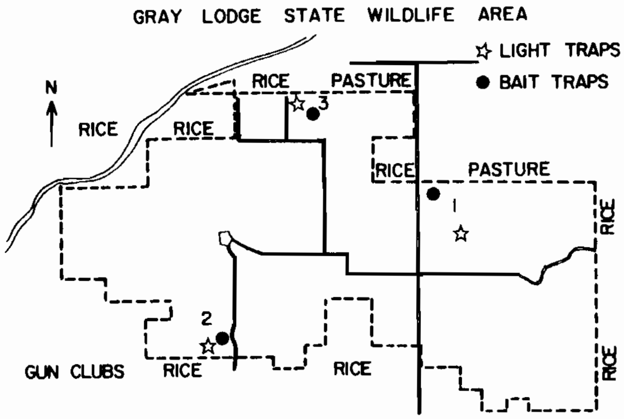

Figure 1. Layout and trap locations for Gray Lodge Wildlife Refuge located 2 miles north of the Sutter Buttes, in Butte county, Calif.

These traps were operated from one to seven days every week from April to November. The period of operation for each week depended in part on the level of mosquito activity. Other monitoring systems such as rabbit-baited CO_2 traps were used but the results are not included in this report. Collections from the three light traps were analyzed separately, however in the final analysis the results were combined to determine the average number of mosquitoes collected per trap-night.

Egg and larval distributions were studied in three fields: 36, 71, and 88, which were known to support large larval populations from previous observations. The distribution of viable *Aedes* spp. eggs was assessed by removing samples of soil, 29 cm x 20 cm x 5 cm (depth), from randomly selected sites located along 10 - 12 transect lines that had been set up in each field. Soil samples were returned to the lab, placed in aluminum pans, submerged with water and held outdoors for seven days. Larvae, when present, were then counted and identified. Soil samples were collected in July and August of 1982 and 1983.

Larval distribution and abundance was surveyed in the three fields in the fall within 24 to 48 hrs of flooding. Three dips were taken in a standardized pattern around each soil sample site and the number of larvae/dip recorded. This distribution of larvae in the field was then compared with the results of the soil samples collected in the summer. The larval survey was conducted in September in all 3 fields in 1982 and in field 71 in 1983. Due to a misunderstanding in flooding schedules, field 36 was flooded in 1983 before a follow-up could be conducted.

[2]Yellow Springs Instrument Company, Inc., Yellow Springs, Ohio.

Larval development times and attrition rates were determined for the fall populations of *Aedes* spp. in another field (75) in areas where large populations of larvae had been recovered from soil samples. Nine strips of aluminum 45 cm in width were used to form circles one meter in diameter. The rings were then placed in the field in clusters of three along a 100 meter transect. They were held in place by wooden stakes which were driven into the soil along the inside and outside walls and then nailed together through the ring. After the field was flooded and the enclosures filled with water the populations of *Aedes* contained inside were followed every 24 hrs at midday using the following standarized dipping procedure: five dips were taken along the inside edge of the rings and five were taken in the interior with at least one minute allowed between dips. Three of the nine enclosures were discarded from analysis because of water overflowing the walls during the flooding of the field. Due to similarities in population sizes, the remaining six enclosures were paired and their results averaged in the final analysis.

The final study reported in this paper compares the presence and densities of larval populations with different types of vegetation. Soil samples predominantly composed of either bermuda grass (*Cynodon dactylon*) or swamp timothy (*Heleochloa schoenoides*) were removed from field 75, returned to the lab and submerged with water as in previous experiments. Grass blade lengths were measured and larval populations analyzed. In addition, at the time soil samples were removed from the field, the following temperatures were recorded using a telethermometer[2]: base of bermuda grass, mid-crown, base of swamp timothy grass and ambient shade. Temperatures were taken 8 times between 10:45 a.m. and 12:30 p.m., September 9, 1983. The same temperature measurements were also taken when the samples were submerged at the laboratory in Albany, California.

