PROCEEDINGS AND PAPERS

of the

Fifty-fourth Annual Conference of the

California Mosquito and Vector Control Association, Inc.

March 16 thru March 19, 1986

Held at the
RED LION
REDDING, CALIFORNIA

Editor — John C. Combs

Business Office
CALIFORNIA MOSQUITO and VECTOR CONTROL ASSOCIATION, INC.
197 Otto Circle
Sacramento, California 95822

Published December 31, 1986

Printed by
Anchor Press
Sacramento, California 95818

1986 OFFICERS AND DIRECTORS OF THE CALIFORNIA MOSQUITO and VECTOR CONTROL ASSOCIATION, INC.

PRESIDENT .... James R. Caton
PRESIDENT ELECT .... Charles P. Hansen
VICE PRESIDENT .... Norman F. Hauret
PAST PRESIDENT .... William C. Hazeleur

REGIONAL REPRESENTATIVES

COASTAL .... Dennis D. Beebe
SACRAMENTO VALLEY .... Kenneth G. Whitesell
N. SAN JOAQUIN VALLEY ....Douglas C. White
S. SAN JOAQUIN VALLEY ....Harmon L. Clement
S. CALIFORNIA ....Lyle M. Stotelmyre

1986 CORPORATE MEMBERS OF THE CALIFORNIA MOSQUITO and VECTOR CONTROL ASSOCIATION, INC.

Alameda County MAD
Antelope Valley MAD
Burney Basin MAD
Butte County MAD
Carpinteria/Goleta Valley MAD
Coachella Valley MAD
Coalinga-Huron MAD
Colusa MAD
Compton Creek MAD
Consolidated MAD
Contra Costa MAD
Corning MAD
Delano MAD
Delta VCD
Diablo Valley MAD
Durham MAD
East Side MAD
El Dorado County Service Area III
Fresno MAD
Fresno Westside MAD
Glenn County MAD
Kern MAD
Kings MAD
Lake County MAD
Los Angeles County West MAD
Los Molinos MAD
Madera County MAD
Marin/Sonoma MAD
Merced County MAD
Moorpark MAD
Napa County MAD
Northern Salinas Valley MAD
Northwest MAD
Orange County VCD
Oroville MAD
Pine Grove MAD
Sacramento County - Yolo County MAD
San Joaquin County MAD
San Mateo County MAD
Shasta MAD
Solano County MAD
Southeast MAD
Sutter-Yuba MAD
Tehama MAD
Tulare MAD
Turlock MAD
Westside MAD
West Valley VCD

1986 EXHIBITORS, SPONSORS and SUSTAINING MEMBERS
CALIFORNIA MOSQUITO and VECTOR CONTROL ASSOCIATION, INC.

ABBOTT LABORATORIES .... Armando Flores, Regional Manager
1540 East Shaw, #123
Fresno, California 93710

BIOQUIP PRODUCTS, INC. .... Richard P. Fall, General Manager
Post Office Box 61
Santa Monica, California 90406

FENNIMORE CHEMICALS .... H. B. Munns, President
Post Office Box 1116
Glendora, California 91740

GOLDEN BEAR DIVISION OF WITCO .... Carter Knowles, Manager, Special Product Sales
Post Office Box 5446
Bakersfield, California 93388

HINDS INTERNATIONAL, INC. .... James Hinds, Sales
Post Office Box 4327
Portland, Oregon 97208

MICROBIAL RESOURCES, INC. .... Chris J. Stafford, Research Scientist
1302-2 Cynwyd Club Drive
Wilmington, Delaware 19808

MOBAY CHEMICAL CORPORATION .... Jim Truslow, Sales Representative
3010 Fairview Drive
Vista, California 92084

PENICK-BIO .... Lee A. Norton, Sales Representative
Two Clark Drive, Apt. 108
San Mateo, California 94401

TARGET CHEMICAL COMPANY .... Dennis Candito, Pest Management Coordinator
1280 North Tenth Street
San Jose, California 95112

VALLEY REGIONAL INSURANCE SERVICES .... Don Simons, Vice President
1322 E. Shaw Avenue #310
Fresno, California 93710

WILBUR-ELLIS COMPANY .... Michael Cline, Sales Representative
Post Office Box 1286
Fresno, California 93715

ZANUS CORPORATION .... David G. Sullivan, President
1259 El Camino Real, #134
Menlo Park, California 94025

ZOECON CORPORATION .... Ted R. Sleek, Representative
12005 Ford Road
Dallas, Texas 75381

TABLE OF CONTENTS

PUBLIC HEALTH AND DISEASE PREVENTION

GENERAL: CMVCA

CHEMICAL AND BIOLOGICAL CONTROL

ECOLOGICAL STUDIES

OTHER VECTORS THAN MOSQUITOES

EQUIPMENT

California Mosquito and Vector Control Association, Inc.

Volume 54 March 16-19, 1986

SURVEILLANCE FOR ARTHROPOD-BORNE VIRAL ACTIVITY AND DISEASE IN CALIFORNIA DURING 1985

Richard W. Emmons[1], Marilyn M. Milby[2], John D. Walsh[3], William C. Reeves[2], Edmond V. Bayer[4], Lucia T. Hui[3], James D. Woodie[1], and Robert A. Murray[5]

This is the sixteenth (since 1969) in a series of annual reports made to the California Mosquito and Vector Control Association which summarizes the cooperative efforts on encephalitis surveillance by local mosquito control agencies, local health departments, the California Department of Food and Agriculture, private physicians and veterinarians, and other groups represented by the authorship of this report or mentioned in the text which follows. In last year's summary we reported the unprecedented occurrence of epidemic St. Louis encephalitis (SLE) virus activity in major urban areas of southern California, with 26 confirmed or probable human SLE cases, 1 fatal -- the largest outbreak in the state since 1959 when there were 40 cases of SLE and 2 cases of western equine encephalomyelitis (WEE). This event prompted more intensive mosquito surveillance and control efforts, increased monitoring for evidence of SLE and WEE viral activity, and a more comprehensive search for human and equine cases of mosquito-transmitted encephalitis in these urban areas of the state as well as in rural, traditionally endemic areas. Fortunately, the 1985 experience was not as severe as in 1984, although viral activity in urban southern California was again documented.

The highlights of the surveillance program are briefly summarized below. No cases of WEE and only three human cases of SLE were documented during the year:

(1) a 17 year old boy from Lake Elsinore, Riverside County, with mosquito exposure apparently at his home site. He had onset of illness July 24, was hospitalized July 29, recovered and returned home by August 10;

(2) a 31 year old man from the Needles area of San Bernardino County, probably infected by mosquito bite on the Arizona side of the Colorado River near Needles. He became ill August 16 and subsequently recovered completely; and

(3) a 61 year old woman from the North Hollywood area of Los Angeles County, apparently exposed near her home, who became ill August 23 and recovered completely.

A total of 635 patients (85 more than in 1984) were tested serologically by the Viral and Rickettsial Disease Laboratory, the 5 collaborating County Health Department Laboratories (Fresno, Los Angeles, Orange, San Bernardino, and San Diego), and the Microbiology Reference Laboratory, Cypress, California, in order to detect these three cases. Tests by the VRDL for evidence of current California encephalitis (CE) viral infection in 6 patients from several counties where CE virus is known to be active showed no cases of illness which could be attributed to the virus; however, 1 patient (a 16 year old boy from Inyo County) had stationary levels of IGG antibody, but no IgM antibody, indicating that viral infection had occurred sometime in the past. Further search for CE in California is warranted. The virus was first discovered in this state, but

---

[1]Viral and Rickettsial Disease Laboratory, California State Department of Health Services.

[2]Department of Biomedical and Environmental Health Sciences School of Public Health, University of California, Berkeley.

[3]Vector Surveillance and Control Branch, California State Department of Health Services.

[4]Veterinary Public Health Unit, Infectious Disease Branch, California State Department of Health Services.

[5]Infectious Disease Branch, California State Department of Health Services.

Table 1.-Number of mosquitoes and pools tested during 1985 by the Viral and Rickettsial Disease Laboratory by county and species.

	Species											
	Aedes melanimon		Other Species		*Culex peus*		*Culex pipiens* complex		*Culex tarsalis*		Total	
	Mosq	Pools	Mosq	Pools	Mosq	Pools	Mosq	Pools	Mosq	Pools	Mosq	Pools
Butte	100	2	.	.	200	4	.	.	3745	76	4045	82
Colusa	.	.	.	.	.	.	.	.	227	5	227	5
Contra Costa	.	.	.	.	.	.	292	6	.	.	292	6
Glenn	.	.	.	.	.	.	.	.	597	12	597	12
Imperial	.	.	60	2	.	.	.	.	11613	264	11673	266
Inyo	1705	36	.	.	.	.	.	.	223	8	1928	44
Kern	8877	205	30	2	28	1	.	.	27898	616	36833	824
Lake	42	1	.	.	.	.	.	.	1017	21	1059	22
Los Angeles	.	.	2494	54	1621	72	16198	382	6846	188	27159	696
Marin	.	.	100	2	.	.	13	1	562	12	675	15
Merced	.	.	.	.	.	.	.	.	632	14	632	14
Orange	.	.	1755	36	140	7	4487	128	25322	594	31704	765
Riverside	.	.	1168	25	1017	28	4380	97	16137	335	22702	485
Sacramento	.	.	.	.	87	5	.	.	14375	296	14462	301
San Bernardino	.	.	.	.	12	1	1673	34	3514	74	5199	109
San Diego	.	.	450	9	533	14	1182	30	4691	103	6856	156
Shasta	.	.	.	.	.	.	.	.	1079	22	1079	22
Sonoma	.	.	.	.	1344	29	167	4	3272	68	4783	101
Stanislaus	.	.	.	.	.	.	99	3	158	6	257	9
Sutter	.	.	.	.	.	.	.	.	3685	79	3685	79
Ventura	.	.	.	.	.	.	.	.	5448	109	5448	109
Yolo	.	.	.	.	.	.	.	.	13335	270	13335	270
Yuba	.	.	.	.	.	.	.	.	1075	25	1075	25
TOTAL	10724	244	6057	130	4982	161	28491	685	145451	3197	195705	4417

Source: Mosquito Virus Pool Data System, Vector Surveillance and Control Branch, Department of Health Services.

CE disease has not been recognized here since the 1940's.

There were 32 clinically suspect equine cases of WEE reported from 17 California counties; 15 of these were tested serologically, 11 were tested for virus in brain tissue, and 6 cases were tested by both serological and virus isolation methods, but none of them could be confirmed as WEE.

Vector surveillance was intensified in 1985, as compared with the 1984 effort. A total of 4,417 mosquito pools (Table 1) were tested in suckling mice or by cell culture inoculation, 1,348 more than were tested in 1984. The majority of these pools were comprised of *Culex tarsalis*, as usual (3,197 pools, 72.4% of the total), but attempts were also made to include *Culex pipiens* complex (15.5%), *Aedes melanimon* (5.5%) and *Culex peus* (3.6%) in greater numbers than in previous years. Viruses isolated from the pools (Table 2) included: 30 SLE, 28 WEE, 18 CE group, 48 Turlock, 122 Hart Park, and 2 Main Drain -- 248 isolates altogether. As usual, nearly all the isolates came from *C. tarsalis*, except for 15 CE isolates from *A. melanimon*, 1 Turlock and 2 Hart Park from *C. pipiens* complex, 1 Turlock from *Culex erythrothorax*, 2 Main Drain from *Aedes taeniorhynchus*, and 1 SLE isolated from a pool of *C. peus* in Encino, Los Angeles County -- a finding of special interest. A detailed listing of individual positive mosquito pools is shown in Table 3.

There were 5,819 serum samples taken from chickens in the 55 sentinel flocks (10 more flocks than in 1984) which were bled monthly from May through October. The only seroconversions for WEE or SLE antibodies occurred in 6 flocks at southern California sites, as shown in Table 4.

The mosquito and sentinel chicken laboratory testing program was supported entirely this year by the VRDL and by a state contract with the California Public Health Foundation (84-84428-A), rather than having to utilize special University of California Mosquito Control Research funds, as in previous years.

Table 2.-Number of viral isolates from mosquitoes tested during 1985 by the Viral and Rickettsial Disease Laboratory by species, county and agent isolated.

Species	County	WEE	SLE	Turlock	California Group	Hart Park	Main Drain	Total
Aedes melanimon	Kern	.	.	.	15	.	.	15
Culex erythrothorax	Orange	.	.	1	.	.	.	1
Culex peus	Los Angeles	.	1	.	.	.	.	1
Aedes taeniorhynchus	San Diego	.	.	.	.	.	2	2
Culex pipiens complex	Orange	.	.	1	.	2	.	3
Culex tarsalis	Butte	.	.	.	.	4	.	4
	Imperial	21	15	3	.	.	.	39
	Kern	.	.	25	3	53	.	81
	Los Angeles	.	.	.	.	7	.	7
	Merced	.	.	.	.	1	.	1
	Orange	.	.	9	.	37	.	46
	Riverside	3	8	1	.	1	.	13
	Sacramento	.	.	4	.	7	.	11
	San Bernardino	4	6	1	.	.	.	11
	Sutter	.	.	1	.	3	.	4
	Yolo	.	.	2	.	7	.	9
	ALL	28	29	46	3	120	2	226
TOTAL ISOLATES		28	30	48	18	122	2	248

Source: Mosquito Virus Pool Data System, Vector Surveillance and Control Branch, Department of Health Services.

In summary, the surveillance program in 1985, as in 1983 and 1984, confirmed the occurrence of SLE and WEE viral activity along the Colorado River and in southern California urban and suburban areas not previously known to be endemic areas, where the potential for a major outbreak in heavily populated areas exists. Continued surveillance and study of the ecology of viral persistence in these areas, and the means for mosquito control and encephalitis prevention, are essential.

ACKNOWLEDGMENTS.-We thank Mary Ann Mahoney, Cynthia Chan and Oscar Armstrong for special assistance in the mosquito testing and chicken serosurvey; we also thank the many staff members of the Viral and Rickettsial Disease Laboratory, the Vector Surveillance and Control Branch, the Infectious Disease Branch, and others in the California Department of Health Services; all participating local Mosquito Abatement agencies and County Health Departments; the California Department of Food and Agriculture; private physicians and veterinarians; the Microbiology Reference Laboratory; and all others who helped in this surveillance program.

Table 3.-Viral isolates from mosquito pools tested during 1985 by the Viral and Rickettsial Disease Laboratory, compiled chronologically and by Mosquito Abatement District (Pool Data System, Vector Surveillance and Control Branch).

DIST	POOLNO	SPECIES	NUMBER MOSQUITO	VIRUS	MONTH	DAY	PLACE	COUNTY
BUCO	23	CX TARS	50	HART	JUL	1	GRIDLEY	BUTTE
	28	" "	50	HART	JUL	8	NORD	BUTTE
	34	" "	50	HART	JUL	8	NORD	BUTTE
	35	" "	50	HART	JUL	8	NORD	BUTTE
CHLV	22	CX TARS	50	SLE	JUN	20	MECCA	RIVERS
	26	" "	50	SLE	JUN	20	MECCA	RIVERS
	31	" "	50	WEE	JUN	20	MECCA	RIVERS
	44	" "	50	TRLK	JUL	18	MECCA	RIVERS
	49	" "	50	SLE	AUG	22	MECCA	RIVERS
IMPR	76	CX TARS	50	TRLK	APR	16	CALEXICO	IMPERL
	101	" "	50	TRLK	MAY	19	CALEXICO	IMPERL
	135	" "	50	WEE	JUN	11	HEBER	IMPERL
	139	" "	50	WEE	JUN	11	SEELY	IMPERL
	140	" "	50	WEE	JUN	11	SEELY	IMPERL
	145	" "	50	WEE	JUN	11	SEELY	IMPERL
	150	" "	50	WEE	JUN	11	SEELY	IMPERL
	152	" "	23	WEE	JUN	11	SEELY	IMPERL
	155	" "	50	WEE	JUN	11	SEELY	IMPERL
	156	" "	50	WEE	JUN	11	SEELY	IMPERL
	157	" "	50	WEE	JUN	11	SEELY	IMPERL
	158	" "	50	WEE	JUN	11	SEELY	IMPERL
	159	" "	50	WEE	JUN	11	SEELY	IMPERL
	160	" "	50	WEE	JUN	11	SEELY	IMPERL
	161	" "	50	WEE	JUN	11	SEELY	IMPERL
	162	" "	50	WEE	JUN	11	SEELY	IMPERL
	164	" "	42	WEE	JUN	11	HEBER	IMPERL
	166	" "	50	SLE	JUN	11	PALO VERDE	IMPERL
	173	" "	50	TRLK	JUN	11	LAGUNA DAM	IMPERL
	188	" "	50	SLE	JUL	18	HEBER	IMPERL
	189	" "	50	WEE	JUL	18	HEBER	IMPERL
	191	" "	50	WEE	JUL	22	SEELY	IMPERL
	192	" "	50	SLE	JUL	22	SEELY	IMPERL
	193	" "	50	WEE	JUL	22	SEELY	IMPERL
	194	" "	50	SLE	JUL	22	SEELY	IMPERL
	195	" "	37	SLE	JUL	22	HEBER	IMPERL
	198	" "	31	SLE	AUG	8	SEELY	IMPERL
	201	" "	50	SLE	AUG	8	SEELY	IMPERL
	202	" "	50	SLE	AUG	8	SEELY	IMPERL
	203	" "	50	SLE	AUG	8	SEELY	IMPERL
	204	" "	50	SLE & WEE	AUG	8	SEELY	IMPERL
	206	" "	50	SLE	AUG	8	SEELY	IMPERL
	208	" "	50	SLE & WEE	AUG	8	SEELY	IMPERL
	209	" "	50	SLE	AUG	8	SEELY	IMPERL
	211	" "	24	SLE	AUG	15	HEBER	IMPERL
	212	" "	11	SLE & WEE	AUG	15	HEBER	IMPERL
KERN	5037	AE MELN	50	CALGP	MAY	14	LOST HILLS	KERN
	5135	CX TARS	50	TRLK	JUN	10	ARVIN	KERN
	5152	" "	50	HART	JUN	11	BAKERSFIELD	KERN
	5159	AE MELN	50	CALGP	JUN	13	LOST HILLS	KERN
	5167	" "	50	CALGP	JUN	12	DELANO	KERN

continued -

Table 3-continued

DIST	POOLNO	SPECIES	NUMBER MOSQUITO	VIRUS	MONTH	DAY	PLACE	COUNTY
KERN	5172	CX TARS	50	HART	JUN	17	ARVIN	KERN
	5176	" "	50	HART	JUN	17	ARVIN	KERN
	5179	" "	50	HART	JUN	17	ARVIN	KERN
	5191	" "	47	HART	JUN	17	BAKERSFIELD	KERN
	5198	AE MELN	50	CALGP	JUN	19	LOST HILLS	KERN
	5205	CX TARS	50	HART	JUN	25	ARVIN	KERN
	5207	" "	50	HART	JUN	25	ARVIN	KERN
	5208	" "	50	HART	JUN	25	ARVIN	KERN
	5209	" "	39	HART	JUN	25	ARVIN	KERN
	5223	AE MELN	50	CALGP	JUN	26	LOST HILLS	KERN
	5231	CX TARS	50	HART	JUL	1	BAKERSFIELD	KERN
	5235	" "	50	HART	JUL	1	ARVIN	KERN
	5236	" "	50	TRLK	JUL	1	ARVIN	KERN
	5237	" "	50	HART	JUL	1	ARVIN	KERN
	5238	" "	50	HART	JUL	1	ARVIN	KERN
	5239	" "	50	HART	JUL	1	ARVIN	KERN
	5241	" "	50	HART	JUL	1	ARVIN	KERN
	5255	AE MELN	50	CALGP	JUL	2	LOST HILLS	KERN
	5267	CX TARS	50	HART	JUL	2	BAKERSFIELD	KERN
	5268	" "	50	HART	JUL	8	ARVIN	KERN
	5270	" "	50	HART	JUL	8	ARVIN	KERN
	5271	" "	50	HART	JUL	8	ARVIN	KERN
	5272	" "	50	HART	JUL	8	ARVIN	KERN
	5279	" "	34	HART	JUL	8	BAKERSFIELD	KERN
	5283	" "	50	HART	JUL	8	BAKERSFIELD	KERN
	5296	" "	9	HART	JUL	10	BUTTONWILLOW	KERN
	5314	" "	50	HART	JUL	15	BAKERSFIELD	KERN
	5320	" "	50	HART	JUL	15	ARVIN	KERN
	5321	" "	50	HART & TRLK	JUL	15	ARVIN	KERN
	5322	" "	50	HART	JUL	15	ARVIN	KERN
	5323	" "	50	HART	JUL	15	ARVIN	KERN
	5324	" "	50	TRLK	JUL	15	ARVIN	KERN
	5326	" "	50	HART	JUL	15	ARVIN	KERN
	5327	" "	50	HART	JUL	15	ARVIN	KERN
	5328	" "	50	HART	JUL	15	ARVIN	KERN
	5337	" "	50	HART	JUL	16	BAKERSFIELD	KERN
	5350	" "	50	HART	JUL	22	ARVIN	KERN
	5351	" "	50	HART	JUL	22	ARVIN	KERN
	5352	" "	50	HART	JUL	22	ARVIN	KERN
	5354	" "	50	HART	JUL	22	ARVIN	KERN
	5375	" "	50	TRLK	JUL	29	ARVIN	KERN
	5377	" "	50	HART & TRLK	JUL	29	ARVIN	KERN
	5380	" "	25	HART	JUL	29	ARVIN	KERN
	5394	" "	50	HART & TRLK	JUL	30	BAKERSFIELD	KERN
	5411	" "	50	TRLK	AUG	5	BAKERSFIELD	KERN
	5416	" "	50	TRLK	AUG	5	BAKERSFIELD	KERN
	5418	" "	50	HART	AUG	6	ARVIN	KERN
	5419	" "	50	HART	AUG	6	ARVIN	KERN
	5421	" "	50	HART	AUG	6	ARVIN	KERN
	5451	" "	50	TRLK	AUG	12	ARVIN	KERN
	5452	" "	50	HART	AUG	12	ARVIN	KERN
	5453	" "	50	HART	AUG	12	ARVIN	KERN
	5465	" "	27	TRLK	AUG	13	BAKERSFIELD	KERN
	5472	" "	50	HART	AUG	13	BAKERSFIELD	KERN
	5476	" "	16	HART & TRLK	AUG	14	BUTTONWILLOW	KERN
	5477	" "	50	HART	AUG	14	BUTTONWILLOW	KERN
	5513	" "	50	TRLK	AUG	21	ARVIN	KERN
	5522	" "	50	HART	AUG	21	ARVIN	KERN
	5527	" "	50	TRLK	AUG	21	BAKERSFIELD	KERN

continued -

Table 3-continued

DIST	POOLNO	SPECIES	NUMBER MOSQUITO	VIRUS	MONTH	DAY	PLACE	COUNTY
KERN	5560	CX TARS	50	HART	AUG	26	BAKERSFIELD	KERN
	5564	" "	50	TRLK	AUG	26	BAKERSFIELD	KERN
	5598	AE MELN	50	CALGP	AUG	28	LOST HILLS	KERN
	5621	CX TARS	50	TRLK	SEP	4	ARVIN	KERN
	5634	" "	50	TRLK	SEP	4	BAKERSFIELD	KERN
	5638	" "	50	HART	SEP	4	BAKERSFIELD	KERN
	5646	" "	50	HART	SEP	4	BAKERSFIELD	KERN
	5651	" "	50	HART	SEP	5	LOST HILLS	KERN
	5655	AE MELN	50	CALGP	SEP	5	LOST HILLS	KERN
	5666	" "	50	CALGP	SEP	11	LOST HILLS	KERN
	5667	" "	50	CALGP	SEP	11	LOST HILLS	KERN
	5682	CX TARS	50	TRLK	SEP	16	BAKERSFIELD	KERN
	5684	" "	50	TRLK	SEP	16	BAKERSFIELD	KERN
	5691	" "	50	TRLK	SEP	17	ARVIN	KERN
	5715	AE MELN	50	CALGP	SEP	18	LOST HILLS	KERN
	5716	" "	50	CALGP	SEP	18	LOST HILLS	KERN
	5724	CX TARS	50	TRLK	SEP	23	BAKERSFIELD	KERN
	5732	" "	11	TRLK	SEP	24	ARVIN	KERN
	5744	" "	50	TRLK	SEP	25	LOST HILLS	KERN
	5745	" "	11	TRLK	SEP	25	LOST HILLS	KERN
	5746	" "	50	CALGP	SEP	25	LOST HILLS	KERN
	5747	" "	50	CALGP	SEP	25	LOST HILLS	KERN
	5749	" "	50	CALGP	SEP	25	LOST HILLS	KERN
	5771	AE MELN	50	CALGP	OCT	2	LOST HILLS	KERN
	5784	CX TARS	9	TRLK	OCT	10	BAKERSFIELD	KERN
	5792	AE MELN	47	CALGP	OCT	15	LOST HILLS	KERN
LONG	91	CX TARS	30	HART	JUN	20	LONG BEACH	L A
	229	" "	22	HART	JUL	27	LONG BEACH	L A
	246	" "	22	HART	AUG	16	LONG BEACH	L A
MERC	9	CX TARS	50	HART	JUL	22	MERCED	MERCED
NWST	97	CX TARS	50	HART	JUN	25	CORONA	RIVERS
ORCO	43	CX TARS	31	HART	MAY	17	IRVINE	ORANGE
	83	" "	43	HART	MAY	22	IRVINE	ORANGE
	100	" "	50	HART	MAY	17	IRVINE	ORANGE
	144	" "	48	HART	JUN	5	IRVINE	ORANGE
	189	" "	28	HART	JUN	19	IRVINE	ORANGE
	199	" "	50	HART	JUN	19	IRVINE	ORANGE
	201	" "	53	HART	JUN	19	IRVINE	ORANGE
	202	" "	50	HART	JUN	19	IRVINE	ORANGE
	210	" "	50	HART	JUN	19	IRVINE	ORANGE
	211	" "	50	HART	JUN	21	SAN CLEMENTE	ORANGE
	219	" "	50	HART	JUN	26	IRVINE	ORANGE
	263	" "	50	HART	JUL	6	IRVINE	ORANGE
	266	" "	50	HART	JUL	8	IRVINE	ORANGE
	267	" "	12	HART	JUL	8	IRVINE	ORANGE
	268	" "	50	HART	JUL	8	IRVINE	ORANGE
	271	" "	18	HART	JUL	8	IRVINE	ORANGE
	274	" "	50	HART	JUL	10	IRVINE	ORANGE
	276	" "	50	HART	JUL	10	IRVINE	ORANGE
	281	" "	50	HART	JUL	10	IRVINE	ORANGE
	282	" "	20	HART	JUL	10	IRVINE	ORANGE
	283	" "	50	HART	JUL	10	IRVINE	ORANGE
	286	" "	50	HART	JUL	10	IRVINE	ORANGE
	290	" "	48	HART	JUL	10	IRVINE	ORANGE
	297	" "	49	HART	JUL	10	IRVINE	ORANGE

continued -

Table 3-continued

DIST	POOLNO	SPECIES	NUMBER MOSQUITO	VIRUS	MONTH	DAY	PLACE	COUNTY
ORCO	353	CX TARS	50	HART	JUL	16	IRVINE	ORANGE
	449	" "	50	HART	JUL	25	IRVINE	ORANGE
	455	" "	50	HART	JUL	31	IRVINE	ORANGE
	460	" "	50	HART	JUL	31	IRVINE	ORANGE
	462	" "	50	HART	JUL	31	IRVINE	ORANGE
	486	" "	50	HART	AUG	6	IRVINE	ORANGE
	528	" "	50	HART	AUG	13	IRVINE	ORANGE
	531	" "	50	HART	AUG	13	IRVINE	ORANGE
	536	" "	50	HART	AUG	13	IRVINE	ORANGE
	537	" "	50	HART	AUG	13	IRVINE	ORANGE
	538	" "	50	TRLK	AUG	13	IRVINE	ORANGE
	593	" "	38	TRLK	SEP	4	IRVINE	ORANGE
	595	" "	22	HART	SEP	4	IRVINE	ORANGE
	596	CX ERYT	42	TRLK	SEP	10	IRVINE	ORANGE
	619	CX TARS	45	TRLK	SEP	11	IRVINE	ORANGE
	648	CX PIPS	41	TRLK	SEP	18	IRVINE	ORANGE
	652	" "	12	HART	SEP	24	IRVINE	ORANGE
	654	CX TARS	50	HART	SEP	24	IRVINE	ORANGE
	665	" "	50	TRLK	SEP	24	IRVINE	ORANGE
	672	" "	48	TRLK	SEP	24	IRVINE	ORANGE
	674	" "	20	TRLK	SEP	24	IRVINE	ORANGE
	683	CX PIPS	43	HART	SEP	25	IRVINE	ORANGE
	702	CX TARS	50	TRLK	OCT	2	IRVINE	ORANGE
	705	" "	50	TRLK	OCT	2	IRVINE	ORANGE
	710	" "	50	TRLK	OCT	2	IRVINE	ORANGE
	725	" "	50	HART	OCT	2	IRVINE	ORANGE
RVRS	7	CX TARS	50	SLE	JUN	24	BLYTHE	RIVERS
	16	" "	48	SLE	JUN	24	BLYTHE	RIVERS
	19	" "	50	SLE	JUN	24	BLYTHE	RIVERS
	20	" "	50	WEE	JUN	24	BLYTHE	RIVERS
	22	" "	50	WEE	JUN	24	BLYTHE	RIVERS
	23	" "	50	WEE	JUN	24	BLYTHE	RIVERS
	26	" "	50	SLE	JUN	24	BLYTHE	RIVERS
SACR	60	CX TARS	50	TRLK	JUN	24	GALT	SACRA
	78	" "	50	TRLK	JUN	24	MERRITT	YOLO
	107	" "	50	HART	JUN	24	DAVIS	YOLO
	113	" "	50	HART	JUN	24	HERALD	SACRA
	198	" "	50	HART	JUL	8	ZAMORA	YOLO
	216	" "	50	HART	JUL	8	YOLO	YOLO
	248	" "	50	HART	JUL	15	WOODLAND	YOLO
	260	" "	50	TRLK	JUL	15	WOODLAND	YOLO
	270	" "	50	HART	JUL	15	DAVIS	YOLO
	271	" "	50	HART	JUL	15	DAVIS	YOLO
	297	" "	50	HART	JUL	22	ELK GROVE	SACRA
	299	" "	50	HART	JUL	22	ELK GROVE	SACRA
	345	" "	50	HART	AUG	5	WOODLAND	YOLO
	419	" "	50	TRLK	AUG	19	RIO LINDA	SACRA
	437	" "	50	HART	AUG	19	ELK GROVE	SACRA
	439	" "	50	HART	AUG	19	ELK GROVE	SACRA
	478	" "	50	HART	AUG	26	WILTON	SACRA
	516	" "	50	HART	SEP	3	GALT	SACRA
	521	" "	50	TRLK	SEP	3	ELK GROVE	SACRA
	545	" "	50	TRLK	SEP	16	ELK GROVE	SACRA
SANB	3	CX TARS	33	SLE	JUN	6	GROMMET	SBERN
	6	" "	50	WEE	JUN	6	NEEDLES	SBERN
	8	" "	50	SLE	JUN	6	NEEDLES	SBERN

continued -

Table 3-continued

DIST	POOLNO	SPECIES	NUMBER MOSQUITO	VIRUS	MONTH	DAY	PLACE	COUNTY
SANB	9	CX TARS	50	WEE	JUN	6	NEEDLES	SBERN
	10	" "	50	SLE	JUN	6	NEEDLES	SBERN
	13	" "	50	WEE	JUN	6	NEEDLES	SBERN
	52	" "	50	WEE	JUL	2	NEEDLES	SBERN
	61	" "	50	TRLK	AUG	28	NEEDLES	SBERN
	63	" "	50	SLE	AUG	28	NEEDLES	SBERN
	65	" "	44	SLE	AUG	28	NEEDLES	SBERN
	68	" "	50	SLE	AUG	20	PARKER DAM	SBERN
SAND	130	AE TAEN	50	MAIN	AUG	20	IMPERIAL BEACH	SDIEGO
	132	" "	50	MAIN	AUG	20	IMPERIAL BEACH	SDIEGO
SOVE	96	CX TARS	50	HART	MAY	25	HARBOR LAKE	L A
	135	CX PEUS	18	SLE	JUL	16	ENCINO	L A
	167	CX TARS	50	HART	AUG	6	HARBOR LAKE	L A
	177	" "	50	HART	AUG	7	WHITTIER	L A
	249	" "	50	HART	SEP	25	WHITTIER	L A
SUYA	10	CX TARS	50	HART	JUN	17	SUTTER	SUTTER
	16	" "	39	TRLK	JUN	17	EAST NICOLAUS	SUTTER
	36	" "	11	HART	JUL	22	EAST NICOLAUS	SUTTER
	44	" "	50	HART	JUL	22	EAST NICOLAUS	SUTTER

Table 4.-Serological conversions to WEE and SLE viruses in sentinel chickens, California, 1985.

Flock location	No. of chicks	Percent with antibody (WEE/SLE)					
		May	June	July	Aug	Sept	Oct
		Sacramento Valley					
15 flocks	295	0	0	0	0	0	NB[a]
		San Joaquin Valley					
12 flocks	223	0	0	0	0	0	NB
Kern County, 8 flocks	141	0	0	0	0	0	0
		Southern California					
Santa Barbara, Isla Vista	20	0	0	0	0	0	0
Ventura, Pt. Mugu	20	0	0	0	0	0	0
Los Angeles, La Brea	18	0	0	0	0	0	0
Los Angeles, Eaton Canyon	19	0	0	0	0	0	0
Southeast, Harbor Lakes	16	0	0	0	0	NB	NB
Southeast, Balboa Golf	20	0	0	0	0/5	0/5	0/5
Southeast, El Dorado	19	0	0	0	0	0	0
Orange, Duck Club	19	0	0	0	0	0	0
Orange, San Mateo Pt.	19	0	0	0	0	0	0
Orange, Featherly Park	20	0	0	0	0	0	0
West Valley, Ontario	17	0	0	0	0	0	0
San Bernardino, Needles	14	0	6/17	47/40	43/46	50/57	NB
Northwest, Corona	10	0	0	0	0	0	0
Coachella Valley, Mecca	17	0	0	0	0/6	0/6	0/6
Imperial, Palo Verde	13	0	0/87	13/100	15/100	15/100	NB
Imperial, Bard	17	0	0	0/78	0/83	0/82	NB
Imperial, El Centro	16	0	47/0	36/14	61/94	56/94	NB
San Diego, Vista	17	0	0	0	0	0	0
San Diego, Lakeside	19	0	0	0	0	0	0
San Diego, San Ysidro	16	0	0	0	0	0	0

a NB = not bled.

A FURTHER EVALUATION OF *CULEX* MOSQUITOES IN THE GREATER LOS ANGELES AREA FOR THEIR ABILITY TO VECTOR ST. LOUIS ENCEPHALITIS VIRUS

James L. Hardy, Richard P. Meyer,

William K. Reisen, and Sally B. Presser

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

ABSTRACT

Following the outbreak of St. Louis encephalitis (SLE) in the Greater Los Angeles area during late summer and early fall of 1984, a vector competence study was undertaken to compare the ability of *Culex* mosquito species from the Los Angeles area and Bakersfield area to vector indigenous and nonindigenous strains of SLE virus (Hardy et al., 1985). The results indicated that *Culex peus* was the most competent vector of all strains of SLE followed, in order, by *Culex tarsalis* and *Culex quinquefasciatus*. However, the possibility existed that August and September 1984 populations of *Cx. quinquefasciatus* were more competent vectors than were the November 1984 populations evaluated for experimental vector competence or that the geographical populations sampled were not representative of other populations in the Los Angeles area. Further, the unusually high ambient temperatures in the Los Angeles area during August and September 1984 could have enchanced the vectorial capacity of *Cx. quinquefasciatus* females for SLE virus. With these thoughts in mind, a more intensive vector competence study was done in Orange and Los Angeles Counties from June through September 1985 to further evaluate the vector competence of *Culex* species for a Los Angeles strain of SLE virus, to compare the vector competence of mosquitoes incubated at 25°C versus 30°C, to determine if seasonal variations in vector competence occurred and to evaluate the mosquito infectivity and transmissibility of two 1984 Los Angeles strains of SLE virus.

Briefly, the procedures used to evaluate vector competence were as follows: Late instar larvae and pupae were collected at field sites and transported to an insectary in Bakersfield where adults emerged. Three- to 7-day old females were allowed to ingest 50 to 200 infectious units of virus per female from gauze pledgets soaked with sucrose-sweetened, defibrinated viremic chicken blood. Fed females were incubated for 14-18 days at 25°C or 30°C before virus transmission attempts were done by the capillary tube method. Results of virus tests on each female and her salivary secretions were used to determine infection and transmission rates, respectively.

Vector competence tests were done on a total of 18 collections made at 9 different sites in Los Angeles and Orange Counties from June 6 to September 6, 1985, including 9 collections of *Cx. quinquefasciatus*, 5 of *Cx. tarsalis*, 3 of *Cx. peus* and 1 of *Culex erythrothorax*. *Culex peus* females were clearly more susceptible to peroral infection with SLE virus and more efficient transmitters of virus than were either *Cx. tarsalis* or *Cx. quinquefasciatus* females. *Culex tarsalis* females were significantly more susceptible to peroral infection with SLE virus than were *Cx. quinquefasciatus* females, but females of both species that became infected after ingestion of virus were equally efficient transmitters of SLE virus. The population of *Cx. erythrothorax* evaluated was highly refractory to *per os* infection. No significant differences were observed in the vector competence of *Culex* mosquitoes collected in Los Angeles County versus Orange County. Incubation of females at 30°C rather than the usual 25°C had surprisingly little affect on virus infection and transmission rates for any *Culex* species. The seasonal infection rates of SLE virus with *Cx. tarsalis* increased from 30% in July to 90% in September; however, transmission rates obtained with infected *Cx. tarsalis* females remained rather low (20 to 40%) throughout the season. No seasonal change was observed in the vector competence of *Cx. quinquefasciatus* for SLE virus. Interestingly, the SOUE 46-84 strain of SLE virus, which produces minute plaques in cell cultures, was significantly less infectious for *Cx. quinquefasciatus* by the peroral route than was the SOUE 16-84 strain. In contrast, the SOUE 46-84 strain was only slightly less infectious for *Cx. tarsalis* females than was the SOUE 16-84 strain. Both SLE virus strains were isolated from pools of *Cx. tarsalis* collected 1 week apart in September 1984 in the Harbor Lake area of Long Beach and both had similar laboratory passage histories.

ACKNOWLEDGMENT.-The authors extend their gratitude to Gilbert L. Challet and James Webb, Orange County Vector Control District, and Frank W. Pelsue and Jack E. Hazelrigg, Southeast Mosquito Abatement District, whose significant collaborative effects made this study possible. This research was funded by Research Grant AI-03028 from the National Institute of Allergy and Infectious Diseases, and by funds for mosquito research allocated annually through the Division of Agriculture and Natural Resources, University of California.

REFERENCES

Hardy, J. L., S. B. Presser, R. P. Meyer, W. K. Reisen, L. D. Kramer, and A. V. Vorndam. 1985. Comparison of a 1984 Los Angeles strain of SLE virus with earlier

California strains of SLE virus: mouse virulence, chicken viremogenic, RNA oligonucleotide and vector competence characteristics. Proc. Calif. Mosq. & Vector Control Assoc., 53:10-15.

USE OF THE *IN SITU* ENZYME IMMUNOASSAY FOR THE RAPID DETECTION OF ARBOVIRUS INFECTIONS IN MOSQUITOES IN CALIFORNIA

Robert R. Graham[1], James L. Hardy, and Sally B. Presser

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

ABSTRACT

Llano Seco (LLS) virus is an orbivirus in the family Reoviridae that was isolated initially in 1971 from a pool of *Culex tarsalis* collected in Butte County in the Sacramento Valley (Karabatsos, 1985). Recently, we became interested in determining the prevalence of this virus in various mosquito species and geographic areas in California. However, this virus is difficult to isolate and study in the laboratory because it is relatively avirulent and noncytopathogenic for suckling mice and cell cultures, respectively, which are the laboratory systems most frequently used to isolate arboviruses from mosquitoes. Therefore, we decided to evaluate the *in situ* enzyme immunoassay (EIA) which can detect the presence of virus antigens in infected cell cultures in the absence of cytopathology.

The *in situ* EIA was modified slightly from that originally described by Yong-He et al. (1984) and Dr. T. F. Tsai (personal communication). An aliquot of each triturated mosquito pool was added to each of two wells in a 96-well polyethylene plate and a standardized suspension of C6/36 *Aedes albopictus* cells was added to all wells in the plate. Then the plates were incubated for 96 hrs at 31°C to allow any virus present in the mosquito pools to be amplified in the cells. After removal of the culture medium, the cells were fixed on the bottom of the wells with 10% formalin before a standard indirect EIA was done to detect LLS virus antigen in the fixed cells. For comparative purposes, an indirect immunofluorescent assay (IFA) was done simultaneously with the *in situ* EIA. The IFA employed C6/36 cells in 8-well, teflon-coated microscopic slides and was adapted from a procedure developed in the Viral and Rickettsial Disease Laboratory at the California State Department of Health Services (Mr. James Woodie, personal communication).

The *in situ* EIA and IFA were found to be highly sensitive since both tests could detect 1 to 2 infectious units of virus per 0.1 ml of a normal mosquito pool suspension mixed with stock LLS virus. Tests were completed on 1,614 pools of *Cx. tarsalis*, 269 pools of *Aedes melanimon*, 43 pools of *Culex quinquefasciatus*, and 16 pools of miscellaneous mosquito species collected throughout California as part of the 1984 encephalitis surveillance program. LLS-like viruses were detected in a total of 54 *Cx. tarsalis* pools representing all of the major geographical areas in California where this species occurs; 46 pools were positive by both *in situ* EIA and IFA, 8 pools by the *in situ* EIA only, and 1 pool by the IFA only. Further tests need to be done to determine if the LLS-like viruses in *Cx. tarsalis* pools are strains of LLS virus or one of the other 2 antigenically-related orbiviruses (Umatilla and CV-73) that are known to occur in California.

In summary, the *in situ* EIA was found to be a rapid and sensitive method for the detection of LLS-like viruses in mosquitoes. Preliminary tests indicate that this test may be equally useful in the detection of western equine encephalomyelitis and St. Louis encephalitis viruses in mosquitoes. However, further evaluations need to be done before a recommendation can be made about whether this test should replace virus detection systems currently being used in the encephalitis surveillance program in California.

ACKNOWLEDGMENT.-This research was funded by Research Grant AI-03028 from the National Institute of Allergy and Infectious Diseases, Biomedical Research Support Grant 5-S07-RR-05441 from the National Institutes of Health, and by special funds for mosquito research allocated annually through the Division of Agriculture and Natural Resources, University of California.

REFERENCES

Karabatsos, N. (Editor). 1985. International Catalogue of arboviruses including certain other viruses of vertebrates. 3rd. ed., American Society of Tropical Medicine and Hygiene, San Antonio, pp. 624-626.

Yong-He, A., Y. Wen-Fang, T. Zhong-Wen, G. Ji-Qian, C. Qin-Sheng, and W. Yi-Min. 1984. A simplified new method for the identification of arboviruses: virus isolation using enzyme immunoassay on cultured cells. J. Virol. Methods 9:45.

---

[1]Present address: DAD, USAMRIID, Ft. Detrick, Frederick, Maryland 21701.

PROCEDURES FOR EVALUATING THE VECTOR COMPETENCE OF MOSQUITOES FOR ARBOVIRUSES[1]

R. P. Meyer[2], J. L. Hardy, S. B. Presser, and W. K. Reisen[2]

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

Within the last decade, extensive laboratory testing has revealed that the capacity of a female mosquito to effectively vector an arbovirus, i.e., vector competence, is dependent upon complex interrelationships between various endogenous and exogenous factors (Takahashi 1976, Hardy et al. 1983). Tests for vector competence quantify the relative capacity of a female mosquito to become infected and transmit an arbovirus by bite. Since these 2 parameters are measured in field mosquito populations over both time and space, testing procedures should be standardized to allow for direct biological and statistical comparisons. This paper describes briefly (i) some of the testing procedures currently being used by the Arbovirus Research Program at the University of California, Berkeley, to assess the vector competence of mosquitoes for arboviruses and (ii) recent improvements in testing procedures that will provide a more reliable and sensitive system for determining the vector competence of mosquitoes in California (Table 1).

A standard vector competence test consists of 6 major steps: 1. collection and/or laboratory colonization of field mosquito strains, 2. exposure of females to an arbovirus source, 3. extrinsic incubation to allow virus to replicate within the female vector, 4. in vivo or in vitro assessment of virus transmission, 5. arboviral assays to detect the presence and titer of virus, and 6. data analyses. Each of these steps will be discussed briefly emphasizing the procedures used by the Arbovirus Research Program.

Source of female mosquitoes.-Adult female mosquitoes tested for vector competence are usually obtained by one of several methods: field collection by CO_2 attractant type traps, artificial and natural shelters, rearing from mature larvae and pupae, or from established laboratory colonies. When 2 or more field strains are tested simultaneously, female mosquitoes should be collected by the same sampling method. Differences in the peroral susceptibility to western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses have been demonstrated for female *Culex tarsalis* Coquillett collected at the same field site by CO_2 traps or as pupae (Meyer et al. 1983a).

Exposure of females to an arbovirus source.-In nature, female mosquitoes, not infected transovarially, are infected perorally by ingesting virus present in the blood of a viremic vertebrate host. This process is reproduced in the laboratory by allowing female mosquitoes to feed on an experimentally infected viremic host or on a mixture of virus and sweetened (2.5% sucrose) defibrinated whole rabbit blood presented in cotton gauze pledgets. The latter method of peroral infection has met with mixed results. Comparative tests with SLE virus have shown that mosquitoes infected perorally on a suspension of mouse brain derived virus in sweetened defibrinated rabbit blood are from 10- to 100- fold less susceptible to infection than females infected perorally feeding on viremic chickens (Meyer et al. 1983b). Recent tests with SLE virus have shown that experimental infection rates comparable to those obtained by feeding mosquitoes on viremic hosts can be obtained when females are allowed to ingest sweetened defibrinated viremic chicken blood presented in pledgets. Since the viremias produced by individual chickens may vary by 10- to 100-fold, the use of pooled defibrinated viremic chicken blood, (i.e., defibrinated viremic blood pooled from a large series of infected wet chickens), is operationally more advantageous towards maintaining testing continuity. The pooled defibrinated viremic chicken blood can be produced in quantity, stored at -70°C, and diluted to the appropriate concentration just prior to mosquito feeding. This procedure eliminates the need for producing viremic chickens for each vector competence test and allows the ingestion of a uniform virus challenge among tests.

Extrinsic incubation to allow virus replication within the female vector.-After an arbovirus has been ingested by a female mosquito, it must first replicate in and escape from the midgut and then infect and replicate in the salivary glands in

[1]This research was funded by Research Grant AI-3228D from the National Institute of Allergy and Infectious Diseases, Biomedical Research Support Grant 5-S07-RR-05441 from the National Institutes of Health and by special funds from mosquito research allocated annually through the Division of Agriculture and Natural Resources, University of California.

[2]Arbovirus Field Station, P.O. Box 1564, Bakersfield, CA 93302.

Table 1.-Sequence of procedures used to determine the experimental vector competence of mosquitoes for arboviruses. Methodologies listed under new procedures will be incorporated into the vector competence testing program at the University of California, Berkeley.