Other weather data used in the analysis of light traps were collected by the Biggs-West Gridley Water District in Gridley, Calif., and consisted of mean daily averages, departures from 30-year norms and precipitation levels.

RESULTS AND DISCUSSION.-Results of light trap collections indicate that between 1982 and 1983 rather marked changes in relative abundance occurred among the four major species of mosquitoes found on the refuge (Table 1). In 1983, *Ae. melanimon* and *Cx. tarsalis* predominated, representing 40% and 35%, respectively, of the season total, while *Ae. nigromaculis* and *An. freeborni* represented 14% and 11%, respectively. These results were in sharp contrast to the previous year where *An. freeborni* and *Ae. nigromaculis* were the most prevalent species.

A previous survey by Rosenfield et al. (1981) indicated that larval *Cx. tarsalis* and *An. freeborni* are relatively rare on the refuge during the spring and summer, probably due largely to

Table 1. Comparative abundance of the 4 major species of mosquitoes[1] found at Gray Lodge Wildlife Refuge, Butte county, Calif., in 1982 and 1983.

	Ae. melanimon		*Ae. nigromaculis*		*Cx. tarsalis*		*An. freeborni*	
	No.	%Tot.	No.	%Tot.	No.	%Tot.	No.	%Tot.
1982	40	19	51	25	44	21	73	35
1983	136	40	47	14	119	35	38	11
	3.4 x↑		---		2.7 x↑		-0.5 x↓	

[1]Collected in light traps: ave. no. mosq./trap-night.

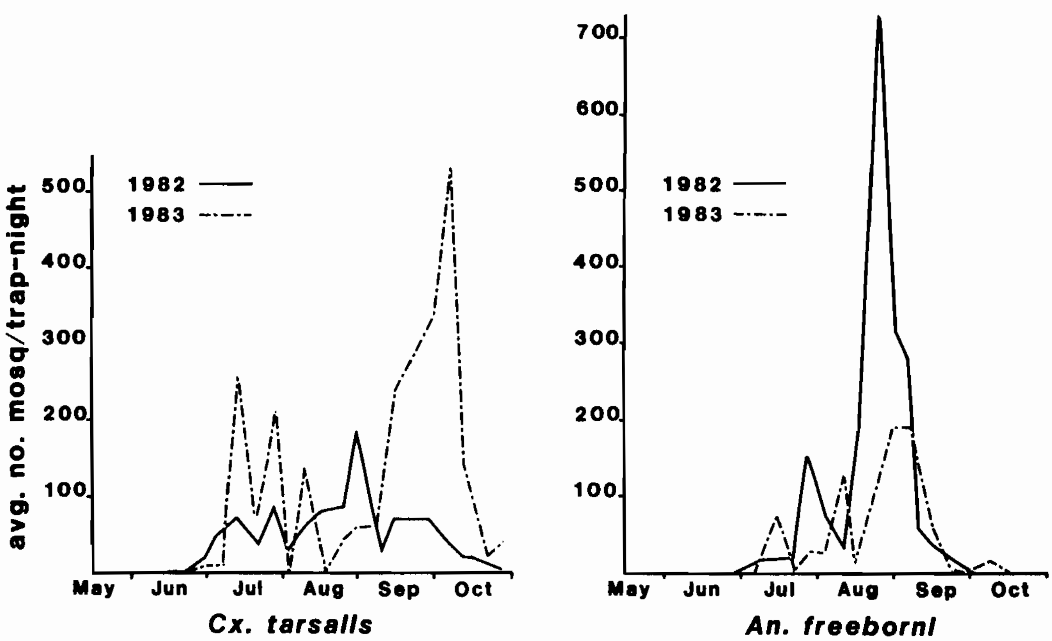

Figure 2. Results of light trap collections in 1982 and 1983 for *Cx. tarsalis* and *An. freeborni*, Gray Lodge Wildlife Refuge, Butte county, Calif.