Standard Procedure	New Procedure
1. Source of female mosquitoes tested for arboviral vector competence.	
Female mosquitoes collected by attractant type trap or from resting shelters, reared from field collected larvae/pupae, or obtained from laboratory colonized strains.	
2. Exposure of female mosquitoes to an arbovirus source.	
Virus ingested while feeding on a suspension of infected mouse brain diluted in sweetened defibrinated whole rabbit blood or while feeding on a viremic chicken	Virus ingested while feeding on pooled sweetened defibrinated viremic chicken blood
3. Extrinsic incubation to allow the virus to replicate within the female vector.	
Females incubated at a at a constant temperature of 25 and 30 degrees for 10, 14, 1nd/or 21 days post feeding	Females incubated using simulated cycling environmental air temperatures for a specified number of degree-days
4. *In vivo* or *in vitro* assessment of virus transmission.	
Females ingesting virus are allowed to feed on normal hosts (*in vivo*) after a suitable period of extrinsic incubation	Females ingesting virus are allowed to salivate into an artificial medium (*in vitro*) after a suitable period of extrinsic incubation
5. Arboviral assays to detect the presence and/or titer of virus.	
Mosquito infection determined by cell culture assay systems	Mosquito infection determined by *in situ* immunoassay
Arbovirus transmission determined by viremia development or sero-conversion in chickens fed upon by potentially infected mosquitoes	Arbovirus transmission determined by *in situ* immunoassay
6. Data analyses.	
Mosquito susceptibility to peroral infection expressed quantitatively as an infection rate, susceptibility profile, and/or ID_{50}	Influence of variations in environmental air temperature on the quantitative expression of mosquito susceptibility and arbovirus transmission parameters
Arbovirus transmission expressed quantitatively as a transmission rate and/or transmission index	

order to be transmitted by bite. The time required for infected females to become infective, (i.e., capable of transmitting virus) is referred to as the period of extrinsic incubation. The time that is required for a female mosquito to become infective is largely dependent upon mosquito genetic factors and environmental temperature (Hardy et al. 1983). Mosquitoes tested for vector competence are usually incubated at constant temperatures, i.e., 25 or 30°C, for periods ranging from 10-21 days post feeding before females are tested for their ability transmit virus. Standardized incubation temperatures and exposure times are necessary to maintain continuity between experiments. However, exposure of experimentally infected mosquitoes to a constant temperature does not provide data that is relevant to the duration of the extrinsic incubation period in nature. Studies are currently in progress to determine the effects of naturally cycling daily air temperatures on the extrinsic incubation of WEE and SLE viruses in *Cx. tarsalis*. The immediate objective will be the development of a temperature model for extrinsic incubation that simulates the cyclic changes in daily ambient air temperature that occur in nature (i.e., mosquito habitats in Kern County, California) during each month of the year. This model will be based upon an understanding of the temporal relationship between the mosquito daily activity cycle and concurrent changes in ambient air temperature (Fig. 1). The daily activity cycle of a nocturnally active species such as *Cx. tarsalis*, exposes the adult female to 2 distinctly different thermal environments: 1. the air temperature of the diurnal resting shelter and 2. the air temperature of the space occupied by mosquitoes at night. Vector competence data obtained by incubating female mosquitoes under stimulated conditions of cycling daily air temperatures as presented by the model will eventually be used to evaluate a degree-day model for quantifying the extrinsic incubation of arboviruses under various seasonal temperature regimens.

In vivo or in vitro assessment of virus transmission.-Until recently, the ability of female mosquitoes to transmit WEE and SLE viruses was determined experimentally by allowing potentially infective mosquitoes to feed individually on a normal week-old chicken and subsequently measuring viremia development or antibody conversion to the virus in those chickens fed upon by infective mosquitoes. Although this method provided the best definitive measure of vector competence, procedural problems frequently precluded the testing of adequate numbers of infective females in order to obtain statistically reliable sample sizes. During the infection process, female mosquitoes ingest blood from a viremic host or blood soaked pledget containing virus and become gravid within several days. Unfortunately, gravid females seldom refeed until after oviposition. Furthermore, gravid females obtained from field strains of some mosquito species, including *Cx. tarsalis* and *Culex peus* Speiser, do not oviposit readily in captivity. Accordingly, testing field strains of these species for their ability to transmit arboviruses experimentally has met with limited success.

Takahashi (1976) and Aitken (1977) both developed in vitro methods for determining mosquito transmission of arboviruses. Virus transmission was determined by inserting the proboscis of a potentially infective female mosquito into a capillary tube containing a artificial medium that induced salivation and protected virus from immediate inactivation. The medium was then tested for the presence of virus by standard

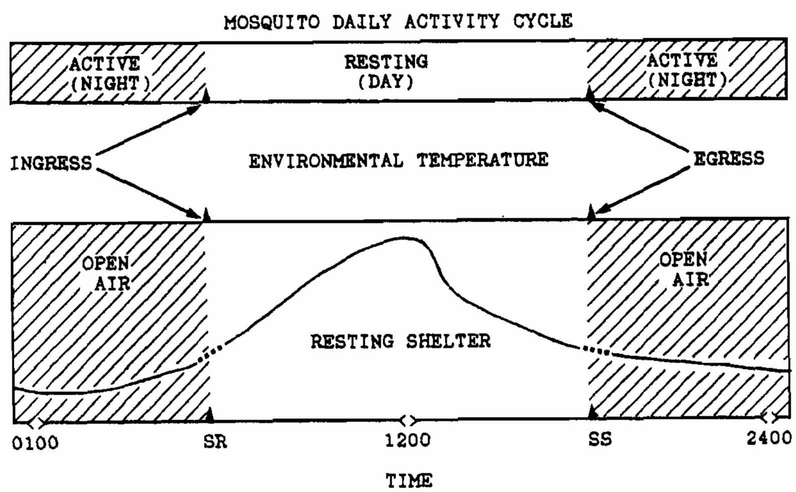


Figure 1.-Ambient air temperature model for the extrinsic incubation of arboviruses in a nocturnally active mosquito vector based upon cyclical changes in environmental temperatures associated with diurnal resting shelters and nocturnal flight activities. SR = sunrise and SS = sunset.

assay procedures. The advantages provided by these in vitro methods are 2-fold: 1. a large number of mosquitoes can be evaluated rapidly for virus transmission potential and 2. the requirement for oviposition is no longer necessary. We are currently evaluating several in vitro methods to determine the most sensitive and reproducable method for routine use in our testing program.

Arboviral assays to determine the presence and/or titer of virus.-Determining the presence of an arbovirus relies upon sensitive in vitro and in vivo assay systems that are highly specific for the detection of infectious virus, virus antigen, or virus neutralizing antibody. The time required to detect virus or neutralizing antibody either directly or indirectly is dependent upon the type of assay system employed. Currently, the time required to determine virus infection and transmission by plaque assays in cell cultures ranges from 1 to 3 weeks. Although our current testing procedures that utilize plaque assays in various diagnostic tissue cultures, (e.g., vero cells) are relatively rapid, special handling requirements still severely limit the overall number of mosquitoes and/or saliva samples that can be tested simultaneously. We are in the process of converting our existing assay system to a newer in situ enzyme immunoassay (EIA) that utilizes micro-cell cultures grown in 96 well plates (Yong-He et al. 1984, Graham et al. 1986). For this system, aliquots of a potentially infected mosquito suspension or contents of a capillary tube are placed in 2 wells each in a 96 well plate and all wells are seeded with a suspension of susceptible cells and then incubated at 36°C. The cells are then fixed with methanol and assayed for virus antigen by standard indirect EIA's. This system shows much promise for the rapid detection of WEE and SLE viruses in experimentally infected mosquitoes. Once operational, the in situ EIA should increase our present laboratory virus tests by at least 2-fold.

Data analyses.-Peroral susceptibility to infection with an arbovirus is quantified as an infection rate calculated as the percentage of females tested that become infected after some period of extrinsic incubation or as an experimental ID_{50} (log probit infection dose 50) calculated by either probit analyses or extrapolated from a dosage response curve. Experimentally, the ID_{50} represents the concentration of virus ingested that will infect 50% of the females tested.

The ability of an infected female mosquito or a mosquito population to transmit an arbovirus by bite is measured quantitatively as a transmission rate or transmission index, respectively. The transmission rate is calculated as the percentage of infected females that transmit virus after some period of extrinsic incubation. By comparison, the transmission index is calculated as the percentage of total females tested that transmit virus.

As discussed previously, mosquitoes experimentally infected with arboviruses are incubated at constant rather than under natural cycling environmental temperatures. We intend to develop degree day models for WEE and SLE viruses that will provide thermal summation data essential to determining precisely the period of extrinsic incubation of both viruses. Hurlbut (1973) has developed a degree-day model for SLE virus in *Culex quinquefasciatus* Say. Our models initially will be developed by determining the optimal time for susceptible laboratory stains of *Cx. tarsalis* to become infective at different temperatures. The number of degree-days required for females to become infected and/or transmit virus by bite is a constant calculated from the rate of thermal summation at or above the lowest temperature (C) that does not preclude the replication of virus (Fig. 2A). Since the rate of viral replication is temperature dependent, the time measured in real days at which females will become infective in nature will vary in accordance with seasonal changes in ambient air temperature. Therefore, the number of degree-days based upon the constant rate will be accrued at a faster rate during the summer when daily ambient air temperatures are warmer and more slowly during the spring or fall when daily air temperatures are cooler (Fig. 2B).

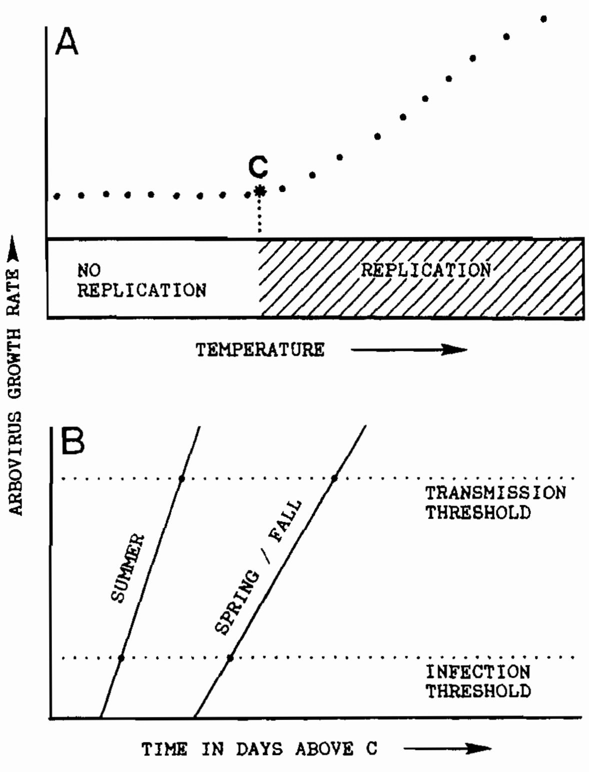


Figure 2.- A. Role of ambient air temperature on the rate of arbovirus replication in mosquitoes. B. The potential effects of seasonal variations in environmental temperatures on the length of the extrinsic incubation of an arbovirus in mosquitoes with similar midgut infection thresholds.

The changes in our vector competence testing program presented herein hopefully will provide a more realistic and efficient system for assessing the vector competence of field mosquito populations for arboviruses. Seasonal temperature effects on the extrinsic incubation of WEE and SLE viruses ultimately will be incorporated into a epidemiological model that closely depicts the dynamics of arbovirus transmission in nature.

REFERENCES

Aitken, T. H. G. 1977. An in vitro feeding technique for artifically demonstrating virus transmission by mosquitoes. Mosq. News 37:130-133.

Hardy, J. L., E. J. Houk. L. D. Kramer and W. C. Reeves. 1983. Intrinsic factors affecting vector competence of mosquitoes for arboviruses. An. Rev. Entomol. 28:229-262.

Graham, R. R., J. L. Hardy and S. B. Presser. 1986. Use of the in situ enzyme immunoassay (EIA) system for rapid detection of arboviruses in mosquitoes of California. Proc. Calif. Mosq. and Vector Contr. Assoc. 54:10.

Hurlbut, H. S. 1973. The effects of environmental temperature upon the transmission of St. Louis encephalitis virus by *Culex pipiens quinquefasciatus*. J. Med. Entomol. 10:1-12.

Meyer, R. P., J. L. Hardy, S. B. Presser and J. P. Bruen. 1983a. Comparative arboviral susceptibility of female *Culex tarsalis* (Diptera: Culicidae) collected in CO_2 - baited traps and reared from field-collected pupae. J. Med. Entomol. 20:56-61.

Meyer, R. P., J. L. Hardy and S. B. Presser. 1983b. Comparative vector competence of *Culex tarsalis* and *Culex quinquefasciatus* from the Coachella, Imperial and San Joaquin Valleys of California for St. Louis encephalitis virus. Am. J. Trop. Med. Hyg. 32:305-311.

Takahashi, M. 1976. The effects of environmental and physiological conditions of *Culex tritaeniorhynchus* on the pattern of transmission of Japanese encephalitis virus. J. Med. Entomol. 13:274-284.

Yong-He, Z., Y. Wen-Fong, T. Zhong-Wen, G. Ji-Qian, C. Qin-Sheng and W. Yi-Min. 1984. A simplified new method for identification of arboviruses: Virus detection using enzyme immunoassay on cultured cells. J. Virol. Methods 9:45-51.

PARTNERS IN MOSQUITO RESEARCH AND CONTROL

James B. Kendrick, Jr.

Vice President--Agriculture and Natural Resources
University of California Office of the President

I would like to open this address by touching briefly on the highlights of the early history of mosquito research and control in California even though some of you in the audience are quite familiar with the story. It is such an illustrious history of the developing partnership involving the University, the mosquito abatement districts, and the State Department Health Services that it deserves a little repetition.

The history began only two years after the turn of the century during a period when two landmark human medical advances were made: first, the discovery that the *Anopheles* mosquito transmitted human malaria; and second, the development of experimental evidence that yellow fever was transmitted by the *Aedes aegypti* mosquito. It was also a period when Gorgas, during the construction of the Panama Canal, demonstrated that malaria and yellow fever could be controlled and prevented by taking measures to eliminate mosquitoes. Another development by Entomologist L. O. Howard during this period was the use of oil for mosquito larval control.

While these great events were making medical history, the University was coping with a serious malarial problem in California's great Central Valley. It also became involved in a saltmarsh pest mosquito problem in the San Francisco Bay Area in 1904 with research undertaken by Entomology Professors L. Woodworth and H. J. Quayle of UC Berkeley. Their research constituted one of the first two mosquito research and control projects in the nation. The other was a saltmarsh mosquito project in New Jersey.

The outstanding event of this period was the launching of a 10-year anti-malarial campaign in the Central Valley in 1910 by Professor W. Herms, UC Berkeley, assisted by Stanley Freeborn and Engineer Harold F. Gray. Freeborn was one of Professor Herm's students, who many years later after an illustrious career in mosquito research became the Provost of the Davis campus of the University of California.

The Malaria Campaign together with the saltmarsh project laid the groundwork for the 1915 passage of the Mosquito Abatement Act by the State Legislature. By 1921 endemic malaria was eliminated in California by the efforts of the Herms team and the half dozen newly formed Mosquito Abatement Districts. However, the vectors of malaria are still present and we still see an occasional case of indigenous malaria. Another historical event was the discovery in 1930 of mosquito-borne encephalitis. It replaced malaria as the dominant vector-disease problem in California.

In this early period of the California mosquito research and control programs, the seeds were planted for a partnership between the Mosquito Abatement Districts, the University, and the State Department of Health Services. The Mosquito Abatement District/University link was apparent 55 years ago when Agriculture Hall, now known as Wellman Hall, at UC Berkeley was the site for the first annual conference of the California Mosquito Control Association. For 18 successive years thereafter Agriculture (Wellman) Hall was the home of the CMCA Annual Conference. Thereafter, the conferences have been held in more commodious surroundings: such as the Sheratons, the Miramars, the Holidays, the Hiltons, and the Red Lion Inns.

Let us now look at the postwar period of pesticide dominance in California mosquito control (also agricultural pest control). I have learned that before the end of World War II in 1945 the basic tools of the mosquito control profession consisted of a hand dipper to collect larvae for mosquito surveys, the pick and shovel for ditching and draining breeding places, the hand sprayer for applying mosquito oil, and the fish net and bucket to collect and carry mosquitofish to stock mosquito breeding places. It was truly an integrated control program of physical, chemical, and biological control, but also a labor intensive one.

When DDT, the miracle organochlorine insecticide, became available at the end of World War II, these multiple approaches to mosquito control were rapidly displaced by near total reliance on DDT applications for control of this insect. It made mosquito control cost-effective for the new and expanding mosquito control districts being formed in California. With DDT, districts were able to satisfy quickly the many community demands for protection from mosquitoes in rural and urban areas. The fact that DDT was an environmental contaminant, destroying beneficial organisms as well as disease vectors, was not fully appreciated at the time. A decade later Rachel Carlson's book "Silent Spring", popularized the food chain interaction from DDT environmental contamination and the use of DDT in the USA came to an end.

But irrespective of Carlson's book the fragile mosquito was not so easily daunted by the miracle insecticide. It had survived millions of years on this planet and it was not about to give up to DDT. Resistance to DDT developed rapidly until the insecticide became worthless even at heavy, uneconomical dosage rates. For a while the only alternative used was to replace DDT with another synthetic insecticide which was soon followed by resistance to it and the need for another replacement. This treadmill scenerio was repeated about every two to three years with no end in sight.

By the mid-1960's, the time had come to get off the treadmill and to rethink and redirect mosquito control strategies. The need for extraordinary measures was eloquently expressed by the late Richard Frolli, manager of the Kings Mosquito Abatement District, at the 1969 Annual Conference of the CMCA. He said, "These are crucial times--our pesticides are failing! Our basic solutions for mosquito control are dying! The resistance phenomenon has matured. The pasture mosquitoes and the encephalitis mosquitoes have triumphed over sprays in many parts of California. We must change our basic strategy, we must change our basic solutions, we must change our district images to ones other than spray districts if we are to be effective in mosquito abatement. In many counties of the State chemical sprays are no longer effective at safe, legal, economical rates. After 25 years of continuous spraying, the mosquitoes have become immune or multi-resistant to all common public health mosquito-cides, including malathion, parathion, EPN, fenthion, ABATE, VAPONA, DIBROM, DURSBAN, and others."

Frolli said it all in a few words. It was a rallying cry for a change, long overdue.

The situation in the late sixties was a "crisis" in mosquito control because practical alternatives to the synthetic pesticides were not available. Apart from mosquitofish, biological control had not advanced beyond the laboratory stage. Our knowledge of mosquito biology and ecology in relation to predators, parasites, and pathogens was superficial. Moreover, research on biological control had lagged seriously during the 20-year preoccupation with chemical insecticides. Research on narrow spectrum insecticides that would kill selectively resistant mosquito larvae, while sparing the natural enemies, was still in the exploratory stage. Methods for counteracting resistance in the field were needed.

The events leading up to the present University of California mosquito research program have been clearly described by Professor Reeves in his paper on the History of Mosquito Control in California published in the 1984 Annual Report on the University's Mosquito Research. He noted that the existing program resulted from the State Legislature's directed transfer of the Department of Health Services' mosquito research activities to the University in July 1965, in order to achieve a single state supported program of mosquito research. This intention, however, was not fully implemented until 1972-73 when a legislative appropriation of $300,000 supported by your association together with the State Department of Health Services was enacted. The joint support was crucial to the success of the appropriation. This sum, together with $100,000 from the State Water Fund, provided the basic funding for the University's mosquito control research. To achieve coordinated oversight and advice for the program, the University appointed an advisory committee, consisting of representatives of the State Department of Health Services, The California Mosquito and Vector Control Association, and a number of University of California representatives including public health professionals and experimental entomologists. I chaired this committee. To achieve improved cooperation between all parties, the University also created the mosquito program coordinator position. I am informed that of all mosquito research programs in this nation and internationally this cooperative partnership of support and oversight is unique.

In Bill Hazeleur's letter of invitation for me to address this conference he asked that I clarify the reason for organizing the program as a University-wide, coordinated, goal-oriented, activity.

Those of us responsible for managing the mosquito research and outreach endeavors felt the objective of the program should be the development of improved strategies, methods, materials, and equipment to control mosquito problems in California. To do so we knew would require the resources of a number of related specialities within the University not necessarily all found at a single campus.

The idea of a University-wide organization was based on the need to assemble a multiagency, multidisciplinary makeup of studies leading to mosquito control. Public health aspects are prominent in the program because mosquitoes affect people. They carry disease as well as irritate and annoy people often times beyond tolerance. Also, mosquitoes are born from water, and water is essential to agriculture, to industry, to cities, to recreation, to environmental enhancement, and everything related to human existence and activity. The study of the mosquitoes themselves by entomologists is essential as are specialties of many other disciplines such as soil and water sciences, engineering, animal science, parasitology, genetics, virology and bacteriology. The program's multiagency links include in addition to the mosquito abatement districts, state, federal and international health agencies, water resources agencies, the Department of Agriculture, EPA, and Fish and Wildlife agencies.

Coordination was considered essential if we were to keep all of the diverse elements united and working effectively toward the overall goal. We believe we have developed a balanced study of the problem with basic as well as applied research covering different categories such as: vector-disease interactions, biology and ecology of the mosquito; and biological, chemical, physical and cultural control methodologies. Coordination is achieved by administering the program centrally and subjecting research proposals to peer review by technical and advisory committees. The mosquito program coordinator facilitates communication, mobilizes resources, monitors progress, assists and serves committees in evaluating research, implements research recommendations, and is responsible for reports on research progress.

Research funding.-President Hazeleur also asked about the program's funding with reference to the continuing security of the special fund now that it is part of the regular University budget, instead of appearing as an annual line item state appropriation. In 1985, the amount was $527,000 up from the original 1973 allocation of $300,000. So the attrition from inflation has not been too severe. This special fund is only part of the total research financial picture. It is greatly augmented from other sources, $100,000 from the state water fund, and about $1 million from the University's general funds. In addition, researchers in the program have attracted an additional $1.2 million from state, federal, and international sources for a total of $2.6 million.

The funding while impressive compared with the original allocation is still insufficient to support all of the high priority mosquito research.

Getting back to Bill Hazeleur's question about maintaining the integrity of the special fund for mosquito research, I know that as long as such a high demand for mosquito research funds exists, there will be no reason to even consider diverting it to other research needs. Furthermore, as long as the fund is responsibly administered by the Office of the Vice President--Agriculture and Natural Resources, I am sure that it will maintain its integrity.

Research committees.-At the beginning of the coordinated program three research committees were appointed:

1. The University-wide Advisory Committee on Mosquito Research is a multiagency body presently chaired by the Assistant Vice President of Agriculture and Natural Resources, Dr. Lowell Lewis. The Committee is principally concerned with mosquito research policy, campus and interagency coordination, and outside participation in the program. It addresses broad issues and questions regarding funding and research emphasis, research progress, and national and international developments in mosquito research.

2. The Mosquito Research Technical Committee is an interdisciplinary body of seven UC scientists each with expertise in at least one or more of the disciplines involved in mosquito research and control. The committee reviews and evaluates research proposals for scientific merit, relevance, feasibility, and potential. It then recommends to the Coordinator the proposals to be considered for funding. The review is help in conjunction with a two-day symposium where all mosquito research assemble to present proposals and progress reports which are critique in an open session.

3. The CMVCA Research Committee participates in the review process and represents the research interest of the Association. It reviews and evaluates all research proposals with special reference to relevance of the research to mosquito abatement districts. The chair of the committee is a member of the University-wide Advisory Committee and submits the research recommendations of the CMVCA committee to the University-wide Advisory Committee and to the mosquito Program Coordinator.

Progress.-Another question raised by your Association concerned research progress and accomplishments. The legislature has also raised a similar question in the past. I am reminded of three definitions applicable to research progress:

"If a thousand things advance, and nine hundred and ninety-nine retreat, that is progress" (Russ Fontaine);

"All progress is precarious, the solution to one problem brings us face to face with another problem!" (Martin Luther);

"There are no gains without pains!" (Adlai Stevenson).

The question is not an easy one to answer because we find that research results are often negative rather than positive, or they represent only incremental advances rather than breakthroughs. However, we must remember that both positive and negative research information is of value in designing strategies to control mosquito populations.

One dramatic statistic which demonstrates progress is the reduced use of broad spectrum synthetic insecticides. The reduction amounts to more than 75 percent since 1972. This reduction in chemical use is not to imply that the need for chemical control has diminished, but it reflects a changing emphasis from primary reliance on chemical control to the utilization of a variety of control strategies which together make up the integrated mosquito control program.

Another measure of progress is the fact that the major mosquito-borne disease encephalitis has been effectively suppressed despite the presence of the virus in birds and mosquitoes, except for an outbreak of Saint Louis encephalitis in 1984 in metropolitan Los Angeles and Orange Counties.

Other examples of important research progress are noted in:

-- the development of indoor systems for overwintering mosquitofish.

-- the development of the mosquito-selective and the environmentally safe biorational insecticide, *Bacillus thuringiensis*.

-- the determination of the efficacy of environmentally safe - selected insect growth regulators.

-- the formulation of strategies to avoid or suppress the development of mosquito resistance to insecticides.

-- the increased understanding of canine heartworm disease transmission.

-- the achievement of environmentally acceptable marsh management for mosquito control.

-- the development of rapid diagnostic field tests for detection of western equine encephalomyelitis virus in mosquitoes.

and the development of field application of the fungus, *Lagenidium giganteum* for potential control of the ricefield mosquito.

What about the future?.-It was Einstein who said, "I never look into the future. It will come soon enough". Nevertheless, a look at the future is essential for planning our research 10 and 20 years hence. In the 1985 annual report of the Mosquito Research Program this topic was addressed by mosquito abatement district managers and by UC researchers. Most of the views expressed indicated that change was necessary because of the continued growth in population resulting in an expansion of urbanization and industrial activities. Agriculture and mosquito populations have always been intimately linked. Although agriculture in California is not likely to expand in the short term the nature of cropping patterns may change for a variety of economic reasons. Decreasing irrigation water supplies would cause a change as would the increasing problems associated with salinity and toxic wastes. Lack of domestic and foreign markets for a number of California-grown crops will also influence the pattern of cropping activities.

There is also the potential of enlarging mosquito problems by creating numerous shallow evaporation reservoirs in the San Joaquin Valley to receive saline and toxic agricultural drainage water in order to reduce runoff of irrigation waste water into natural basins such as the Kesterson Reservoir.

There is increasing evidence of agreement that the rising demand for water can only be met by water conservation measures and through reclamation and reuse of wastewater from municipal, industrial, and agricultural sources. The processes involved in reclamation are frequently prime sources of mosquito breeding grounds. Also the uses made of wastewater for creation of wetlands, marshlands, parklands, and ponds for environmental enhancement are popular attractions for city dwellers, birds and aquatic wildlife. These are also rich sources of encephalitis vector mosquitoes as well as the nuisance mosquitoes. This trend of environmental enchancement has strong public support and it is not likely to diminish. Research on the intricate biological and ecological processes influencing mosquito production in these new and expanding uses of waste water is underway but underfunded.

The wastewater-related mosquito problem is seen as the highest priority need for mosquito research for the final years of this century and possibly during the early years of the twenty-first century. The challenge is to control mosquitoes effectively and economically utilizing measures which do not alter adversely the environments and the biota of these wastewater reservoirs.

Is the program entering a new phase?-The foregoing discussion should indicate that California's need for research in mosquito control is not about to diminish in the future. The outbreak of Saint Louis encephalitis in southern California's largest urban area has exposed serious gaps in the knowledge of encephalitis transmission and in the biology, ecology and behavioral traits of the suspected urban vectors.

The dynamic and variable nature of mosquito problems in California reflect the influence of continuous population growth, and the human need and desire for water in all aspects of our daily existence.

Mosquito control measures in California must not be permitted to succumb to complacency and adoption of monotomous routines and cookbook solutions, because the wiley mosquito can be counted upon to resist extinction.

Quoting from Bill Reeves' talk before your annual conference of 1985, he said "If one compares the projects currently being carried out with those carried out in 1978, it is obvious that the research effort is constantly changing its focus in an effort to face today's problems."

There are signs that mosquito control is entering a new phase in its long history. The period of pesticide dominance of control is over. The resistance problem has diminished and will continue to wane as the new environmentally safe mosquito selective insect growth regulators become available.

The bond of the partnership between the University, the Mosquito Abatement Districts of California, and the State Department of Health Services has been one of the great strengths of the mosquito research program. The progress and success we have achieved to date represents a collaborative efforts of many people and agencies. All Californians whether they recognize it or not are endebted to our effective partnership. I am proud to have played a part in its development. I wish it and all of you well as you strive to strengthen this relationship for the challenges of the future.

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

January 1985 - January 1986

Charles P. Hansen[1]
San Mateo County Mosquito Abatement District
1351 Rollins Road
Burlingame, California 94010

BIOLOGICAL CONTROL.-R. L. Coykendall, Chairman. Our 1985 Committee charge was to disseminate information on biological control to Association members. To further these goals, work was initiated on a series of one-sheet handouts on various biological control agents which were named Bio Notes. By the end of the year, final drafts of three Bio Notes had been completed. Drafts of these Bio Notes on mosquitofish, guppies and dragonflies were reviewed by committee members and forwarded to the Publications Committee for final editing and printing. Work on other Bio Notes will be continued in 1986. Additionally, Bio Briefs - our Committee newsletter, was edited this year by Craig Downs of the Contra Costa M.A.D. and three issues were published and disseminated. One meeting of our Committee was scheduled for Monterey on April 25, 1985; but no committee members were able to attend. Consequently, further committee business was conducted through informal telephone conversations. We request in the future that Districts having personnel serving on this Committee authorize their travel and if necessary lodging so we may conduct Committee business more effectively.

CHEMICAL CONTROL COMMITTEE.-L. Lino Luna, Chairman. The Committee's task was to work with the Mosquitoborne Encephalitis Virus Surveillance and Control Program Steering Group on encephalitis virus surveillance and control to identify insecticides and means of application for encephalitis mosquito control in each region of the State; consider if the findings of this Committee should be made a part of the "California's Mosquitoborne Encephalitis Virus Surveillance and Control Program" manual; keep abreast of all chemicals being used and in turn inform member agencies of successful tests and varied formulations.

The Committee developed a Questionnaire consisting of six questions pertaining to our task. The survey was mailed to the 46 MAD/VCDs listed in the 1985 CMVCA Yearbook. A total of 45 MAD/VCDs, one County Health Department, and one Service Area responded. Only one MAD did not respond.

The respondents indicated additional work was desirable upon the following: ranking of the major mosquito species in order of importance in each agency; and the listing of the species tested for resistance, to what chemicals, as well as the magnitude or extent of the resistance problem, if known.

Therefore, the Committee recommends that consideration be given to the above two points. The results of the survey and questionnaire were published as part of the CMVCA minutes dated January 24, 1986. For additional information please contact the Northwest MAD.

COOPERATIVE PLANNING.-Charles H. Dill, Chairman. Over the past year the Committee has continued to push for district implementation of the Cooperative Agreement with Cal Fish & Game. One of the stumbling blocks to general acceptance seems to be that there is no reciprocal responsibilities for the Resources Agency. Concern with this is understandable but our philosophy was to take the first giant step by agreeing to report our activities and work for reciprocity later. Well, later is now. Brain Hunter, Region II, Regional Manager for Cal Fish & Game contacted the Chair and stated that he felt his agency should do no less than ours in striving for a higher level of cooperation. He wants to work with the Committee to include a protocol for Cal Fish & Games's reporting of acquisitional and restorative projects so that the Coastal Region MAD's can have input that would prevent mosquito problems. I would hope that we can have a working model for the CMVCA Board in short order.

Additionally this year, with the able assistance of Don Womeldorf and Jack Walsh, we have re-established the Wildlife Management Mosquito Suppression Committee. The Committee has decided that we should set up a "traveling road show" to visit each region - convene a meeting of each member agency's colleagues for the purpose of discussing a problem of local importance, e.g., salt marshes, duck clubs, wetlands, etc. The first region we plan to visit is the Sacramento Valley. The problem of wetlands, such as the Butte Sink, would be the primary topic and rice field conversion to duck clubs a secondary one.

In the coming year, the newly appointed Committee should meet regularly in order to better push for Federal involvement with the Cooperative Agreement. Mr. Felix Smith representing U.S. Fish & Wildlife Service on the WMMS Committee stated at our last meeting that he was continuing to push for acceptance of the Agreement by the Service in California and he needs our encouragement and support.

---

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

ENTOMOLOGY RESEARCH.-J. E. Hazelrigg, Chairman. In 1985, Russell E. Fontaine, Coordinator of Mosquito Research, University of California, sent a call to approximately 40 University of California researchers to submit research proposals pertinent to California mosquito control. Over thirty proposals were received for review. The Committee (ERC) first met in Davis, March 12-13 to begin the initial review of these proposals.

The submitted proposals covered a broad base of statewide mosquito problems, both basic and applied. The principal research categories were chemical control, testing, and enhancement; mosquito biology, ecology, genetics and ethology; techniques and strategies with mosquitofish; vector disease control; marshland and wastewater management; and other fish as biological control agents. All proposals were reviewed "with the relevance to the needs of California mosquito control and that which has near-term prospects for applied uses" in mind. The Committee was more supportive of researchers collaborating with other agencies, particularly local mosquito abatement districts, or who sought additional or outside funding, or were historically productive and good at meeting research commitments and disseminating results.

As usual, the ERC compiled its report of comments, ratings and recommendations. The report included an average numerical rating of funding priority, comments and recommendations on each of the submitted proposals. The Report made available to the Mosquito Research and Technical Committee of the University of California, is used in their subsequent review of the same proposals. This report was submitted to the Board of Directors of the CMVCA in mid-year.

EQUIPMENT.-Claude L. Watson, Chairman. The Committee met on several occasions this past year to discuss various types of equipment that may be of interest to mosquito and pest abatement districts throughout the state.

After extensive discussion the Committee members felt that it would be worthwhile to put together an equipment show around the first week of April 1986.

This would give each district a chance to look at all of the different types of equipment currently in use.

The equipment show was a great success. The Committee wishes to thank all who attended.

LEGISLATIVE.-Allen R. Hubbard, Chairman. Some of the primary activities the Committee worked on in 1985 included the following: Health and Safety Code recodification; the exotic vector importation bill; support for Health and Safety Code clean-up language drafted by Peter Detwilder; review and proposed changes to the Katz bill; kept informed on the Southern California Encephalitis bill AB-2537; and the amendment to the Fair Labor Standards Act. In addition, the Committee reviewed legislation that could potentially have an impact on mosquito abatement districts, as well as upon local government.

PHYSICAL CONTROL.-Dennis D. Beebe, Chairman. President Hazeleur charged the Committee with compiling mosquito prevention criteria that could be used by mosquito and vector abatement district's throughout the state.

For that purpose the Committee recommends that the Guidelines, Standards and Checklists for Vector Prevention, available through the California Department of Health Services, Vector Surveillance and Control Branch, be adopted by the CMVCA as the guidelines for mosquito prevention in California.

These guidelines were compiled by a joint committee of the California Conference of Directors of Environmental Health, California Mosquito and Vector Control Association, and California State Department of Health Services, Vector Surveillance and Control Branch.

PUBLICATIONS.-John C. Combs, Chairman. The Committee met once, formally, and frequently, informally, on a wide range of editing and publications planning assignments. Among the accomplishments of the Committee were: production of the Proceedings and Papers and Yearbook on schedule, the setting up of the Salary Survey on the "File Pro" computer program, and plans for the production of two new major publishing undertakings, the Reeves monograph and completely revised training manuals.

STATEWIDE ACCREDITATION.-Charles P. Hansen, Chairman. This Committee has been in existence only one year, but it experienced a very successful and productive beginning. Three out of the five CMVCA regions provided continuing education programs in 1985 with the other two regions working on the start-up details. Some of the major topics covered were: mosquito biology and identification; pesticide worker and safety; cholinesterase testing; CMVCA conference and scientific workshops; mosquitoes of California, their behavior and habitats; mosquito control research projects in California; and general safety and first aid. Our goal was to have all five regions providing continuing education program by 1986 and it appears that goal will be met. The Committee is already working on exciting programs for 1986. Another major goal of the Committee is the coordination of a standardized statewide program. It is our hope that within the next three to four years this program will be in place and in full operation.

EFFICIENCY AND COST ANALYSIS OF CHEMICAL VS. INTEGRATED *CULEX TARSALIS* CONTROL IN CENTRAL CALIFORNIA RICE FIELDS

T. Miura, D. E. Reed[1], and R. M. Takahashi

University of California
Mosquito Control Research Laboratory
9240 S. Riverbend Avenue
Parlier, California 93648

ABSTRACT

Larval collection, treatment and cost records for the control of *Culex tarsalis* in Fresno County rice fields were analyzed to compare cost and benefits between chemical control and integrated control strategies. Results indicated that integrated control had cost less and was more effective than chemical control alone. Integrated control reduced the average number of chemical treatments necessary for control as well as the densities of mosquito larvae.

Integrated pest management (IPM) has advanced, during the last decades, from theory to actual applications practiced by both public and private institutions as well as the academia. Its concept has been around for even more years and its acceptance now seems to be generally pervasive among those responsible for pest control (Huffaker 1971, World Health Organization 1982).

Since there are more things to do in order to implement it, one can deduce that it will necessarily cost more. However, it is the objective of this paper to show that the implementation of one IPM strategy, in relation to mosquito control in rice fields, not only achieves more desirable results but also monetarily costs less to carry out.

BACKGROUND AND METHODS.-In past years about 10 to 15 thousand acres of rice were planted annually within the jurisdiction of the Fresno Westside Mosquito Abatement District (FWMAD) in central California. Rice fields normally were flooded during the two month period between April 15 to June 15.

Two main control strategies, biological and chemical, have been used to control rice field mosquitoes within the district. The goal of the district is to abate immature stages of mosquitoes especially *Culex tarsalis* Coquillett larvae by stocking fields with the mosquitofish, *Gambusia affinis* (Baird and Girard). This method has been demonstrated to be effective in reducing mosquito larval densities (Davey et al. 1974, Hoy et al. 1971, Hoy and Reed 1970, 1971) and is easy to apply because the mosquitofish multiply and disperse themselves through the field (Davey and Meisch 1977). Other fish have been tested (Davey et al. 1974, Washino 1968, Washino et al. 1967) but *Gambusia* is adaptable to a wider range of adverse habitats and environmental conditions (Emerick 1942, Harrington and Harrington 1961, Mulligan et al. 1983, Sjogren 1972).

[1]Fresno Westside Mosquito Abatement District, Post Office Box 125, Firebaugh, California 93622.

When possible, rice fields were normally stocked with mosquitofish at a rate of 0.2 lbs per acre (200 to 400 fish) 7 to 10 days after permanently flooding the fields for the season (Hoy and Reed 1971). Where mosquitofish was not able to be planted and/or mosquito larval (i.e., *Cx. tarsalis* 3rd and 4th instar) counts equalled or exceeded 0.1 per dip sample (Hoy and Reed 1971), chemical control strategies were considered. When this method was used as an adjunct to biological control, integrated control was created in part. The density and species of mosquitoes sampled, the density of predators and potential harm to the human and wildlife were considerations deliberated before chemical treatments were initiated either alone or as an adjunct. When this course was decided, however, aerial (Piper PA 36-300 Brave) applications of parathion at 0.1/acre was the method of choice because empirically the method kills quickly and effectively and apparently is not detrimental to fish at low rates used for mosquito control (Davey and Meish 1977).

the rice plants broke the water surface. Inspections were made at two week intervals by spot sampling fields with a standard mosquito dipper. When larvae were observed in a dip they were saved in a hand-held concentrator (Reed 1970) and 9 additional dips were collected from a transect in the immediate vicinity (Reed and Bryant 1972). The concentrated samples were then transferred to vials containing 50% alcohol preservative and transported to the laboratory for identification. The procedure was then repeated along transects in different parts of the field. The data collected were then analyzed to estimate mosquito population densities from which control strategies were planned.

The assembled records of 2 control strategies (chemical and integrated) in 1984 afforded us to examine and compare the differences in the cost and benefit between the two. During the 1984 season 7,130 acres of rice fields were stocked with mosquitofish and 19,259 acres were sprayed with chemicals to control *Cx. tarsalis* larvae.

Efficacy of mosquito control was determined by comparing larval collection records between the integrated and chemical control fields. The data were grouped and calculated to determine which field type had higher densities of larvae and higher incidences of positive larval collections during inspections. Percentages were calculated within each type for the former.

Monetary costs were calculated from money actually spent during the 1984 season. Treatment acres were calculated by multiplying the number of acres in fields by the number of times they were treated and summing the products.

RESULTS.-Table 1 shows that a higher percentage within unstocked fields generally required more multiple chemical treatments than the fields stocked with mosquitofish. The average number of treatments necessary for control in stocked fields was 1.6 per field. The average for the unstocked field was 2.3 per field, or about 1.5 times more than the fields with fish.

In Table 2 (bottom) there was a larger percentage within chemical control fields which had high larval densities of mosquito than integrated control fields. Moreover, the chemical-only fields produced 8.5% more positive observations during inspections. This reinforces the notion that mosquitofish can reduce mosquito populations even though most studies show that crustaceans, chironomids and small corixids are the most abundant invertebrates in the *Gambusia* diet (Ahmed et al. 1970, Farley 1980, Miura et al. 1979, 1984, Washino 1968, Washino and Hokama 1967).

The actual number of inspections required for integrated control fields were greater (Table 2) simply because there were more of them. The average number of inspections per field was about the same because all fields needed to be checked whether they were stocked with fish or not.

Table 3 shows an itemization of the actual costs needed during the 1984 season using the two control strategies. The cost per acre for chemical control ($5.16/acre) alone was about 1.5 times more than for integrated control ($4.22/-acre).

DISCUSSION AND SUMMARY.-Integrated pest management involves much more than we have sketched above. Socio-economic, environmental, political, philosophical aspects as well as long term goals are also considered in planning integrated control strategies. Nevertheless, we have presented several important aspects of this rather complex system in very concrete terms.

Integrated control, combining mosquitofish and chemical treatments, saved the district $0.94 per acre for a total savings of $6,715 during the 1984 season. Had integrated control been able to be used throughout the districts 9,528 acres of rice, the potential savings could have been about $9,000. Integrated control also reduced the average number of chemical treatments as well as the densities of mosquito larvae. Shortage of fish supply and some rice cultural practices not conductive for fish survival have precluded the use of fish on some fields.

It was not intended to focus only on the monetary cost aspect of integrated control (since the amount saved is somewhat trivial when compared to the total normal operating budgets for mosquito abatement districts), nor was it intended to strictly test the efficacy of any method. What was intended was to generally demonstrate the advantages and feasibility of integrated control in California rice fields over using chemical control practices alone. The results of this analysis indicated that integrated control had cost less and was more effective than chemical control alone.

Table 1.-Comparison of multiple chemical treatments required for fields stocked with mosquitofish and unstocked fields.

	Stocked Fields		Unstocked Fields	
No. Treatments	No. Fields	% of Treatments	No. Fields	% of Treatments
0	19	0	6	0
1	42	23	5	6
2	23	25	9	20
3	22	36	11	38
4	7	16	5	23
5	0	0	1	6
6	0	0	1	7
Ave. Treatment/Field	1.6		2.3	

Table 2.-Comparison of numbers of inspections and larval densities between integrated and chemical control fields in the FWMAD during 1984.

	Integrated	Chemical
	Inspections	
Total during 1984	649.0	190.0
No. of Fields	113.0	38.0
Avg. Per Field	5.7	5.0
Percent Positive	43.6	52.1
	Larval Density	
>0.2	16.5%	39.7%
0.1-0.2	18.5%	16.8%
<0.1	10.2%	0.5%
0	56.4%	47.9%

Table 3.-Cost of mosquito control strategies in the FWMAD during 1984.

Cost Factor	Rate	Chemical Control	Integrated Control
Chemical (Parathion)	$0.25/acre	$ 1,749.25	$ 3,065.55
Airspray (Plane+Labor)	$1.49/acre	10,425.53	18,270.40
Groundspray (Vehicle+Labor)	$8.10/hour	194.00	379.00
Fish Storage		0.00	0.00
Fish Harvest		0.00	0.00
Fish Stocking		0.00	0.00
Total		$12,368.78	$30,080.90
Acres		2,398	7,130
Cost/acre		$5.16	$4.22

ACKNOWLEDGMENT.-This study was funded in part by a U.S.D.A. cooperative agreement no. 82CRSR-2-1010 and by a special State of California appropriation for mosquito control research.

REFERENCES

Ahmed, W., R. K. Washino and P. A. Gieke. 1970. Further biological and chemical studies on *Gambusia affinis* (Baird and Girard) in California. Proc. Calif. Mosq. and Vector Contr. Assoc. 38:95-97.

Davey, R. B. and M. V. Meisch. 1977. Dispersal of mosquitofish (*Gambusia affinis*) in Arkansas rice fields. Mosq. News 37:777-778.

Davey, R. B., M. V. Meisch, D. L. Gray, J. M. Martin, D. E. Sneed and F. J. Williams. 1974. Various fish species as biological control agents for the dark ricefield mosquito in Arkansas rice fields. Envir. Entomol. 3:823-826.

Emerick, A. M. 1942. *Gambusia* in sewage ponds. Proc. Calif. Mosq. and Vector Contr. Assoc. 12:128-131.

Farley, D. G. 1980. Prey selection by the mosquitofish *Gambusia affinis* in Fresno County rice fields. Proc. Calif. Mosq. and Vector Contr. Assoc. 48:51-55.

Harrington, R. W., Jr. and E. S. Harrington. 1961. Food selection among fishes invading a high subtropical salt marsh: From onset of flooding through the progress of a mosquito brood. Ecology 42:646-665.

Hoy, J. B., A. G. O'Berg and E. E. Kauffman. 1971. The mosquitofish as a biological control agent against *Culex tarsalis* and *Anopheles freeborni* in Sacramento Valley rice fields. Mosq. News 31:146-152.

Hoy, J. B. and D. E. Reed. 1970. Biological control of *Culex tarsalis* in a California rice field. Mosq. News 30:222-230.

Hoy, J. B. and D. E. Reed. 1971. The efficacy of mosquitofish for control of *Culex tarsalis* in California rice fields. Mosq. News 31:567-572.

Huffaker, C. B. 1971. Biological Control. Plenum Press N. Y., London. 511 pp.

Miura, R., R. M. Takahashi and R. J. Stewart. 1979. Habitat and food selection by the mosquitofish *Gambusia affinis*. Proc. Calif. Mosq. and Vector Contr. Assoc. 47:46-50.

Miura, R., R. M. Takahashi and W. H. Wilder. 1984. Impact of the mosquitofish (*Gambusia affinis*) on a rice field ecosystem when used as a mosquito control agent. Mosq. News 44:510-517.

Mulligan, III, F. S., D. G. Farley, J. R. Caton and C. H. Schaefer. 1983. Survival and predatory efficiency of *Gambusia affinis* for control of mosquitoes in underground drains. Mosq. News 43:318-321.

Reed, D. E. 1970. Operational use of an improved mosquito larvae concentrator. Mosq. News 30:274.

Reed, D. E. and T. J. Bryant. 1972. Interrelation between water depths and the distribution of *Gambusia affinis* and immature *Culex tarsalis* in Fresno County rice fields. Proc. Calif. Mosq. and Vector Contr. Assoc. 40:122-123.

Sjogren, R. D. 1972. Minimum oxygen thresholds of *Gambusia affinis* (Baird and Girard) and *Peocilia reticulata* Peters. Proc. Calif. Mosq. and Vector Contr. Assoc. 40:125-126.

Washino, R. K. 1968. Predator prey studies in relation to an integrated mosquito control program, a progress report. Proc. Calif. Mosq. and Vector Contr. Assoc. 36:33-34.

Washino, R. K. and Y. Hokama. 1967. Preliminary report on the feeding pattern of two species of fish in a rice field habitat. Proc. Calif. Mosq. and Vector Contr. Assoc. 35:84-87.

World Health Organization. 1982. Manual on Environmental Management for Mosquito Control. WHO offset Publ. No. 66. Switzerland. 284 pp.

GROUND AND AERIAL APPLICATION OF THE FUNGUS *LAGENIDIUM GIGANTEUM* IN CALIFORNIA RICE FIELDS

James L. Kerwin and Robert K. Washino

Department of Entomology
University of California
Davis, California 95616

ABSTRACT

The aquatic fungus *Lagenidium giganteum* produces biflagellate motile spores either sexually or asexually which are capable of recognizing, encysting upon and infecting mosquito larvae in a variety of habitats. Both reproductive stages have been cultured in vitro using yeast extract - based media in 12 liter fermentation tanks. During the 1985 mosquito breeding season in the northern Sacramento Valley the fungus was applied in 400 m^2 plots using backpack sprayers and in 1 hectare plots by aerial spraying. In either ground or aerial treatments, the asexual stage resulted in immediate larval mortality as monitored using sentinel mosquitoes and dipping of the indigenous population. Some degree of recycling of the fungus was documented for several months following a single application. Activation of the sexual stage, the oospore, was erratic but resulted in significant mortality of *Culex tarsalis* and *Anopheles freeborni* when oospores were dried in the field rather than in the laboratory prior to application.

Subsequent modifications in the growth media used to culture the fungus have unexpectedly resulted in enchanced germination of both reproductive stages. Further investigation of the precise factors in the complex culture media now being used which regulate spore production and activation will lead to enchanced yields and predictable germination of the spores, resulting in the availability of an effective and economical alternative to chemical control of mosquitoes.

FIELD EVALUATION OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* IN VARIOUS HABITATS OF SUTTER-YUBA MOSQUITO ABATEMENT DISTRICT

Michael R. Kimball, Susan D. Bauer, and Eugene E. Kauffman

Sutter-Yuba Mosquito Abatement District
Post Office Box 726, Yuba City, California 95992

INTRODUCTION.-The Sutter-Yuba Mosquito Abatement District encompasses a major portion of Sutter and Yuba Counties, California, and contains approximately 660 square miles of primarily agricultural land. Within this area there is a diversity of mosquito habitats. These habitats include rice (approximately 120,000 acres in 1985), two major waterfowl refuges and numerous irrigated pastures.

Presently, wide area adulticiding is being used as a control method in these environmentally sensitive areas. There is a growing concern about the use of ULV chemicals and the associated drift into non-target areas. With the recent insurance coverage problems and liability involved with the use of these chemicals, there is a desire at the Sutter-Yuba Mosquito Abatement District to focus an increasing amount of attention toward the biological control of mosquitoes. At this time the mosquitofish, *Gambusia affinis*, is our major biological agent. However, there exists a need for additional biological agents and integrated management methods.

To address this need, the operational application of *Bacillus thuringiensis* var. *israelensis* (Bti) was initiated at the beginning of the 1985 mosquito season on a limited basis for field evaluation. The objectives of this study were to examine the relationships between efficacy and application rates, environmental factors and timing of treatments. The cost effectiveness of this treatment for our District was then determined.

MATERIALS AND METHODS. Spray Equipment.-For the aerial application of Bti it was necessary to contract for a pilot and airplane. Considering the extremely high cost of aerial application with conventional aircraft, a two-passenger Piper Super Cub® and an experienced agricultural pilot were chosen at a cost of $122 per hour.

The airplane was fitted with two Mini Micronair® rotary atomizers, attached at both sides of the craft, one on each wing strut. The rotary atomizer consisted of a propeller-driven, 20-mesh wire gauze. As material impacted the wire mesh, the liquid was sheared into fine droplets. The pitch of the blades was adjustable which permitted the rpm of the atomizer to be varied. The faster this unit spun, the finer the droplets became. A Mini Micronair® propeller-driven pump was mounted in front of the stationary landing gear. The output of the pump could be increased by adjusting the pitch of the propeller blades. A 15-gallon plastic barrel was strapped under the fuselage between the landing gear to serve as a spray tank, from which the pump drew material. Two valves were mounted within easy reach of the pilot, in line between the pump and the rotary atomizers. One valve was a simple on-off flow regulator. The other valve permitted very fine pressure adjustment in the system. A pressure gauge was installed in line between the pressure adjustment valve and one of the atomizers, easily visible to the pilot. A flow regulator with a rotating plate having six different orifice settings was installed in line, immediately preceding each atomizer (Figure 1). Another interchangeable plate could be selected, allowing a total of 12 different orifice settings for flow rate adjustment. After initial testing it was discovered that the smallest orifice settings (#1-#3) clogged easily with certain Bti formulations available to the District. A 40-mesh, cartridge filter was installed down line from the pump, alleviating the problem.

Fuel capacity limited the duration of flights to 2.5 hours, which allowed the spraying of approximately 600 acres. At a cost of $122 per hour, the basic cost for the airplane and pilot was $0.50 per acre.

Study Areas.-The study area included varied mosquito habitats in the Sutter-Yuba Mosquito Abatement District with an emphasis on rice agriculture. One block of rice fields located in Sutter County contained approximately 4,000 acres and encompassed 50 fields which ranged in size from 20-130 acres. An area this size was chosen to permit sampling of each field once a week by a crew of six to seven research personnel. All fields in this block were sampled for larvae commencing in mid-June. Fields indentified as having larvae present at a pre-selected threshold of a least eight larvae per 100 dips (0.08/dip) were placed randomly into three treatment regimes: a single application of Bti in June; an application of Bti in June immediately followed by stocking with mosquitofish at a rate of 0.2 lbs per acre; and an application of Bti in June followed with a July application of Bti. All three treatment regimes and the control were replicated three times. Selected control fields had larvae occurring at or above the 0.08/dip threshold level. Treatment dates were chosen to coincide with historical peaks in the numbers of *Culex tarsalis* and *Anopheles freeborni*, respectively. Sampling sites were established for each field and 25 dips were taken in a semicircular route at each site using a standard white plastic (473ml) dipper (Stewart et al. 1983). The number of sampling sites ranged from three for the smallest field to eight for the largest field. On each sampling date instar and density of *Cx.* and *An.* larvae were recorded as were rice heights, water temperatures and water depths. Sampling was

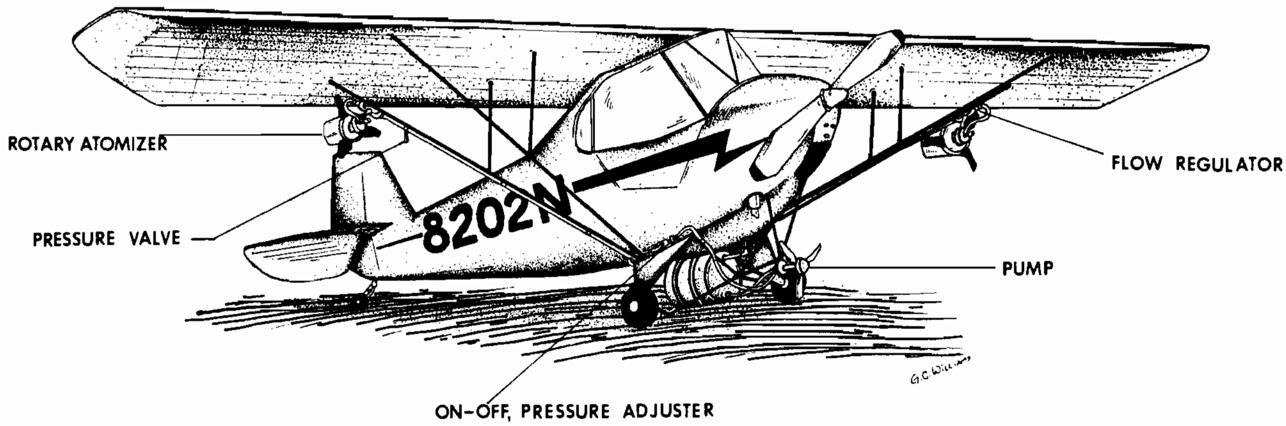


Figure 1

conducted on a weekly schedule from June 10 thru August 23, 1985. Rice fields to be treated were sampled the day before and the day after treatments to assess treatment efficacy. Additional caged sentinel larvae were used in approximately 33% of the fields treated. Sentinel buckets were set out at least 30 minutes before treatment, using lab-reared second and third instar *Culex tarsalis* larvae with 5 larvae per bucket. This density was chosen to approximate the natural density of larvae usually found clumped in the field, and to reduce the possibility of competition among larvae for Bti (personal communication Dr. Richard Garcia, U.C. Berkeley). Lids were placed on the buckets one half hour after treatment to exclude predators. Buckets were left in the field for 24 hours, at which time larvae were numerated to obtain mortality results.

Rice fields throughout the district, though not included in the aforementioned research block, were sampled during the season by operational field technicians on their individual schedules. Fields located with larvae occurring above a 0.08/dip threshold were treated as aircraft availability and funding permitted. Fields were sampled before and after treatments to assess treatment efficacy. Caged sentinel larvae were employed in a small percentage of the fields. District-wide, a total of 6,251 acres of rice were treated in 1985.

Refuge Studies.-Starting in mid-August each year, ponds in both the Sutter Wildlife Refuge and Graylodge State Wildlife Area are flooded for migrating waterfowl. The two refuges are capable of producing large numbers of *Aedes nigromaculis* and *Aedes melanimon*, which often impact nearby residential and agricultural areas.

Seventeen ponds within these two wildlife areas were selected for treatment based on availability of Bti, aircraft and funding. The ponds were sampled by field technicians before and after applications to determine efficacy.

Pastures Studies.-Only one irrigated pasture in north Sutter County was selected for Bti treatment. There had been limited success with adulticiding there due to limited accessibility for terrestrial vehicles and highly variable wind patterns. Two treatments which totaled 360 acres were made during the season. The pasture was sampled by field technicians before and after applications to determine treatment efficacy.

The Bti used during the season was provided by four manufacturers. They included: Abbott Laboratories (Vectobac-AS®, 600 International Toxic Units/mg); Biochem Products (Bactimos®, 1000 ITU/mg); Microbial Resources (Skeetal®, 1400 ITU/mg); and Sandoz (Teknar®, 600 ITU/mg and Teknar HP-D®, 1200 ITU/mg). All formulations were an aqueous suspension of Bti. Vectobac-AS® was used exclusively on the intensively sampled block of rice in Sutter County.

Treatments.-A determination of swath width was made using Tee-jet® spray cards and cups of lab-reared, third instar *Culex pipien* larvae. The aircraft made passes over the cards and larvae at both 25- and 50-foot altitudes, using orifice setting #3 at 20 lbs pressure. The results of the tests using larvae showed an effective swath width of 100 feet at both altitudes. The wind conditions during all tests were calm. It was noted on several replications that the spray cards showed no sign of material impingement while the cups of larvae immediately adjacent to the spray cards showed very high mortality. The spray droplet size for the Bti materials was never measured. A droplet size for water of 70 to 110 microns in diameter was estimated from tables in the Micronair handbook using the aircraft speed, blade pitch of the rotary atomizer, and flow rate.

The application rate was determined on each treatment date by loading a known amount of material into the spray tank, setting the desired orifice size on the flow regulator, adjusting the system to a given pressure, then spraying a known acreage. After the treatment, the spray tank and lines were drained and the amount of remaining material was measured. A simple mathematical calculation yielded the application rate. Temperature, humidity, wind speed and wind direction were measured and recorded for each treatment.

The first Bti treatments for the rice fields divided into the three treatment regimes occurred on June 20 and 22, 1985 and involved fields in all categories. After reviewing the mortality data for the June 20 treatments, the application rates for the June 22 treatments were increased (Table 1). The second Bti treatments occurred on July 17, 1985 and involved the fields that were to be treated twice. An application rate of 4.5 oz/acre was chosen for these treatments, as satisfactory control had been achieved at this rate in other areas of the District during the summer.

Rice fields throughout the District, excluding the aforementioned group, were treated between July 11 and August 23 and totaled 4,882 acres. Application rates ranged from 2.0 oz/acre to 5.7 oz/acre.

Bti treatments in refuge habitats took place between August 12 and September 10, 1985. Application rates ranged from 8.2 oz/acre to 16.0 oz/acre (Table 2).

The two Bti treatments to the pasture occurred on July 29 and August 22, 1985. The application rates were 7.5 oz/acre and 7.0 oz/-acre, respectively (Table 2).

RESULTS AND DISCUSSION.-Overall treatment mortality for the group of rice fields divided into three treatment regimes was encouraging. On the first day of treatments in June, a 76.6% mortality rate was calculated from pre- and post-treatment dipping data and a 52.0% rate from sentinel bucket data (Table 1). There were wind gusts up to 12 mph during the applications which caused some drift problems. Subsequent treatments were cancelled when wind speeds exceeded 5 mph. In addition, one of the rotary atomizers jammed causing reductions in material delivery during the treatment of the last three fields, one of which contained sentinel buckets. Mortality rates might have been higher had these problems not occurred. The second day of treatments in June resulted in 95.4% mortality calculated from pre- and post-treatment dipping data and 98.3% mortality from sentinel bucket data. The applications in July (fields to be treated twice during the summer) resulted in 72.9% mortality as indicated by pre- and post-treatment dipping data, and 99.2% mortality in the sentinel buckets. The rice height had reached 30 inches in many fields by this time. It was encouraging that the Bti was able to penetrate a canopy of this height.

The number of *Cx. tarsalis* and *An. freeborni* larvae over the sampling period from June to August did not differ significantly (t-tests) among treatment regimes and controls (Figures 2 & 3). In the case of the Bti and mosquitofish treatments, this was contrary to results from other similar studies (Stewart et al. 1983a). It appears that the District's wide area adulticiding program with spray routes throughout much of the study area depressed the mosquito populations significantly. Consequently, this may have minimized any differences among treatment regimes. One study field in the control group completely outside the adulticiding area had much higher numbers of larvae throughout the summer in comparison to control fields located inside the adulticiding area.

Another factor influencing these results was the timing of Bti treatments in June and July. The targeted *Cx. tarsalis* larval peak in June actually took place two weeks before our June 20 and 22 treatments. These treatments were late due to lack of sampling personnel and the inability to sample most rice fields due to shallow water levels for herbicide applications. Our July 17 treatments preceded the actual mid-August peak in numbers of *An. freeborni* larvae. These treatments were performed early because of concern about the ability of the material to penetrate the tall, extremely dense rice stands in August and unfounded grower's concerns that the material would disrupt the flowering processes in rice that takes place at this time. Another explanation for the depressed larval populations (*Cx. tarsalis*) in June may be a period of very hot weather (101-109°F) from June 6 to June 18, 1985. This may also explain why the control fields declined along with the treated fields.

Finally, the Bti formulations applied have little residual activity, and only last from 36-48 hours. This lack of residual activity may necessitate more frequent applications to effect a measurable change in overall larval population densities.

Treatment mortalities for rice fields, not included in the group test with the three treatment regimes, were also encouraging. Application rates as low as 2.0 oz/acre (1200 ITU) were used with mortality in sentinel buckets averaging 78.8% (0.0% in controls). The pilot initially felt uncomfortable applying the material at this low rate because the material was not visible as it exited the rotary atomizers. Applications on July 17 at a rate of 5.7 oz/acre (600 ITU) resulted in 90.3% mortality in pre- and post-treatment dipping and 95.0% mortality in sentinel buckets (Table 1).

The mortality results as presented in Table 2 are highly variable in relationship to application rates and their associated toxicity. There are two factors we experienced that affect this relationship. At a given dosage rate, the greater the density of larvae and/or the more advanced the larvae, the lower the resulting mortality. This is especially true in our refuge habitats where greater than 50 larvae per dip are found pre-treatment. The second factor is the presence or absence of dry grasses and other organic material. The aqueous suspension of Bti adsorbs readily in these conditions. Recommendations for future treatments would be an application rate of 16.0 oz/acre (600 ITU) or more for the two conditions mentioned above.

Mortality for the two pasture treatments are presented in Table 2. On the August 22 application the air temperature was 84°F. It was noted during the treatment that the Bti material was crystallizing on the gauze of the rotary atomizer. This raised the question as to whether the mater-

Table 1

INTENSIVELY DIPPED RICE

APPLICATION RATE	INT. TOXIC UNITS	$\bar{X}$ % MORTALITY	$\bar{X}$ % MORTALITY SENTINELS	ACRES	DATE
5.9 OZ./ACRE	600	76.6 RANGE 54.8-100	52.0 NO CONTROLS equip. malfunction	484	6-20-85
8.4 OZ./ACRE	600	95.4 RANGE 85.7-100	98.3 11.0 IN CONTROLS	494	6-22-85
5.7 OZ./ACRE	600	90.3 RANGE 83.3-100	95.0 5.0 IN CONTROLS	287	7-11-85
4.5 OZ./ACRE	600	72.9 RANGE 66.6-100	99.2 5.7 IN CONTROLS	391	7-17-85

Table 2

REFUGES

APPLICATION RATE	INT. TOXIC UNITS	% MORTALITY	ACRES	DATE
16.0 OZ./ACRE	600	33.3	80	8-14-85
15.0 OZ./ACRE	1000	$\bar{X}$-71.7 RANGE 50.0 - 92.0	430	8-28-85 TO 9-5-85
15.0 OZ./ACRE	600	$\bar{X}$-79.7 RANGE 66.6 - 100	555	8-30-85 TO 9 10 85
12.0 OZ./ACRE	600	20.0	30	9-4-85
8.5 OZ./ACRE	600	76.7	75	8-12-85
8.2 OZ./ACRE	600	40.0	70	8-20-85
		PASTURES		
7.5 OZ./ACRE	600	75.0	180	7-29-85
7.0 OZ./ACRE	1000	0 HIGH TEMPERATURE	180	8-22-85

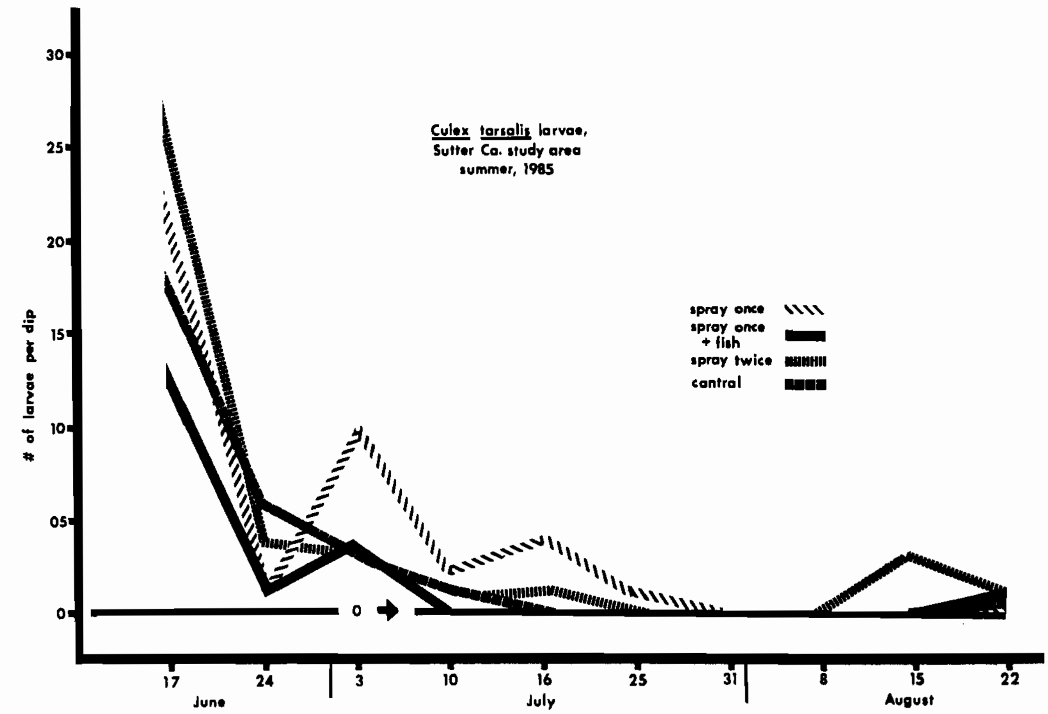


Figure 2

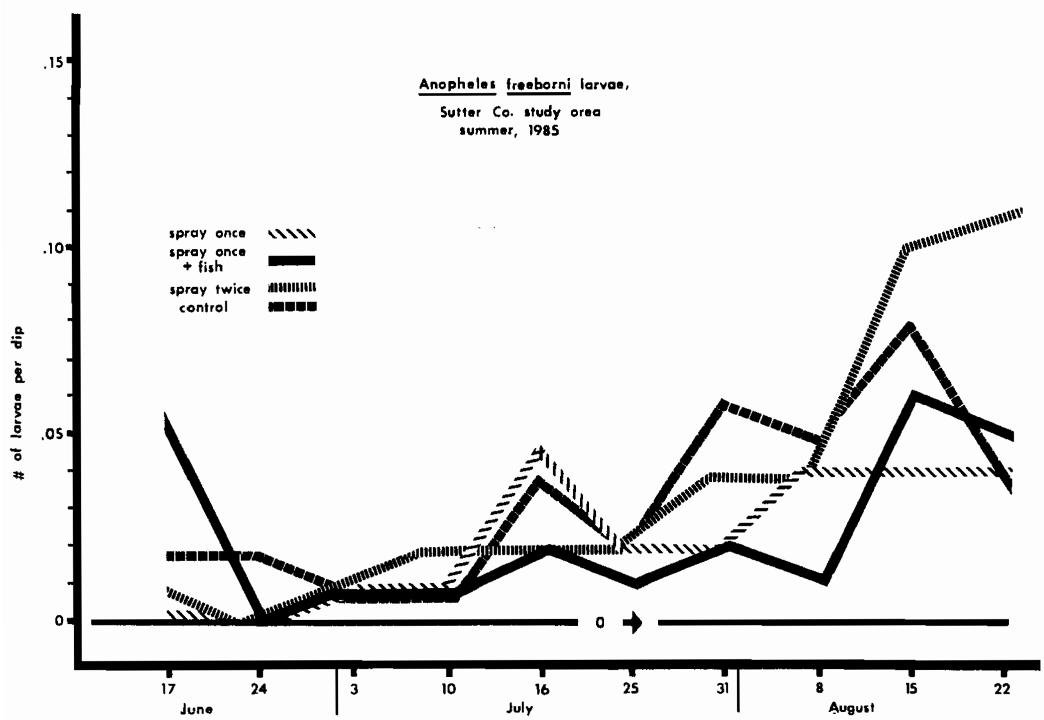


Figure 3

ial was actually reaching the targeted pasture; possibly dried particles were being blown downwind. Subsequent applications were discontinued when the air temperature reached 80°F, with most work being done in the early morning.

As mentioned previously, there were problems with the rotary atomizers binding. This situation occurred several times during the summer spray operations. Usually a copious application of light oil around the sealed bearing alleviated this problem. The recommendation from Micronair, Inc. was to remove and discard the metal shield on one side of the bearing, then replace the false grease nipple with a standard nipple. The unit could be lubricated with one stroke of a grease gun after one to two days operation.

During the treatment of ponds at the two wildlife refuges, waterfowl were sometimes a problem. The aircraft struck and was damaged by a duck during one treatment. Attempts were made to discourage waterfowl from targeted ponds with little success. When loud noises from truck horns or percussion devices were used, the waterfowl would retreat only a short distance and usually flew to another location on the same pond. Treatments under these potentially hazardous conditions were sometimes cancelled - at the pilot's discretion.

The results of the work done during the 1985 season were favorable. Future plans for the use of Bti in 1986 include the treatment of approximately 80,000 acres of rice, along with a small amount of wildlife refuge and pasture mosquito habitat.

REFERENCES

Stewart, R. J., C. J. Schaefer and T. Miura. 1983a. Sampling *Culex tarsalis* immatures on rice fields treated with combinations of mosquitofish and *Bacillus thuringiensis* H-14 toxin. J. Econ. Entomol. 76(1):91-95.

Stewart, R. J., T. Miura and R. B. Parman. 1983b. Comparison of sample patterns for *Culex tarsalis* in rice fields. Proc. Calif. Mosq. and Vector Contr. Assoc. 51:54-58.

SAMPLING OF *CULEX SPP.* LARVAE IN URBAN CATCH BASINS AND THEIR CONTROL USING *BACILLUS THURINGIENSIS* SEROTYPE H-14

Russell B. Parman

Kern Mosquito Abatement District
Post Office Box 9428, Bakersfield, California 93389

ABSTRACT

A presence-absence sampling procedure was developed which provided weekly indices of larval *Culex spp.* populations in urban catch basins and was more amenable to statistical analysis than standard counts. Weekly treatments using 50 ml per liter tank mixes of *Bacillus thuringiensis* serotype H-14 formulations were as effective as GB-1356 for larval control.

INTRODUCTION.-During 1984 the Kern Mosquito Abatement District evaluated several *Bacillus thuringiensis* serotype H-14 commercial larvicidal formulations under various rural operational conditions and found them to be both efficacious and economical, especially for ground based operations (Parman 1985). Cost reductions of 73% per treated source were achieved when these compounds were used in place of Golden Bear-1356 larvicide oil. However, urban operators reported control failures against *Culex spp.* larvae in catch basins when using 25 ml/l liquid concentrate (LC) B.t. H-14 tank mixes (2 pts/10gal). Since records of larval populations in catch basins had not been kept, the present study was undertaken to develop a simple sampling program for urban *Culex* larval populations which could be utilized by field personnel and to compare the control efficacy of B.t. H-14 vs that of GB-1356 LO.

METHODS AND MATERIALS.-Weekly larval sampling was conducted in 32 catch basins divided equally among the treatment zones of eight operators within the Bakersfield, California city limits. Basins were chosen which could be sampled directly from their curb entrance. In each basin three dips were taken consecutively from left, right, and center next to the basin walls. Numbers of immatures were recorded as instars I-II, III-IV, pupae and egg-rafts. To evaluate treatment effects, sampling was conducted five to six days after curbing operations within each of the eight operator zones for a period of eight weeks during late May to early July. The two control agents were alternated at two-week intervals between zones 1-4 and 5-8. Both GB-1356 LO and B.t. H-14 were applied to gutters and catch basins using vehicle mounted sprayers with an average output of 6.8 liters/min. B.t. H-14 (Teknar®, Sandoz Inc; Vectobac® AS, Abbott Laboratories) was applied as aqueous tank mixes of 50 ml/liter (4pts/10 gal).

Analysis of preliminary samples indicated a negative binomial distribution (Elliott 1977) with very low mean values and k approximately 0.05. Taylor's power law and $\log_{10}(X+1)$ transformations failed to satisfy basic ANOVA assumptions (Sokal and Rohlf 1969), so dips were evaluated instead as either positive or negative, and proportions of these classes for each treatment were analyzed using 2x2 chi-square contingency tables with Yate's correction for continuity.

RESULTS.-Analysis of larval counts over a 13 week period indicated a good correlation between the proportion of dips positive and the mean value (Instars I-II, r = .79, $\bar{x}$ = 15.98 p - 0.52, n = 13; Instars III-IV, r = .85, $\bar{x}$ = 15.85 p - 0.05, n = 6). Estimated mean values for the entire 1985 season are presented in Figure 1. Pupae and egg rafts were taken in very low numbers and were not presented.

Weekly mean values of instars I-II in zones 1-4 and 5, 6, 8, 9, were significantly correlated (r = .510, p<.01, n = 27) when partial control or control-free periods (Figure 1, arrows) were included. When these periods were excluded from the analysis, weekly mean values for the two zones were significantly correlated through August (r = .680, p<.02, n = 13) but not through the entire season (r = .301, p>0.1, n = 20).

Treatment comparisons are presented in Table 1. There were no differences between the effects of B.t. H-14 and GB-1356 on either instar group during the eight week test period. Significantly more dips were positive following partial control or control-free periods than following control periods. Estimated mean values five to six days post-treatment were 62% and 75% lower than those following control-free periods for instars I-II and III-IV, respectively.

The use of 50 ml/l tank mixes of B.t. H-14 resulted in a 41% savings in material cost when compared with GB-1356 LO, with costs averaging $0.86 and $1.45/curbing mile for the two respectively materials. Total volume for Bt H-14 tank mixes and GB-1356 LO averaged 1.1 and 0.9 gal/curbing mile, respectively.

DISCUSSION.-The present study indicated that 50 ml/liter tank mixes of B.t. H-14 LC were as effective as GB-1356 LO in controlling larval *Culex spp.* populations in catch basins. Twenty-five ml/l tank mixes applied later in the season appeared to be effective also, but data suggested a greater proportion of dips positive for instars III-IV when compared to GB-1356 LO - treated basins.

Pfunter (1978) reported a 2-3 day residual activity for GB-1356 in two catch basins when applied at 15 ml basin. Our vehicle applied an estimated 111 to 354 ml/basin, but the larger

Table 1.-Number of positive and negative dips taken from catch basins during the test period (A), and for the entire season (B).

		INSTARS					
		I,II			III,IV		
A.) TREATMENT	+	−	CHI-SQUARE	+	−	CHI-SQUARE	
BT H-14	36	348		7	377		
GB-1356	41	343	.0003	4	380	.3689	
AREAS							
1-4	46	338		11	373		
5,6,8,9	31	353	2.8291	0	384	9.2230*	
WEEKS							
1-4	37	347		6	378		
5-8	40	344	.0577	5	379	0	
B.) COMPARISON							
TREATED	217	1,658		62	1,813		
UNTREATED	125	369	58.5661*	63	431	68.5393*	

*$p < .005$

number of basins introduced a much greater degree of variability in water flow and debris content. It was not expected that B.t. H-14 and GB-1356 treated basins would show similar instar distributions since the former product is not considered to have residual activity beyond 24 hours. However, it was estimated that the initial concentrations of B.t. H-14 LC within basins ranged from 2-175 ppm, depending upon basin water volume (40 - 2,000 liters) and spray volume applied per basin (111 - 354 ml). These initial concentrations are well above those achieved on a per-area basis; B.t. H-14 applied to one acre-foot at 2.3 liters/ha produces approximately 0.8 ppm. Thus it is conceivable that in some basins sufficient residual may be present 24 hours post-treatment to cause mortality in newly hatched larvae, even assuming a 99% loss of toxin.

The presence-absence sampling used during this study holds several advantages over conventional larval counts. Time required to gain mean values is reduced, especially as populations increase. Where large amounts of silt or debris are present, only one individual needs to be observed. In addition, the type of data generated are much more suitable for statistical analysis than are counts; Andersen (1965) demonstrated that when mean and k of the negative binomial distribution were low ($\bar{x}<3$ and k approaching zero) that transformations were inadequate to satisfy the requirements for analysis of variance. Southwood (1978) provides a detailed account of presence-absence sampling.

Weekly urban surveillance of *Culex* larvae provides two important pieces of information. First, sampling first and second-instar larvae five to six days post-treatment presumably provides an index of the gravid female adult population, so long as sampling occurs after any residual activity of treatment has past. The positive inter-zone correlations observed post-treatment through August indicated that the same seasonal population trends were recorded from separate urban areas. During September, treatment schedules were erratic due to personnel changes and it was difficult to adhere strictly to the post-treatment sampling schedule. Preliminary studies using manure-baited ovitraps failed to show a positive correlation between numbers of egg rafts in traps and numbers of instars I-II in catch basins but the number of traps used (8) was relatively small.

Secondly, failure to treat catch basins consistently produced distinct increases in larval counts. Any control failures due to insecticide resistance or product deficiencies should thus be readily detected.

The need for the above kinds of information is underscored by current trends toward increased urbanization, and by the recent St. Louis encephalomyelitis cases in metropolitan Los Angeles (Reeves 1985). Further study in Kern

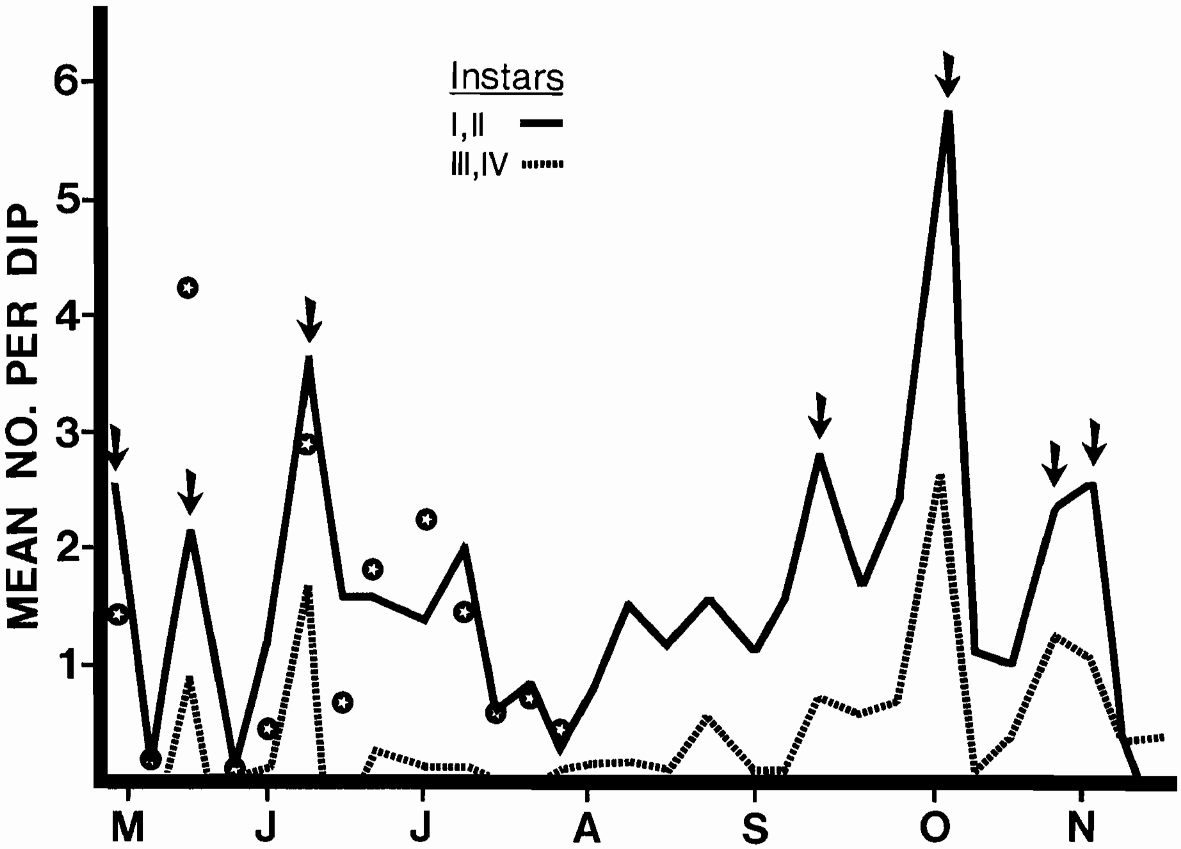


Figure 1.-Mean numbers of *Culex spp.* larvae per dip in urban catch basins during 1985. Values were estimated from actual counts (stars) and from the proportion of dips positive (lines). Arrows indicate samples following partial or non-control periods.

County will include the use of lower B.t. H-14 rates and an evaluation of Dimilin®25 WP for larval control. Surveillance of adult and larval *Culex spp.* populations using manure-baited ovitraps and catch-basin sampling will also be expanded.

ACKNOWLEDGMENTS.-The assistance of Cary Welch, Foreman, and the Urban operators of the Kern Mosquito Abatement District is gratefully acknowledged.

REFERENCES

Andersen, F. S. 1965. The negative binomial distribution and the sampling of insect populations. Proc. XII int. Congr. Ent. 395. Sited in Southwood, T.R.E. 1978. Ecological Methods. Chapman and Hall, New York. 524 pp.

Elliott, J. M. 1977. Some Methods for the Statistical Analysis of Samples of Benthic Invertebrates. Freshwater Biological Association, Scientific Publication no. 25. 2nd ed. 155 pp.

Parman, R. B. 1985. Operations with commercially available *Bacillus thuringiensis* serotype H-14 formulations in Kern County, California. Proc. Calif. Mosq. and Vector Contr. Assoc. 53:66-70.

Pfunter, A. R. 1978. The development and control of *Culex quinquefasciatus* Say and *Culex peus* Speiser in urban catch basins. Proc. Calif. Mosq. and Vector Contr. Assoc. 46:126-129.

Reeves, W. C. 1985. Perspectives and predictions following the St. Louis encephalitis outbreak in Southern California. Proc. Calif. Mosq. and Vector Contr. Assoc. 53:30-31.

Sokal, R. R. and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Co., San Francisco, CA. 776 pp.

Southwood, T.R.E. 1978. Ecological Methods. Chapman and Hall, New York. 534 pp.

IMPACT OF CARBAMATE INSECT GROWTH REGULATORS ON THE SELECTED AQUATIC ORGANISMS: A PRELIMINARY STUDY

T. Miura, C. H. Schaefer, and R. J. Stewart

University of California
Mosquito Control Research Laboratory
9240 South Riverbend Avenue
Parlier, California 93648

ABSTRACT

Static acute toxicity tests were conducted to determine effects of R016-1294 (2-[p-(m-chlorophenoxy)phenoxy]ethyl ethylcarbamate), R016-1295 (2-[p-(m-flurophenoxy)phenoxy]ethyl ethylcarbamate) and fenoxycarb (ethyl[2-(4-phenoxyphenoxy)ethyl]carbamate) on mosquitofish. Concentrations used in the tests ranged from 1.0 to 0.001 ppm. There was no mortality of fish in either treated and control chambers at 96 h observation except some mortality at higher treatment rates of R016-1295. At the rates of 0.015, 0.02 and 0.03 lb/acre applied, fenoxycarb did not exhibit any apparent effects on cladocerans, copepods, ostracods and mayfly nymphs in the treated pastures.

In recent years, several new carbamates which have different modes of action, such as insect growth regulators, have received much attention as potential means of controlling various insect pests (Kramer et al. 1981, Robertson 1982, Parrella et al. 1982). Schaefer et al. 1985 reported that R016-1294 (2-[p-(m-chlorophenoxy) phenoxy]ethyl ethylcarbamate) and R016-1295 (2-[p-(mfluorophenoxy)phenoxy]ethyl ethylcarbamate) were highly effective against immature mosquitoes (*Aedes spp.* and *Culex spp.*). Mulla et al. 1985 reported that fenoxycarb (ethyl[2-(4-phenoxyphenoxy)ethyl]carbamate) showed very high efficacy against mosquito larvae in both laboratory and field tests. The objective of this report is to evaluate the impact of these compounds on selected aquatic organisms commonly found in mosquito breeding habitats.

MATERIALS AND METHODS. Chemical and Condition.-Toxicants used were R016-1294, R016-1295 and fenoxycarb supplied by MAAG Agricultural Research and Development. For laboratory tests, serial dilutions were made from technical materials in acetone solution using temperature adjusted (27±1°C) tap water as diluent. The test chambers (6 liter glass aquaria) were held at 27±1°C under a photoperiod of 14:10 LD regimes. For field tests, EC formulations (125g/liter) were used.

Laboratory Tests.-The mosquitofish used were from laboratory colonies. For testing R016-1294 and R016-1295, adult and juvenile fish were separately exposed to the chemicals. Adult fish used averaged 2.7 cm SL and 0.29 g weight; sexes were mixed with a ratio of 2 males to 5 females. Juvenile fish used were 2 to 3 weeks old; average size was 1.2 cm SL and 0.04 g in weight. Treatment rates used for adult fish were 1.0, 0.56, 0.32, 0.18 and 0.1 ppm but for juveniles, they were 0.1, 0.07, 0.02 and 0.01 ppm. Tap water and acetone controls were prepared for all tests. To test fenoxycarb male and female fish were separately exposed to treatment rates of 1.0, 0.1, 0.01 and 0.001 ppm. For each test 5 fish were used in test chamber and repeated 2 times. Observations were made daily for 4 days after treatment. The criteria for effect was death, defined as a lack of body and opercular movement and failure to respond to probing.

Laboratory colonies of Amphipods (*Hyallela azteca*) and field collected damselfly nymphs (*Ishnura spp.*) were also exposed to R016-1294 at rates of 1, 0.1, 0.01 and 0.001 ppm.

Field Tests.-Various sized pastures in Modesto area were treated with EC formulation (125 g/liter) of fenoxycarb against *Aedes nigromaculis* by aircraft. Procedures used are explained in detail by Schaefer et al. 1975. In brief, fixed-wing aircraft was used. The spray systems were calibrated to deliver 2 gal/acre. Spray was done by a pilot and aircraft of the East Side MAD. Instructions were given to the pilot to apply the material as would normally be done under operational use. Applications were made during the early morning hours to avoid winds over 5 mph and higher temperatures.

Effects on aquatic, nontarget organisms were monitored by taking 10 dips prior to treatment and at indicated intervals during the post-treatment observation period (Miura and Takahashi 1975).

RESULTS AND DISCUSSION. Laboratory Tests.-R016-1295. Table 1 summarizes the results of the static tests with adult fish. At treatment of 1.0 ppm, 40% of fish exposed were killed, but no mortality was observed at 0.1 ppm. Juvenile fish exposed at 0.1 ppm or below showed no mortality at 96 h.

R016-1294. No effect of the chemical on adult fish was found at 96 h in any of the treatments of controls (Table 1). Occasional observations made beyond 96 h revealed no mortality to 168 h. The rates tested for juvenile fish were safe to fish, no noticeable changes were observed during the test period. No deleterious effect was observed in either amphipods or damselfly nymphs during the 4-day observation period.

Fenoxycarb. No chemical caused mortality was seen during the 9-day observation period.

Field Tests.-At all rates (0.015, 0.02 and 0.03 lb/acre) applied, fenoxycarb did not exhibit any apparent deleterious effects on aquatic non-

Table 1.-Effects of R016-1295 and R016-1294 on adult mosquitofish, a static test.[a/]

Concentration (ppm)	Post treatment (h) 24	48	72	96	Post treatment (h) 24	48	72	96
	R016-1295				R016-1294			
1.0	0	20	40	40	0	0	0	0
0.56	0	0	10	20	0	0	0	0
0.32	0	0	10	10	0	0	0	0
0.18	0	0	10	10	0	0	0	0
0.10	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0

[a/] Numbers indicate mortality in percent.

Table 2.-Effects of fenoxycarb on nontarget organisms applied against pasture mosquito *Aedes nigromaculis*, July 20, 1985.

Organism	July 19	20	21	22	23
	Sorrentino Pasture	:	Rate 0.03 lb/acre		
Cladocera	198	258	394	500	350
Copepod	8	16	13	20	56
Ostracod	2	6	14	10	55
Mayfly	28	22	20	20	15
Tropisternus L.[a/]	0	0	1	3	3
	Hollowman Pasture	:	Rate 0.02 lb/acre		
Cladoceran	1	1		15	30
Copepod	1	123		182	260
Ostracod	90	58		66	222

[a/] L = larvae

target organisms prevailing in the irrigated pastures (Table 2). The dominant organisms found in the treated pastures were cladocerns, copepods, ostracods and mayfly nymphs. No mortality or abnormal behavioral changes were observed during the study period.

SUMMARY.-Overall assessment is that the chemicals R016-1294, R016-1295 and fenoxycarb are safe to mosquitofish and other organisms commonly found in irrigated pastures. The rate of 0.02 to 0.01 ppm represents a highly effective rate for mosquito larval control (Schaefer et al. 1985, Mulla et al. 1985). It would be expected that the use of these compounds to mosquito control probably pose no problems on the environment.

ACKNOWLEDGMENT.-This study was founded, in part, by USDA Cooperative Agreement No. 82CSRS-2-1010 and by a Special California State Appropriation for mosquito control research. We wish to express our appreciation to the personnel of the East Side MAD for their cooperation.

REFERENCES

Jones, S. C. 1984. Evaluation of two insect growth regulators for bait-block method of subterranean termite (Isoptera: Rhinotermitidae) control. J. Econ. Entomol. 77:1086-91.

Kramer, K. J., R. W. Beeman and L. H. Hendricks. 1981. Activity of R013-5223 and R013-7744 against stored-product insects. J. Econ. Entomol. 74:678-80.

Masner, P., G. Husler, A. Pryde and S. Dorn. 1983. Interactions between a non-terpenoid carbamate with juvenile hormone activity of *Adoxophyes reticulana* Huebn. J. Insect Physiol. 29:569-74.

Miura, T. and R. M. Takahashi. 1975. Effects of the IGR, TH6040, on non-target organisms when utilized as a mosquito control agent. Mosquito News 35:154-59.

Mulla, M. S., H. A. Darwazeh, L. Ede and B. Kennedy. 1985. Laboratory and field evaluation of the IGR Fenoxycarb against mosquitoes. J. Am. Mosq. Contr. Assoc. 1:442-48.

Parrella, M. P., K. L. Robb, G. D. Christie and J. A. Bethlee. 1982. Control of *Liriomyza trifolii* with biological agents and insect growth regulators. Calif. Agr. (Nov.-Dec.) 17-19.

Robertson, J. L. 1982. Effect of body weight on lethal effectiveness of a juvenile hormone analygue applied to male and female Western spruce budworm (*Choristoneura occidentalis*) (*Lepidoptera: Tortricidae*). Can. Entomol. 1063-68.

Schaefer, C. H., W. H. Wilder and F. W. Mulligan, III. 1975. A practical evaluation of TH6040 as a mosquito control agent in California. J. Econ. Entomol. 68:183-85.

Schaefer, C.H., W.H. Wilder, E.F. Dupras, Jr. and R. J. Stewart. 1985. Efficacy and chemical resistance of two highly-active carbamate developmental inhibitors. J. Agric. Food Chem. 33:1045-48.

APPLICATION OF DIFLUBENZURON SAND GRANULES FOR CONTROLLING *CULEX TARSALIS* LARVAE BREEDING UNDER MATURE COTTON STANDS

C. H. Schaefer, H. L. Clement[1], W. H. Wilder,

F. S Mulligan, III and E. F. Dupras, Jr.

University of California
Mosquito Control Research Laboratory
9240 South Riverbend Avenue
Parlier, California 93648

ABSTRACT

Application of 0.5% diflubenzuron and granules to cotton fields in August and September allowed control of *Culex tarsalis* immatures when liquid sprays could not penetrate the vegetative canopy. No detectable residues occurred on the vegetation, even following six multiple aerial applications.

INTRODUCTION.-Diflubenzuron, or Dimilin[TM], is well established as a mosquito control agent in California; extensive field tests have shown high degree of efficacy at a rate of 0.025 lb. AI/acre (Schaefer et al. 1975). Studies on the chemical persistence of diflubenzuron showed that deposits on vegetation from aqueous sprays are relatively long-lasting but such residues can be avoided by using granular formulations (Schaefer et al. 1976, 1977). In 1985, diflubenzuron became available for use by California mosquito abatement districts on limited sources.

Even though diflubenzuron is registered for the application of aqueous sprays on cotton for control of the boll weevil, the compound is not

Table 1.-Summary of Treatments of Cotton Fields Treated with Diflubenzuron 0.5% Sand Granules.

Field	No. Acres Treated	Date (1985)	Actual Treatment Rate (lb AI/acre)
Sill	7.0	8/27	0.039
Sill	7.0	9/ 3	0.039
Sill	7.0	9/ 5	0.027
Sill	7.0	9/10	0.034
SVG[a/]	7.6	8/15	0.036
SVG	7.6	8/20	0.027
SVG	7.6	8/27	0.039
SVG	7.6	9/ 3	0.039
SVG	7.6	9/ 5	0.027
SVG	7.6	9/10	0.034

a/ SVG is Shafter Vegetables Growers

[1]Kern Mosquito Abatement District, Post Office Box 9428, Bakersfield, California 93389.

Table 2.-Summary of Cotton Leaf Samples Treated with Diflubenzuron 0.5% Sand Granules.