Table 2. Ambient temperatures recorded at Biggs-West Gridley Water District, Gridley, Calif.

	1982			1983		
	$\bar{x}$ hi	$\bar{x}$ lo	Daily $\bar{x}$	$\bar{x}$ hi	$\bar{x}$ lo	Daily $\bar{x}$
May	30.4	11.9	21.1	28.5	11.8	20.1
June	30.4	13.9	22.1	32.4	15.0	23.7
July	35.4	17.0	26.2	34.0	15.8	24.5
August	34.0	16.2	25.1	35.1	17.6	26.3
September	29.8	15.5	22.6	32.9	15.2	24.1
October	24.5	10.1	17.3	28.3	11.3	19.8

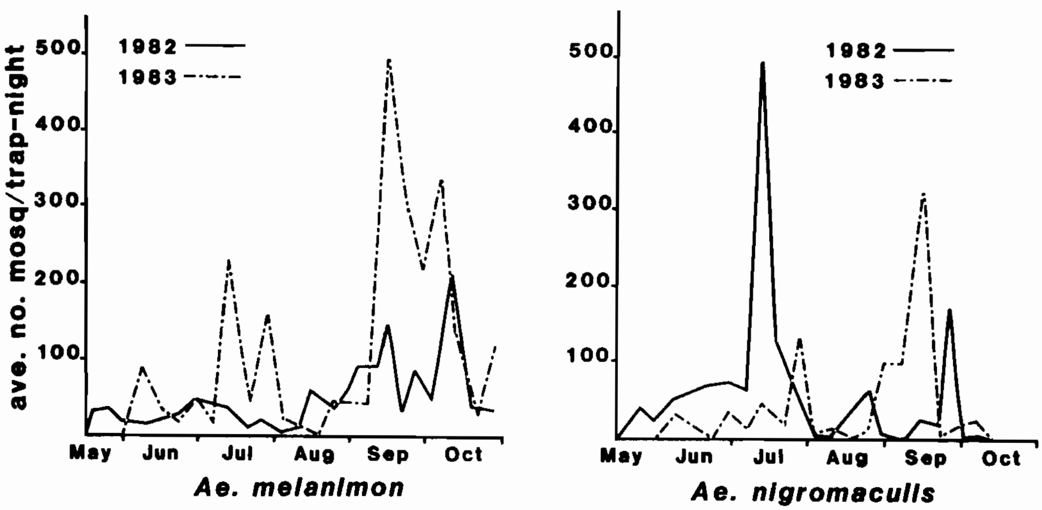

Figure 3. Results of light trap collections in 1982 and 1983 for *Ae. melanimon* and *Ae. nigromaculis*, Gray Lodge Wildlife Refuge, Butte county, Calif.

the lack of suitable breeding sources, but are fairly common from mid-September through October after the fall flooding of the fields. In 1982, light trap indices showed *Cx. tarsalis* peaking in numbers between late August and early September, when the fall floodings were just getting underway (Fig. 2). This species declined thereafter suggesting the flooded fields on the refuge were not contributing significantly to its production. In 1983 this pattern of events was different, with the major increase in population numbers shifting to September and October. This correlated well with the presence of the standing water in the flooded refuge fields. The reasons for this increase in activity over the previous year are not entirely clear but a contributing factor may have been the warmer temperatures in the late summer and early fall: the average temperatures in August, September, and October were, respectively, 1.2°, 1.5°, and 2.5°C/day greater than in 1982 (Table 2). In contrast to *Cx. tarsalis*, populations of *An. freeborni* were markedly depressed in 1983, particularly in the late summer and early fall. A number of factors may have accounted for this change including the reduction in rice acreage surrounding the refuge.