Field	Date (1985)	Sampling time	Area/75 leaves (in^2)	Residues found ng/in^2
Sill	8/27	pre No. 1	2,637.4	N.D.[a/]
Sill	8/28	24 hrs. post	2,393.0	N.D.
Sill	9/ 3	pre No. 2	2,236.1	N.D.
Sill	9/ 4	24 hrs. post	2,089.4	N.D.
Sill	9/ 5	pre No. 3	2,168.2	N.D.
Sill	9/ 6	24 hrs. post	2,589.2	N.D.
Sill	9/10	pre No. 4	2,651.9	N.D.
Sill	9/11	24 hrs. post	2,412.4	N.D.
SVG[b/]	8/14	pre No. 1	2,338.5	N.D.
SVG	8/15	24 hrs. post	2,141.6	N.D.
SVG	8/20	pre No. 2	2,170.7	N.D.
SVG	8/21	24 hrs. post	2,071.5	N.D.
SVG	8/27	pre No. 3	1,974.5	N.D.
SVG	8/28	24 hrs. post	2,014.9	N.D.
SVG	9/ 3	pre No. 4	2,077.3	N.D.
SVG	9/ 4	24 hrs. post	2,105.5	N.D.
SVG	9/ 5	pre No. 5	1,983.0	N.D.
SVG	9/ 6	24 hrs. post	2,260.6	N.D.
SVG	9/10	pre No. 6	2,294.8	N.D.
SVG	9/11	24 hrs. post	2,585.0	N.D.

a/ N.D. is not detected (less than 0.000009 ng/in^2

b/ SVG is Shafter Vegetable Growers

approved for use on this crop for mosquito control. As the breeding of *Culex tarsalis* beneath dense cotton stands is an annual problem in the San Joaquin Valley, a study was conducted to determine the efficacy of diflubenzuron granules for controlling this source and measurements were made to determine the extent of any residues that might result from the treatments.

MATERIALS AND METHODS.-Two cotton fields in western Kern County were selected to represent both furrow and sprinkler irrigation conditions. The Shafter Vegetable Growers (SVG) field was located on rolling ground and was irrigated by sprinklers; standing water which was retained in the lower areas resulted in the breeding of *Culex tarsalis* during August and September. A second, level cotton field, Sill field, was irrigated by furrows. This latter field was not known to allow breeding of mosquitoes but was used as a comparison for residues which might result from treatments in the absence of any sprinkling.

Diflubenzuron granules, 0.5%, were prepared as follows: a 200 lb. lot of 16 mesh sand was placed in a cement mixer and mixed for 30 minutes with 20 fluid oz. of Golden Bear 1356 larvicide oil; 4 lbs, of 25% diflubenzuron wet-table powder was added and mixing was continued for 15 minutes; 0.25 lb. of amorphous silica (Hi-Sil) was added as a drying agent and the mixing was continued for an additional 15 minutes.

For aerial application, the Kern M.A.D. Ayres/Thrush was equipped with a Swathmaster spreader and flown at 130 mph at an altitude of 30 feet. This provided an effective swath width of 60 feet and an application rate of ca. 8 lbs. granule/acre for a desired dose of 0.04 lb. AI/acre. For each application, pre-weighed granules were placed in the aircraft hopper, each pass was flagged and the granules remaining were collected and re-weighed to allow calculation of the actual rate applied. The immature population of *Culex tarsalis* in the SVG field was sampled by dipping before and then daily for several days following treatment. In addition, six water samples were collected from the SVG field before and following each treatment and these were bioassayed with laboratory-reared, fourth-instar *Culex quinquefasciatus* larvae to verify biological activity.

The Sill cotton field was treated four times and the SVG field six times, all during August and September 1985.

Before and 24 hours following each application a random sample of cotton leaves (2-3 lb. lot) was collected from each field. These were placed in an ice chest and transported to the laboratory where they were stored at -20°C or lower until analysis for diflubenzuron residues.

For residue analysis, the cotton leaves were thawed at room temperature and groups of 75 leaves each were carefully rinsed (both sides) with redistilled hexane. The combined total leaf surface area was determined by passing each group of leaves through a portable area meter (Li-Cor Model LI-3000). The hexane rinse from each group was reduced to dryness; the residue was dissolved in acetonitrile and analyzed by high-pressure, liquid chromatography using a Perkin-Elmer series 400 liquid chromatograph and the procedures described by van Rossum et al. 1984.

RESULTS AND DISCUSSION.-A summary of the treatment schedules for these two cotton fields is shown in Table 1. In all cases the actual rates applied were close to the suggested dose rate for diflubenzuron against mosquitoes (0.02-0.04 lb. AI/acre).

Bioassay of the water samples after application of the granules to the SVG field showed 95 to 100% activity following the treatments. The dipper sampling confirmed the bioassay data; while numerous larvae were present during the treatment times they did not successfully develop to the pupal stage. Thus, the treatments resulted in operational control of these populations.

Data on the leaf samples are summarized in Table 2. No measurable residues were observed after any of the six applications on the SVG field nor after the four applications on the Sill field. There was no rainfall during the course of the study period. Thus, 0.5% sand granules of diflubenzuron can be applied in multiple applications in order to achieve reductions of *Culex tarsalis* immature populations and no apparent residues on cotton foliage result.

REFERENCES

Schaefer, C. H. and E. F. Dupras, Jr. 1976. Factors affecting the stability of Dimilin in water and there persistence of Dimilin in field waters. Jour. Agric. Food Chem. 24:733-739.

Schaefer, C. H. and E. F. Dupras, Jr. 1977. Residues of diflubenzuron in pasture soil, vegetation and water following aerial application. Jour. Agric. Food Chem. 25:1026-1030.

Schaefer, C. H., W. H. Wilder and F. S. Mulligan, III. 1975. A practical evaluation of TH-6040 as a mosquito control agent in California. Jour. Econ. Entomol. 68:183-185.

van Rossum, A., A. de Reijke and J. Zeeman. 1984. Diflubenzuron. In Analytical Methods for Pesticides and Plant Growth Regulators, XIII. Ed. Zweig/Sherma. Academic Press. Pg. 165-169.

MICROMETEOROLOGICAL INSTRUMENTATION TO ASSIST WITH EFFECTIVE MOSQUITO CONTROL

Norman B. Akesson and Richard Gibbs

University of California, Davis

The dispersion of mosquito control materials released into the atmosphere from aircraft or ground equipment becomes highly dependent on local weather conditions. These include wind direction and velocity, temperature and temperature difference with height and to lesser extent humidity or moisture content of the atmosphere. Barometric pressure is more a tool for projecting weather patterns on an area-wide scale and as such can aid the mosquito district personnel in predicting favorable weather patterns or possible significant changes in a normal local pattern that would affect their operation.

Figure 1 shows a diagram of the typical summer daily pattern of temperature difference with height that occurs in the California interior and coastal valleys. This temperature difference or inversion with warmer air overhead is essential to successful aerosol applications and will also greatly influence the dispersion of larger drop size sprays. This diagram merely shows how temperature difference taken at two heights changes (horizontal scale lower, is for temp., vertical scale is height) during a 24-hr daily period. The normal decrease in temperature with height is shown as the slightly tilted vertical line passing through the apex of the several lines and down through noon and 5 p.m. on the circular scale. Between approximately these two points on the daily circle (to the right) the ground level is warmer than at a higher elevation indicating that air is moving upward and circulating any airborne materials released at ground level into the upper air. As we progress on the top circular route from 10 a.m. to the right we pass noon and 2 p.m. to 4 p.m. where maximum lapse (warm air near the ground) conditions normally occur. Now as we move on the bottom circle (to the left) passing 5 p.m. to 6 p.m. we begin to see the temperature difference shifting to warmer air overhead which corresponds to a temperature inversion. Under typical valley conditions this inversion may maintain itself throughout the night and will breakup or change to lapse sometime during the period 8 to 10 a.m. the following morning.

The change that occurs each day in the temperature difference is related to and accompanied by (a) sunrise and set, (b) wind direction and velocity and (c) occurrence and movement of area-wide (synoptic) atmospheric pressure changes. Heating of the earth by the sun and cooling by radiation to a cool sky will change the temperature of the ground and air close to the ground. This heating and cooling also aids in producing mountain slope winds causing air to move up-slope in the daytime and down-slope at night. Large-scale highs and lows of atmospheric pressure produce clockwise rotation of the air around a "high" and counterflow around a "low". This interacts still further with hills and valleys and along with the daily heating and cooling pattern helps to move coastal cool marine air into the valley during the afternoon and evening and out at night and early morning. All of these combine to produce the strong temperature inversion conditions in the evening and early morning widely noted in California valleys.

The two basic weather factors that identify good mosquito control application weather are the temperature inversions and the wind velocity. These two elements may be combined into a single number called the stability ratio or Barad Number which is graphed in Figure 2. The SR is simply the temp. diff. at two heights (lower temp. is subtracted from the upper) divided by wind velocity squared at half way in height between the temperature sensors. The units are metric, degrees centigrade and velocity in cm/sec and require multiplication by 10^5 to bring into whole numbers.

In order to effectively use Figure 2, accurate wind velocity (to ½ mi/hr) and temp. diff. (0.1 degree F) should be available which requires rather sophisticated meteorological instrumentation. Obviously Figure 2 is also limited to inversion conditions and low winds which of course again define the useful weather for mosquito control work. As an example of the use of Figure 2, the temp. diff. is located on the vertical (right) scale either in F or C degrees. For the example shown at 2.9°F we move to the right until the observed wind velocity (lower, horizontal) scale projection line upward is noted. We have taken as an example, 3.8 mi/hr, which then intersects the temp. diff. at the slanted line 5 which is the stability ratio under these conditions. For suitable application weather condtions the SR should be greater than 0 and in order to have this the wind velocity should not exceed 5 mi/hr and the temp. diff. be greater than + 0.1°F. A very practical way to observe temperature inversion and wind velocity as well as direction is to create a smokey fire in which velocity can be observed moving downwind as well as how rapidly mixing is taking place vertically.

Figure 3 shows an actual aerosol or cold fog released at ground level and gradually being dispersed along the ground as well as upward by the breaking of the temperature inversion. Obviously, mosquitoes in the air and grass under such application conditions are not going to be contacted by the material contained in the visible cloud. However, due to the wide range of drop sizes inherently produced by all of the customary application machines, a portion of the cloud, largely invisible, does settle toward the ground and will provide a certain amount of airborne

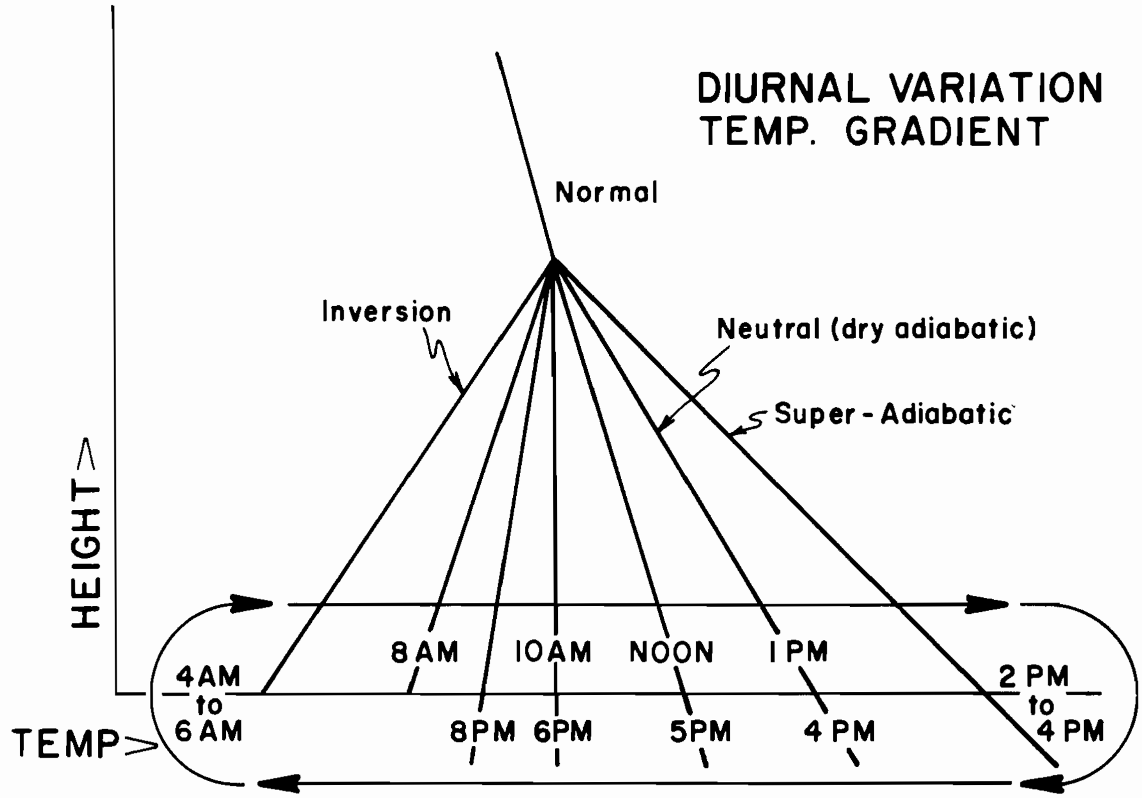


Figure 1.

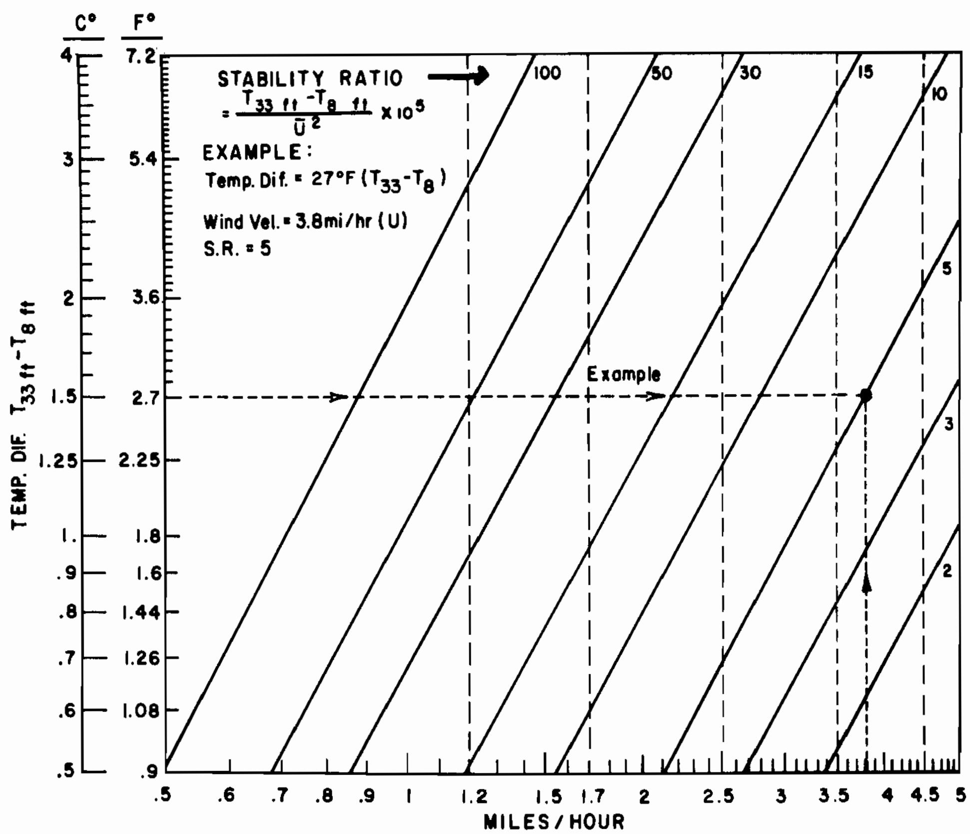


Figure 2.

Figure 3.-Aerosol Cloud.

contact as well as fallout on the ground under the cloud. Details on the effect of weather conditions on mosquito control and measurements of airborne and fallout are discussed in the accompanying two papers by Akesson and Yoshimura.

INSTRUMENTATION.-A considerable variety of weather station systems are being offered commercially, but as would be expected these range from the low priced "home and hobby" machines which are generally minimal for wind velocity and temp. diff. to highly sophisticated and expensive systems suited for accurate weather measurement. Figure 4 shows a typical utility or minimal station with the visual read out console which can be located relatively near to the weather sensors. The wind vane, velometer and one temp. unit (shielded) are shown as well. The degree of accuracy or minimum threshold response for the low-cost instruments is of the order of 1-2 mi/hr and 1°F which is quite adequate for utility use, but can be improved (for a nominal cost) to go to 0.5 mi/hr and 0.1°F, the latter generally requiring a matched thermistor bridge circuit.

Figure 5 shows a portable field type station suitable for transport to a specific spray site for close and accurate monitoring of application weather conditions. The unit has a threshold of 0.5 mi/hr velocity and 0.2°F temp. diff. Data are collected from the sensors and stored in a micro-chip unit from which they can be retrieved and delivered to a small computer coupled printer. This unit has storage for about 24 hrs of data. This unit as well as any of the utility or more sensitive instruments can be coupled to a telephone or radio transmitter modem so that the station can be left in the field and data "phoned back" on request by a home-based telephone. Data can be dumped into a computer and in turn used to produce hard copy of the information gathered.

Figure 6 showns a close-up view of the solar powered double cylinder aspirator used on the weather tower of Figure 5. Some form of powered aspiration is needed in order to keep air moving over the sensor and to insure that the sun heat will not affect the temp. reading. The gravity flow unit shown in Figure 4 is minimal in this

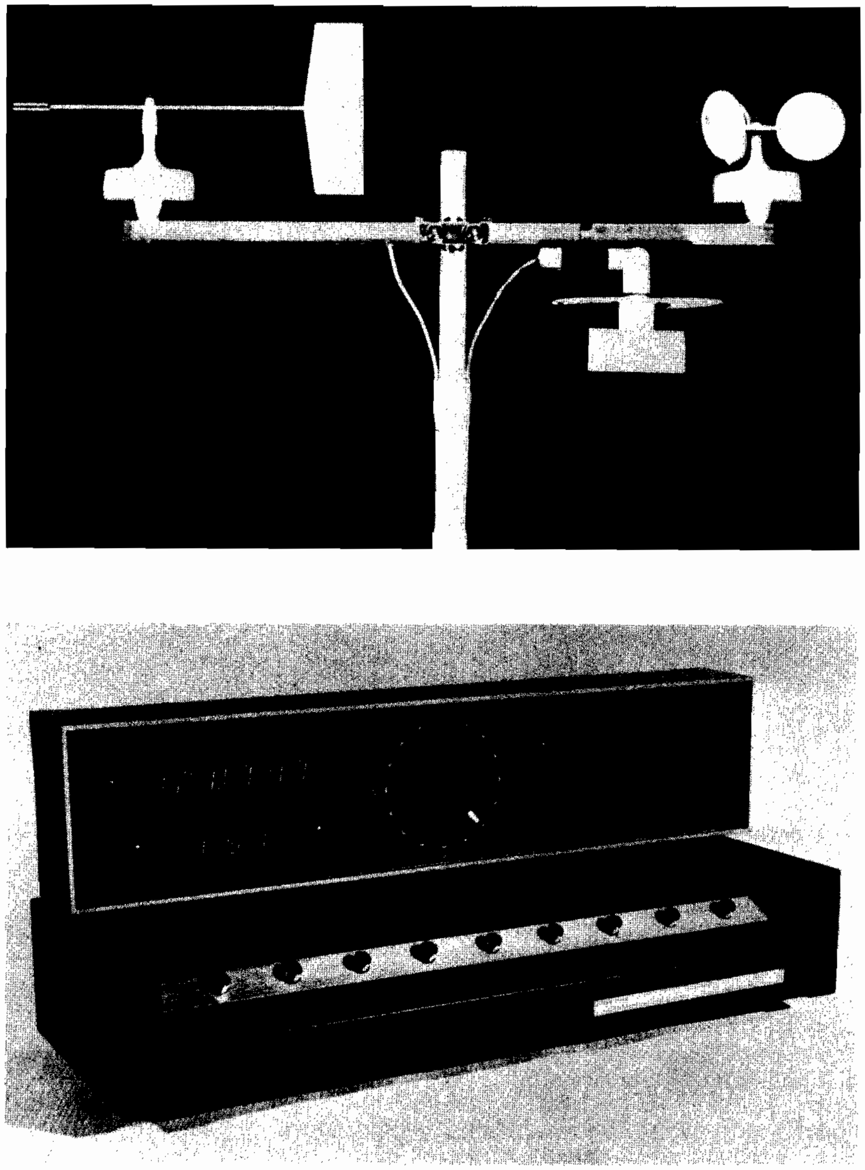


Figure 4.-Utility-type station.

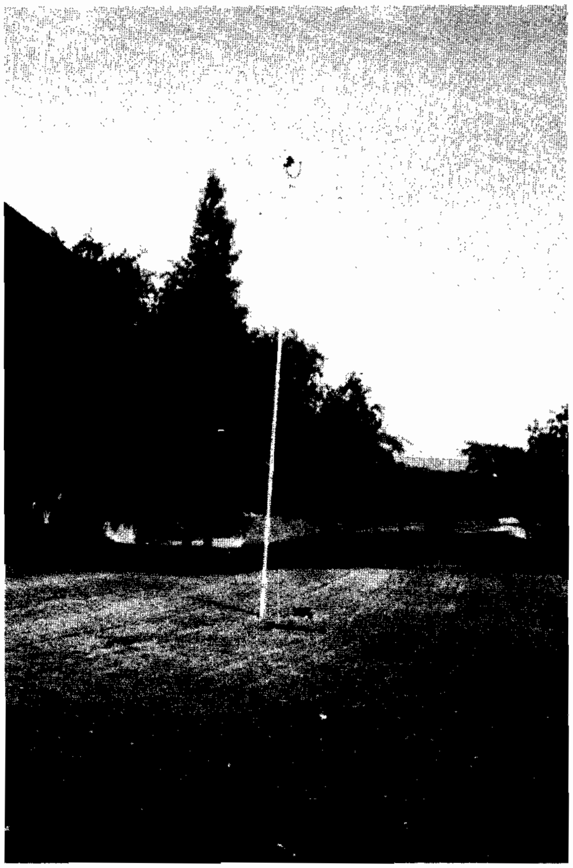


Figure 5.-Sensitive weather station.

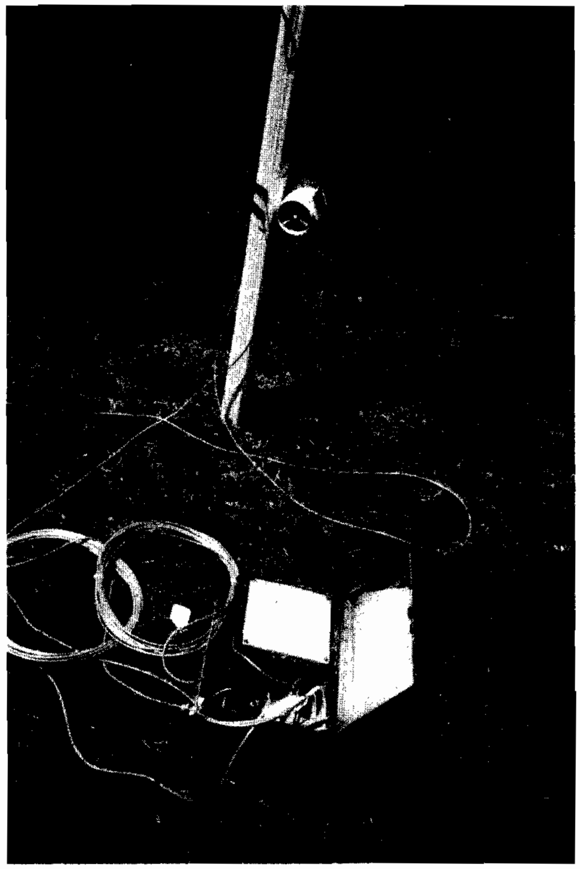


Figure 6.-Solar powered aspirator.

respect and is one of the shortcomings of the customary utility station. However, powered aspirators can be purchased and should be used for increasing temp. diff. accuracy in all weather station systems.

In summary, in order to develop an efficient and effective mosquito control program, especially if it involves aerosol application, it is essential to have weather monitoring stations which provide a least minimal threshold accuracy to both aid in prediction and to provide "hard copy" monitoring of actual weather conditions during a given application. Such equipment has long been available from the well-known meteorological instrument manufacturers, but has also been costly for district offices to use and maintain. There are now at least a half dozen manufacturers of utility or hobby type Instruments which can provide the basic requirements at minimal sensitivity. Today the microcomputer has added a new dimension to usefulness of weather station equipment and along with basic weather sensors can be purchases as off-the-shelf units which will aid greatly in increasing effectiveness of mosquito control operations.

OPTIMIZING APPLICATION CONDITIONS

FOR MOSQUITO CONTROL WITH SCOURGE®

Norman B. Akesson, Nancy Raubach and Richard Gibbs

University of California, Davis

Field studies relating the application factors of formulations, drop size and weather conditions have been made for several years by the Agricultural Engineering Dept. of the University of California at Davis with the cooperation of mosquito control entomologists at Davis and various California Mosquito Control District personnel and California Dept. of Health Bureau of Vector Control personnel. Application equipment, both aircraft and ground, has been used with a variety of formulations, chemical and biological, with different drop sizes produced, various release rates and under a considerable range of weather conditions. The basic objectives of these tests were to (1) evaluate the usefulness and capabilities of various application machines for mosquito control (2) to determine dispersion characteristics and effectiveness of different drop sizes, (3) to determine the dispersion and mosquito control effectiveness in relation to weather conditions during application and (4) to correlate all of these application factors and determine in as far as possible how they affect both larvae and adult mosquito control.

Drop size and effect on mosquitoes with different formulations of widely used chemicals has been studied for many years at the USDA Gainesville, Florida laboratories by Mount and Weidhaus and more recently by Haile and others. Their work as well as that of other observers has indicated that drop sizes of various chemical materials of 10-15 microns (micrometers) diameter are most effective in controlling adult mosquitoes for both field and laboratory testing. However, their work is based heavily on wind tunnel or confined atmosphere testing which leaves a considerable gap for correlation with aircraft application. Thus, we have viewed the practical minimum size as being somewhat larger or in the 20-30 micron diameter range when the overall operational system including weather conditions have to be considered.

But that has only been one element of the problem of conducting field scale tests to correlate material, drop size, weather factors and mosquito control. There are only three known atomizer devices that will produce drops below about 75 microns as a volume median (vmd) size range. These are, (1) two fluid (air and liquid) atomizers, (2) vortical nozzle, cold foggers and (3) certain high peripheral velocity rotating devices such as the Micron-X®. Tests run on a number of district cold foggers, both shop built and commercial, showed that many of these were being operated with high flow rates that produced drop spectra in the 50-60 vmd micron range, while most aircraft equipment was being operated in the 100-150 vmd micron range. On this note we decided to see if studies using smaller drop size might not be worth pursuing to find if possible what the optimum size for different applications might be and specifically to see if California Districts might be using too large drop size for optimum results.

Our first large scale correlation studies were made in the Goose Valley, Burney Basin MAD in 1977. These were done with a commercial cold fogger ground rig applying Dursban at about 4 oz/acre and were conducted under both morning and evening inversion conditions. We did not get our drop size Particle Measuring Systems unit until sometime after these tests (1980), but data on samples collected and counted from coated glass slides and KromKote paper indicated a probable size range of 15-20 microns vmd. Later tests with the PMS confirmed these results.

The results of these 3 tests of 2-4 passes each on a single line gave us a good correlation of downwind aerosol dispersion and caged adult mosquito control to a distance of 2000 ft. from the application line. We were able to note that evening tests provided temperature inversion conditions of greater total time, which however did not show up any differences in relation to control of native or caged mosquitoes. Although we learned a good deal about dispersion of aerosols, we weren't satisfied that our need for correlation to mosquito control was met.

Another large plot study was conducted in 1982 with the material *Bacillus thuringiensis, israelensis* formulated as Teknar®. Here, we again tried to relate drop size, dispersion, weather factors and control effectiveness with Bti which was applied to a rice field area for larval control. Three different machines were used in these studies. The first was an aircraft flying ten 50 ft. swaths to cover an area of 500 ft. wide by ½ mile long. Six 8004 LP nozzles were used producing an approximate drop size of 300 microns vmd and applying 3 gal of Teknar ai to 30 acres or at 0.1 gal/ac. The second machine was an air carrier or blower rig which we operated on a single path along the upwind side of our rice paddy plot. Two passes were made on a single line with this machine at 5 mi/hr applying 3.25 gal. of Teknar ai. Four D2-13 type hollow cone nozzles were used producing drop size of about 150 microns vmd.

The third machine was a cold fogger unit of shop-built type. The drop size produced was of the order of 50 microns, vmd and 6 passes were made a 5 mi/hr to release a total of 4 gal. Teknar ai. Local weather was monitored for all runs made on the same evening and showed good inversion conditions and low but positive wind drift.

The results of these tests (Figure 1) were impressive to us in that control of larvae in cups

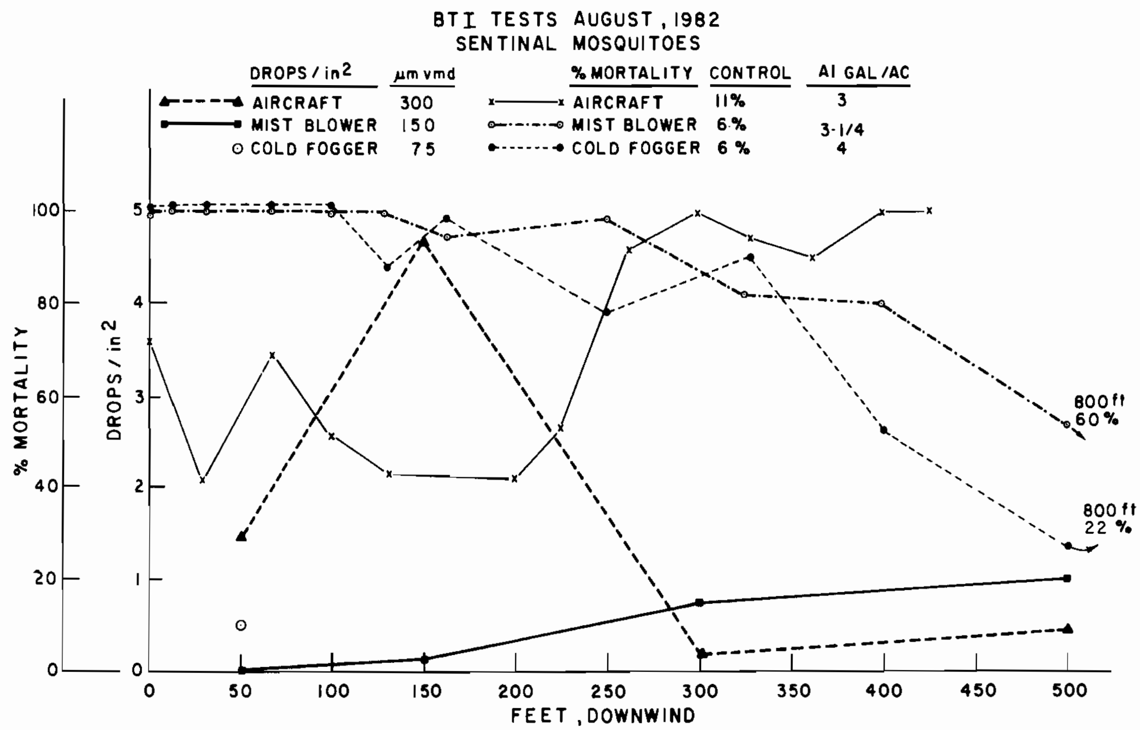


Figure 1.

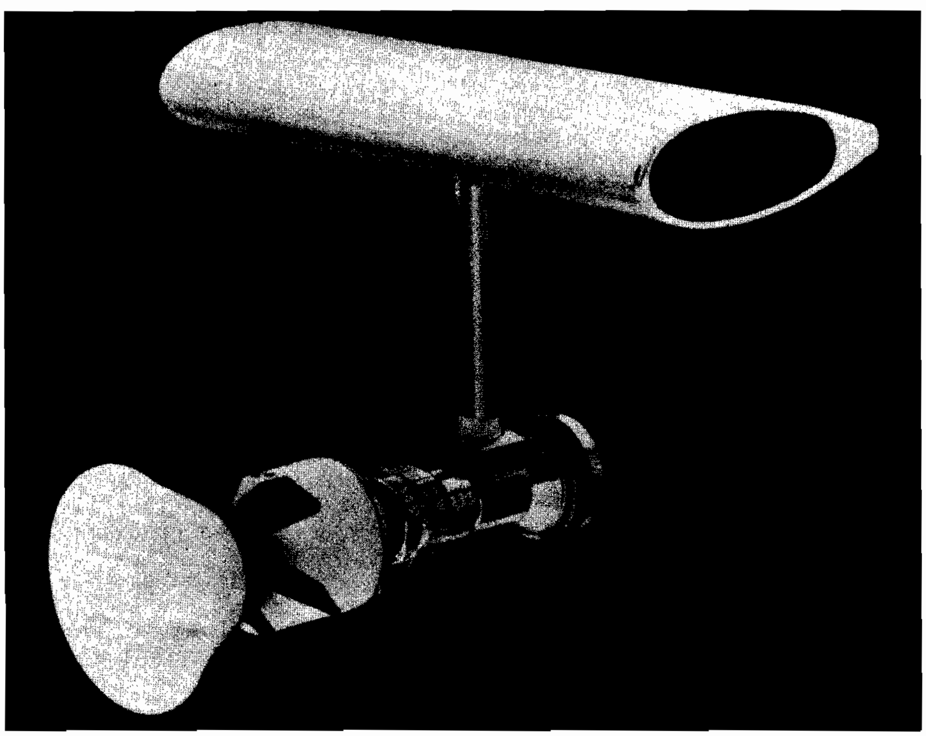


Figure 2.

placed at rice level was 100% at 125 ft. down wind and 80% out to about 400 ft. with the air blower and the cold fogger machines. It also shows that results with the aircraft, even though we made ten 50 ft. swath width passes out to 500 ft., were erratic out to about 250 ft. but rose to 95% or better from 300 to 450 ft. Figure 1 shows a graphical picture of the results. The response of the larvae in cups for the 3 runs is shown as solid (aircraft) dashed (cold fogger) and dot-dash (air carrier) narrow width. We also tried to get correlation to drops caught on KromKote cards placed downwind for each test with limited results. These are shown as dashed (aircraft) and solid (air carrier) heavy lines. Only one point was found with drops from the cold fogger shown as a circle at 50 ft. Even; though we could not get reliable drops collection data, the effective control on larvae prompted us to pursue these tests with a new material Scourge® for 1984 and 1985 seasons.

1984 Scourge® Tests.-Our third and fourth large field plot tests were run with Scourge®, a resmethrin material mixed 1/9 with cottonseed oil, in our 1984 tests and with Witco Golden Bear oil in 1985. Actual ai was 0.151 lbs/gal of the formulated mix. The aircraft we used was a Gruman Agcat biplane, 450 hp engine, flown at about 20 ft. elevation at 100 mi/hr. We used the Micron-X® rotary cup atomizer (Figure 2) which is driven by a small multi-blade fan. Drop size was controlled by using flow rate control with different flow discs in the line behind the atomizers. From smallest (10-15 micron vmd) drops a #15 disc held the flow rate at about 3 oz/min (15 lb/in^2 pressure) for each of the 40 spinners. A #32 disc at about 25 lb/in^2 was used on each of 10 spinners to obtain a flow rate of 12 oz/min, 120 oz total, and drop size of 20-30 microns vmd. All dosage rates from 1984 were aimed at .0035 lbs/ac. Figure 3 shows the drop size characteristics for the rotary Micron-X® atomizer. Smallest drops are not shown here hence this figure only gives a general picture of drop size, rpm and flow rate for this device. The relationship between rpm and blade angle is also shown, we used the faster spinning 20° blade for our tests and obtained the drop size results as noted, operating the spinner at about 25,000 rpm.

The first test was done with caged sentinel mosquitoes, applying two drop sizes at same application rates to two different plots. From these tests we determine the swath width obtained which showed 1000 ft. for the smaller drops and about 600 ft. for the larger. Weather was monitored and indicated slightly turbulent (no inversion) weather. We also took fallout (mylar sheets 6 x 18 in.) and air sampler (25 ft^3/min on 4" dia. glass glass fiber filters) information which confirmed swath widths for the two drop sizes as indicated by the caged mosquitoes.

The 2nd test was again run with two drop sizes with single passes on two different plots. Confirmation of the 900-1000 ft. swath for 10-15 microns vmd and 600-700 ft. for the 20-30 micron vmd.

The weather was variable and although control of sentinels in the area was good, the control of native mosquitoes, while showing a sharp drop for about 24 hrs, was then rapidly reinfested and populations went up.

The 3rd run was made again with the two

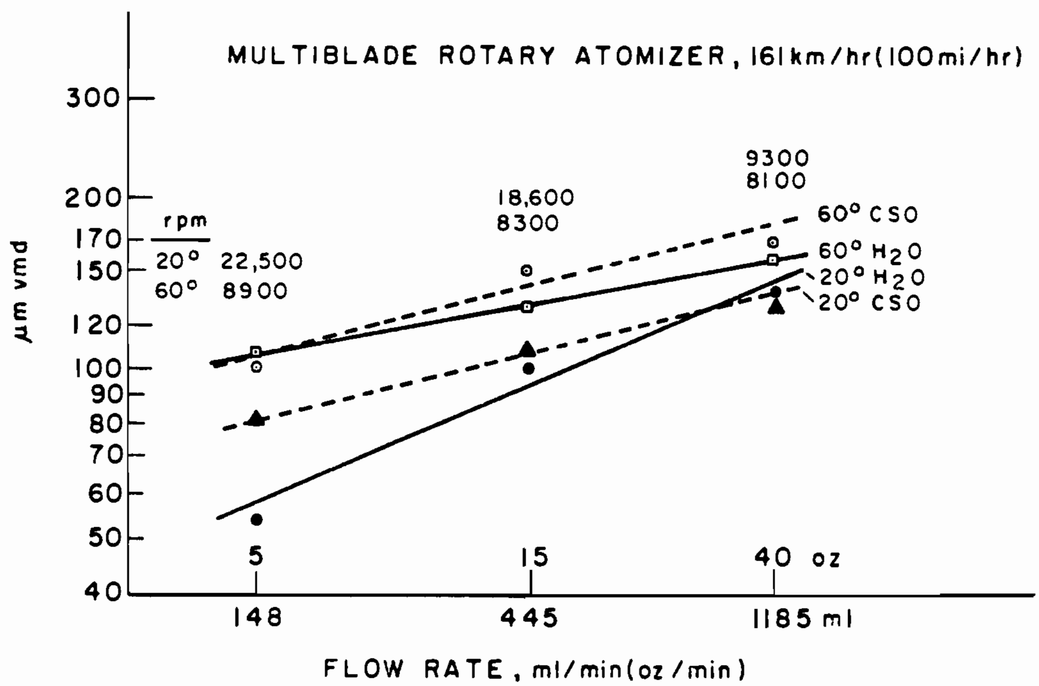


Figure 3.

drop sizes and the Micron-X® rotary atomizers. Here we had good strong inversion weather, and we made three passes with each test to see if a larger area would give us a longer residual effectiveness of the Scourge®. But data taken by Yoshimura (see paper following) on leg counts and in EVS traps showed rapid mosquito re-infestation within 24 hrs time. Drop size difference were still of the same order with smaller drops producing wider swath.

Our last test for 1984 (Oct. 25) was on a different basis than the previous three tests. Here, we operated over a single application line applying 10 passes with the aircraft allowing the material to drift down wind. The weather was very good with a strong temperature inversion and a light but positive breeze. The results are shown in Figure 4. The sentinel mosquitoes (picked up after one pass of the aircraft) were placed downwind at 8 stations as shown, 0 to 2640 ft. distant from the aircraft path. Only the large (20-30 microns vmd) drop size was used and as can be seen the control of the sentinels was above 80% out to 660 ft. and dropped to very low response on out to 2640 ft. It is interesting to note the air sampler data, which was collected at ambient air speed on glass fiber air filters at 5 stations from 165 ft. out to 2640 ft. The effect on sentinel mosquitoes is paralleled by the collection of resmethrin on the air filters. The other information is shown as fallout data under the aircraft and down wind to 2640 ft. Here the level remains high all the way out to the end of the sampled area.

1985 Tests with Scourge®.-During the 1985 season three further tests were run with Scourge® and GB oil 1/9 mix. The same aircraft was used and only the small drop size 10-15 microns vmd was applied. Here we again had sentinel mosquitoes out in cages in the sprayed area and also took air and fallout samples down wind from the last aircraft pass.

The first test was made with 8 passes applied to about a 320 acre plot; the weather was slightly turbulent, no inversion. We took 100 ft. swath intervals and calculated the resmethrin dosage at about .001 lbs/acre in 0.8 oz/ac. Air and fallout samples were taken and sentinels as well as EVS trap data. Effect on caged mosquitoes was high, but very little effect on natives was observed (see Yoshimura paper).

Our second run set (August 29, 1985) was on a larger plot of about 885 acres. Five passes were made with the aircraft on 1000 ft. pass

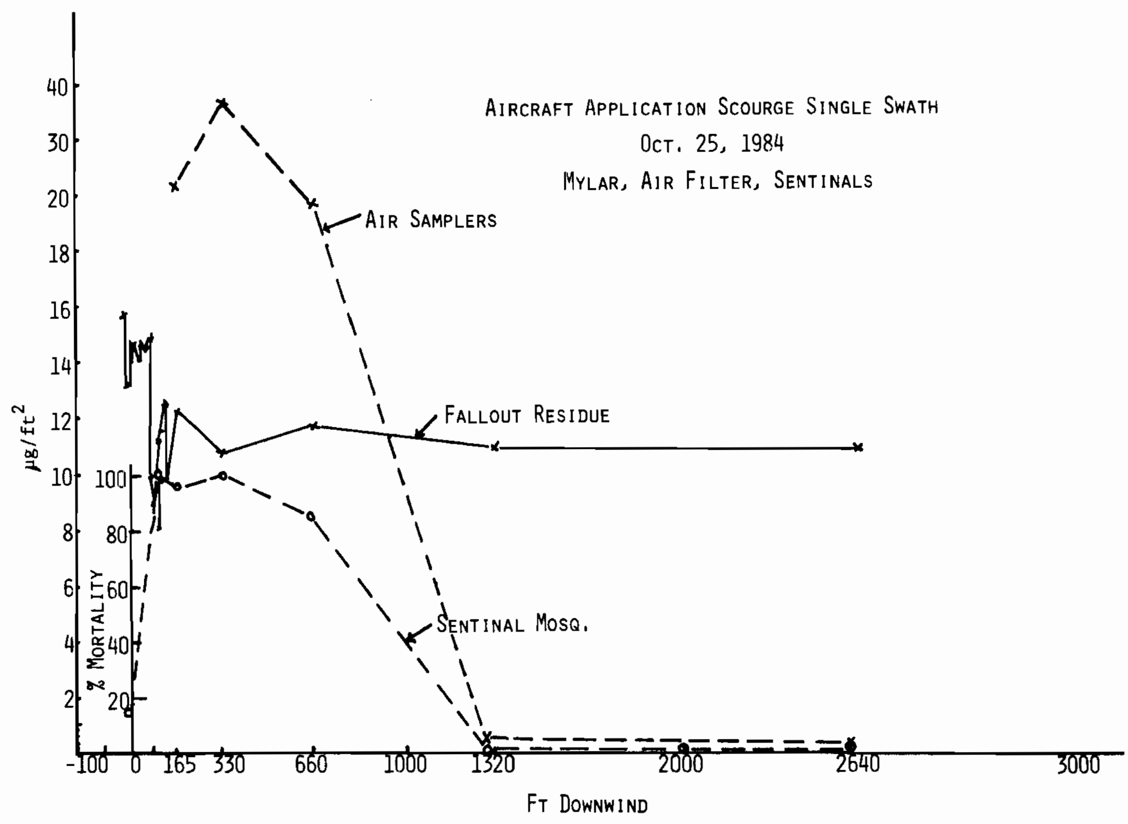


Figure 4.

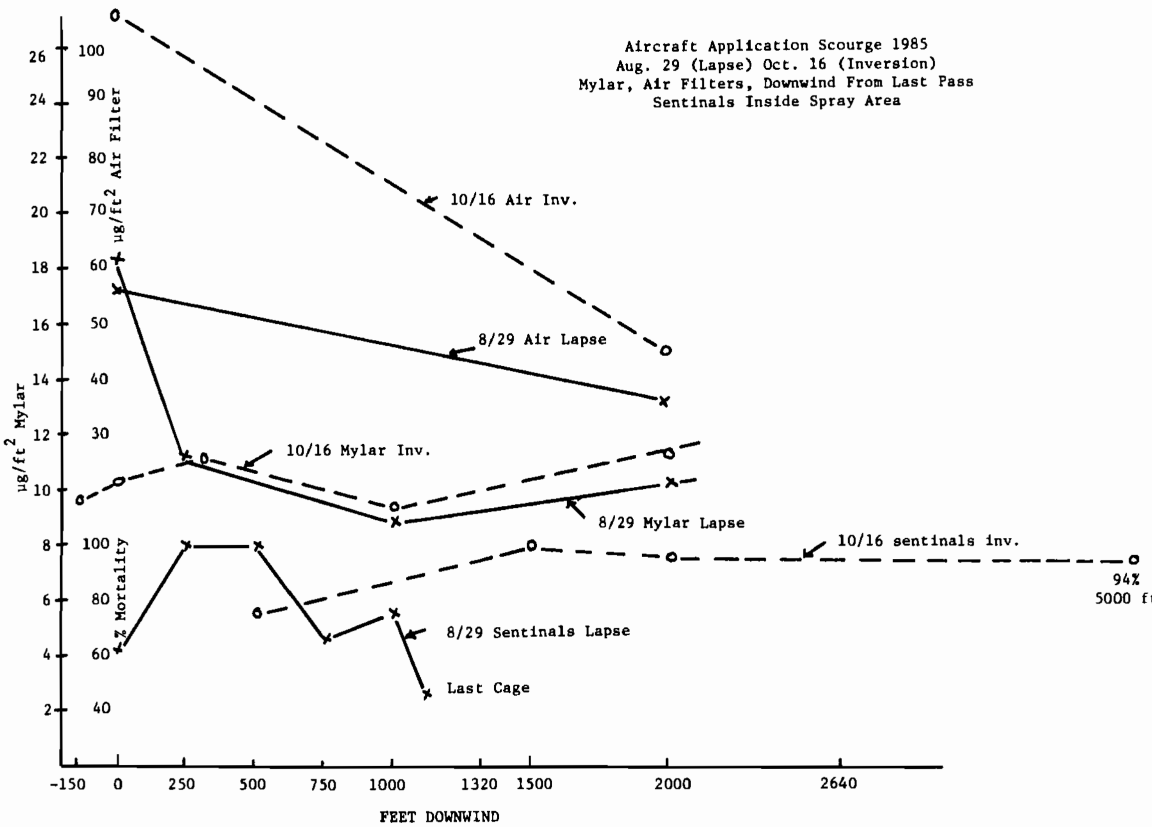


Figure 5.

width. Weather data indicates we had lapse (no inversion) type weather conditions. Sentinel mosquito cages were placed in the spray area at 5 stations 0 to 1100 ft. from the wind edge of the sprayed field. Air samplers and mylar fallout sheets were placed on the down wind edge of the field out to 2000 ft. Figure 5 shows the plots of the results of the August 29 (lapse) tests as well as for our last run in 1985 on October 16. This latter test was run on the same field as August 29 with an extra 1000 ft. pass width on the down wind (North wide) for a total of 6 passes. The sentinels were again set out inside the sprayed field from one edge (up wind) to the farther most down wind edge. Air samplers and fallout sheet data are also shown and were placed down wind from the last aircraft pass and not in the sprayed field.

Noteable here is the effect of the inversion type weather which prevailed for the October 16 test. Note the almost 2X levels of resmethrin found in the air samplers for the lapse (8/29) vs. the inversion (10/16) tests. The fallout data shown next does not show this strong an effect; but only a small increase for inversion above the lapse run. Effect on sentinel mosquitoes is also very positive for the inversion run. Here, the first cage at 500 ft. shows about a 75% mortality, but the remaining cages out to 5000 ft. show 94 to 100% control for the inversion run. The lapse run had cages only to 1100 ft. but showed an erratic pattern which can logically be attributed to the poorer lapse type weather which dispersed the aerosol size drops.

SUMMARY.-All of the applications made for these tests on application machine and weather functions were made with oil base (except Teknar®) type materials which minimizes evaporation and drop size shrinkage. Effects of weather factors are positive showing particularly the need for inversion weather in order to obtain optimum results. The drop size studies show little correlation to the Bti tests runs with a range of 50 to 300 microns vmd used. With the Scourge® runs small drops will produce wider effective swaths on sentinel (caged) mosquitoes. But the results with resmethrin on native mosquitoes is extremely variable and no clear relationship could be established between effective native population control and drop size or weather conditions.

The effectiveness of small drops 10-30 microns vmd in terms of dispersion and coverage has widely acknowledged. For our tests there was no reduction in sentinel mosquito control observed because of use of small drops, and in fact other researchers believe increased drop size would likely reduce effectiveness. We have no difficulty obtaining the 10-30 micron vmd with properly operated ground cold foggers. But there is no aircraft aerosol system presently available which can adequately produce even a 60-75 micron vmd aerosol. This would include the Micron-X® units we used, which although they produced the small drop size range, were found to be inadequate in terms of durability. No other commercial spinning devices have been found to produce the small size range. Until a suitable aircraft mounted atomizer is found, aircraft applications of cold fog or aerosols will remain less than optimal.

Scourge® appears to be useful as a rapid knock-down material for adulticiding, but there is virtually no residual effect beyond 24 hrs., perhaps less. For further comments on the Scourge® tests see Yoshimura's paper following.

EFFICACY OF SCOURGE™ ON MOSQUITO POPULATIONS

Glenn Yoshimura

Sacramento County/Yolo County Mosquito Abatement District
1650 Silica Avenue, Sacramento, California 95815

INTRODUCTION.-Outbreaks of encephalitis in Canada (Mahdy et al. 1979, Sekla 1982) and Texas (Mitchell et al. 1969) have been successfully suppressed through reduction of the adult mosquito population. In preparation for such an event as occurred in Kern County in 1952 and more recently in Los Angeles and Orange Counties in 1984, studies utilizing low volume application equipment and insecticides have taken on a new perspective in California (Reisen et al. 1985, Schaefer et al. 1985).

It has been shown what the optimum droplet spectra should be for adulticiding (Mount et al. 1970, Lofgren et al. 1973) and that there is a direct relationship between the droplet size and the insecticide used which affects the resultant mortality of the adults (Weidhaas et al. 1970). To attain this balance, the application volume and types of spray nozzles have also been tested (Yates et al. 1984). The theory being that with equipment capable of producing smaller and more uniform droplet sizes of the effective range, there would also be a better dispersion and coverage of the treatment area by the insecticide. The equipment and insecticides used in each case may have differed, but research still continues in trying to find the combination that is most effective.

This portion of the report deals with the impact of the insecticide, Scourge™, on mosquito populations when aerially applied at low volume rates using the Micron-X® rotary atomizer nozzles.

STUDY AREAS. T.S. Glide Tule Ranch.-The study area at the T.S. Glide Tule Ranch is located in the S. Yolo Bypass ca. 9 miles SE of Davis, CA. The 920 acres of pastures on the ranch property range in size from 5 to 116 A with a 40-70 A duck club located in the NW corner of the ranch property. The acreage of the duck club varies as it is also used as an irrigation holding pond for those pastures within its vicinity.

Heidrick Farms.-Heidrick Farms is located ca. 7 miles NE of Davis in the N. Yolo Bypass and is primarily a rice growing region. A fallow field was used to confirm the swath width of the 20-30 micron Vmd droplets as the weather is generally more conducive for low volume applications than in the S. Yolo Bypass.

C.F. Kearney Ranch.-The 320 A study area of the C.F. Kearney Ranch that was treated consisted of 160 A of irrigated pastures, 70 A of duck club and the remaining 90 A of land left in its natural state. The pastures were bordered on the west and south by corn fields and on the north by fallow ground. Although the pastures produced very few mosquitoes, the adjoining duck club which received the irrigation runoff from the pastures was the principal source of the mosquito problem. Kearney's is located in the S. Yolo Bypass, ca. 8 miles SE of Davis.

The first through third tests were conducted at the T.S. Glide Ranch while the fourth test was done at Heidrick Farms during 1984. The first and fourth tests were strictly to determine the effective swath width with the use of adult mosquitoes held in disposable cages. The field tests were conducted at the Glide and Kearney Ranches during 1985.

METHODS AND MATERIALS.-Scourge™, a pyrethroid manufactured by Penick-Bio UCLAF Corporation, consists of a mixture of 1 part resmethrin and 3 parts piperonyl butoxide. It was diluted with cottonseed oil at a 1:9 ratio for the tests conducted in 1984 and with Golden Bear 1356 mosquito larvicide oil at the same ratio for the 1985 tests.

Laboratory reared adult mosquitoes in disposable cages (Townzen & Navtig 1973) were used as sentinels for the swath width determinations. Organophosphate susceptible and OP-resistant populations were used simultaneously to compare differences in mortality if any occurred. Approximately 20 adults of each population were placed separately in disposable cages that were suspended from a 4 foot stake. An hour after the aircraft sprayed the sentinel line, the cages were retrieved and individually placed in a plastic bag containing a damp paper towel. The adults were held in this manner in the laboratory until mortality counts were recorded 24 hours posttreatment. The effective swath width was then determined by interpolation of the mortality noted compared to the distance of the sentinels from the flight path.

The efficacy of Scourge™ during the 1984 field tests was monitored with the use of modified CO_2-baited CDC traps and leg counts taken pre- and posttreatment. The CO_2-baited CDC traps and sentinel cages containing laboratory reared and native mosquito populations were used during 1985. Per cent reduction of the field population (in Tables) was based on the formula by Fleming and Retnakaran 1985. Species other than *Aedes* collected in the CO_2-baited traps during the 1984 field tests were eliminated from the counts as comparisons with leg counts could not be made. Assessment of control through the use of leg counts was dispensed with for 1985 as it was felt that biting activity varies with the time of day and therefore is not indicative of the effect that the insecticide has on the field population.

FIELD TESTS AND RESULTS. Test 1 - 1984.-The swath width tests using Scourge™ were conducted on July 24. Two lines of sentinel stakes with cages containing ca. 20 adult mosquitoes were set out in a N-S direction ca. perpendicular to the wind direction. The aircraft

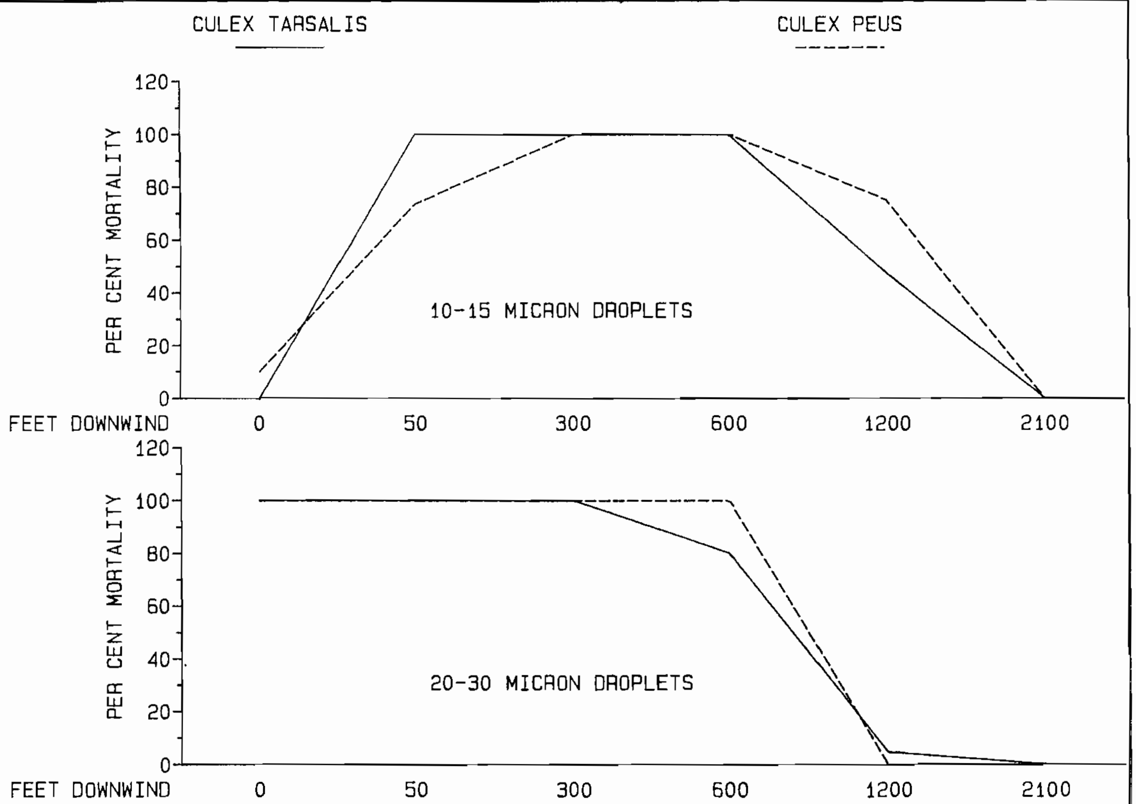


FIG. 1. SENTINEL MORTALITY OF CAGED ADULTS

flew over a flagged flight path at an altitude of 20 feet. The sentinel stakes were spaced at 0, 50, 300, 600, 1,200 and 2,100 feet downwind from the line of flight with each stake having 2 cages, one containing *Culex tarsalis* Coquillett and the other *Cx. peus* Speiser adults. The results of the applications (Fig. 1), 24 hours posttreatment, were as follows: with the 10-15 µ droplets, there was hardly any mortality in the sentinels beneath the aircraft but there was 100% mortality up to the 600 foot sentinel stake. At the 1,200 foot stake there was 47.4% mortality of the *Cx. tarsalis* sentinels and 75% mortality of the *Cx. peus* sentinels. No mortality was noted in the cages located 2,100 feet downwind from the flight path. From the results obtained, it was decided that a 1,000 foot swath width would be reasonable for adequate coverage of a treatment area.

There was 100% mortality of both species up to the 300 foot sentinel stake with the 20-30 µ droplets. At 600 feet, the mortality of *Cx. tarsalis* adults was 80% and for *Cx. peus* was 100%. Mortality at 1,200 feet was 4.7% for *Cx. tarsalis* and 0% for *Cx. peus*. No mortality was noted at the 2,100 foot sentinel stake. The mortality for *Cx. peus* was corrected using Abbott's formula as there was a 4.7% mortality in an untreated cage used as a check. With the larger drops, a swath width of 600 feet was chosen (Fig. 1).

Test 2 - 1984.-Presampling of the *Aedes* adult mosquito population using modified CO_2-baited CDC traps started on July 24 for the first field test. Six traps were used to monitor the population in each of the test plots. Trap counts in the 60 A plot designated as plot 1 for the 10-15 µ drops averaged 196.1 females/trap/night over a 3 night collection period. Counts in the 40 A plot, plot 2, for the 20-30 µ drops averaged 177.3 females/trap/night over the same collection period. Four traps were used to monitor the adult population in an untreated area located to the west of plot 2. The average number of mosquitoes collected was 240.9 females/trap/night. The species that emerged from the 3 sampling areas were *Aedes melanimon* Dyar and *Ae. nigromaculis* (Ludlow).

Prior to treatment, on the morning of July 27, leg counts were taken in all 3 plots. Pretreatment counts at designated stations in plot 1 showed a total count of 117 females, the count for plot 2 was 147 females and the count for plot 3 - the untreated area was 22 females. An hour after the 2 plots were treated, leg counts were taken to determine the efficacy of Scourge™. Plot 1 had a total count of 31 females or a 74% reduction, plot 2 a count of 96 females or a 45% reduction and plot 3 a total count of 10 females or a 55% reduction.

Trap collections on the night of July 27-28 showed an average count of 14.4 females/trap in plot 1 - a 92.6% reduction; an average count of 181.9 females/trap in plot 2 - an increase of a little over 1% and an average count of 20.8 females/trap in plot 3 - a 91.4% reduction. The number of adults collected in the untreated area showed a greater reduction than that achieved in plot 2 treated with the larger droplet sizes (Table 1).

Continued trapping on the night of July 28-29 showed a resurgence of the adult population in plots 1 and 2. The average counts in plots 1 and 2 exceeded the pretreatment counts with 577 females/trap and 1,673.8 females/trap respectively. The untreated area had an average count of 143.2 females/trap or approximately 40.5% less females collected than at pretreatment levels.

Test 3 - 1984.-The third field trials for Scourge^{TM} commenced on August 6. Four CO_2-baited traps were used to monitor the adult female populations in the designated treatment plots and 2 traps were used in the comparison area. Plot 1 for the 10-15 µ drop application consisted of 80 A while plot for the 20-30 µ drop application was 135 A.

Pretreatment counts in plot 1 averaged 5,590 *Aedes* females/trap/night, plot 2 averaged 3,568 females/trap/night and the comparison area averaged 3,305 females/trap/night over a 2 night sampling period. Prior to treatment on August 8, leg counts were taken in the 3 plots. Plot 1 had a total landing count of 354 females, plot 2 a count of 272 females and plot 3 a total of 750+ females. Counts taken an hour after treatment showed that plot 1 had a landing count of 30, a 92% reduction; plot 2 a count of 93, a 66% reduction and the untreated comparison area a count of 500+ or approximately a 33% reduction.

Posttreatment trap collections on August 8-9 for plot 1 had an average of 3,065.5 *Aedes* females/trap, a 45.2% reduction from pretreatment levels. Plot 2 an average of 2,906 females/trap an 18.6% reduction and the comparison area an average of 2,616 females/trap - a 20.9% reduction (Table 2). Once again the untreated area had a greater reduction in numbers than plot 2 treated with the larger drops. Trap collections on August 9-10 showed a resurgence of adults in plots 1 and 2 which more than doubled the pretreatment count averages. Plot 1 had an average of 11,357 females/trap, plot 2 an average of 7,095 females/trap while the untreated area had an average of 2,182 females/trap.

Test 4 - 1984.-The final test of 1984 was run on the evening of October 25 at Heidrick Farms. Two strains of *Cx. p. pipiens* L. adults were placed in sentinel cages to check the swath width of the 20-30 µ drop application. Eight sentinel stakes, each with cages of an OP-susceptible and an OP-resistant strain of adults were spaced over a mile interval in an N-S direction.

Table 1.-Comparison of adult mosquito control on field plots treated with Scourge^{TM} at 2 different droplet spectra. July 27, 1984.

	Avg. No. ♀♀/trap/nt.			Leg counts		
	Pre	Post	% red	Pre	Post	% red
Plot 1 (10-15µ)	196.1	14.4	19	117	31	59
Plot 2 (20-30µ)	177.3	181.9	-1,084	147	96	0
Untreated	240.9	20.8		22	10	

Table 2.-Comparison of adult mosquito control on field plots treated with Scourge^{TM} at 2 different droplet spectra. August 8, 1984.

	Avg. No. ♀♀/trap/nt.			Leg counts		
	Pre	Post	% red	Pre	Post	% red
Plot 1 (10-15µ)	5,590	3,065.5	31	354	30	87
Plot 2 (20-30µ)	3,568	2,906	-2	272	93	49
Untreated	3,305	2,616		750+	500+	

After the aircraft sprayed the sentinel line, the cages of adults were retrieved and handled as in the first swath width determinations. Mortality counts at 24 hours indicated drift of the insecticide out to the 660 foot stake downwind. Mortality of the OP-susceptible strain at 660 feet was 100% while the mortality of the resistant strain was 86.4%. There was not mortality in the cages located beyond the 660 foot sentinel stake.

Test 1 - 1985.-Pretreatment sampling of the adult mosquito population at the Kearney Ranch with CO_2-baited CDC traps started on July 22. Six traps were located within the 320 A study area and 3 traps were located from ½ to 1 mile upwind in an untreated area. The number of mosquitoes collected during the 3 day sampling period averaged 914.9 females/trap/night in the designated spray area and 1,245.6 females/trap/night in the comparison area. The species collected in decreasing order of prevalence were: *Ae. melanimon, Ae. nigromaculis, Cx. tarsalis, Cx. erythrothorax* Dyar, *Cx. pipiens, An. freeborni* Aitken and *Ae. dorsalis* (Meigen).

Prior to spraying on July 25, a sentinel line was established in the middle of the treatment zone with the sentinel stakes spaced 530 feet apart in a N-S direction. Each stake held 2 cages of adults; the first, a laboratory reared OP-susceptible strain of *Cx. pipiens* and the second, field collected *Ae. melanimon*. The sentinel mosquitoes were used to correlate the efficacy of Scourge™ when sprayed under unfavorable weather conditions. The first 2 sentinel stakes on the upwind side of the treatment zone spaced at 0 feet or directly beneath the flight path of the aircraft and at 530 feet received at single treatment; the rest of the sentinels located further downwind received multiple treatments with the overlapping swaths. Results after 24 hours showed that there was no mortality in the sentinels at 0 feet. The wind gusting to 10 mph at the time of application did not allow the droplets to settle directly beneath the aircraft. Mortality of the sentinels at 530 feet showed a low of 33.6% with *Ae. melanimon* to a high of 58.6% with *Cx. pipiens*. Mortality of the sentinels receiving multiple treatments ranged from 68.4% to 100% with the highest mortality being furthest downwind.

Posttreatment trap collections on July 25-26 yielded an average count of 674.8 females/trap within the spray zone and 458.3 females/trap within the comparison area. Although the number of adults collected was less than pretreatment levels, the reduction is more likely due to the windy conditions that prevailed (Table 3).

Test 2 - 1985.-The pretreatment sampling for the second adulticiding test commenced on August 26 at the T.S. Glide Ranch. Pretreatment trap collections averaged 5,564.8 females/trap/night on

Table 3.-Population monitoring and sentinel mortality at the C.F. Kearney Ranch sprayed with Scourge™ on July 25, 1985.

	Avg. No. ♀♀/trap/nt.		
	Pretreatment	Posttreatment	% red
Spray	914.9	674.8	-101
Untreated	1,245.6	458.3	

Station No.	Distance Downwind (ft.)	% Sentinel mortality	
		Ae. melanimon *	*Cx. pipiens*
1[a]	0	0	0
2[a]	530	34	59
3[b]	1,060	68	81
4[b]	1,590	77	76
5[c]	2,210	100	94
6[c]	2,650	93	96
Untreated		9	0

a = single treatment; b and c = multiple treatments from overlapping swaths; * = sentinel mortality corrected by Abbott's formula.

Table 4.-Population monitoring and sentinel mortality at the T.S. Glide Ranch sprayed with Scourge™ on August 29, 1985.

	Avg. No. ♀♀/trap/nt.		
	Pretreatment	Posttreatment	% red
Spray	2,750.3	4,082	-356
Untreated	5,564.8	12,557.6	

		% Sentinel mortality	
Station No.	Distance Downwind (ft.)	*Ae. melanimon* *	*Cx. pipiens*
1	10	82	35
2	250	100	100
3	500	100	100
4	750	85	70
5	1,000	85	69
6	1,220	71	30
Untreated		3	0

* = sentinel mortality corrected by Abbott's formula.

Table 5.-Population monitoring and sentinel mortality at the T.S. Glide Ranch sprayed with Scourge™ on October 16, 1985.

	Avg. No. ♀♀/trap/nt.		
	Pretreatment	Posttreatment	% red
Spray	913	301.8	-3
Untreated	2,092.7	669.7	

	% Sentinel mortality	
Station No.*	*Ae. melanimon*	*Cx. pipiens*
1	0	0
2	92	96
4	100	91
5	100	100
6	91	58
Untreated	0	0

* = sentinels omitted from station 3.

the Glide Ranch while the comparison area averaged 2,750.3 females/trap/night. The species collected were the same as in the previous test at Kearney's.

Approximately 885 A were designated for spraying with the aircraft making 5 passes at 1,000 foot intervals. The comparison area was the Kearney Ranch at which the previous test was held.

A sentinel line was set in a N-S direction on August 29 with the stakes spaced at 10, 250, 500, 750, 1,000 and 1,220 feet downwind from the flight path. Each stake held a cage of OP-resistant *Cx. pipiens* and field collected *Ae. melanimon*. The results of the 24 hour counts are as follows: at 10 feet, mortality ranged from a low of 34.5% for *Cx. pipiens* to a high of 82.1% for *Ae. melanimon*. There was 100% mortality of all sentinels at 250 and 500 feet. Sentinel mortality decreased past the 500 foot mark with a low of 68.8% for *Cx. pipiens* to a high of 85.2% for *Ae. melanimon*. At 1,220 feet, the mortality range was 29.7% for *Cx. pipiens* and 71.4% for *Ae. melanimon*.

Posttreatment trapping on August 29-30 resulted in an increase in the number of females collected in the spray zone. The number of females/trap averaged 12,557.6. The number of females collected in the comparison zone decreased to an average of 1,360.7 females/trap (Table 4).

Wind conditions were within range for effective spraying but the low air temperature which was less than 63 degrees Fahrenheit at the time of spraying, might have affected the flight activity of the adults (Bidlingmayer 1985), thus decreasing exposure. There was also a lapse condition, or no inversion at the time of spraying.

Test 3 - 1985.-The third pretreatment sampling commenced on October 14 at the Glide Ranch. Trap collections for the two prespray sampling nights averaged 913 females/trap/night at the Glide Ranch and 2,092.7 females/trap/night at the comparison area which was a duck club located ½ mile S of Glide's.

Sentinel cages containing *Cx. pipiens* and field collected *Ae. melanimon* adults were located at 5 of the sampling stations in the spray area on October 16 to check on spray coverage rather than the swath width. A sentinel stake was omitted from a sampling site as cattle were moved into the pasture location.

The acreage sprayed was ca. 1,000 A with the aircraft making 6 passes at 1,000 foot intervals. Weather data at the time of spraying showed a temperature inversion of 9 degrees F., a stability ratio of 1.7, wind direction predominantly from the WSW and wind velocity which varied between 0 to 6.4 mph. Relative humidity averaged 51.7%.

Swarming activity of adults had started at 1755 h and was still in progress when the aircraft made its first spray pass at 1825 h. The aircraft sprayed in a W-E direction at an altitude of 20 feet with the sixth and final pass ending at 1840 h.

The sentinel cages were left in the field overnight and retrieved the following morning. Final mortality counts taken 36 h posttreatment showed fairly consistent coverage throughout the spray area. The only sentinels with no or low mortality occurred at the station that was situated beneath the canopy of eucalyptus trees. There is a discrepancy in the data when comparing mortality of the sentinels to the posttreatment trap results.

Posttreatment trapping on October 16-17 showed a decrease in the number of mosquitoes in both the spray and comparison areas. There was a reduction of 67% in the treated area while that of the untreated area was 68%. The net result is a negative suppression of the adult population due to the spray effort (Table 5).

DISCUSSION.-The concept of low volume applications of insecticides while in theory is a good one, the feasibility of the procedure in actual field treatments may be limited by the conditions that have to be met for a successful program. The factors that have to be considered which affect any insecticide application are: 1) the meteorological conditions at the time of application, 2) the vegetative canopy, 3) the biology of the target species and 4) the insecticide being applied.

When dealing with small droplet sizes, wind velocity, a temperature gradient or inversion, the relative humidity and the wind direction become critical factors which affect the outcome of the insecticide application. The weather conditions should be optimal to get maximum coverage on to the target area.

The second factor is the vegetative canopy. Although it was not a major factor in our field trials, the height and density of the vegetation may impede penetration of the insecticide to the target area.

The timing of insecticide application in the case of adulticiding should also be considered. The peak activity would be the prime time for application of the insecticide. Unfortunately, the duration of these activities when an aircraft is capable of spraying is minimal. The adult population that is newly emerged, engorged either with a sugar or blood meal, or females that are gravid may be resting or in harborage at the time of spraying, thereby lessening its chances to insecticide exposure.

Insecticides that degrade rapidly decrease the chances of exposure to the segment of the population that is inactive at the time of application. Multiple insecticide treatments over a period of time, increasing the dosage rate, or an insecticide with greater residual activity may have to be used to reduce the adult population to tolerable levels.

ACKNOWLEDGMENTS.-I wish to thank K. Boyce, Susan Critchfield, C. Deidrick, S. Wright and Annett Eiffert who assisted in setting and retrieving the traps.

REFERENCES

Bidlingmayer, W.L. 1985. The measurement of adult mosquito population changes - some considerations. J. Am. Mosq. Control Assoc. 1:328-348.

Fleming R. and A. Retnakaran. 1985. Evaluating single treatment data using Abbott's formula with reference to insecticides. J. Econ. Entomol. 78:1179-1181.

Lofgren, C.S., D.W. Anthony and G.A. Mount. 1973. Size of aerosol droplets impinging on mosquitoes as determined with a scanning electron microscope. J. Econ. Entomol. 66:1085-1088.

Mahdy, M.S., L. Spence and J.M. Joshua, eds. 1979. Arboviral encephalitides in Ontario with special reference to St. Louis encephalitis. Ontario Ministry of Health. 365 pp.

Mitchell, C.J., J.W. Kilpatrick, R.O. Hayes and H.W. Curry. 1969. Effects of ultra low volume applications of malathion in Hale County, Texas. I. Western encephalitis virus activity in treated and untreated towns. J. Med. Entomol. 6:155-162.

Mount, G.A., C.S. Lofgren, K.F. Baldwin and N.W. Pierce. 1970. Droplet size and mosquito kill with ultralow volume aerial sprays dispersed from a rotary-disc nozzle Part II. Mosq. News 30:331-334.

Reisen, W.K., M.M. Milby, W.C. Reeves, M.W. Eberle, R.P. Meyer, C.H. Schaefer, R.B. Parman and H.L. Clement. 1985. Aerial adulticiding for the suppression of *Culex tarsalis* in Kern County, California, using low volume propoxur: 2. Impact on natural populations in foothills and valley habitats. J. Am. Mosq. Control Assoc. 1:154-163.

Schaefer, C.H., H.L. Clement, W.K. Reisen, F.S. Mulligan, III, R.B. Parman and W.H. Wilder. 1985. Aerial adulticiding for the suppression of *Culex tarsalis* in Kern County, California, using low volume propoxur: 1. Selection and evaluation of the application system. J. Am. Mosq. Control Assoc. 1:148-153.

Sekla, L., ed. 1982. Western equine encephalitis in Manitoba. Manitoba Health Service Commission, Winnipeg. 295 pp.

Townzen, K.R. and H.L. Navtig. 1973. A disposable adult mosquito bioassay cage. Mosq. News 33:113-114.

Weidhaas, D.E., M.C. Bowman, G.A. Mount, C.S. Lofgren and H.R. Ford. 1970. Relationship of minimum lethal dose to the optimum size of droplets of insecticides for mosquito control. Mosq. News 30:195-200.

Yates, W.E., N.B. Akesson and R.E. Cowden. 1984. Measurement of drop size frequency from nozzles used for aerial applications of pesticides in forests. Forest Pest Management. 221 pp.

INSECTICIDE SUSCEPTIBILITY OF MOSQUITOES IN CALIFORNIA: STATUS OF ORGANOPHOSPHORUS RESISTANCE IN LARVAL *CULEX TARSALIS* THROUGH 1985

Malcolm A. Thompson

California Department of Health Services
Vector Surveillance and Control Branch
714 P Street, Room 616, Sacramento, California 95814

INTRODUCTION.-Organophosphorus (OP) resistance surveillance of larval *Culex tarsalis* mosquito populations has been a priority activity of this agency since the resistance surveillance program was restored in 1984. Our objective has been to test representative *Cx. tarsalis* populations from local control agencies in each geographic region of the State.

There is a need to identify OP resistance trends which may have occurred since testing was discontinued in 1978. Data are essential to planning in advance of a disease outbreak in California. Application of the information gathered by surveillance is pertinent at both the local agency level where disease transmitting mosquitoes are being controlled, and at the State level to provide information needed to coordinate activities of local control programs if it becomes necessary to avert a disease outbreak.

Table 1 is reproduced as it was published by Zboray and Gutierrez (1979) and is the standard to which current resistance levels will be compared. This table summarizes the susceptibility of *Cx. tarsalis* populations from 46 control agencies during 1963 through 1978.

The purpose of this report is to present resistance levels obtained during 1983-85, and to compare these findings to levels documented in Table 1. Test data from twice as many agencies are needed to identify current trends and to complete this project; consequently surveillance will be continuing.

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

RESULTS AND DISCUSSION.-Although many bioassays may have been completed at an agency, only the highest LC_{50} for each OP is listed for that agency in both Tables 1 and 2. This method of organization enables review of test data by means of the worst possible scenario as laboratory determinations are presented in their most resistant profile.

Table 2 lists by agency the highest resistance level of the present survey together with the highest recorded through 1978 (from Table 1) for comparison. The year the highest LC_{50} was obtained and the resistance status of that LC_{50} are also included. Resistance status is determined by criteria previously set forth (Thompson, 1985). Briefly, LC_{50}s are categorized as susceptible, incipient or resistant depending upon thresholds and the LC_{90}/LC_{50} ratio. However, for the purpose of discussion and comparisons in this report, the LC_{50} values of Table 2 are segregated into only two categories, susceptible or resistant. If the LC_{50} is greater than 0.1 ppm for malathion, 0.005 ppm for parathion and fenthion, or 0.0025 ppm for temephos and chlorpyrifos, it is resistant.

Although the purpose of Table 2 is to display the highest LC_{50}s obtained at each agency, many of these LC_{50}s fall within the susceptible range. Examples are the four current LC_{50}s reported for Consolidated MAD. These nicely susceptible values document references of baseline susceptibility which could be used for comparison to the LC_{50}s of other larval *Cx. tarsalis* populations. All four LC_{50}s came from a single larval population collected from spring sources located in the Sierra Nevada foothills, apparently isolated from the influence of OP-pressured populations of the San Joaquin Valley.

In contrast to the Consolidated MAD data, Table 2 provides many examples of highly resistant *Cx. tarsalis* populations. Of notoriety is a once heavily pressured and now operationally resistant population reported for Northwest MAD. OP larvicides would be ineffective against this population if applied at their recommended dosage rates.

Table 3 contrasts present survey results to those reported through 1978 by comparing percentages of the LC_{50}s listed in Table 2 which are resistant. Only the agencies of Table 2 listing a current LC_{50} together with an LC_{50} from the 1978 table are referred to in this comparison. Of significance is the decrease in the number of fenthion resistant larval populations encountered in the present survey (71% to 43%), and an increase in chlorpyrifos resistant populations (33%

Table 1.-Highest laboratory demonstrated level of organophosphorous tolerance in larvae of *Culex tarsalis* through 1978.

	MALATHION				PARATHION				FENTHION				CHLORPYRIFOS			
Agency	Year	LC_{90}/LC_{50} ratio	LC_{50}	95% limits	Year	LC_{90}/LC_{50} ratio	LC_{50}	95% limits	Year	LC_{90}/LC_{50} ratio	LC_{50}	95% limits	Year	LC_{90}/LC_{50} ratio	LC_{50}	95% limits
El Dorado					1969	1.69	.0026	.0019 - .0036	1966	1.25	.0022	.0020 - .0025	1969	1.79	.00022	.00016 - .00031
Pine Grove	1965	1.95	.020	.018 - .023	1973	2.20	.0018	.0014 - .0023	1974	1.70	.0056	.0045 - .0083	1975	3.00	.00065	.00052 - .00084
Burney Basin	1974	2.30	.015	.01 - .021					1974	1.60	.0026	.0022 - .0029				
Shasta	1967	11.70	.087	.054 - .14	1977	1.50	.0024	.0021 - .0027	1977	2.50	.013	.010 - .016	1977	5.10	.0028	.002 - .0047
Tehama Co.	1968	9.85	.053	.032 - .083	1964	1.86	.0031	.0027 - .0035	1971	1.93	.0056	.0047 - .0073	1972	1.51	.00053	.00048 - .00058
Los Molinos	1963	2.53	.057	.045 - .071	1974	2.90	.0019	.0013 - .0027	1974	1.60	.0038	.0033 - .0044	1975	1.63	.0003	.00025 - .00037
Corning	1967	7.65	.092	.061 - .14	1963	1.85	.0024	.0020 - .0028	1977	2.80	.0039	.0026 - .0054	1977	6.80	.00062	.00026 - .0010
Butte Co.	1966	7.55	.10	.069 - .25	1972	2.53	.0045	.0040 - .0052	1971	2.83	.0079	.0068 - .0091	1968	1.51	.00028	.00026 - .00030
Glenn Co.									1976	3.00	.0012	.00031-.0018				
Colusa	1971	2.25	.045	.035 - .058	1968	2.04	.0015	.0011 - .0018	1976	2.60	.0043	.0033 - .0069	1977	1.30	.0016	.0010 - .0025
Sutter-Yuba	1966	6.05	.41	.26 - 1.20	1977	7.70	.0035	.0018 - .0053	1976	1.70	.0045	.0039 - .0053	1971	2.14	.00090	.00070 - .0012
Sacramento-Yolo	1966	6.63	.11	.081 - .19	1966	1.49	.0032	.0027 - .0040	1968	1.55	.0031	.0027 - .0035				
Solano Co.	1965	12.73	.11	.066 - .17	1965	1.58	.0023	.0025 - .0033	1969	1.30	.0027	.0023 - .0031				
Diablo Valley	1977	2.40	.10	.079 - .12	1977	5.60	.0041	.0031 - .0054	1977	2.00	.023	.019 - .029	1977	4.00	.0021	.0016 - .0028
No. San Joaquin	1967	8.48	.068	.051 - .091	1969	1.41	.0014	.0012 - .0016	1972	1.93	.0044	.0038 - .0054	1967	1.50	.00043	.00038 - .00049
San Joaquin	1967	3.76	.071	.052 - .096	1977	3.40	.0082	.0051 - .013	1972	1.87	.0058	.0047 - .0072	1975	3.90	.0012	.00054 - .0017
East Side					1966	1.27	.0017	.0015 - .0019	1972	4.28	.0033	.0024 - .0045				
Turlock					1966	1.72	.0021	.0017 - .0025	1970	3.65	.016	.013 - .022	1973	7.90	.0029	.0021 - .0040
Merced Co.	1966	13.01	.47	.26 - 1.8	1966	1.28	.0038	.0035 - .0043	1975	4.00	.0074	.0034 - .011	1969	2.01	.00051	.00045 - .00057
Madera Co.	1969	11.47	.25	.15 - 1.3	1970	2.05	.011	.0092 - .014	1970	3.03	.013	.0099 - .017	1970	8.48	.019	.0093 - .14
Fresno Westside	1972	4.34	.23	.14 - .30	1969	1.65	.022	.018 - .028	1969	1.60	.023	.021 - .027	1969	2.27	.0089	.0074 - .011
Fresno	1963	18.05	.095	.049 - .15	1969	3.85	.0053	.0045 - .0063	1969	1.98	.015	.013 - .017	1969	5.40	.0017	.0012 - .0029
Consolidated	1969	2.56	.24	.19 - .36	1970	2.44	.023	.018 - .028	1970	1.51	.053	.047 - .060				
Delta	1964	6.40	.080	.056 - .12	1964	2.00	.0030	.0025 - .0036	1964	1.55	.0049	.0043 - .0056				
Kings	1972	8.48	.75	.55 - 1.06	1972	2.94	.054	.045 - .065	1972	2.48	.040	.034 - .046	1972	2.85	.027	.023 - .032
Tulare	1975	8.19	.52	.34 - .71	1971	2.90	.0099	.0052 - .013	1969	2.09	.017	.012 - .023	1971	7.45	.011	.0060 - .015
Delano	1965	3.95	.058	.044 - .077	1977	3.77	.0053	.0043 - .0066	1977	2.88	.0059	.0046 - .0072	1977	3.81	.0021	.0009 - .0031
Kern	1964	5.45	.065	.046 - .088	1978	3.77	.026	.018 - .038	1976	4.92	.013	.0093 - .020	1978	4.91	.0061	.0046 - .0078
West Side	1969	13.01	2.1	1.3 - 4.8	1965	1.63	.0032	.0028 - .0036	1970	1.61	.0062	.0045 - .0085	1966	1.36	.00049	.00045 - .00053
Alameda Co.									1973	1.90	.014	.012 - .017				
San Mateo Co.	1977	6.60	.28	.20 - .47	1975	5.00	.002	.0012 - .0029	1977	2.60	.018	.016 - .021	1975	2.70	.0016	.0012 - .002
N. Salinas Vy.					1976	3.60	.0033	.0024 - .0044					1978	4.20	.0037	.0024 - .0051
Goleta Vy.	1966	1.40	.054	.048 - .060	1966	1.50	.0024	.0023 - .0027	1969	1.97	.0044	.0037 - .0054	1966	1.56	.00063	.00057 - .00069
Carpinteria	1966	2.17	.030	.027 - .034					1966	1.78	.0072	.0060 - .011				
Ventura Co.									1976	1.69	.0035	.0032 - .0040				
L. A. Co. West	1978	2.20	.016	.013 - .019					1978	2.20	.0035	.003 - .0042	1978	1.70	.00035	.0003 - .00041
Southeast	1967	2.63	.069	.059 - .080					1969	2.21	.042	.036 - .053	1970	2.39	.00083	.00069 - .00099
Long Beach									1972	3.47	.012	.0097 - .015	1972	6.11	.0019	.0014 - .0026
Orange Co.	1973	2.50	.060	.047 - .080					1973	2.65	.0078	.0062 - .012				
Riverside City	1972	3.10	.033	.028 - .040	1972	2.74	.0026	.0021 - .0032								
Northwest	1971	1.81	.016	.014 - .018	1971	1.55	.0016	.0015 - .0018	1971	1.45	.0026	.0023 - .0029	1971	2.24	.00024	.00022 - .00028
San Diego Co.	1972	3.49	.12	.099 - .15	1972	2.15	.0038	.0033 - .0047	1972	2.51	.0070	.0061 - .0082				
Imperial Co.	1976	3.23	.065	.043 - .084	1976	2.20	.0069	.0057 - .0087	1976	2.10	.0046	.0034 - .0057	1977	5.00	.005	.0034 - .0073
Coachella Vy.	1972	1.94	.073	.062 - .086	1970	2.62	.016	.013 - .020	1972	4.54	.0086	.0058 - .011	1971	4.19	.026	.019 - .046
Antelope Vy.	1968	2.59	.024	.016 - .032					1971	1.51	.0040	.0033 - .0049				
Inyo Co.	1969	1.34	.026	.024 - .029					1972	2.03	.0059	.0047 - .0081	1969	1.35	.00045	.00041 - .00048

Table 2.-Highest laboratory demonstrated levels of organophosphorous resistance (in ppm), and resistance status[1] of *Culex tarsalis* larval populations recorded through 1978[2] compared to highest recorded 1983 through 1985.

AGENCY	MALATHION			PARATHION			FENTHION			CHLORPYRIFOS		
	Year	LC_{50}/LC_{90}	Status	Year	LC_{50}/LC_{90}	Status	Year	LC_{50}/LC_{90}	Status	Year	LC_{50}/LC_{90}	Status
Marin-Sonoma	1985	0.092/0.84	I	—	—	-	1985	0.0042/0.012	I	1985	0.0018/0.0028	S
	—	—	-	—	—	-	—	—	-	—	—	-
Burney Basin	1984	0.025/0.069	S	1984	0.0025/0.0082	S	1984	0.0038/0.0092	S	1984	0.0014/0.0046	S
	1974	0.015/0.034	S	—	—	-	1974	0.0026/0.0042	S	—	—	-
Shasta[3]	1985	0.069/0.22	I	1984	0.0021/0.003	S	1984	0.0096/0.032	R	1985	0.003/0.011	R
	1967	0.087/1.0	I	1977	0.0024/0.0036	S	1977	0.013/0.032	R	1977	0.0028/0.014	R
Tehama Co.	1984	0.058/0.53	I	1984	0.0042/0.0096	S	1984	0.0036/0.012	I	—	—	-
	1968	0.053/0.53	I	1964	0.0031/0.0058	S	1971	0.0056/0.011	R	1972	0.00053/0.0008	S
Butte Co.[4]	—	—	-	1984	0.0012/0.0035	S	—	—	-	1984	0.00061/0.0016	S
	1966	0.1/0.75	I	1972	0.0045/0.011	I	1971	0.0079/0.022	R	1968	0.00028/0.00042	S
Consolidated	1985	0.01/0.021	S	1985	0.002/0.003	S	1985	0.0016/0.0021	S	1985	0.00088/0.0015	S
	1969	0.24/0.61	R	1970	0.023/0.056	R	1970	0.053/0.081	R	—	—	-
Kings	—	—	-	1984	0.012/0.043	R	1984	0.014/0.037	R	—	—	-
	1972	0.75/6.4	R	1972	0.054/0.16	R	1972	0.04/0.1	R	1972	0.027/0.077	R
Northwest	1984	0.27/0.5	R	1984	0.044/0.16	R	1984	0.084/0.21	R	1984	0.11/0.25	R
	1971	0.016/0.029	S	1971	0.0016/0.0025	S	1971	0.0026/0.0038	S	1971	0.00024/0.00054	S
Owens Valley	1985	0.017/0.034	S	—	—	-	1985	0.0033/0.0057	S	1985	0.0013/0.0021	S
	1969	0.026/0.035	S	—	—	-	1972	0.0059/0.012	R	1969	0.00045/0.00061	S
Coachella Valley	—	—	-	1984	0.011/0.028	R	—	—	-	1984	0.0052/0.015	R
	1972	0.073/0.14	S	1970	0.016/0.042	R	1972	0.0086/0.039	R	1971	0.026/0.11	R
San Bernardino Co.[5]	1984	0.44/1.9	R	1984	0.056/0.11	R	—	—	-	1983	0.0093/0.039	R
	1972	0.057/0.096	S	1972	0.0018/0.0058	S	1972	0.0043/0.022	I	1972	0.0004/0.0006	S
Riverside Co.[5]	—	—	-	1984	0.018/0.058	R	—	—	-	—	—	-
	1972	0.097/0.58	I	1972	0.0054/0.014	R	1972	0.0058/0.017	R	—	—	-
Imperial Co.[5]	1985	0.14/0.38	R	1983	0.009/0.024	R	—	—	-	—	—	-
	1972	0.21/0.36	R	1972	0.018/0.036	R	1972	0.017/0.034	R	1972	0.0082/0.041	R

1. S = Susceptible; I = Incipient; R = Resistant.

2. Zboray et al., 1979.

3. Local mosquito control agency data, 1984 and 1985.

4. Local mosquito control agency data, 1984 unpublished.

5. Unpublished data, 1972.

Table 3.-Comparison of larval *Culex tarsalis* populations from same agencies by percentage of LC_{50}s indicating resistance to specified larvicides.

Larvicide	1963-78	1983-85
Malathion	25%	38%
Parathion	50%	60%
Fenthion	71%	43%
Chlorpyrifos	33%	67%

Table 4.-Comparison of the LC_{50}s of larval *Culex tarsalis* populations of Table 1 (recorded through 1978) by percentage of those indicating resistance to specific larvicides.

Larvicide	All Agencies	Present Survey Agencies
Malathion	32%	25%
Parathion	31%	50%
Fenthion	59%	71%
Chlorpyrifos	31%	33%

to 67%). Percentages for malathion and parathion remain reasonably similar, thus no real change has occurred since 1978.

Most of the larval *Cx. tarsalis* populations of the present survey are susceptible to malathion (38%) and fenthion (43%) whereas most are resistant to parathion (60%) and chlorpyrifos (67%).

However, the resistance trends identified in Table 3 are applicable only to the agencies comprising the present survey (those of Table 2 as specified) and may not reflect the resistance profile of the entire state. For example, of the LC_{50}s recorded through 1978, the percentage of those which are resistant for the agencies of the present survey did not follow the statewide percentages of all 46 agencies in the Zboray-Gutierrez table. Table 4 illustrates these differences. Those agencies in Table 1 involved in the present survey, as a group had substantially more parathion resistant populations, and a greater percentage of fenthion resistant populations than the entire group of 46 agencies at the conclusion of testing in 1978. It would be unwise to assume that present findings would reflect current statewide resistance levels.

CONCLUSION.-Further testing of *Cx. tarsalis* in some areas of the State is necessary to fill-in data gaps. Targeted areas include metropolitan southern California, the bay area, and the San Joaquin Valley. When data has been gathered representative of all major regions of the State (probably during the 1986 mosquito season) this survey will be complete. Its summary will provide a skeletal overview of current "worst case" *Cx. tarsalis* resistance which can be used for comparison to past information, and between geographic regions to identify trends which may have been in operation since 1978. It will also give us information on what materials are available for control in the event of an encephalitis outbreak.

ACKNOWLEDGMENT.-I would like to thank agency managers for providing our program with the assistance that I have needed. I am grateful to the staff of those agencies who supplied the data noted in Table 2.

REFERENCES

Gillies, P. A., and D. J. Womeldorf. 1968. Methodology employed in the California mosquito larvicide resistance surveillance program. Vector Views 15:45-50.

Thompson, M. A. 1985. Insecticide susceptibility of mosquitoes in California: Status of organophosphorus resistance in larval *Culex tarsalis* through 1984, with notes on restoration of surveillance program. Proc. Calif. Mosq. and Vector Contr. Assoc. 53:71-73.

Zboray, E. P., and M. C. Gutierrez. 1979. Insecticide susceptibility of mosquitoes in California: Status: of organophosphorus resistance in larval *Culex tarsalis* through 1978, with notes on mitigating the problem. Proc. Calif. Mosq. and Vector Contr. Assoc. 47:26-28.

GB 1356 - FIELD AND LABORATORY FAILURES

Robert Schoeppner

San Mateo County Mosquito Abatement District
1351 Rollins Road, Burlingame, California 94010

Oils have long been relied upon to control mosquito larvae. An early description of the use of oil to control mosquito larvae was found in an editorial in the American Daily Advertiser of Philadelphia. It stated as follows:

> "As the late rains will produce a great increase of mosquitoes in the city, distressing to the sick and troublesome to those who are well, I imagine it will be agreeable to the citizens to know that the increase of those poisonous insects may be much diminished by a very simple and cheap mode, which accident discovered. Whoever will take the trouble to examine their rain water tubs will find millions of the mosquitoes fishing about the water with great agility, in a state not quite prepared to emerge and fly off.
>
> A gill of oil (4 oz.) poured into a common rain water cask will quickly diffuse over the surface, and by excluding the air, will destroy the whole brood."

The occasion was a yellow-fever outbreak in Philadelphia. The date - August 29, 1793. This recommendation was published 100 years before Dr. L. O. Howard recommended kerosene for mosquito control. Although credit for developing and promoting kerosene is attributed to Dr. Howard, the idea and demonstration of oil for mosquito control belongs to the editor of the American Daily Advertiser, (Richards, 1941).

Our district relies heavily on larviciding oils for mosquito control, in fact we depend on oil for approximately 95% of our control work.