There were some major differences in the activity patterns of the *Aedes* species on the refuge between 1982 and 1983. In 1983 neither species appeared in the light traps until early June, almost a month later than in 1982. This was probably due to the long wet spring which resulted in a delay in the irrigation of pastures outside the refuge which produce the first brood of *Aedes*. As the season progressed, *Ae. melanimon* was unusually active in the midsummer in July while *Ae. nigromaculis* did not peak in numbers until late August and early September - activity patterns that were almost completely the reverse for these two species in 1982. These changes may be partly explained by the cool July temperatures which averaged 1.7°C/day less in 1983 than in 1982 and the warm late summer and early fall temperatures mentioned previously. Several workers have reported that *Ae. nigromaculis* is more thermophilic than *Ae. melanimon* and as such is more prevalent in the warmer, generally mid-summer, months while *Ae. melanimon* is more active in the "cooler" periods of early spring and late summer (Bohart and Washino, 1978). It can be speculated that in 1983 the delay of the really warm temperatures until the late summer contributed to the shift in activity for *Ae. nigromaculis*, while the cool temperatures in July allowed *Ae. melanimon* to predominate.

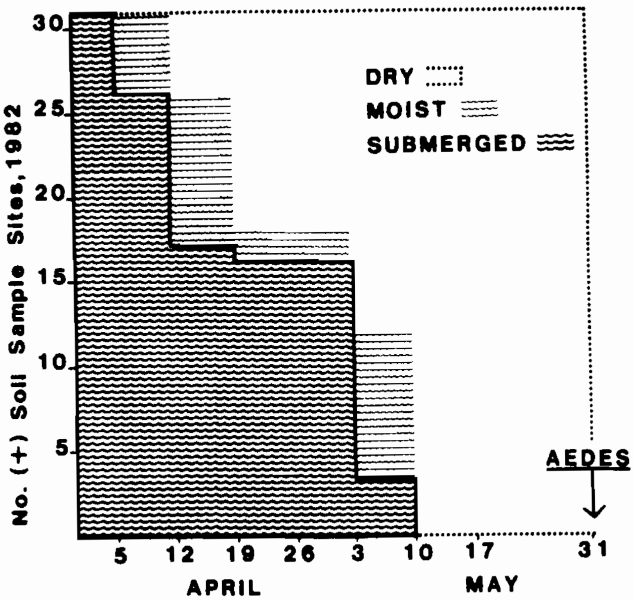

Figure 4. Appearance of adult *Aedes* spp. and availability of suitable oviposition sites in 1983 (based on 1982 soil sample sites positive for larvae in field 71).

The tremendous increase in the numbers of *Ae. melanimon* in the fall of 1983 are probably the result of eggs laid in the summer in fields that had been temporarily flooded for the production of millet. Both light trap data and field observations confirm the presence of large numbers of adult *Ae. melanimon* on the refuge at this time.

In the spring of 1983, all fields were completely dry for approximately two weeks before the first *Aedes* spp. were either noted in light traps or observed landing on investigators (Fig. 4). As a result, it is speculated that fields on the refuge did not serve as suitable sites for oviposition at this time. However, since weather can vary from year to year, this is probably not always the case. For example, in 1982 the spring was warmer, outside fields were flooded earlier, and *Aedes* spp. appeared in light traps in early May. As some of the refuge fields were still damp at this time, it is assumed that oviposition could have occurred.

Soil samples removed from fields 36 and 71 in July and August of 1983 showed a major reduction in the number that were positive for larvae as compared with those that had been collected in 1982 (Table 3). It is assumed that the most likely reason for this great reduction in 1983 was the lack of suitable moist substrates available for oviposition in the spring. However, further studies would be required to clarify these observations.

The fall larval surveys conducted in fields 71 and 36 in 1982 showed a correlation between the positive and negative soil sample sites and the presence or absence of larvae. In 1983, however, the larval survey following the fall flooding of field 71 revealed large numbers of larvae which were not expected because of the low numbers of larvae present in the August soil samples. This apparent inconsistency is difficult to explain in view of the positive correlations observed in 1982.