During the early winter of 1981, our technicians reported problems of field failures with GB 1356[1] when applied at the recommended rate of 5 gals/acre against a mixed population of *Aedes squamiger* Coquillett and *Culiseta inornata* Williston. The location, Kavanaugh field in East Menlo Park, was a 40 acre site - a brackish water marsh composed of pickleweed and brass buttons. Because of the uneven terrain, unstable blocky soil and the dense vegetation, an all terrain vehicle with a power sprayer was utilized for the application. The water temperature was 50°F and the air temperature reached 56°F.

A 48 hour post treatment inspection of the site revealed that the *Culiseta inornata* population was totally eliminated, while the *Aedes squamiger* larvae were observed moving uneffected beneath the oil slick.

A second application of GB 1356 at 5 gals/acre was sprayed on the marsh to control the *Ae. squamiger*. This application represented a total of 10 gallons of larviciding oil per acre and reflected an approximate material cost of $18.00/acre, exclusive of the additional application costs. This operation was completely unsound economically. Ideal weather conditions, free of wind or rain, prevailed throughout the test period. The consideration of application technique or incomplete coverage was ruled out when we observed the second application of GB 1356.

Again, a 24 hour post-treatment inspection revealed uneffected *Ae. squamiger* larvae moving beneath an oil slick.

A third application, this time with Fenthion 7.0 E.C. at 2 ozs/acre produced the desired total mortality.

Routinely, post-treatment inspections had been conducted at all breeding sites that had been treated with insecticides other than petroleum oils. Based upon previous experience, and too, the mode of killing by petroleum oils, post-treatment inspections of oil treated breeding sites was considered unwarranted. Based upon our experience of recent field failures with *Ae. squamiger*, I would suspect that, in previous years, many populations of adult mosquitoes came-off from those oil treated breeding sites.

Following our control failures with GB 1356, other districts within the coastal region were asked to conduct field trials using GB 1356 at 5 gals/acre against *Ae. squamiger*. Similar field failures were exhibited. Where integrated populations of *Ae. squamiger* and *Cs. inornata* were encountered, only the *Cs. inornata* larvae were killed with GB 1356.

The influence of salinity to reduce the effectiveness of larviciding oils was tested, using GB 1356 at the rate of 5 gals/acre in the laboratory starting with the ambient water, 12 parts per thousand, dilutions to 6, 3, and 1.5 parts per thousand were made using tap water as the diluent (Table 1). Each dilution was replicated twice with 20 - 2nd through 4th instar larvae per sample. An evaluation of the percent mortality after 24 hours indicated that salinity had little influence upon the effectiveness of GB 1356 as a larviciding oil to control *Ae. squamiger* when maintained in the laboratory at 72°F.

Additional problems were encountered. In January 1982, our technicians reported field failures with GB 1356 when applied at the recommended rate of 3 gals/acre against *Ae. increpitus* Dyar in fresh water marshes.

[1]Witco Chemical Company, Bakersfield, California.

Table 1.-The influence of decreased salinity upon *Ae. squamiger* when treated with GB 1356 at 5 gals/acre.

Salinity	Percent Mortality
12.0%	60
6.0%	45
3.0%	62
1.5%	52
tap water	58

3rd - 4th instar larvae

A 24 hour post-treatment check revealed that *Ae. increpitus* larvae were relatively uneffected by the GB 1356. Where *Cs. inornata* occurred in mixed populations, the oil affected a complete mortality of the *Cs. inornata* population.

The breeding sites were retreated with Fenthion 7.0 at 2 ozs/acre. A 24 hour post-treatment inspection revealed total mortality of the *Ae. increpitus* population.

At this point, laboratory studies were considered essential. Three oils, GB 1356, GB 1111[2] and Flit MLO[3], used at the rates of 3 and 6 gals/acre, were tested against *Ae. squamiger, Ae. increpitus,* and *Ae. sierrensis* Ludlow. *Ae. sierrensis* was included in the series to determine whether it, too, exhibited similar resistance to oil as shown by *Ae. squamiger* and *Ae. increpitus*. Each test, replicated three times, consisted of 20 - 3rd and 4th instar larvae per sample. The series was maintained in the incubator at 52°F, a temperature that closely corresponded to the temperature of the water in which the larvae developed (Table 2).

The oils tested at rates of 3 and 6 gals/acre failed to provide even marginal levels of control when used against the three *Aedes* species discussed. From the data, it appears that doubling the application rate did not provide a comparable increase in the percent mortality, particularly as indicated by Flit MLO against the three species.

A second series of tests were treated in a similar manner and maintained in the laboratory at 72°F (Table 3). GB 1111 and Flit MLO exhibited an increased mortality against *Ae. squamiger* and *Ae. increpitus*. GB 1356, at either rate, failed to offer increased mortality of 72°F, relative to the 52°F trials. *Ae. sierrensis* basically, was uneffected by either of the oils or influenced by temperature variations at which tests were conducted.

Unfortunately, I failed to recognize, until

Table 2.-Three petroleum oils tested against larvae of three *Aedes* species. Tests maintained at 52°F.

Oil and Rate		Percent Mortality[1] *Ae. squamiger*	*Ae. increpitus*	*Ae. sierrensis*
GB 1356				
	3 gals	11.7	30.0	2.5
	6 gals	16.7	36.7	5.0
GB 1111				
	3 gals	18.3	18.3	3.3
	6 gals	33.3	51.7	8.3
Flit MLO				
	3 gals	21.7	58.3	29.2
	6 gals	20.0	66.7	15.8

[1]24 hour post-treatment inspection

[2]Witco Chemical Company, Bakersfield, California.

[3]Exxon Oil Company, Houston, Texas.

Table 3.-Three petroleum oils tested against larvae of three *Aedes* species. Tests maintained at 72°F.

Oil and Rate		Percent Mortality[1] *Ae. squamiger*	*Ae. increpitus*	*Ae. sierrensis*
GB 1356				
	3 gals	13.3	31.7	5.0
	6 gals	26.7	31.7	5.8
GB 111				
	3 gals	65.0	56.7	14.2
	6 gals	71.7	62.5	4.1
Flit MLO				
	3 gals	95.0	86.7	5.0
	6 gals	98.3	86.7	20.0

[1]24 hour post-treatment inspection

midway through the study, that latter instar mosquito larvae exhibited an increased susceptibility to oils. In some cases, mortality data was irregular, particularly where samples contained an abundance of 4th instar larvae. Tests with *Ae. nigromaculis* Ludlow and *Ae. dorsalis* Meigen, treated with GB 1356 at 3 and 6 gals/acre and maintained at 72°F, primarily utilized 4th instar larvae. As a result, the percent mortality in the samples was relatively high.

In the laboratory, after oil treatment, *Aedes* larvae were observed to move rapidly up and down in the water column. Several hours post-treatment, the larvae became quiescent on the bottom, many resting with their ventral side upward. In the field, this survival adaptation permitted *Aedes* larvae to avoid contact with the water surface for long periods when unfavorable surface conditions existed. *Culex pipiens* and *Cs. inornata* larvae did not exhibit this same avoidance mechanism to larviciding oils.

As a result of our laboratory and field tests, our district has completely discontinued the use of larviciding oils against larvae of any *Aedes* species.

REFERENCES

Richards, Jr., A. G. 1941. Differentiation between toxic and suffocating effects of petroleum oils on larvae of the house mosquito (*Culex pipiens* L.) Trans. Am. Ent. Soc. 67:161-196.

LARVICIDAL ACTIVITY OF PLANT EXTRACTS IN PONDS

Abdessalam Sherif and R. G. Hall

School of Medicine, Loma Linda University
Department of Physiology and Pharmacology
Loma Linda, California 92354

INTRODUCTION.-Mosquito populations are often influenced by vegetation associated with the mosquito habitat (Bates, 1949). Plants sometime physically restrict oviposition while in other cases they contribute to the success or demise of the immature stages. Plants conceal and protect immature forms from predators as well as influence water temperature, evaporation rate and chemical content of the water (Angerilli and Beirne, 1974). Substances released from certain plants, including the bladderwort (*Utricularia*), stonewort (*Chara*) and duckweed (*Lemna*), are toxic to immature mosquitoes (Boyd 1949). Observations of natural and experimental ponds in which contain *Elodea nuttalli* show fewer mosquito larvae than ponds without plants or with other species of plants (Sherif and Hall, unpublished data).

In the laboratory, extracts from a fresh water flowering plant *Elodea nuttallii*, a marine alga *Macrocystis pyrifera*, and a terrestrial sage brush *Artemisia cana* produce high larval mortality (Sherif and Hall 1984, 1985).

Are such extracts larvicidal in field conditions also? Reported here are preliminary studies of extracts from four plants on mosquito populations in natural ponds.

METHODS AND MATERIALS.-Ponds in Riverside County, California, with an average surface area of 4 m^2 and depth of .5 m, and known to support breeding of mosquito populations were chosen.

Four plants: *Artemisia cana* (Pursh), *Elodea nuttallii* (Planch), *Macrocystis pyrifera* (Linnaeus) and *Spriogyra nitida* (Dillw) were collected in southern California, washed several times with tap water, dried at 60° C and powdered. The powder was then refluxed for 8 h with absolute methanol and petroleum ether (3:1 vol/vol). The eluate was collected and evaporated to dryness.

An aqueous extract of each plant was prepared by dissolving dried plant powder in water. The solution was filtered and freeze dried.

One g of each aqueous and methanol-petroleum ether extract was dissolved in 10 ml of acetone and diluted to one l with distilled water. Each extract solution was spraved on the surface of three ponds randomly chosen. Three control ponds received only water containing the same amount of acetone as used to prepare the extract solutions.

The commercial larvicides Golden Bear 1356 (11 1/4000 m) and Altosid®(1 tablet/9 m), were applied to another three ponds each.

Water samples were collected with a 400 ml dipper every other day beginning four days before treatment and continuing for 24 days. Mosquito larval density was determined for each pond by collecting five dips, four around the edge and one from the middle of each pond.

RESULTS AND DISCUSSION.-The majority of larvae collected from the ponds were identified as *Culex* spp.

Extracts from *Elodea, Artemisia* and *Macrocystis* gave 80% or more mortality within two days of treatment (Figure 1). The methanol-petroleum ether extracts were more larvicidal than the aqueous extracts. The toxicity of the methanol-petroleum ether extracts of *Elodea* and *Macrocystis* as well as the aqueous extract of *Macrocystis* remained high through the 24 day period following treatment.

Golden Bear oil produced a mortality near 100% within 2 days following treatment but then the number of mosquitoes began to increase rapidly with 4 to 6 days of treatment (Figure 2). Ponds treated with Altosid showed a large decrease in larval density beginning 8 days following treatment but within another 8 days the number of larvae in these ponds increased rapidly.

The number of larvae in the control ponds remained high and relatively constant throughout the experimental period.

This study substantiates our previous laboratory findings that extracts of several plants are larvicidal. The most encouraging finding is that the activity of some of these extracts remain highly active for up to 24 days or possibly longer under field conditions. This suggests the possibility of control with less frequent application than necessary with presently-used larvicides.

REFERENCES

Angerilli, P. and B. Beirne. 1974. Influence of some fresh water plants on the development and survival of mosquito larvae in British Columbia. Can J. Zool. 52:812-815.

Bates, M. 1949. The nature history of mosquitoes. The MacMillon Company. New York pp. 1-379.

Boyd, M. 1949. Malariology. Vol. I and II. W. B. Saunders Co. Philadelphia.

Bradley, G. 1932. Some factors associated with the breeding of *Anopheles* mosquitoes. J. Agr. Res. 44:381-399.

Buhot, E. 1927. Effects of Mosquito larvae of a *Queensland Nitella*. Roy. Soc. Qd. Proc. 38:59-61.

Cabellero, A. 1922. Otras espacies larvicidas del genevo *Chara*. Soc. Esp. Hist. Nat. Biol. 22:418-421.

Muirhead-Thomson, R. 1964. Studies on the breeding places and control of *Anopheles gambiae* var. *melas* in coastal district of

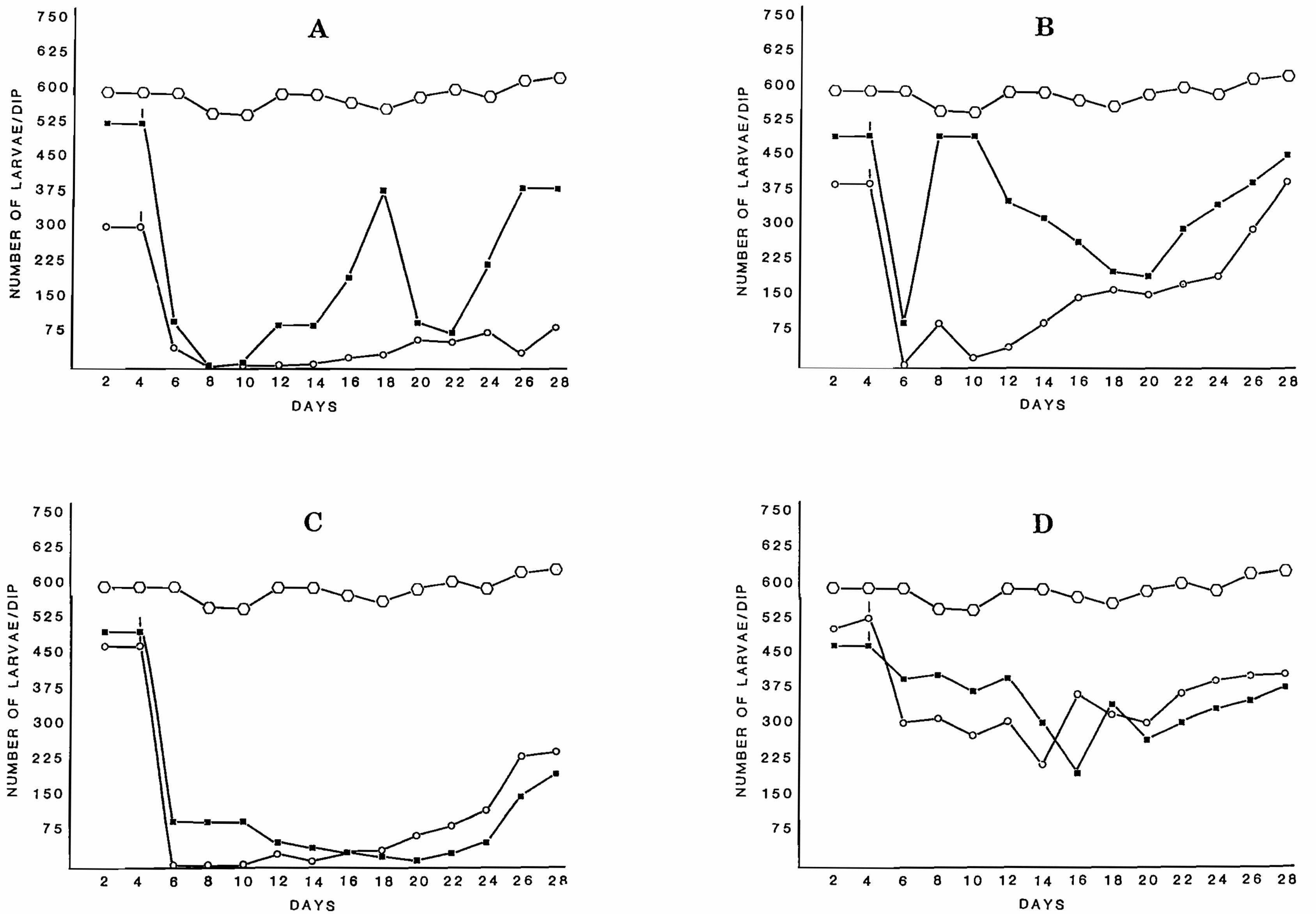


Figure 1.-Number of mosquito larvae collected from natural ponds treated with methanol-petroleum ether extract o and aqueous extract ■ of *Elodea* (A), *Artemisia* (B), *Macrocystis* (C) and *Spirogyra* (D); untreated controls ⬡. Each point is the mean of 15 dips (5 dips per pond X 3 replicates). Extracts were applied on day 4.

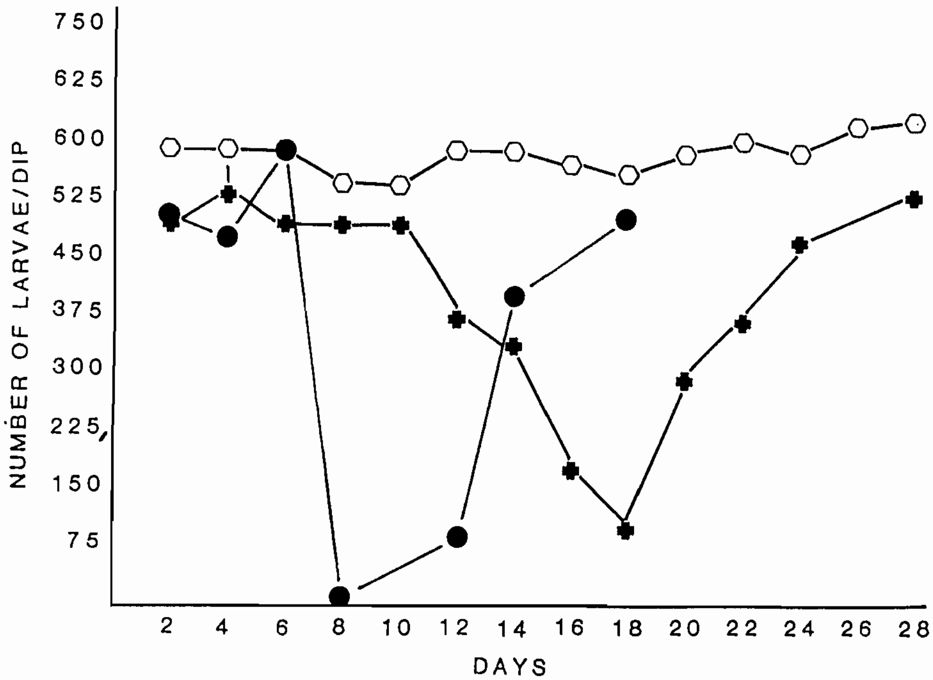


Figure 2.-Number of mosquito larvae collected from natural ponds treated with Golden Bear oil ● and Altosid ✚; untreated controls ○. Each point is the mean of 15 dips (5 dips per pond X 3 replicates). Treatments were applied on day 4.

Liberia. Entom. Res. Bull. 36:185-252.

Sherif, A., and R. Hall. 1984. Effects of *Artemisia cana* extract on the development of *Culex quinquefasciatus*. Proc. Calif. Mosq. and Vector Contr. Assoc. 52:76-79.

Sherif, A., and R. Hall. 1985. Possible vector control substances from *Macrocystis pyrifera*. Soc. Vect. Ecol. 10(1):42-44.

Sherif, A., and R. Hall. 1986. The utilization of plant ingredients as aids in mosquito control. Proc. Calif. Mosq. Vector Contr. Assoc. 53:113-116.

Sherif, A., and R. Hall. 1985. Plants detrimental to mosquito development. Proc. New. Jersey Mosq. Contr. Assoc. 72.

PHOTOTOXICITY OF HEMATOPORPHYRIN DERIVATIVE IN LARVAE OF *CULEX QUINQUEFASCIATUS*

J. Tosk, Abdessalam Sherif,

R. G. Hall and Benjamin H. S. Lau

Departments of Biology, Physiology and Microbiology
Loma Linda University
Loma Linda, California 92350

ABSTRACT

The combined effects of hematoporphyrin derivative (HPD) and visible light on the survival of *Culex quinquefasciatus* larvae were determined. It is observed that the combination of HPD and photoirradiation is toxic to the larvae as a function of HPD dosage and photoirradiation time.

INTRODUCTION.-Hematoporphyrin derivative (HPD) is a photosensitizing dye which exhibits phototoxicity in several biological systems (1-3). This substance has been used in conjunction with photoirradiation in the treatment of cancer because of its ability to localize in tumor cells which are subsequently rendered photosensitive (3-5). The structure of HPD is shown in Figure 1. The mechanism of HPD-induced phototoxicity appears to involve the generation of singlet oxygen upon photoirradiation via the triplet photoexcited state of the dye (2,6). This pathway to the production of singlet oxygen is illustrated in Figure 2.

Photosensitization of mosquito larvae has been reported by Kagan, et al. (7) who employed 2,5-diphenyloxazole (POP) and 1,4-bis(5-phenyloxazol-2-yl)benzene (POPOP) as the photosensitizers in *Aedes aegypti* larvae. Kagan's group found that POP and POPOP exhibited significant toxicity in the dark as well. HPD appears to be non-toxic except in the presence of light.

In the present study we have exposed *Culex quinquefasciatus* larvae to HPD in the dark and observed potent phototoxicity upon subsequent exposure to incandescent and solar irradiation.

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. HPD was synthesized according to Lipton, et al. (8). Second instar larvae were treated with various concentrations of HPD dissolved in fresh tap water for 18 hours in the dark prior to photoirradiation. Food was withheld from the larvae from the time of HPD exposure until after photoirradiation. Control larvae were exposed to HPD without subsequent photoirradiation. Additional control larvae were irradiated without prior exposure to HPD. Just prior to photoirradiation the larvae were removed from the water containing HPD and transferred to fresh tap water. Ten larvae were irradiated per treatment in triplicate. Photoirradiation was performed using either a quartz-halogen lamp with an incident intensity of 20 mW/cm^2 or via solar irradiation. After photoirradiation all larvae were returned to the dark and fed. Viability was determined at 24 hour intervals following irradiation.

RESULTS AND DISCUSSION.-The phototoxicity of HPD in the mosquito larvae is shown in Figure 3 as a function of HPD dosage. It was observed that the lowest effective concentration of HPD was 1 μg/ml with a constant photoirradiation time of 20 minutes. The survival time was observed to decrease as the concentration of HPD was increased. At an HPD concentration of 20 μg/ml there was not survival at 24 hours after irradiation. Photoirradiation alone was non-toxic to the larvae.

Figure 4 illustrates the phototoxicity of a constant concentration of HPD (20 μg/ml) with subsequent solar irradiation (0-20 min). In this experiment it was observed that the survival decreased as the irradiation time was increased. It is important to note that at even the highest dosage of HPD studied (20 μg/ml), no toxicity was observed when larvae remain in the dark.

This study points the way toward a novel method of mosquito control via agents which photosensitize the larvae. HPD is of interest in this regard as it appears to exhibit no toxicity of its own unless there is subsequent photoirradiation. At a minimum effective dose of 1 μg/ml one might be concerned that this concentration is high when compared with conventional mosquito control agents. This is, however a preliminary study and other substances are under evaluation which appear to possess even greated potency as photosensitizers toward mosquito larvae.

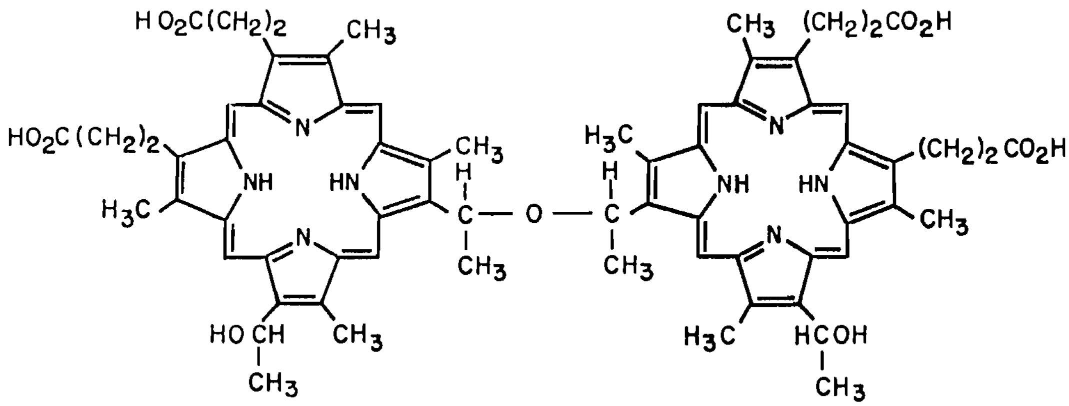
We are also studying the effects of HPD on adult mosquitoes which were challenged with this substance as larvae and reared in the dark. This is an important question as the situation may be found in the field where adults will emerge from larvae which grew in low levels of light.

REFERENCES

Benson, R. C., Jr. 1985. Treatment of diffuse transitional cell carcinoma in situ by whole bladder hematoporphyrin derivative photodyamic therapy. J. Urol. 134:675-678.

Cowled, P. A. and I. J. Forbes. 1985. Phototoxicity in vivo of haematoporphyrin derivative components. Cancer Lett. 28:111-118.

Structure of Hpd-Active Component



Bis - 1 - [8-(1 - hydroxyethyl) deuteroporphyrin - 3 - yl] ethyl ether

FIGURE 1.

MECHANISM OF SINGLET OXYGEN GENERATION BY HEMATOPORPHYRIN DERIVATIVE AND LIGHT

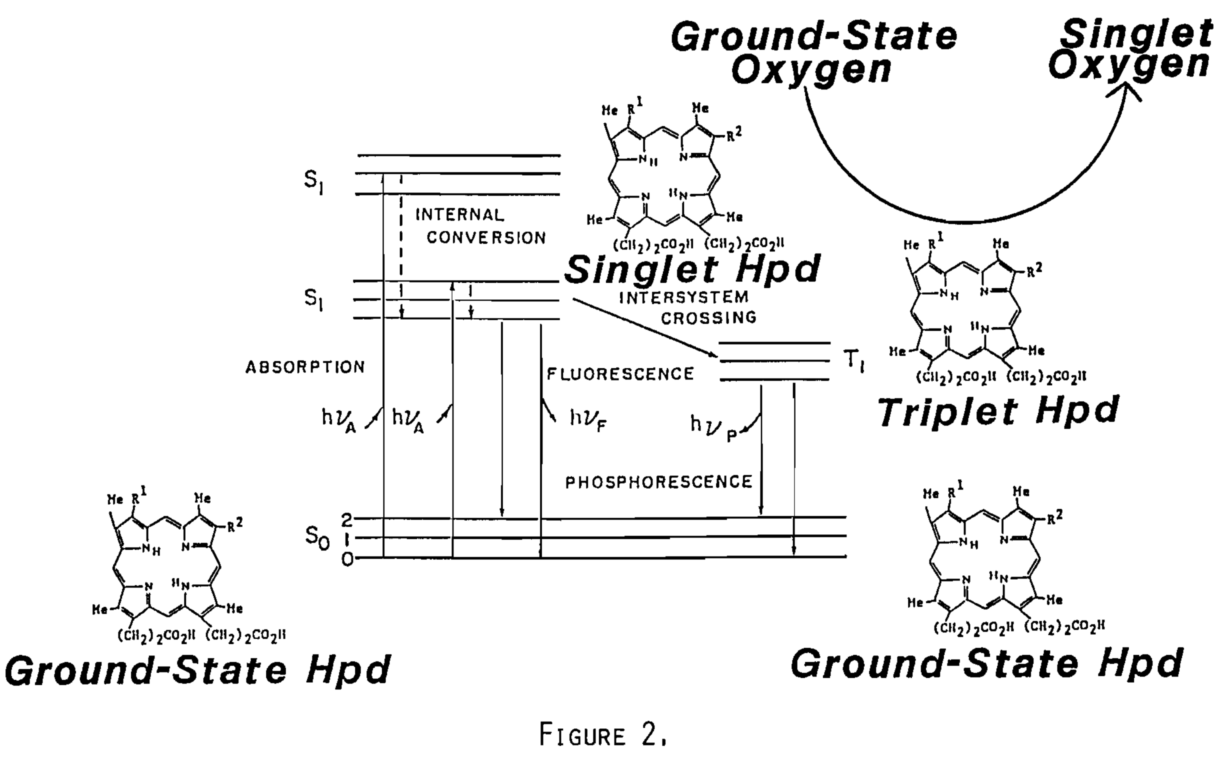


FIGURE 2.

PHOTOTOXICITY OF HPD (0-20 μg/ml) AND 20 MINUTES OF PHOTOIRRADIATION TIME IN CULEX LARVAE

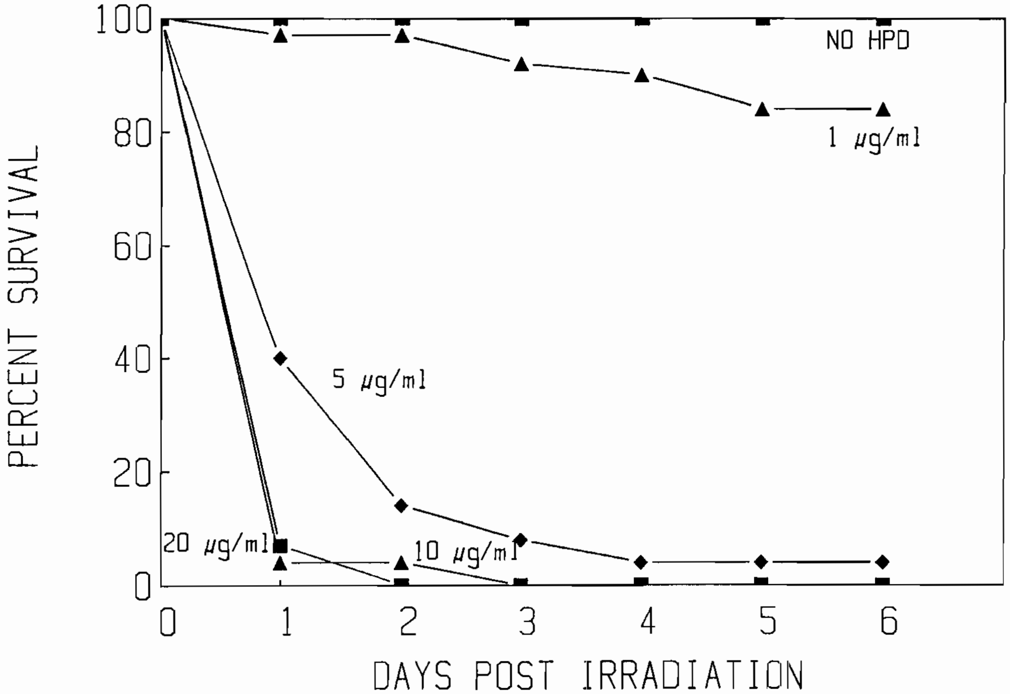


FIGURE 3.

PHOTOTOXICITY OF HPD (20μg/ml) AND SOLAR IRRADIATION IN CULEX QUINQUEFASCIATUS LARVAE

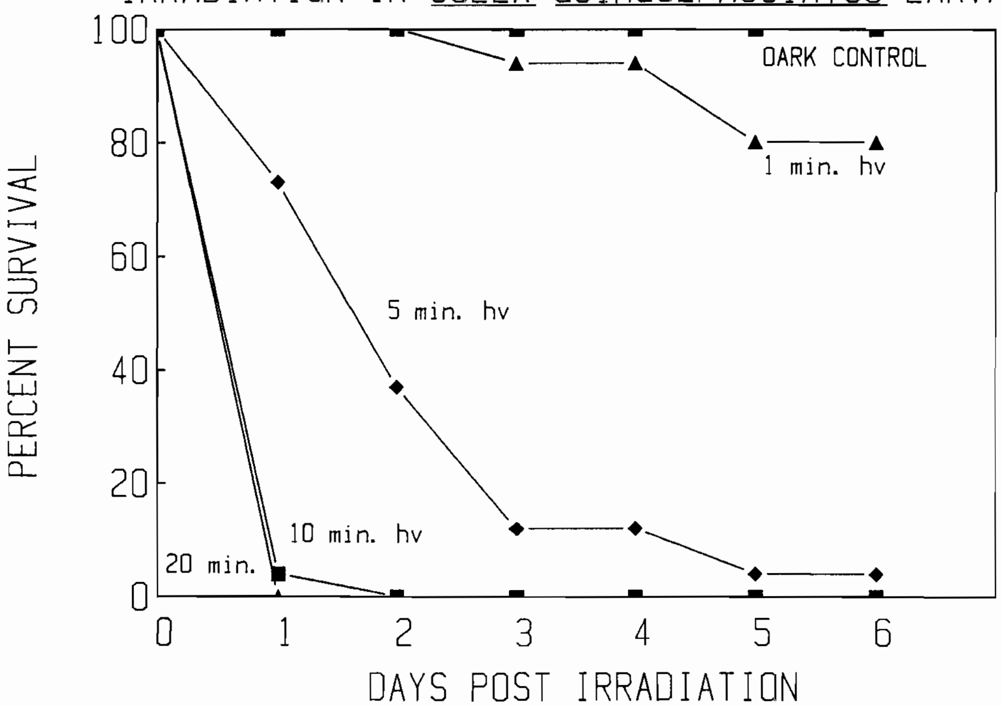


FIGURE 4.

Dougherty, T. J., J. E. Kaufman, A. Goldfarb, K.R. Weishaupt, D. Boyle and A. Mittleman. 1978. Photoradiation therapy for the treatment of malignant tumors. Cancer Res. 38:2628-2635.

Kagan, J., C. P. Kolyvas, J. A. Jaworski, E. D. Kagan, I. A. Kagan and L. H. Zang. 1984. The phototoxicity of 2,5-diphenyloxazole (POP) and 1,4-bis(5-phenyloxazol-2-yl)-benzene (POPOP). Photochem. Photobiol. 40:479-483.

Keene, J. P., D. Kessel, E. J. Land, R. W. Redmond and T. G. Teuscott. 1986. Direct detection of singlet oxygen sensitized by haematoporphyrin and related compounds. Photochem. Photobiol. 43:117-120.

Kessel, D. 1984. Hematoporphyrin and HPD: photophysics, photochemistry and phototherapy. Photochem. Photobiol. 39:851-859.

Lewin, A. A., L. E. Schnipper and C. S. Crumpacker. 1980. Photodynamic inactivation of Herpes Simplex Virus by hematoporphyrin derivative and light. Proc. Soc. Exp. Med. Biol. 163:81-90.

Lipson, R. L., E. J. Baldes and A. M. Olsen. 1961. The use of a derivative of hematoporphyrin in tumor detection. J. Natl. Cancer Inst. 26:1-11.

HATCHERY MANAGEMENT TECHNIQUES FOR *GAMBUSIA AFFINIS*

Werner P. Schon

Sacramento County/Yolo County Mosquito Abatement District
1650 Silica Avenue, Sacramento, California 95815

ABSTRACT

Observation and careful testing of the best mosquitofish sources in the Sacramento County/Yolo County Mosquito Abatement District provided much of the information required for rearing mosquitofish. Water quality similarities were used as guidelines in our hatchery ponds, as well as dense zooplankton and phytoplankton populations.

Using a natural drain (Laguna Creek) for much of our pond water, we built and evaluated a filtering system to remove gamefish and their eggs.

Heavy metal concentrations in our soil dictated that we increase and maintain a high pH in our hatchery ponds. Through the use of buffers (Sodium Bicarbonate), and phytoplankton blooms we were able to do so.

Chemical and organic fertilizers were used with varying application rates, combinations, and then evaluated.

Zooplankton were collected from our most productive fish sources, identified and cultured in our ponds before adding fish in the spring. Zooplankton and phytoplankton populations were also evaluated in our over-wintering ponds.

Pond production, in pounds of fish harvested per pond was difficult to determine because all ponds were connected with culverts. This allowed the fish to move from pond to pond freely in the spring.

The culverts going between ponds were screened with 1/8" hardware cloth to better evaluate over-wintering data. Water levels in the over-wintering ponds were maintained with our deep-well pump.

INTRODUCTION.-Many of the district's fish sources became unreliable and many others were lost completely with the addition of the new Regional Sewage Treatment Plant. The district purchased the old Elk Grove sewer ponds in 1978 to develop a district mosquito fish hatchery. Here we could have control of water quality, evaluate pond design, production and over-wintering parameters.

In the spring of 1984, testing of water quality in all of the best producing mosquitofish sources was begun. pH, NH_3-NH_4, pond fertility (turbidity), water temperature, and dissolved oxygen were checked. The collection of zoo-plank ton was begun for identification.

The hatchery ponds were not producing as well as many other fish sources. Soil and water samples were taken to the Sacramento County Department of Agriculture for analysis. Laguna Creek water was not tested because it was not static. Storm drains as well as agricultural waste water contributed to its flow, changing pH and ammonia levels throughout the day.

The County Department of Agriculture found high concentrations of heavy metals in our hatch-ery soil: Cr 33.8 ppm, Cu 19.2 ppm, Pb 4.2 ppm. The well water was free of heavy metals and other toxins.

Unsure of what to do about the heavy metals in the soil, the Department of Fish and Game, Water Pollution Control Laboratory was contacted. Mr. Kim McCleneghan, the Water Quality Biolo-gist, suggested that, at a pH of 8.2 -- 8.8 in the rearing ponds none of the metals should enter solution (toxicity results from dissolved metals). The metals should all remain in a complexed form unavailable to fish.

MATERIALS AND METHODS.-In the fall of 1984 all of the ponds were drained; the fish removed and the remaining water chlorinated. The chlorine was used to kill any remaining gamefish, their eggs, tadpoles, fish parasites and pathogens. The chlorine was very effective at 5 ppm. All of the district's hatchery ponds are drained and chlorinated at least once a year, some in late summer and the others in early spring.

A total of 20 ponds were used for production in the spring of 1985. Eighteen of them were approximately .1 surface acres, two of them were .26 surface acres. A total of 2.32 surface acres and 7.85 acre feet of water.

When filling the rearing ponds, the water was buffered with sodium bicarbonate, 40 lbs/acre foot of water. Phytoplankon (blue green algae from Knight Landing sewer ponds) were also added, 150 gallons -- 1200 gallons per pond. The ponds were fertilized, in hope of maintaining the algae bloom, and thus our right pH. All ponds were filled with well water at a pH of 7.4.

Two days after the rearing ponds were full, the collection of zooplankton from two of the best producing fish sources, Courtland sewer ponds and Bryton Station (vernal pond) was begun. The crustaceans collected from Courtland sewer ponds were Daphnia, Cyclops, and Hyalella. Bryton Station provided Daphnia, Cyclops, Eu-cypris, and Branchinecta. Phytoplankton as well as zooplankton were collected from both sources.

Bryton Station plankton provided a poor phytoplankton bloom, even though the ponds were fertilized with organic (chicken manure) and inorganic (20-20-5) fertilizers, with varying application rates. Bryton Station did provide a very abundant zooplankton bloom in all ponds. Daphnia numbered over 30/liter and Cyclops 22/liter after only 19 days. Additionally, within the same time frame, the Secchi Disk exceeded

Table 1.-Bond Road Fish Hatchery 1985.

POND #	DATE	STOCKING RATE (LBS.)	DATE	FISH HARVESTED (LBS.)
#1	3-19	40 LBS.	6-29	216 LBS.
#2	3-19	48 LBS.	6-29	224 LBS.
#4	3-15	20 LBS.	6-22	124 LBS.
#5	3-19	20 LBS.	6-24	88 LBS.
#6	3-19	20 LBS.	6-29	22 LBS.
#7	3-28	20 LBS.	6-21	38 LBS.
#8	3-28	20 LBS.	6-21	38 LBS.
#9	3-28	20 LBS.	7-1	44 LBS.
#10	4-1	40 LBS.	7-1	54 LBS.
#11	4-1	20 LBS.	7-1	52 LBS.
#12	4-1	20 LBS.	6-24	70 LBS.
#13	5-1	2 LBS.	6-24	62 LBS.
#14	5-1	2 LBS.	6-24	110 LBS.
#15	5-1	2 LBS.	6-24	54 LBS.
#16	5-1	2 LBS.	6-24	62 LBS.
#17	5-1	2 LBS.	--	0 LBS.1/
#18	--	0 LBS.	7-1	118 LBS.
#19	4-11	2 LBS.	6-22	58 LBS.
#20	4-01	20 LBS.	6-22	60 LBS.
#21	4-11	20 LBS.	--	0 LBS.1/
TOTAL STOCKED		340 LBS.	TOTAL HARVESTED	1494 LBS.

1/ Ponds treated with Aquazine® (algicide) for control of filamentous algae.

the maximum pond depth of 36".

The Courtland sewer ponds provided the best phytoplankton bloom. After 19 days, pond fertility (turbidity) was eight inches, with the ponds having a bright green color.

Combination plankton source introductions provided a fair phytoplankton bloom averaging 16" in 19 days.

After adding the fish to the ponds, the zooplankton populations diminished quickly and all ponds had a phytoplankton bloom of 6 - 12 inches after 30 days.

In four of the ponds that were slow to green, we developed a filamentous algae problem. In two of the ponds, the algae was physically removed and refertilized. This worked very well. The ponds were quick to develop a good phytoplankton bloom. The other two ponds were treated with Aquazine® (Simazine) according to the label. The fish quickly left those two ponds and never returned during that season. The Aquazine® was also very slow in controlling the filamentous algae.

All of our hatchery ponds were originally of a long, raceway-type design, 25 ft. wide and 280 ft. long, with an average depth of 4.5 ft. The slope of the ponds was 1:1. These ponds were very difficult to seine and provided little harborage for fry from the adult fish.

The first two experimental ponds were built by removing the center levee from between two raceway ponds. The slope was changed from 1:1 to 4:1, making it much easier to seine fish and maintain levees. A 3" to 6" river rock ripwrap was used to provide harborage for fry. These ponds are very similar to sewage oxidation ponds, but the rock is raked out approximately 18" from the bank providing shallow warm areas in the ponds for the fish. As mentioned before, all ponds had open culverts between them allowing the fish to move freely from pond to pond. Pond design production could not be evaluated accurately. This coming season, all ponds have been separated and this pond design will be better evaluated.

In Pond #1 a low pressure air line was used commencing September 14, 1985. The air line was 120' long plastic 3/8 tubing. The pump was set on a timer and ran from 4:00 a.m. -- 8:00 a.m., seven days a week. Although Pond #1 had the air line, dissolved oxygen and pH levels were almost always lower than in Pond #2.

Both ponds were stocked with 250 lbs of fish, from the same source and on the same dates. On February 8, 1986 both ponds were pumped down and seined. Pond #1 was seined 82 lbs, or 32.8% of stocking rate. Pond #2 was seined for 127 lbs or 50.8%.

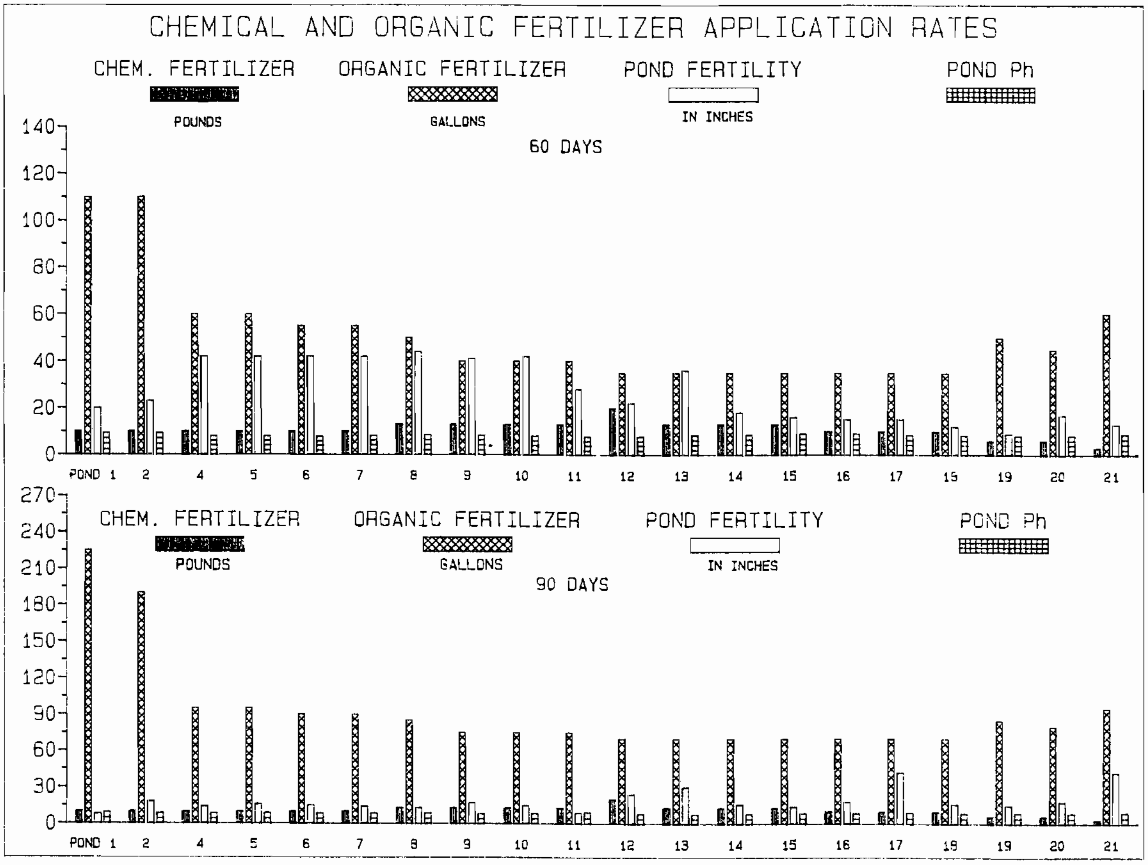


Figure 1.

Table 2.

	POND 1				POND 2			
WEEKLY DATE	-pH	TEMP	D.O. (PPM)	FERTILITY	-pH	TEMP	D.O. (PPM)	FERTILITY
9/17/85	8.21	69°F	-	18"	8.71	69°F	-	16"
9/26/85	8.83	25°C	-	12"	9.02	25°C	-	10"
10/02/85	8.43	18°C	2.1	10"	8.94	18°C	4.2	7"
10/08/85	8.24	20°C	3.4	12"	8.72	20°C	4.1	10"
10/15/85	8.16	19°C	-	12"	8.23	19°C	-	10"
10/22/85	8.03	16°C	4.5	12"	8.51	15°C	5.0	13"
11/05/85	8.10	16°C	4.6	10"	8.83	16°C	7.2	9"
11/12/85	8.57	10°C	6.8	10"	9.80	9.5°C	9.5	10"
11/18/85	7.97	10°C	11.1	11"	9.66	10°C	11.9	10"
11/21/85	8.19	9°C	10.1	9"	9.28	9°C	11.4	10"
11/25/85	8.20	10°C	7.2	9"	9.48	10°C	7.6	9"
12/04/85	8.36	11°C	9.7	9"	9.14	11°C	9.5	9"
12/12/85	8.49	11°C	9.0	10"	8.42	11°C	7.9	12"
12/12/85	8.61	9°C	7.2	12"	8.49	8°C	7.6	13"
12/22/85	8.12	10°C	10.7	12"	9.61	9°C	10.7	11"
01/03/86	8.55	12°C	8.5	13"	8.76	11°C	9.4	17"
01/13/86	8.89	9°C	-	15"	9.72	9°C	-	15"
01/23/86	8.55	13°C	10.1	15"	9.61	13°C	11.4	14"
01/30/86	8.51	14°C	9.14	13"	9.51	14°C	10.7	13"
02/02/86	8.49	14°C	9.0	14"	9.65	14°C	10.1	13"
	LOW PRESSURE AIR LINE						CONTROL POND	

Zooplankton, cultured in Pond #3 were pumped into Pond #2 twice during the winter months, on December 5, 1985 and on January 3, 1986. Pond #2 still had good Daphnia and Cyclops populations when we seined it on February 8, 1986. In all of our zooplankton fed ponds over-wintering success was better than 50%. A dense phytoplankton bloom 6" -- 12" was not a significant factor. Pond #31 was never less than 26". Over-wintering success in this zooplankton fed pond was 68%. Pond fertility of Pond #20 was never less than 22". Over-wintering success in this zooplankton fed pond was 132 lbs or 52.8%.

RESULTS AND DISCUSSION.-Mosquitofish production in all of the Sacramento County/Yolo County Mosquito Abatement District's most productive sources was always accompanied by dense zooplankton populations.

Phytoplankton blooms were also found in many of our fish sources. These we stimulated in our hatchery with chemical and organic fertilizers. Phytoplankton, an important part of the pond food web, was also used to control aquatic plants and filamentous algae, that would interfere with the seining of our ponds.

The rock pond design will provide spawning area's for the gravid females as well as harborage for the newly spawned fry. Mosquitofish production in Ponds #1 & #2 (rock ponds) for 1985 indicates that we are moving in the right direction.

Before breaking ground for any fish hatchery, complete soil and water analyses should be done. The Sacramento County Department of Agriculture was very cooperative and did analyses for the district on several occasions.

When developing a mosquitofish hatchery do not be afraid to seek guidance. Find out what problems others have had and where and how they were overcome. Everyone we spoke to was eager to help.

ACKNOWLEDGMENTS.-Success in the district's rearing and over-wintering ponds was made possible with the help and encouragement of many people and agencies. Robert L. Coykendall, Sutter-Yuba Mosquito Abatement District, suggested fish food, sump cover (to shade out filamentis algae), the importance of phytoplankton bloom, and parameters in minimum and maximum pond depth. Debbie Thompson, Senior Agricultural Biologist, State Department of Fish and

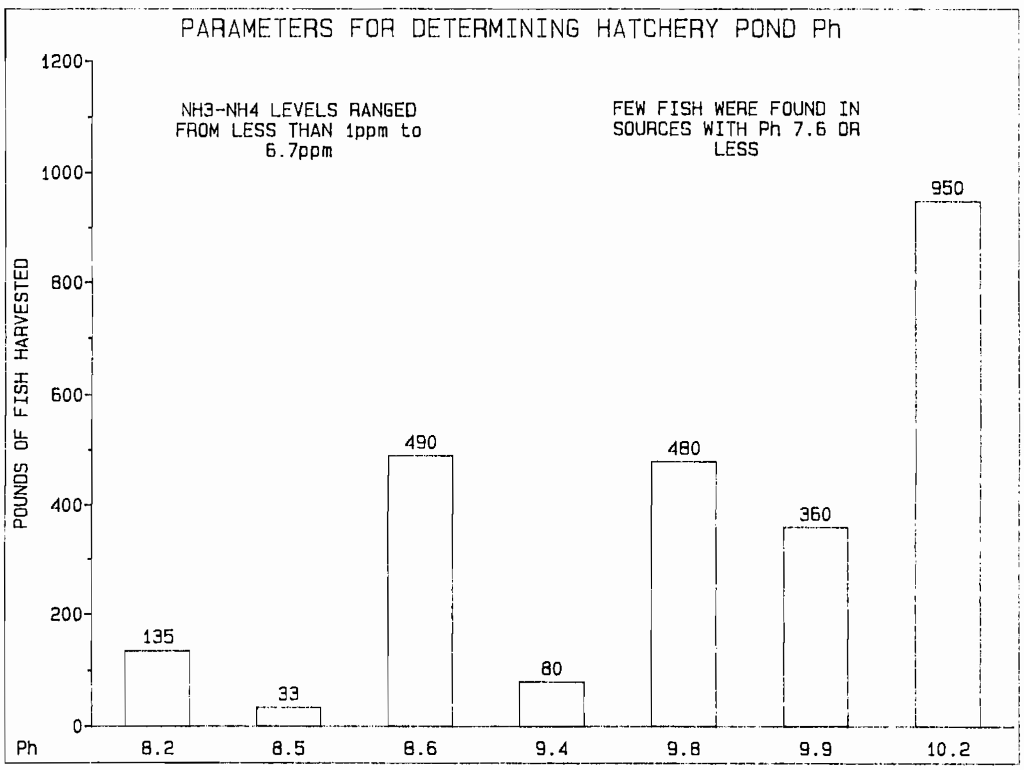


Figure 2.

Game, provided soil and water analysis. Mike Cockran, Central Valley Fish Hatchery, stressed the importance of zooplankton populations, use of chlorine to sterilize ponds. Charlie Young, district employee, helped with the design of the filtering system. Kim McCleneghan, Water Pollution Control Laboratory, suggested high pH for keeping heavy metals out of food web.

REFERENCES

Coykendall, R. L. 1980, Fishes in California Mosquito Control, CMVCA press.

Dupree, H. K. and Huner, J. V. 1984. Third report to the fish farmers, U.S. Department of the Interior Fish and Wildlife Service.

Boyd, C. E. 1979. Water Quality in Warmwater Fish Ponds, Department of Fisheries and Allied Aquacultures.

Fontaine, R. E. 1983. Coordinator of Mosquito Research. Proceeding of the Mosquitofish Workshop.

Davison, V. E. 1955. Managing Farm Fishponds for Bass and Bluegills. Farmers Bulletin No. 2094.

Schauber, P. 1983. Aquarium Digest International No. 18. Breeding Daphnia. Printed in West Germany.

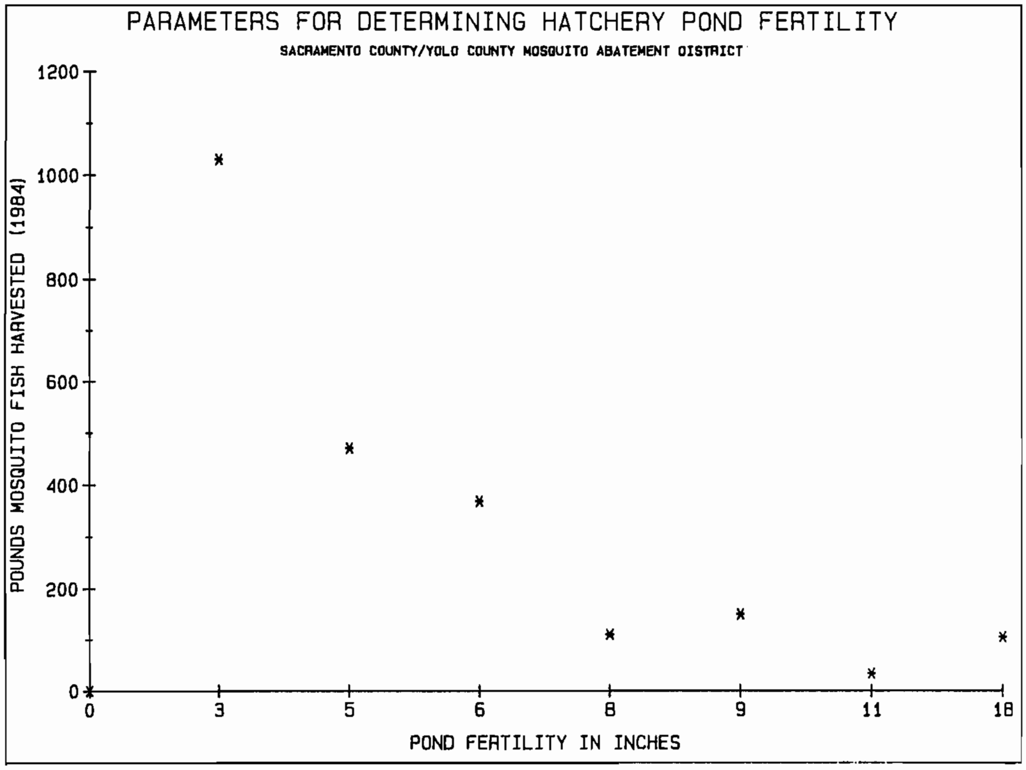


Figure 3.

A SURVEY OF MOSQUITOFISH *GAMBUSIA AFFINIS* PARASITES IN SACRAMENTO COUNTY

S. A. Wright and K. W. Boyce

Sacramento County/Yolo County Mosquito Abatement District
1650 Silica Avenue, Sacramento, California 95815

ABSTRACT

Mosquitofish health is of critical importance to the *Gambusia affinis* aquaculturist. Water quality and nutrition are of primary importance in raising fish, but knowledge of parasitism is also important in maintaining the health of cultured fish. In this study mosquitofish have been sampled from three heavily populated sources in Sacramento County. Parasite infection levels were found to be higher in the fall sample than in the spring sample taken from the three sites. It was also noted that parasite infection levels were higher and more persistent in cultured pond sites. From 180 *G. affinis* dissected, five protozoans, seven helmenthes, and one saprophytic fungus were collected. Outside the sample, two other protozoans and a crustacean parasite were found infecting mosquitofish.

INTRODUCTION.-Parasites are characteristically efficient organisms that generally do not kill their hosts in natural conditions. It is possible, however, that the situation is different for the mosquitofish, *Gambusia affinis* (Baird and Girard), in confined cultured environments. Excessive handling abrades the fishes' natural protective mucus coating, leaving them especially susceptible to repeated exposure of parasitic eggs, larvae and bacterial infections associated with overcrowding.

The objective of this study was to collect the various types of parasites found infecting mosquitofish, noting their site location and habitat. Our purpose was also to identify and compare these parasites using our own observations and those of others. It is hoped that this information may be useful in the future for those working with cultured *G. affinis* in disease prevention and parasite control.

SITE DESCRIPTION.-Collections were made from three different locations representing three distinct habitat types. Sample sites in Sacramento County were as follows: 1. Morrison Creek near Florin Road, 2. Rio Linda Water Treatment ponds on Rio Linda Way, and 3. The Fishery, a catfish hatchery on Elk Grove-Florin Road.

Rio Linda and the catfish hatchery are both pond environments with turbid water and some vegetation covering the bottoms. The margins of the ponds are all heavily vegetated. The Rio Linda ponds are about half the size and are much shallower than the hatchery ponds. Morrison Creek is fairly swift running and has relatively clear water with little vegetation along the bottom. The water is colder at Morrison Creek than at either Rio Linda or the Fishery ponds.

The Fishery, a co-op catfish and sturgeon hatchery consists of eight approximately one acre man-made impoundments adjacent to Florin-Elk Grove Road. The ponds are designed to serve as pond environments to rear catfish, and are also heavily populated with *G. affinis*. Temperature fluctuations were found to be less in these ponds during the winter months, relative to other environments sampled.

The smaller Rio Linda ponds consist of the remains of the Rio Linda waste-water treatment and reclamation ponds, and are now rented for *G. affinis* rearing by the Sacramento County/Yolo County Mosquito Abatement District. Temperature levels were found to be lower during the winter in these ponds likely due to their shallower waters and smaller size.

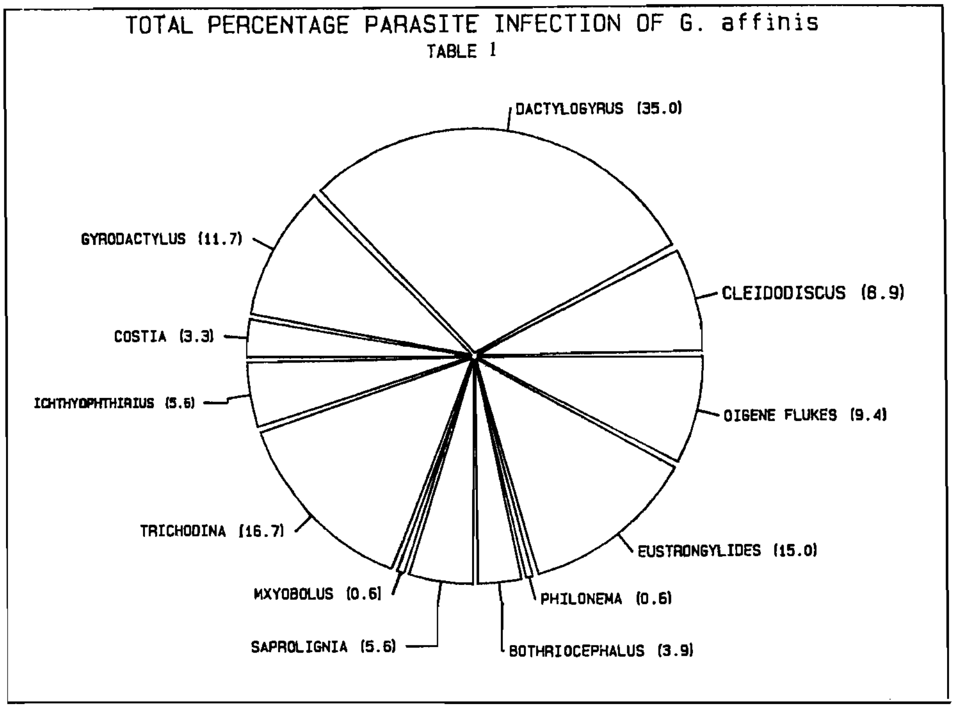
Morrison Creek is presently used as a city street drain run-off and flood control. During summer months, movement is reduced to a near stagnant state. In the winter months, water rapidly moves through the drain system, though remaining relatively shallow. Water temperature remained relatively constant during winter despite fluctuations in ambient temperatures.

Presently all three sites are used for the rearing and collecting of *G. affinis* for biological control projects.

Other fish present in the collection sites include carp *Cyprinis carpo* L., golden shiner *Natemigonus crysaleueas* (Mitchell), flat head *Pimpephales oromelas* (Rafineque), Tui chub *Gila bicolor* (Girard), speckled dace *Rhinichys osculus* (Girard), and bluegill *Lepomis macrachirus* (Rafineque).

MATERIALS AND METHODS.-Fish were obtained by seining and netting with aquatic nets. Captured fish were transported to the laboratory in buckets containing water from the site of collection. Upon return to the laboratory, fish were held in aquariums.

In October 1985, 30 fish from each site were examined and in February 1986, a second set of 30 fish from each site were examined. Specimens were randomly selected from the aquariums, sexed, and given careful external and internal examinations. The fish were killed by pithing just prior to examination. Intestine, liver, spleen, gall bladder, kidney, and gonads, were examined fresh. The scales, mucus, and intestinal lining were inspected from scrapings. Gill lamellae were examined directly under cover glass and viewed using a phase contrast microscope. Measurements were made using an ocular micrometer scale. Dissection was accomplished using



iris scissors to cut a short section ventrally from anterior just under the operculum to the vent posteriorly. Dissecting needles were used to tease free the organs from the peritoneal mesentaries. The peritoneal cavity was carefully washed out with water and also examined.

RESULTS AND DISCUSSIONS.-Compiled in Table 1 are the results of the external and internal examinations of *G. affinis* along with percentage evaluation of data from numbers of fish with parasites. Data concerning parasites represent only infected individual fish and do not show numbers of individual parasites collected. Male and female fish were not separated and therefore cannot be used for any evaluations, as unequal numbers were dissected.

A total of 180 fish were examined for parasites. From those, 114 were females and 66 were males. Ninety-one point two percent of the female fish and 80.3% of the males were afflicted with some type of parasite. Overall, 87.2% of the fish examined contained or harbored some type of parasite.

From the 180 fish examined, thirteen types of parasites were collected. Five protozoan and 7 helmenthic parasites were recovered along with a fungal saprophyte.

Table 1 represents the percentage of parasites found infecting *G. affinis*. Both single and multiple infections are included in this pie chart.

Monogenetic trematode parasites were by far the most prevalent. Of those fish parasitized, 55.6% were infected with this ectoparasitic fluke. The most commonly collected of these flukes was *Dactylogyrus sp.* (Diesing), which represents over 35% of all parasite varieties found. *Gyrodactylus sp.* (Nordmann), was discovered on 11.7% and *Cleidodiscus sp.* (Mueller), the least common, existed in small but persistant numbers making up 8.9%.

The nematode parasites were the most remarkable. Both varieties encountered were peritoneal encysters. By far the most common was *Eustrongylides sp.* (Jägerskiöld), infecting almost 15.0% *Philonema sp.* (Kuitunen-Ekbaum), was collected in only one fish which represents only 0.6% of those infected.

Peritoneal digenetic trematodes were collected in just over 9% of the fish.

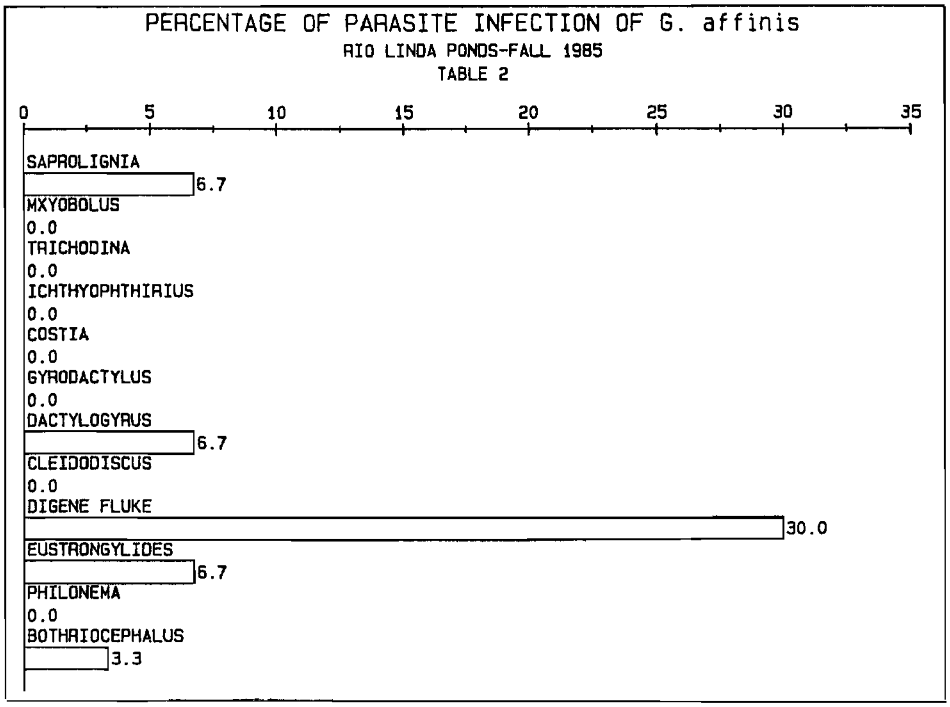
The cestode *Bothriocephalus sp.* (Blanchard), was infrequently collected in only 3.9% of those infected, though usually existing in multiples per fish.

From the available data collected on protozoan parasites, infection numbers appear to occur as isolated in time and location. Two good examples are: 1. the outbreak of *Trichodina sp.* (Ehrenberg), representing 16.7% of the overall, but occurring only at one site and only during one season, and 2. *Ichthyophthirius sp.* (Fouquet), at 5.6%, also occurring only at one place and time.

These and other noted protozoan outbreaks occurred in the spring after a long winter with reduced water temperatures.

The phycomycetes *Saprolignia sp.* (Nees Von Esenbeck), represented only 5.6% of those parasitic infections noted, however, this fungus was always associated with previously infected fish of poor health and is suspected to be the ultimate cause of mortality.

In Tables 2 - 7, spring and fall collections



are separated for each site and show percentage differences in *G. affinis* parasitism.

Table 2 graphs horizontally the results collected at Rio Linda in the fall of 1985 collection. The most predominate parasite collected was the visceral digenetic trematode which was found in 30% of the fish examined. The peritoneal nematode *Eustrongylides*, and the gill fluke *Dactylogyrus* were both collected in 6.7%. The cestode *Bothriocephalus* was found in 3.3% of the fish. Also noted was the *Saprolignia* fungus in 6.7% of the fish.

In Table 3 the Rio Linda spring 1986 sample is graphed. There were no nematodes or internal flukes collected, but every fish in this sample was heavily infected with the ectoparasitic protozoan *Trichodina*. The epithelial trematode *Gyrodactylus* was found to cover 70% of the fish surveyed, and *Saprolignia* was found on 20%.

Shown in Table 4 is the Beer Fishery fall 1985 sample. All the fish collected from these ponds were parasitized by the monogenetic gill fluke *Dactylogyrus*. Most fish hosted dozens of these trematodes. These same fish were also infected at 16.7% with the nematode *Eustrongylides* as well as the visceral flukes also at 16.7%.

Table 5 shows an increase in parasitism at the Beer Fishery in spring 1986. The same parasites are represented as those collected from the site in the fall sample. A new monogenetic trematode *Cleidodiscus* was found on the gills of 53.3% of the fish while *Dactylogyrus* was still found on 40% of the fish. The digenetic trematode infection level dropped to 10% after the winter while the *Eustrongylides* parasite population increased to 36.6%. There was also noted a fairly heavy mixed protozoan infestation.

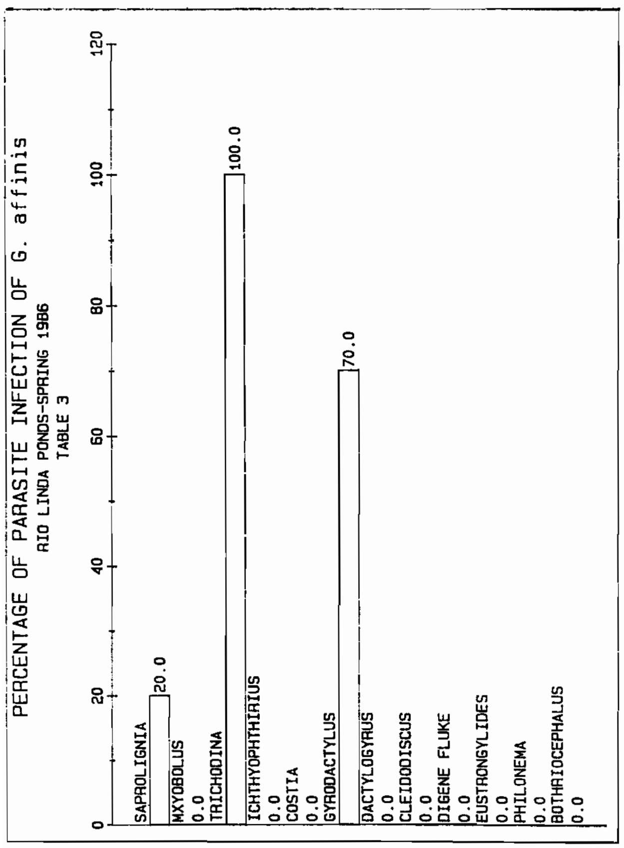
Table 6 outlines the fall parasitic level from the Morrison Creek sample taken in October 1985. A majority of the fish, over 63%, were infested with *Dactylogyrus*. These fish were also parasitized with *Eustrongylides* at 16.7% of the sample. Fish were also found to contain *Bothriocephalus* in 16.7% of the sample. The fall Morrison Creek sample contained the only specimen of *Philonema* collected. A small percent of the fish were infected with *Saprolignia*.

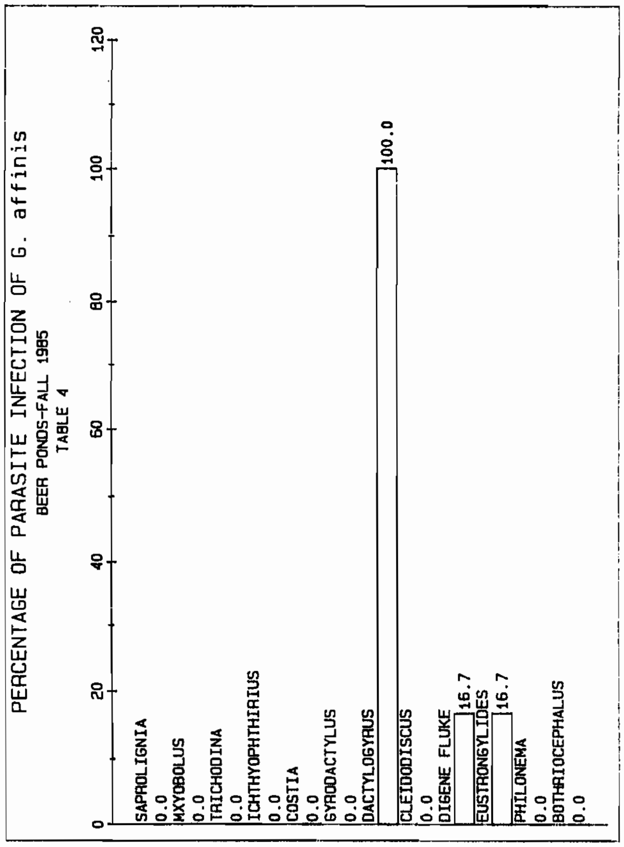
In Table 7 the spring sample taken from Morrison Creek showns a change in the variety of parasites collected. Two protozoans were collected. *Ichthyophthirius* infection was found to be 33.3% and one fish was infected with *Mxyoblus sp.* (Bütschli). The *Eustrongylides* level was found to be lower at 16.7% as well as the *Bothriocephalus* infection level at 3.3%.

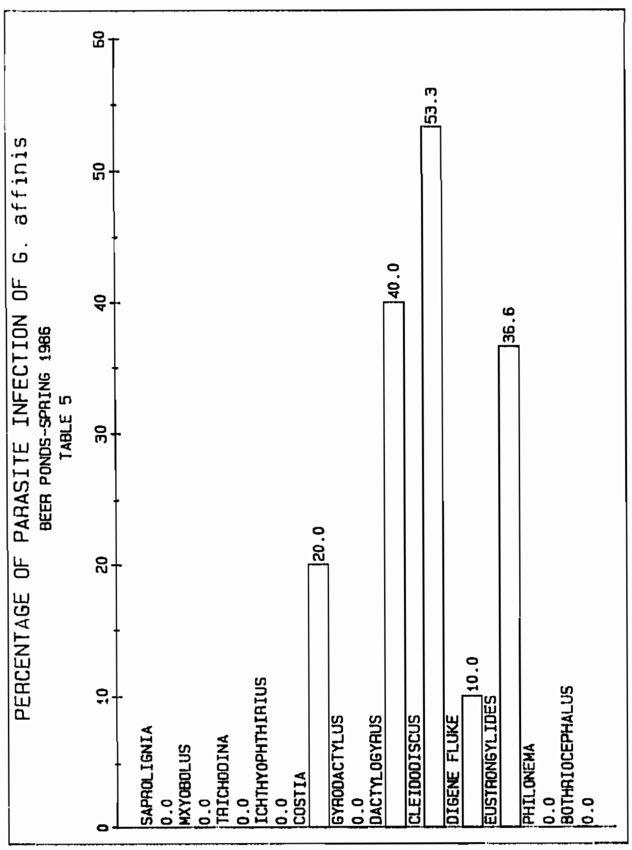
In Tables 8 - 11 the four major helmenthic parasite varieties are compared spring and fall for all three sites. The verticle bar graphs show percentage infection level.

In Table 8 the two gill monogenetic trematodes *Dactylogyrus* and *Cleidodiscus* are combined and graphed. The combined populations of these two flukes was always higher in the fall decreasing to zero at both Morrison Creek and Rio Linda sites. Winter conditions at the Beer Fishery were less severe than the other two sites and gill fluke populations were observed to drop only 10% through the winter.

Table 9 shows the same comparison for *Bothriocephalus*. In the two sites where this cestode was collected, it was found that population levels decreased from fall to spring. There were no cestodes collected at the Beer Fishery.


PERCENTAGE OF PARASITE INFECTION OF G. affinis
RIO LINDA PONDS-SPRING 1986
TABLE 3
0
20
40
60
80
100
120
SAPROLIGNIA
20.0
MXYOBOLUS
0.0
TRICHODINA
100.0
ICHTHYOPHTHIRIUS
0.0
COSTIA
0.0
GYRODACTYLUS
70.0
DACTYLOGYRUS
0.0
CLEIDODISCUS
0.0
DIGENE FLUKE
0.0
EUSTRONGYLIDES
0.0
PHILONEMA
0.0
BOTHRIOCEPHALUS
0.0


PERCENTAGE OF PARASITE INFECTION OF G. affinis
BEER PONDS-FALL 1985
TABLE 4
0
20
40
60
80
100
120
SAPROLIGNIA
0.0
MXYOBOLUS
0.0
TRICHODINA
0.0
ICHTHYOPHTHIRIUS
0.0
COSTIA
0.0
GYRODACTYLUS
0.0
DACTYLOGYRUS
100.0
CLEIDODISCUS
0.0
DIGENE FLUKE
16.7
EUSTRONGYLIDES
16.7
PHILONEMA
0.0
BOTHRIOCEPHALUS
0.0


PERCENTAGE OF PARASITE INFECTION OF G. affinis
BEER PONDS-SPRING 1986
TABLE 5
0
10
20
30
40
50
60
SAPROLIGNIA
0.0
MXYOBOLUS
0.0
TRICHODINA
0.0
ICHTHYOPHTHIRIUS
0.0
COSTIA
20.0
GYRODACTYLUS
0.0
DACTYLOGYRUS
40.0
CLEIDODISCUS
53.3
DIGENE FLUKE
10.0
EUSTRONGYLIDES
36.6
PHILONEMA
0.0
BOTHRIOCEPHALUS
0.0

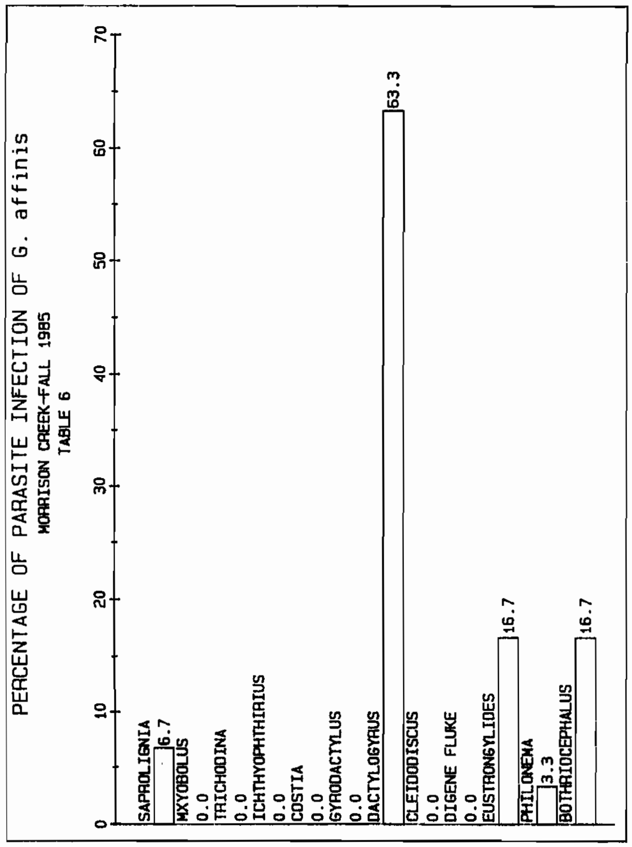

PERCENTAGE OF PARASITE INFECTION OF G. affinis
MORRISON CREEK-FALL 1985
TABLE 6
0
10
20
30
40
50
60
70
SAPROLIGNIA
6.7
MXYOBOLUS
0.0
TRICHODINA
0.0
ICHTHYOPHTHIRIUS
0.0
COSTIA
0.0
GYRODACTYLUS
0.0
DACTYLOGYRUS
63.3
CLEIDODISCUS
0.0
DIGENE FLUKE
0.0
EUSTRONGYLIDES
16.7
PHILONEMA
3.3
BOTHRIOCEPHALUS
16.7

Table 10 graphs the frequency of the digenetic trematode collected at two sites. Once again, the sample sites showed a decline of parasite population through winter. At Rio Linda the fluke population declined from 30% infection in the fall to zero in the spring. A less drastic decline occurred at the Beer Fishery, where infection level dropped from 16.6% to 10% over winter.

In Table 11 the frequency of *Eustrongylides* collected is graphed. In two of the three sites, Morrison Creek and Rio Linda, the *Eustrongylides* populations declined through winter. Morrison Creek declined from 16.7% in the fall to 13.3% in the spring. Rio Linda fell from 6.6% to zero after winter. But, this trend was broken at the Beer Fishery where the nematode population were found in increase by over 100%.

As seen in Table 12 which compares all four of these helmenthic groups, there appears to be a relationship between the winter months and the reduction of fish which contained helmenthic parasites. This relationship may suggest that the pressures of parasitism and winter stresses could contribute to fish mortality. Though this observation may not be completely reflected by the *Eustrongylides* nematode.

A few parasites were excluded from the above statistics due to their infrequent numbers, but warrant mention here to complete the list of organisms found to parasitize *G. affinis*.

Protozoans.-*Ambiphrya sp.* (Raabe), were found on the gills of four fish dissected from the spring sample taken at Rio Linda.

One fish collected from Morrison Creek contained specimens of *Vohlkamphia sp.* (Chatton and La Lung-Bonnaire).

A few fish were found to host infestation of *Ichtyobodo sp.*, formerly *Costia sp.*, from the Beer Fishery.

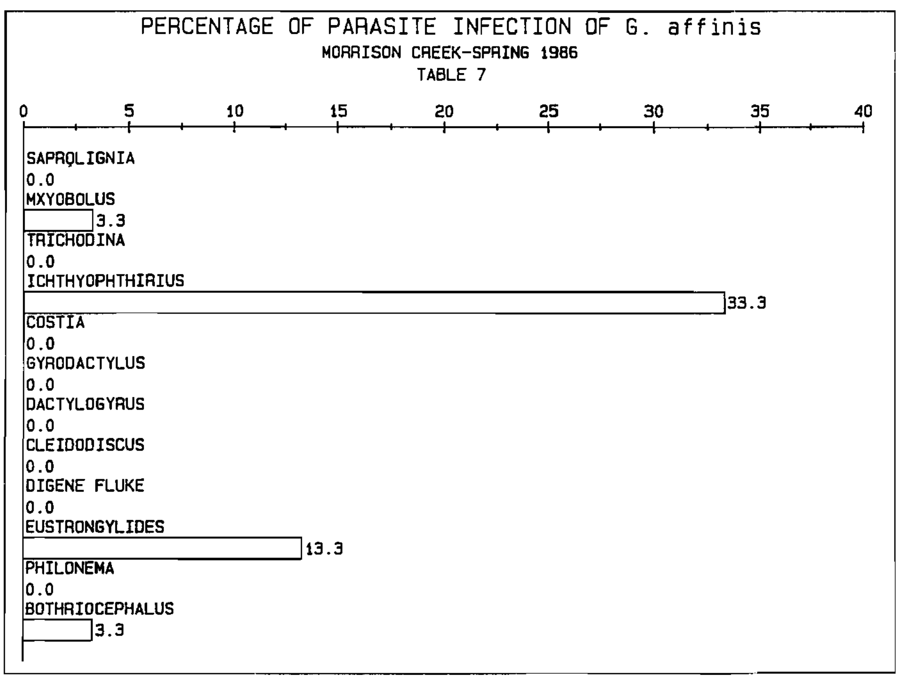
A single fish collected from Morrison Creek was found to be infected with an unidentified microsporidian.

Crustacea.-No fish contained in the sample inspected were infected with the parasitic copepod *Lernaea sp.* L., however, five mosquitofish collected with the sample were infected. *Lernaea* is an important fish parasite and therefore warranted mention in this study.

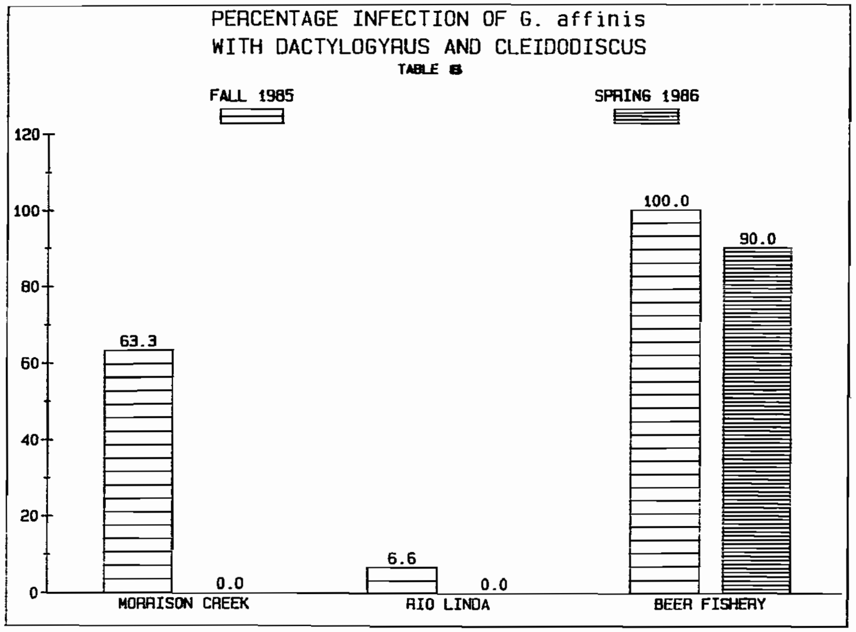
CONCLUSIONS.-An overall view of the statistics show lower parasite population dynamics in cultured pond environments. An overall higher parasite population level was observed in cultured ponds relative to natural creek or small overwintering pond environments.

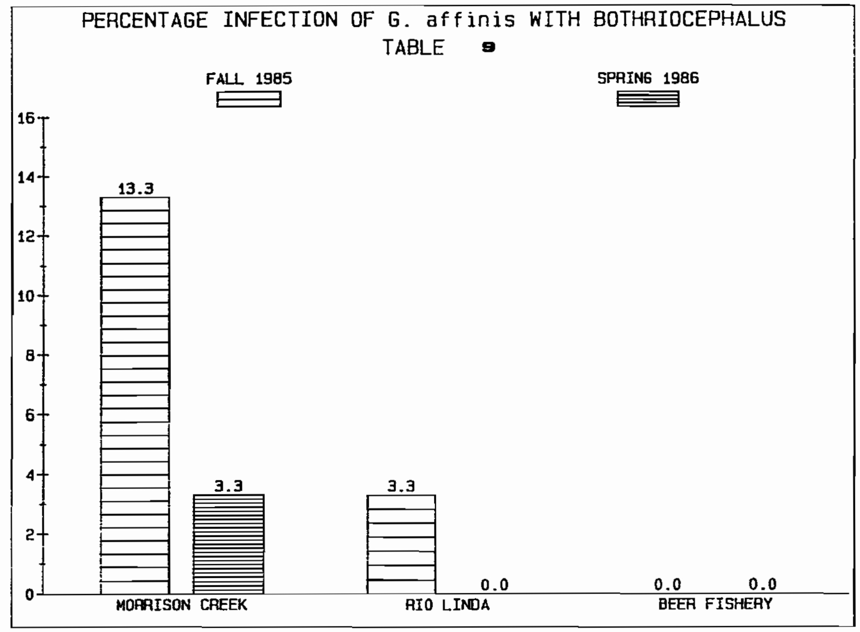
This observation suggests that those populations subjected to the natural stresses of overwintering lose those fish of poor health including those parasitized. This cleansing of the fish population assures a strong reproductive population for the spring.

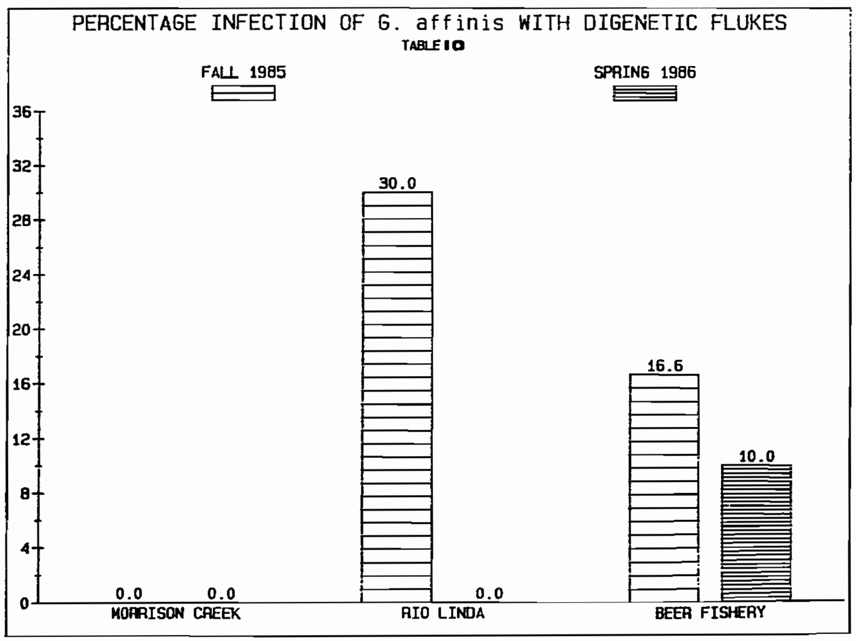
In the cultured pond environment where normal winter stresses are reduced, fish of ill health are more likely to survive the winter. Survival of the host fish allows the parasite to continue its life cycle thus reproducing and contributing to the increase in parasitic population.

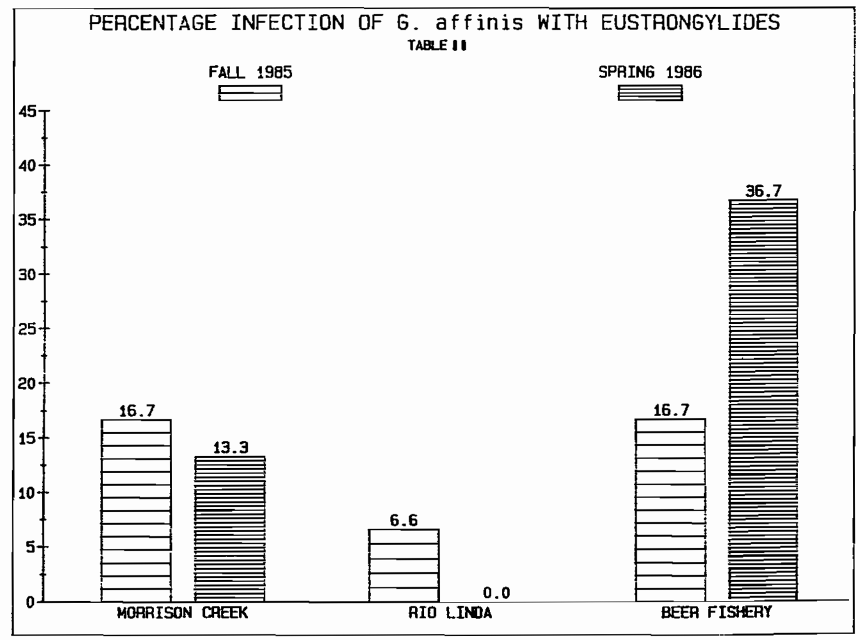


PERCENTAGE OF PARASITE INFECTION OF G. affinis
MORRISON CREEK-SPRING 1986
TABLE 7


PERCENTAGE INFECTION OF G. affinis
WITH DACTYLOGYRUS AND CLEIDODISCUS
TABLE 8
FALL 1985
SPRING 1986
120
100
80
60
40
20
0
63.3
0.0
6.6
0.0
100.0
90.0
MORRISON CREEK
RIO LINDA
BEER FISHERY


PERCENTAGE INFECTION OF G. affinis WITH BOTHRIOCEPHALUS
TABLE 9
FALL 1985
SPRING 1986
16
14
12
10
8
6
4
2
0
13.3
3.3
3.3
0.0
0.0
0.0
MORRISON CREEK
RIO LINDA
BEER FISHERY


PERCENTAGE INFECTION OF G. affinis WITH DIGENETIC FLUKES
TABLE 10
FALL 1985
SPRING 1986
36
32
28
24
20
16
12
8
4
0
0.0
0.0
30.0
0.0
16.6
10.0
MORRISON CREEK
RIO LINDA
BEER FISHERY


PERCENTAGE INFECTION OF G. affinis WITH EUSTRONGYLIDES
TABLE 11
FALL 1985
SPRING 1986
45
40
35
30
25
20
15
10
5
0
16.7
13.3
6.6
0.0
16.7
36.7
MORRISON CREEK
RIO LINDA
BEER FISHERY

The richness of parasitic organisms on fish is evident when even the smallest of samples are taken. Determination of the extent of which *G. affinis* is parasitized has only been started with this small sample. However, from this study it may be suggested that parasitism plays an important role in the growth and reproduction of healthy fish. It is also quite apparent that the importance of parasitism becomes far greater in the cultivation of fishes in confined environments.

In this survey we have only recognized some of the parasites infecting *G. affinis*. Further dissections should be done to normalize these statistics, and the beginning of investigations into the control of parasites through interruption of their life cycles should be pursued.

ACKNOWLEDGMENTS.-Many thanks to Dr. John Modon, Fish and Game pathologist at the Nimbus Dam Fish Hatchery for his invaluable help in both identification of parasites and supplying assistance in finding reference and identification material. Also, thanks to Dr. Ron Hendrick and his staff at U.C. Davis for assistance to identification of parasites. And, special thanks to Mr. Robert Coykendall, Fishery biologist, Sutter-Yuba M.A.D., for his generous effort in locating information of freshwater fish parasitism and his willingness to share knowledge of the subject.

REFERENCES

Amos, K. H. 1985. Procedures for the Detection and Identification of Certain Fish Pathogens. Fish health section, American Fisheries Society, Third edition.

Brown, E. E. 1980. Fish Farming Handbook. AVI Publishing Company, Inc. Westport, Connecticut.

Davis, H. S. 1967. Culture and Disease of Game Fishes. Univ. of California Press, Berkeley and Los Angeles.

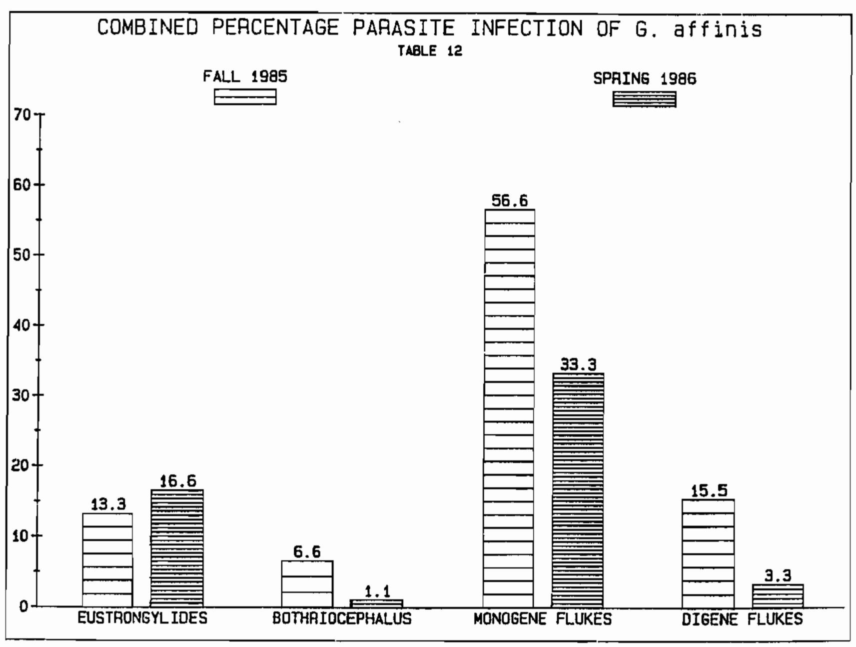
Dupree, H. K. and J. B. Huner, 1984. Third Report to Fish Farmers. The Status of Warmwater Fish Farming and Progress in Fish Farming Research. Publication U.S. Fish and Wildlife Service, Washington, D.C.

Haderlie, E. C. 1954. Parasites of Freshwater Fishes in California. Univ. of California Publications in Zoology, Vol. 57.

Heckmann, R. A., P. D. Greger, J. E. Deacon. 1985. The Asian Fish Tapeworm, *Bothriocephalus acheilognathi*, Infecting Indangered Fish Species From the Virgin River, Utah, Nevada, and Arizona. FHS/AFS Newsletter Vol. 14(1) pp.5.

Hoffman, G. L. 1967. Parasites of North American Freshwater Fishes. Univ. of California Press, Berkeley and Los Angeles.

Kudo, R. R. 1977. Protozoology. Fifth edition. Charles C. Thomas Pub. Springfield Illinois, USA.