Table 3. Comparison of soil samples collected in 1982 and 1983. Gray Lodge Wildlife Refuge, Butte county, Calif.

	Field 71		Field 36	
	1982	1983	1982	1983
No. Sampled	100	50	84	42
No. Positive	43	4	25	7
% Positive	43	8	30	6
Ave. No. Larvae/				
⊕ Soil Sample	41	15	55	25
(Range)	(1-269)	(2-33)	(1-183)	(1-81)

One possible explanation is the 0.18 cm of rain that fell on August 19, 1983, one week after the soil samples had been collected. It was a light rain but it dampened the fields and this might have been sufficient to activate the necessary cues that trigger oviposition. The light trap data show few adult aedines present at this time in August and this would seem to discount this hypothesis; however, it is not known to what extent gravid females enter light traps.

The rate of larval development and attrition observed in the three paired enclosures during the fall flooding are presented in Figure 5. The increase in population size seen in this figure was due to a staggered hatch that resulted from the slow filling of the field and enclosures. Regardless of the initial larval densities, each enclosure contained about the same number of pupae suggesting that intraspecific competition may be an important mortality factor at higher densities. Larval attrition averaged 85% with most of the mortality occurring in the first four days. Pupae were first noted on day 6 and by day 8 accounted for 86.5% of the population. There was no mortality noted among pupae placed in floating screened cages and 97% of these had emerged between days 8 and 9.

The high attrition rate observed during the early instars suggests that a larvicidal treatment with a material such as BTI would be more effective if applied three to four days after the field was flooded.

Table 4 shows the differences in the numbers of larvae recovered from soil samples taken from sites containing bermuda grass versus swamp timothy grass. In this particular situation, a positive association was found between the presence of bermuda grass and large numbers of larvae. Routine dipping in other fields known to be heavy *Aedes* producers has also demonstrated this relationship. Relatively mild temperatures occur at the root - soil interface of bermuda grass stands as compared to other vegetation and this may be an important reason for the above association. Temperatures recorded in early September in field 75 showed an average of 28°C for the base of bermuda grass, while the base of swamp timothy grass averaged 37°C (Table 5). Temperatures as much as 6°C lower than ambient shade were recorded at the base of bermuda grass in another field where the grass was greener and taller.

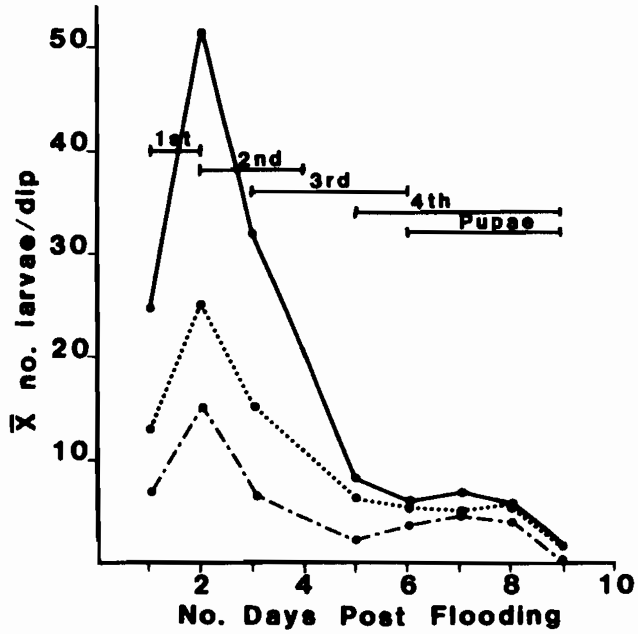

Figure 5. Development of three populations of *Ae. melanimon* under field conditions and appearance of each instar and pupae. Water temperatures: $\bar{x}$ high: 25.6°C, $\bar{x}$ low: 17.5°C.

The base of bermuda grass, then, has a rather mild microclimate which may serve to protect aedine eggs from the harsh drying temperatures, and to provide the adults with a long-term cool and quiet habitat for oviposition. However, bermuda grass does not appear to be the only reason for *Aedes* oviposition: there are fields containing other vegetative types that have populations of *Aedes* and there are fields containing stands of bermuda grass with sections lacking larvae. Although further studies are needed to clarify these observations, it would appear that if all conditions for oviposition are met, the presence of bermuda grass can provide an ideal habitat for *Aedes*.

Table 4. Differences in recovery of larval *Aedes* from soil samples taken from bermuda grass versus swamp timothy grass sites. Field 75, Gray Lodge Wildlife Refuge, September, 1983.

	No. soil samples	No. with larvae	$\bar{x}$ no. larvae	Range	Ave. blade length
Bermuda grass	8	8	651	392-1460	12.4 cm
Swamp timothy	12	0	0		4.0 cm

Table 5. A comparison of temperatures between different locations in vegetation and ambient shade[1].

Location	$\bar{x}$ °C	Range
Bermuda grass: base	28.1	25 - 30
Bermuda grass: mid-crown	39.2	30 - 43
Swamp timothy: base	36.9	30 - 41
Ambient shade	25.9	25 - 27

[1]Recorded between 10:45 a.m. and 12:30 p.m., Sept. 9, 1983.

CONCLUSIONS.-*Ae. melanimon*, *Ae. nigromaculis*, *Cx. tarsalis* and *An. freeborni* are the four important species found on the refuge. The first adult *Aedes* collected in light traps in the spring come from sources outside the refuge, whereas midsummer and fall populations are derived from the refuge as well as from outside pastures. The extent to which the drying refuge fields serve as good oviposition sites in the spring is probably highly variable: in 1983, by the time the first brood of females arrived, the refuge fields were completely dry.

Observations over the last 2-1/2 years indicate only a few fields support large populations of floodwater *Aedes*. Many of these fields are among those that are normally flooded during the late spring and early summer for millet seed production. The flooding of these fields with the subsequent formation of ideal oviposition sites during the drying-up period appears to benefit *Ae. melanimon* and *Ae. nigromaculis* as both are present as adults on the refuge at this time.

Receding water levels, noted in certain fields after the fall flooding, may be providing important oviposition sites for *Aedes* spp., particularly along the shallow margins of the fields.

In fields known to support large larval populations, the *Aedes* spp. are frequently found in association with dense concentrations of bermuda grass. Bermuda grass stands may be providing a quiet moist habitat attractive to ovipositing females and a protected environment for the aedine eggs.

Adult *An. freeborni* populations seen in August are apparently from surrounding rice fields while the *Cx. tarsalis* adults come from outside sources during the midsummer and inside and outside sources during the fall.

RECOMMENDATIONS:

1. *Aedes* larvae are largely restricted to specific areas in a few fields. Using simple dipping methods maps could be developed delineating problem fields and major production sites. Granular formulations of *Bacillus thuringiensis* var. *israelensis* serotype H14 have been shown to be extremely effective against both floodwater *Aedes* at Gray Lodge (Garcia et al. 1982, Garcia et al. 1983). Many larval sources are not that extensive and could be treated with a minimum of cost and effort.

2. Maintenance of constant water levels after the fall flooding would probably reduce suitable oviposition sites during a period when adult *Aedes* are active.

3. Control of certain plant species such as bermuda grass to eliminate attractive and protective oviposition sites may be appropriate but this would require further research.

4. Control of *Cx. tarsalis* and *An. freeborni* would be difficult. The use of *Gambusia affinis* recovered from rice field drainage may be possible but more research would be necessary if these species were to be included for control.

Management practices related to summer flooding for millet seed and the timing of the fall flooding in late August are important reasons for the level of *Aedes* production on the refuge. However, it is assumed that these practices, as they are currently carried-out, are vital to the goals of the refuge and as such have not been considered for alteration in these recommendations.

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