OPERATIONAL MOSQUITOFISH PRODUCTION:

BROOD STOCK MANAGEMENT

C. W. Downs[1], C. Beesley[1], R. E. Fontaine[2],

and J. J. Cech, Jr.[2]

ABSTRACT

Mature mosquitofish were held at three sex ratios; 1:1, 1:2, 1:5 males to females for 95 days. Females were induced to drop young at 65 and 95 days and fry production recorded. Fish held at the ratio of 1:1 produced more fry than the other two ratios.

INTRODUCTION.-The mosquitofish, *Gambusia affinis*, has been used in mosquito control for many years (Haas and Pal, 1984). Due to their chronic shortage during peak mosquito breeding seasons, artificial culture of mosquitofish has been employed with various results.

Culture in ponds has met with limited success. Cold water temperatures, bird predation and the need for persistent aquatic weed control are major constraints. Fortunately, intensive culture systems have shown promise.

Important growth factors for intensive culture of mosquitofish have been investigated. Systems stocked at one fish per liter of water have optimal fish biomass gain (Drazba and Gall, 1980; Downs and Beesley, 1983). Mosquitofish grown at a water temperature of 25°C exhibit optimal growth efficiencies (Wurtsbaugh and Cech, 1983; Downs and Beesley, 1983) and Sawasa (1974) reported a 13:11 L:D photoperiod to be optimal for growth while Gall (1983) reports 16:8 L:D to be optimal for growth and reproduction. Though recent strides have been made in intensive culture other aspects such as reproduction still need examination.

Our intent is to develop operational technology for mosquitofish production. A production system will have two components. One component being a brood stock system to produce gravid females; the other, a hatchery, to obtain fry from gravid females.

The basic parameters to investigate for a brood stock system are sex ratios of brood stock and brood stock density. This work focuses on the first parameter. The proper sex ratio is important as fertilization of all brood females may not take place if the ratio of males to females is too low. Conversely if the ratio is too high the possibility of male-male aggression (Itzkowitz, 1971) may influence male-female interaction.

MATERIALS AND METHODS.-Mosquitofish were harvested from a local stock pond. Large females who did not pass through a 5 mm bar grader were excluded from spawning trials. Selected females were separated into three groups of 256. Each group was individually isolated in spawning baskets in separate tanks in the hatchery described below.

The hatchery consists of two identical systems. Each system consists of four fiberglass culture tanks (.91 m x 2.13 m x .46 m), one cylindrical polypropylene filter (208 liters) filled with 5 cm plastic bio-rings and fiberglass sump (.56 m).

Water was circulated via a 1/10 hp. pump and took 2.1 hours to cycle through the system. A 10 gallon water heater maintained water temperature at 25°C and water loss due to evaporation was replaced via a float valve. A 14:10 L:D photoperiod was maintained by overhead fluorescent lights.

The spawning baskets are plastic strawberry baskets lined with 8 x 8 mesh fiberglass screen. These baskets retain females while allowing newborn fry to escape.

Mature females void of fertilized embryos were desired to begin the experiment. To ensure this, females were initially isolated for one week in the hatchery and induced to drop fry by raising the water temperature to 30°C. Thev were then moved to a greenhouse system for three weeks to further develop ova. Thev were isolated for one week in the hatchery a second time and the process was repeated.

The greenhouse system consists of a post and truss greenhouse (15.25 m x 5.49 m) covered with greenhouse corrugated fiberglass. Contained within are three fiberglass circular tanks (3.66 m x .91 m) connected to a common biofilter, two cylindrical polypropylene tanks (568 liters) filled with 7.5 cm bio-rings and a 1.07 m wooden sump.

Water temperature was maintained at 24°C±2 by a 125,000 B.T.U. swimming pool heater. A 14:10 photoperiod was maintained with fluorescent lights. Water was circulated at 303 liters per minute by a .4 hp. sump.

No fry were observed in the hatchery after the second isolation nor did females show any signs of being gravid. They were now introduced to males to begin the mating trials.

Mature males were introduced into the greenhouse tanks at ratios of 1:1, 1:2 and 1:5, males to females (Table 1). Fish were allowed to mate for three weeks, females were collected, placed in the hatchery and the above spawning procedures were used. Number of males in the greenhouse

---

[1]Contra Costa Mosquito Abatement District, Concord, California 94520.

[2]University of California, Davis, California 95616.

were recorded at each isolation. After one week in the hatchery, females were returned to the greenhouse with the males. Fry production was recorded and the cycle was repeated for one additional spawn.

Fish were fed six times daily by automatic feeders at a daily rate of 5% of their biomass. Salmon starter (Rangen Mfg.) was used throughout the trials.

All tanks were scrubbed and siphoned monthly accounting for approximately 50% makeup water being added monthly.

A Hach DREL water quality kit was used weekly to monitor ammonia, nitrate, nitrite, dissolved oxygen, pH, carbon dioxide and chlorine levels in the greenhouse.

RESULTS.-Number of fry produced in each group was directly related to the ratio of males to females (Table 1). The first isolation produced 1764, 1604 and 1248 fry at the sex ratios 1:1, 1:2 and 1:5 respectively. The second isolation produced 520, 450, and 400 fry. Whether there was a higher proportion of gravid females in the 1:1 group or individual females actually dropped a higher average number of fry was not determined. On the basis of informal observations it is suspected that there was a higher proportion of gravid females. Regardless, there was a direct relationship between the number of males per tank and the number of fry produced.

Mortality of fish over the experiment was greater than expected. Female survival was 61%, 70% and 66% for the sex ratios 1:1, 1:2 and 1:5 respectively. Male survival was 46%, 64% and 64%.

Water quality was good throughout the experiment. Ammonia levels varied between .02-.37 mg/1. Dissolved oxygen ranged between 7.3 - 10.8 ppm. No significant variations in water quality were noted between tanks.

Females averaged .88 g at the start of the experiment and averaged 1.5 g at the conclusion. Males remained relatively the same size, .30 g at the start and .38 g at the end. This translates to a daily growth rate of 1.2% for females. Male growth rates are insignificant as they cease growth upon maturation (Kromholtz, 1948).

DISCUSSION.-The goal of the mosquitofish aquaculture facility is to produce the maximum number of fish. The female mosquitofish held at a 1:1 ratio with males clearly produced the most fry. Based on our previous fish density research (Downs and Beesley, 1983) it is projected that our greenhouse system can support 35 times the number of females. If fry production for each sex ratio is multiplied by this factor the difference in fry production between the ratios becomes more significant. It is expected as hatchery efficiency is improved this difference will be even greater.

The high mortality realized in this experiment may be due to senescence. We originally started this experiment with females retained by the 5 mm bar grader ($\bar{x}$ = 1.3 grams). After one month 50% of the females had died when there was no indication of infection and water quality parameters were excellent. We therefore selected smaller females to alleviate the possibility of senescence yet their growth was sufficient to exceed that of our previous set up. The rapid growth and reproduction demand placed on these fish may "burn out" the fish faster than normal which may lead to a continual need to replace brood stock every two to three months. There was no way to determine age of males selected and mortality may simply be attributed to the old age of male stock we selected.

On the basis of the above data and our stated goal of maximal fry production a 1:1 male-female ratio seems desirable. Future work will focus on brood stock densities, brood stock mortality, female isolation for dropping fry and brood stock replacement schedules.

REFERENCES

Downs, C. and C. Beesley. 1983. Continuous production of *Gambusia affinis*. Proc. Calif. Mosq. and Vector Contr. Assoc. 51:44-46.

Drazba, L. M. and G. A. Gall. 1980. Intensive culture of *Gambusia affinis* under conditions of minimal space. Mosquito Research Highlights, University of California June, 1980.

Table 1.-Male:Female Brood Stock Ratio's and Related Fry Production.

	1:1			1:2			1:5		
days	m	f	fry	m	f	fry	m	f	fry
0 pre-mating	-	256	-	-	256	-	-	256	-
35 males intro.	219	219	-	112	225	-	44	218	-
65 1st isolation	162	196	1764	90	205	1604	30	198	124
95 2nd isolation	104	162	520	70	182	450	28	157	40
Survival (%)	47	63		63	71		64	61	

Gall, G. A. E. 1983. Technology for the mass production of *Gambusia*. University of Calif. Davis. 83 pp.

Hass, R., and R. Pal. 1984. Mosquito larvivorous fishes. Bull. Entomol. Soc. Amer. 30(1): 17-25.

Itzkowitz, M. 1971. Preliminary study of the social behavior of male *Gambusia affinis* (Baird and Girard) (Pisces: Pocciliidae) in aquaria. Chesapeake Sci. 12:219-224.

Krumholz, L. A. 1948. Reproduction in the western mosquitofish, *Gambusia affinis* (Baird and Girard), and its use in mosquito-fish control. Ecol. Monographs 18:1-43.

Wurtsbaugh, W. A., and J. J. Cech, Jr. 1983. Growth and activity of juvenile mosquitofish: Temperature and ration effect. Trans. Amer. Fisheries Soc. 112:653-660.

COMPARATIVE MOSOUITO PREDATION EFFICIENCY OF MOSQUITOFISH AND JUVENILE SACRAMENTO BLACKFISH IN EXPERIMENTAL RICE PADDIES

Joseph J. Cech, Jr. and Alison L. Linden

Department of Wildlife and Fisheries Biology
University of California
Davis, California 95616

ABSTRACT

Twenty-one experimental rice paddies, 30.5 m x 3.5 m, at the UCD Rice Research Facility were stocked at the time of the first permanent water with either mosquitofish, *Gambusia affinis*, or Sacramento blackfish, *Orthodon microlepidotus*, or with both species in various combinations. Three paddies were stocked with 1,320 Sacramento blackfish; three were stocked with 10 mosquitofish; three with 30 mosquitofish; three with a 30:30 combination; one with a 64 blackfish: 30 mosquitofish combination; three with a 300 blackfish: 30 mosquitofish combination; and five controls had no fish stocked. In the data analysis, data from the 64:30 combination were combined with those from the 30:30 combination. Three thousand to 10,000 cultured *Culex tarsalis* mosquito larvae were stocked weekly into each paddy. Dipper samples for mosquito larvae were made weekly during the rice season, 60 dips per paddy, two days after mosquito stocking, but counts were low (i.e. less than ten larvae per 60 dips) until the last two weeks when many wild *Anopheles freeborni* larvae were also found. Larval counts and volumetric measurements of filamentous algae (*Cladophora*, *Spirogyra* and *Zygnema*) were made three times weekly during the last two weeks. At the end of the 12 week session, paddies were drained and the remaining fish and visible invertebrates counted.

Data from the last four dipper-sampling dates show the highest number of mosquito larvae in the Sacramento blackfish-stocked rice paddies ($p < 0.05$, ANOVA). Mean larval numbers were lowest in the "heavily stocked" mosquitofish paddies (57 ± 22, SEM) and the next lowest in the 30:30 combination paddies (74 ± 22); these numbers were not significantly different from the control paddies (82 ± 20), the 30:300 combination paddies (89 ± 25) or the "lightly stocked" mosquitofish paddies (104 ± 25). This low larvivorous ability of blackfish may concern their rapid growth to ca. 70 mm SL and a consequent shift to a filtering mode of feeding. The quantity of algae was inversely related to the number of mosquito larvae in the rice paddies. With the two paddies at the ends of the experimental rice field excluded from the analysis, there was significantly more algae in the "heavily stocked" mosquitofish paddies than in any of the other paddy categories ($p < 0.05$, ANOVA). The lowest mean algae volume was found in the Sacramento blackfish paddies; the next lowest algae volume was found in the "heavily stocked" blackfish plus mosquitofish combination paddies. Thus, the paddies containing the most blackfish seemed to grow the least algae. It is possible that juvenile blackfish, known to grow on strictly plant-based diets, cropped algae in their rice paddies.

At the end of the experiment, the control fields were virtually devoid of fish, indicating that our inflow and outflow screens and levees had remained intact. It was also noted that blackfish populations were at one-third to one-half of their stocked levels. In contrast, mosquitofish populations increased to very high levels; paddies stocked with 30 mosquitofish and those stocked with 30 mosquitofish plus 30 Sacramento blackfish had the most: 1,720 and 1,840 respectively. Mosquito control seemed to be a function of species rather than just the number of predatory fish because there was no significant difference between total fish numbers in the blackfish paddies and both the 300:30 combination paddies and the "lightly stocked" mosquitofish paddies, yet mosquito larval numbers were significantly higher in the blackfish paddies.

The lower number of larvae in the control paddies compared with the blackfish paddies might be attributable to the predatory invertebrate communities that developed there. The control and blackfish paddies had significantly more water striders than all other paddies ($p < 0.05$ by ANOVA). Importantly, the control paddies had significantly more notonectids than all of the other paddies, including the blackfish paddies ($p < 0.05$ by ANOVA). Notonectids are known invertebrate predators on mosquito larvae, and mosquitofish are known to prey upon notonectids. It seems that the notonectids provided predation equivalent to that of the mosquitofish and combination paddies in these experiments.

GREEN SUNFISH: FRIEND OR FOE OF RICE FIELD MOSQUITOES?

Leon Blaustein

Department of Entomology
University of California
Davis, California 95616

ABSTRACT

Studies over a four year period indicate that the impact of green sunfish (*Lepomis cyanellus*) on mosquito abundance in rice fields depends on factors such as abundance of alternative prey, abundance of green sunfish, the size of green sunfish, the species of mosquito and the presence of the mosquitofish, *Gambusia affinis*.

Correlational information suggests that alternative prey is very important. When alternative prey (such as ostracods, cladocerans and chironomids) densities were high, sunfish failed to reduce populations of either *Culex tarsalis* or *Anopheles freeborni*. When alternative prey densities were low, immature green sunfish were effective predators of *C. tarsalis* but were ineffective at reducing *A. freeborni* populations except when green sunfish immature densities were very high. Large green sunfish (ca 8 cm TL) did not reduce abundances of either species of mosquito.

Green sunfish depressed mosquitofish and invertebrate predator populations. Green sunfish populations were also depressed by mosquitofish. Despite a reduction of mosquitofish and invertebrate predator populations, and despite being an inefficient predator of *A. freeborni*, the addition of green sunfish enhanced control of *A. freeborni* provided by mosquitofish. In the presence of green sunfish, mosquitofish restricted themselves more to the rice stand where mosquitoes are present, and spend less time in the borrow pit. This altered habitat utilization may be a mechanism to explain how control of *A. freeborni* in rice fields by mosquitofish is enhanced when green sunfish are present.

THE INFLUENCE OF MOSQUITO CONTROL DITCHES ON THE GEOMORPHOLOGY OF TIDAL MARSHES IN THE SAN FRANCISCO BAY AREA: EVOLUTION OF SALT MARSH MOSQUITO HABITATS

Joshua N. Collins[1], Laurel M. Collins[2], Luna B. Leopold[3] and Vincent H. Resh[1]

ABSTRACT

Geomorphic studies of Petaluma marsh, a large remnant of the historical tidal marshlands of San Francisco Bay, suggest that ditching to control mosquitoes can influence basic processes of marsh development and maintenance. In unditched marshland, dynamic interactions between the erosion and sedimentation of small natural channels and the marsh surface adjust the geomorphology of the marsh to accommodate regional changes in sediment and water supplies. The addition of ditches to tidal drainage systems disrupts these natural processes of geomorphic adjustment. The primary effects of ditching are substantial decreases in tidal heights and currents in small headward channels. Secondarily, ditching prevents the headward erosion of small channels, reduces the rate of tidal inundation of interior marsh plains, and accelerates the retrogression of small channels into new salt marsh mosquito habitats. Ditching generally exempts the marsh surface from the direct geomorphic influence of the tides and promotes the sedimention of the headward reaches of tidal channel networks. These effects of mosquito control ditches and the costs of ditch maintenance can be minimized if natural geomorphic processes are considered in ditch design.

INTRODUCTION.-The continued success of mosquito abatement programs for tidal marshes depends upon the addition and maintenance of ditches that convey tidal water to and from mosquito habitat. Ditching produces rapid results and is more cost-effective than chemical control methods in both economic and environmental terms (Balling and Resh 1983a).

In the tidal marshlands of San Francisco Bay, mosquito control ditches are extensions of natural channel systems. Ditches are dug mechanically, are uniformly 45 cm wide and 60 cm deep, but range from just a few meters to more than 100 m in length. They connect small natural channels to isolated sites on the marsh surface that otherwise retain water and tend to support larval populations of the salt marsh mosquitoes *Aedes dorsalis* (Meigen) and *A. squamiger* (Coquillet). Abatement is achieved by tidal disruption of oviposition substrate (Resh and Balling 1983), exportation of larvae during ebb tide (Smith 1904), and predation by fish that gain access to sites through ditches (Ferrigno and Jobbins 1968). Since mosquito habitats are abundant, ditches are necessarily numerous. Even if the number of ditches is minimized, ditching will more than double the total length of all existing channels in some marshes.

Environmental studies of mosquito control ditches in Bay Area marshes have mainly examined the ecological effects of ditching. Patterns of abundance and distribution of terrestrial vascular plants (Balling and Resh 1983b), intertidal algae (Barnby et al. 1985), terrestrial invertebrates (Garcia and Schlinger 1972, Balling and Resh 1982, Barnby and Resh 1984), aquatic invertebrates (Barnby et al. 1985), intertidal fishes (Balling et al. 1980), and resident birds (Collins and Resh 1985) may be affected by ditching. These patterns vary with ditch age, distance from ditch banks, site-specific tidal regimen, and time of year. Perhaps the most fundamental fact that can be inferred from the literature about ecological effects of ditching is that, at least in the short-term, these effects are restricted to ditch margins. Based upon these ecological studies, Resh and Balling (1983) formulated recommendations for ditch design to reduce disturbances. Geomorphic effects were not considered in these designs. Whereas ecological effects appear to be localized, geomorphic effects of ditching are expected to be much more extensive, because ditching modifies the distribution of tidal flows through environments that are essentially intertidal in nature.

Recent investigations of the geomorphology of Petaluma Marsh, an 1100 ha remnant of historical estuarine marshland located along the Petaluma River about 10 km north of San Francisco Bay, have begun to reveal the evolutionary histories of a variety of common physiographic features (Collins et al. in press). Such studies can provide a basis to predict the long-term functional relationships between ditching and natural processes of marsh development and

---

[1]Division of Entomology and Parasitology, University of California, Berkeley, CA 94720.

[2]Department of Geology and Geophysics, University of California, Berkeley, CA 94720. Present address: East Bay Regional Park District, 11500 Skyline Blvd., Oakland, CA 94619.

[3]Department of Geology and Geophysics, University of California, Berkeley, CA 94720.

maintenance. The purpose of this paper is to introduce the geomorphic concepts that are principally involved in the analysis of ditch effects, and to describe how the evolution of mosquito habitats can be influenced by mosquito control ditches. The following discussions are largely based upon unpublished information from ongoing studies of tidal marsh geomorphology in the San Francisco Bay area. The documentation of these topics will be included in future publications.

THE DYNAMIC NATURE OF TIDAL MARSHES.-Estuarine marshes as physical systems tend to maintain quasi-equilibrium between tidal (i.e., marine) and non-tidal (i.e., upland) influences. An exact equilibrium cannot be achieved because the factors that influence marshes are dynamic. For example, throughout a tidal marsh the rate and magnitude of transport and storage of water and sediment adjust to regional changes in sediment supply and tidal regimen. Continuous adjustments on a small scale over long periods of time cause substantial changes in marsh structure (i.e, the substrate and associated sessile organisms) and configuration (i.e., the overall form, size, and spatial arrangements of storage and transport features). Young marshes gain elevation more rapidly than mature marshes, which through millenia tend to maintain the same average elevation relative to sea level. Mature marshes continue to build upward as sea level rises, unless limited by insufficient peat production or upland sediment sources.

The physiography of tidal marshes is both the cause and also the result of the distribution of tidal energy. For example, the changes in channel morphology that are caused by erosion, transport, and deposition are accomplished by the tidal energy that channels convey. This energy enters and departs a tidal drainage system through a single terminous, referred to as the tidal source. For each tidal cycle, the energy potential (and the potential for morphological change) is proportional to the tidal amplitude. Over time, the average potential is proportional to the average tidal range. Therefore, the amount of tidal energy that is available for geomorphic work tends to be greatest at the marsh margins, where tidal range is largest, and least at the marsh interior, where tidal range is smallest. In contrast, the relative geomorphic influence of non-tidal factors increases with distance away from a tidal source, such that interior marsh plains between drainage systems are transitional between tidal and non-tidal influences (Figure 1). In this sense, the headward regions of tidal drainages within a marsh are analogous to a tidal marsh as a whole.

EVOLUTION OF MOSQUITO HABITATS.-Zones of transition between antagonistic geomorphic processes, such as erosion and sedimentation, are subject to frequent physical change. In the most headward regions of natural tidal drainages, substantial changes in sedimentary processes are initiated by structural

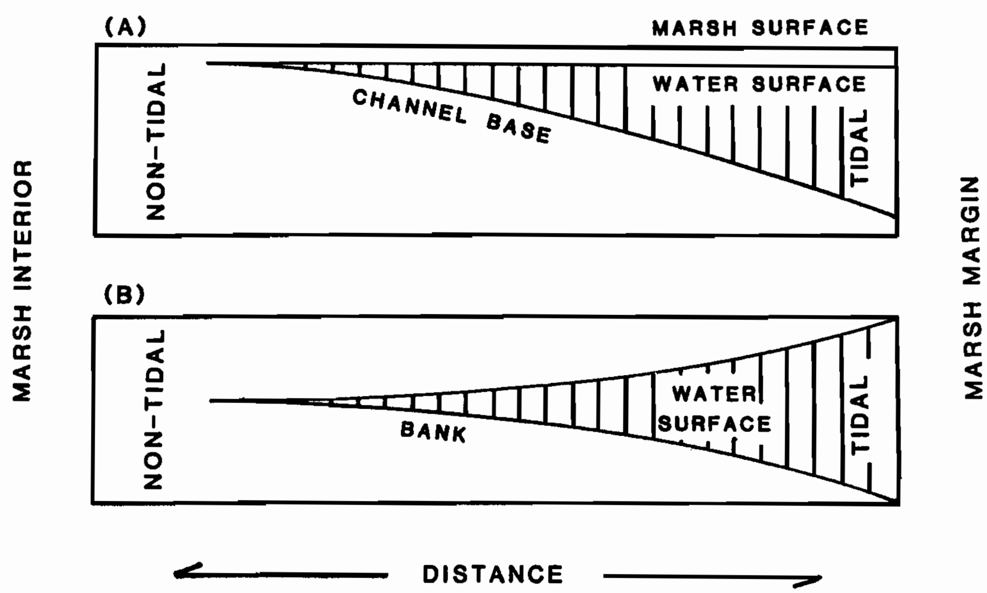


Figure 1.-Schematic diagram of the relative influences of tidal and non-tidal processes on estuarine marsh geomorphology. A generalized tidal drainage system is shown (A) in crossection and (B) as viewed from above (i.e, in plan form).

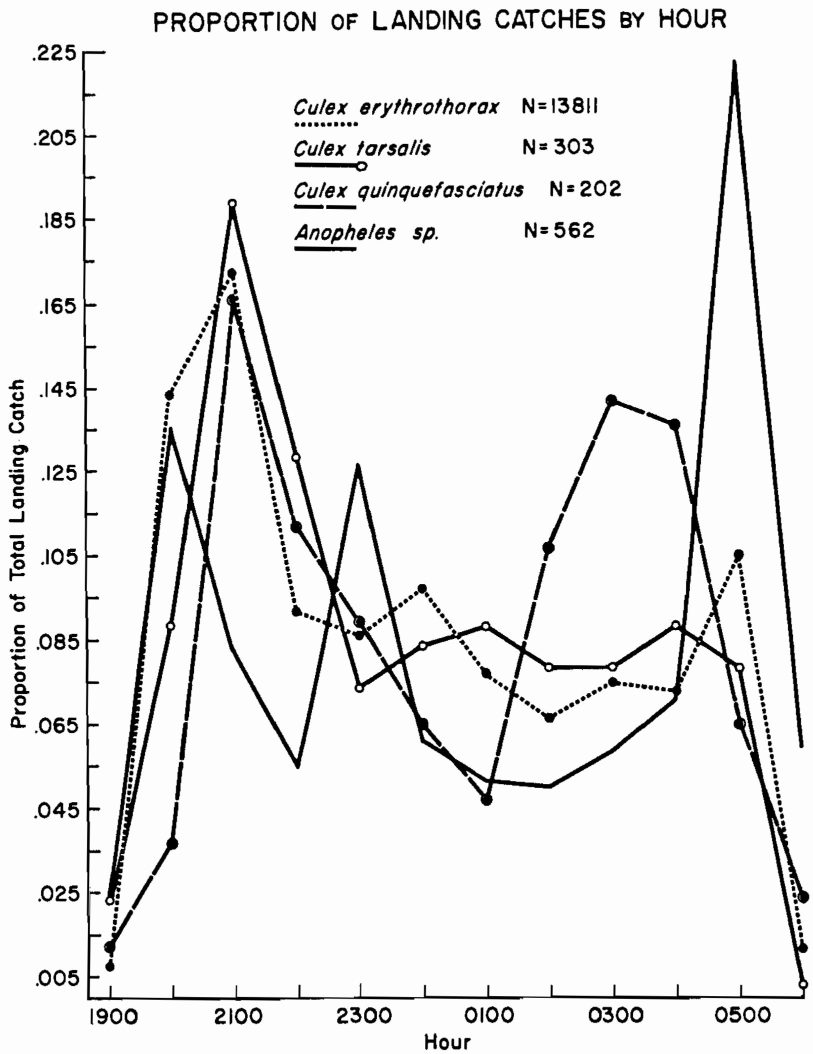


FIGURE 2

pattern is presumably governed by physiological rhythms of the species as well as reactions to cycle of light, temperature, and humidity.

In addition to these general factors that influence blood feeding, there also must be specific stimuli which lead the mosquito to its host. It is clear that some hosts, especially people, are bitten more frequently than are others. Odor, age, race, health, and proximity to a mosquito breeding site are just some of the reasons given to explain these differential biting rates (Lounibos, Rey, and Frank 1985).

Landing collections, although not economical in terms of time, do assess the importance of putative vector species in terms of their relative attraction to man. Although we have no absolute measure of the size of populations studied, it is clear that landing catches, particularly for *Cx. quinquefasciatus*, could be especially useful for adult mosquito surveillance in areas of urban transmission of St. Louis encephalitis virus.

ACKNOWLEDGMENTS.-The authors thank Orange County Vector Control District and Southeast Mosquito Abatement District for their help in this study. We also thank Sylvia Barr for preparing the graphics.

REFERENCES

Lounibos, L. P., J. R. Rey, and J. H. Frank. 1985. Ecology of Mosquitoes: Proceedings of Workshop. Florida Medical Entomology Laboratory, Vero Beach: pp. xxii + 579.

Monath, T. P. (Editor). 1980. St. Louis Encephalitis. Washington, D.C.: American Public Health Association, pp. xx + 680.

Reeves, W. C. and W. McD. Hammon. 1962. Epidemiology of the Arthropod-Borne Viral Encephalitides in Kern County, California 1943-1952. Berkeley, CA.: University of California Press, pp. x + 257.

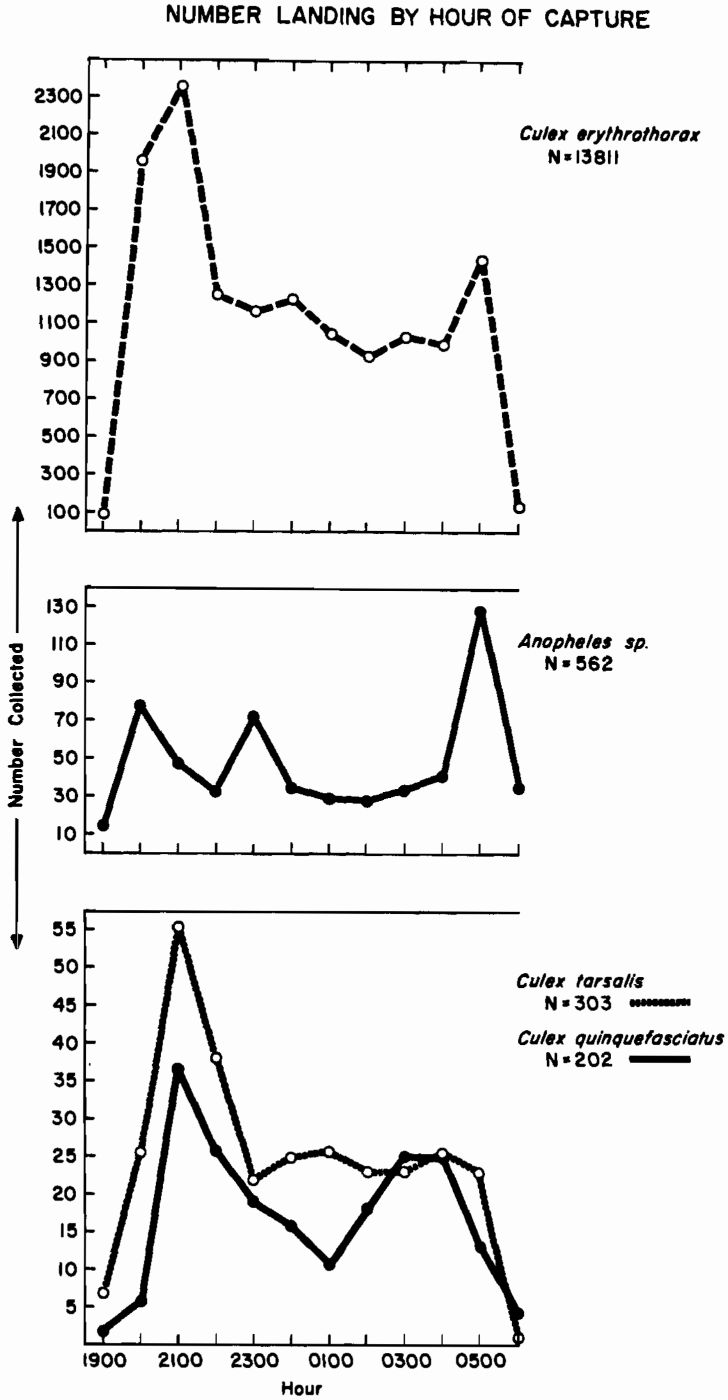


FIGURE 1

activity around 0300 while activity of the other three species is suppressed at this time.

The biting pattern of the *Anopheles* is quite different (Figure 2). Thirteen point five percent were captured at 2000, one hour before peak activity of the three other species. A second peak, 12.5%, occurred at 2300, two hours after peak activity of the three other species. Biting was then reduced to a level below the other species until 0500, when maximum *Anopheles* activity occurred. Twenty-two percent of the total *Anopheles* collected were taken at this hour.

Every mosquito species characteristically bites at certain times of day. This activity

HUMAN BAIT COLLECTIONS OF MOSQUITOES IN A SOUTHERN CALIFORNIA FRESHWATER MARSH

Stanton E. Cope, A. Ralph Barr, Michael J. Bangs,

Amy C. Morrison, and Pensri Guptavanij

School of Public Health
University of California
Los Angeles, California 90024

INTRODUCTION.-The blood feeding habit of mosquitoes, particularly in reference to parasite transmission, has been the subject of a considerable amount of study. Yet, our knowledge is still incomplete, especially in regard to specific stimuli that govern host attraction and biting behavior of particular species.

Culex tarsalis and *Culex quinquefasciatus* have been implicated as vectors of several arboviruses, including St. Louis encephalitis and Western equine encephalomyelitis (Monath 1980, Reeves and Hammon 1962). If we are interested in the transmission of disease-producing agents to man, or the annoyance of man by vectors, it is imperative that we study the attraction of vectors to man. This study was designed to assess the relative man-biting behavior of various mosquito species present in a southern California freshwater marsh.

STUDY SITE.-The area chosen for study was the San Joaquin Marsh in Irvine, California, located approximately 45 miles southeast of Los Angeles and 1.25 miles upstream from the Upper Newport Bay. This 202 acre wildlife reserve is home to over 200 species of birds, half of which are migratory, 24 species of mammals, numerous reptiles and amphibians, and roughly 160 species of plants.

The marsh contains 5 natural ponds and 13 shallow duck ponds designed to attract waterfowl. A system of earth-fill dikes, topped by trails, separates the 18 ponds and provides access throughout the site. Deposits of densely packed peat and fine clay underlie the pond system, providing a highly compressible barrier that lets through little water. Dry bluffs, with remnant coastal sage community and numerous rodent burrows, lie just north and west of the reserve.

METHODS.-Weekly collecting trips were made to the marsh from June 3 to September 30, 1985. On each trip, two workers, sitting on stools approximately 10 meters apart along a road, made bare-leg collections of mosquitoes landing on them. Collections were made for 15 minutes each hour of the night from 1900 hours until 0600 hours the next morning, for a total of six collecting hours per night. Temperature and humidity were measured by psychrometer for each collecting period. Mosquitoes were mouth aspirated, transferred to holding vials, placed on ice, and taken to our laboratory at the UCLA School of Public Health where they were identified and counted.

RESULTS AND DISCUSSION.-A total of 14,899 mosquitoes, representing five different species, was collected, with an average per collecting night of 828 (Table 1). The range per collecting night was 37-2084 while the range of one 15 minute collecting period was 0-893.

Table 1.-Landing collections-San Joaquin Marsh-1985.

Total mosquitoes collected	14899
Average per collecting night	828
Range per collecting night	37-2084
Range-one 15 minute period	0-893

Only 21 *Culiseta inornata* females were collected so this species will not be further considered in this paper. *Culex erythrothorax* (Figure 1, top graph), showing a bimodal biting pattern, accounted for 13,811 or 92.7% of the total collected. Peak biting activity occurred in early evening, followed by relatively lower levels from 2200 until 0400, and finally, a smaller peak at 0500.

Five hundred sixty-two *Anopheles* (Figure 1, middle graph) were collected which represent 3.8% of the total. We are currently calling this mosquito the southern form of *An. occidentalis*. This species exhibited a trimodal biting pattern, with small peaks around 2000 and 2300, reduced activity from midnight until 0400, and then a sharp peak at 0500 hours.

Culex tarsalis accounted for 2.0% of the total, or 303 mosquitoes (Figure 1, bottom graph). This species exhibited a strong peak around 2100 followed by a relatively constant biting rate throughout the night until 0600.

Culex quinquefasciatus, with a total of 202 mosquitoes or 1.4%, also showed a peak around 2100 (Figure 1, bottom graph). The biting then declined showly until 0100, peaked again around 0400, and then dropped off.

Figure 2 shows the relative proportions of the total landing catch for each species by hour of capture. *Cx. tarsalis, erythrothorax,* and *quinquefasciatus* all show peak biting activity around 2100 with 19%, 17%, and 16.6% of each species respectively, biting at this time. *Cx. quinquefasciatus* also shows increased biting

USDA, U.S. Govt. Printing Off., Wash., D.C. 545 pp.

Balling, S. S. and V. H. Resh. 1984. Seasonal patterns of pondweed standing crop and *Anopheles occidentalis* densities in Coyote Hills Marsh. Proc. Calif. Mosq. and Vector Contr. Assoc. 52:122-125.

Benke, A.C., P.H. Crowley, and D.M. Johnson. 1982. Interactions among coexisting larval Odonata: an in situ experiment using small enclosures. Hydrobiologia 94:121-130.

Collins, J. N. and V. H. Resh. 1985. Factors that limit the role of immature damselflies as natural control agents at Coyote Hills Marsh. Proc. Calif. Mosq. and Vector Contr. Assoc. 53:87-92.

Collins, J. N., S. S. Balling, and V. H. Resh. 1983. The Coyote Hills Marsh Model: calibration of interactions among floating vegetation, waterfowl, invertebrate predators, alternate prey, and *Anopheles* mosquitoes. Proc. Calif. Mosq. and Vector Contr. Assoc. 51:69-73.

Collins, J. N., K. D. Gallagher, and V. H. Resh. 1985. Thermal characteristics of aquatic habitats at Coyote Hills Marsh: implications for simulation and control of *Anopheles* populations. Proc. Calif. Mosq. and Vector Contr. Assoc. 53:83-86.

Cook, S. F. 1964. The potential of two native California fish in the biological control of chironomid midges (Diptera: Chironomidae). Mosq. News 24:332-333.

Cook, S. F., J. D. Conners, and R. L. Moore. 1964. The impact of the fishery upon the midge populations of Clear Lake, Lake County, California. Ann. Entomol. Soc. Amer. 57:701-707.

Feminella, J. and V. H. Resh. 1986. The effects of crayfish grazing on mosquito habitat at Coyote Hills Marsh. Proc. Calif. Mosq. and Vector Contr. Assoc. 54:101-104.

Fleming, K. J. and J. K. Schooley. 1984. Foraging patterns and prey selection by marsh fish. Proc. Calif. Mosq. and Vector Contr. Assoc. 52:139.

Gall, G., J. Cech, R. Garcia, V. Resh, and R. Washino. 1980. Mosquitofish - an established predator. Calif. Agric. 34(3):21-22.

Lamberti, G. A. and V. H. Resh. 1984. Seasonal patterns of invertebrate predators and prey in Coyote Hills Marsh. Proc. Calif. Mosq. and Vector Contr. Assoc. 52:126-128.

Legner, E. F. and R. D. Sjogren. 1984. Biological mosquito control: broader recognition of past successes and future research emphases. Proc. Calif. Mosq. and Vector Contr. Assoc. 52:102-107.

McGinnis, S. M. 1984. Freshwater Fishes of California. University of California Press, Berkeley, CA. 316 pp.

Wiens, J. A., J. F. Addicott, T. J. Case, and J. Diamond. 1986. Overview: the importance of spatial and temporal scale in ecological investigations. In: Community ecology, J. Diamond and T. J. Case (eds.), pp. 145-153, Harper & Row, New York, N.Y. 665 pp.

Zar, J. H. 1974. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J. 620 pp.

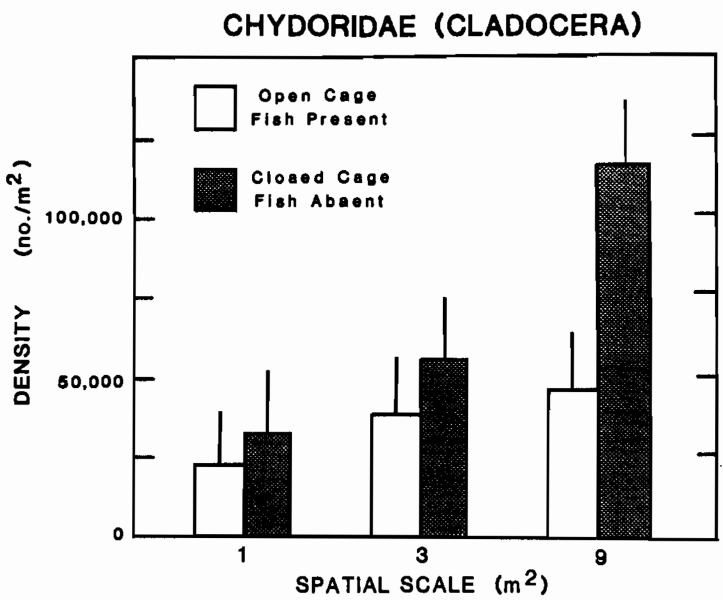


Figure 2.-Mean density of Chydoridae (number/m^2) in the presence (open cages) and absence (closed cages) of fish. Vertical lines indicate one standard error.

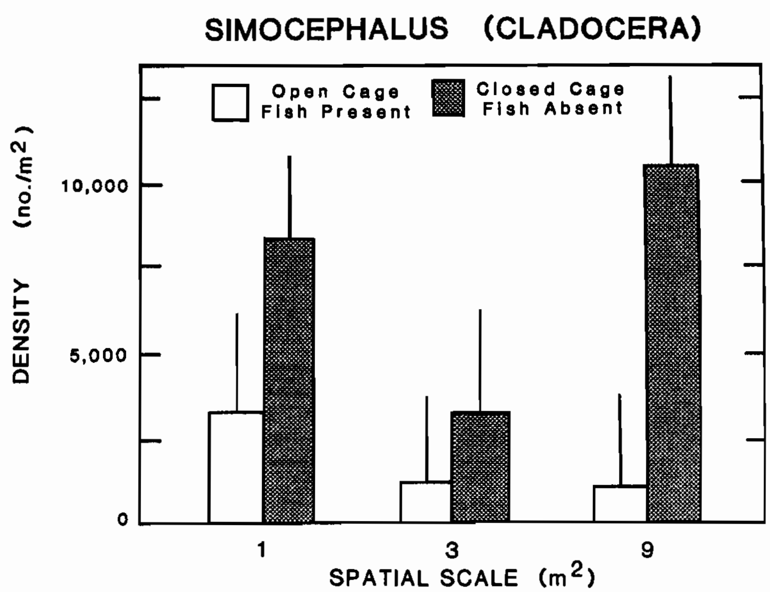


Figure 3.-Mean density of *Simocephalus vetulus* (number /m^2) in the presence (open cages) and absence (closed cages) of fish. Vertical lines indicate one standard error.

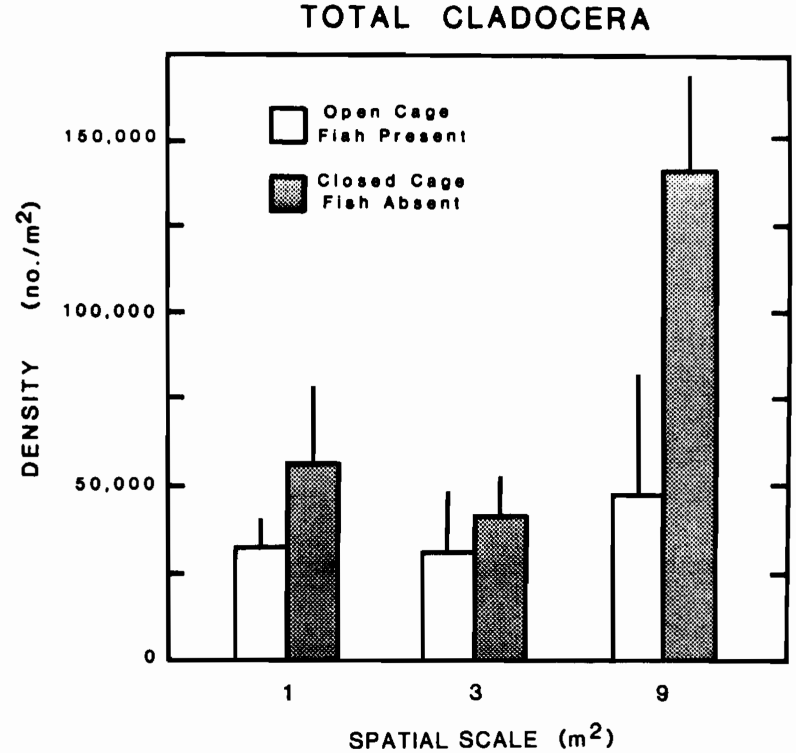


Figure 1.-Mean density of Cladocera (number/m^2) in the presence (open cages) and absence (closed cages) of fish. Vertical lines indicate one standard error.

As with total Cladocera, there was a trend towards increased density of both Chydoridae and *Simocephalus* when fish were removed (Figures 2 and 3). Again, this effect was statistically significant only at the largest spatial scale for Chydoridae (t-test $p < 0.05$) and nearly significant for *Simocephalus* ($p = 0.06$).

One explanation for the relationship between size of exclosure and the strength of measured response is the increased influence of edge effects (due to the higher ratio of exclosure surface area to volume) at smaller spatial scales. For example, the relatively large mesh size of the screen walls allowed smaller size classes of cladocerans to move into or out of the exclosures. High rates of emmigration or immigration of prey could mask the positive (or negative) effects of predator removal on local prey density (Allan 1983). Thus, statistically significant effects might be measured only above a minimum threshold size of exclosure. Below this threshold size any density response to predator removal could be obscured by the flux of prey through the walls of the exclosure.

In summary, the importance of spatial scale must be considered in the design of field experiments. The appropriate experimental scale varies with the biology of the species under study and the nature of the question being addressed (Wiens et al. 1986). The results of our experiment suggest that the following "rule of thumb" may be appropriate for studying the efficacy of fish predators as mosquito control agents in freshwater marshes: use the largest possible size of experimental unit, within the constraints imposed by practical considerations of time, labor, and expense.

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.

REFERENCES

Allan, J. D. 1983. Predator-prey relationships in streams. In: Stream Ecology: Application and Testing of General Ecological Theory, J. R. Barnes and G. W. Minshall (eds.), pp. 191-229, Plenum Press, New York, N.Y. 399 pp.

Bay, E. C. 1976. Part I: Parasites and predators introduced against arthropod pests. Culicidae. In: Introduced Parasites and Predators of Arthropod Pests and Weeds: a World Review, C. P. Clausen (ed.), pp. 339-346. Agric. Handbk. No. 480, ARS,

Table 1.-Effects of cage treatments on relative abundance of fish. Numbers given are mean number of fish trapped in 24 h, with 1 standard error in parentheses. Only data from the large scale (9 m^2) plots are included ($n = 3$); results were similar for small and medium scale plots.

Species	Closed Cage	Open Cage	No Cage
Cottus asper (prickly sculpin)	1.5 (1.1)	10.7 (1.2)	5.8 (1.4)
Cyprinus carpio (carp)	0 (0)	0 (0)	0.2 (0.2)
Gambusia affinis (mosquitofish)	0 (0)	3.3 (1.2)	3.8 (1.3)
Gasterosteus aculeatus (threespine stickleback)	0 (0)	17.3 (7.9)	28.0 (13.7)
Lepomis microlophus (redear sunfish)	0 (0)	22.7 (6.4)	21.5 (11.6)

cage effects) that can affect the density of predators and prey (Benke et al. 1982). Cage effects were measured by comparing the abundance of fish and prey in the open cage treatment with uncaged control plots. Fish were present in both the open cage and uncaged control plots; thus, if any differences in cladoceran densities were observed between the two treatments this would be due to the influence of the cage itself (i.e. a cage effect).

The influence of the size, or spatial scale, of experimental unit on the outcome of the fish removal experiment was tested by crossing the three treatments discussed above with three sizes of plots or cages: 1 m^2 (1 m x 1 m), 3 m^2 (1.73 m x 1.73 m), and 9 m^2 (3 m x 3 m). The resulting nine treatment combinations were each replicated three times using a randomized block design (Zar 1974).

The experiment began on 24 June 1985. Invertebrates were sampled on 23-25 July. The relative abundance of fish was measured on 29 July - 1 August using minnow traps lined with galvanized window screen. A smaller version (0.01 m^2) of the pull-up sampler described by Balling and Resh (1984) was used to collect invertebrates in the water column and pondweed canopy. Three samples were taken per plot, with the location of samples determined by randomly chosen coordinates. To reduce the influence of potential edge effects, no samples were taken within 10 cm of cage walls. Samples were processed following the methods given in Lamberti and Resh (1984). Two of the three samples from each plot were randomly selected for identification and enumeration of Cladocera.

RESULTS AND DISCUSSION.-Closed cages were effective in excluding fish; only a few sculpins were found in the these cages (Table 1). Fish densities and species composition in the open cages were similar to those in the uncaged control plots (Table 1), suggesting that fish moved freely through the vertical openings in the walls of the open cages. There were, however, significantly more *Cottus* caught in the open cages than in the uncaged plots (t-test, $p < 0.05$). The increased relative abundance of *Cottus* in the open cage plots should have had a minimal effect on cladoceran populations for two reasons: (1) *C. asper* is a bottom-dwelling fish that feeds primarily on midge larvae (Diptera: Chironomidae) and other benthic insects (Cook 1964, Cook et al. 1964, McGinnis 1984), and (2) the higher relative abundance of *Cottus* in open cage plots compared with uncaged plots represented less than 10% of the total number of fish present.

There were no significant differences between the open cage treatment and the uncaged treatment in the densities of total Cladocera, Chydoridae, or *Simocephalus* at any of the three spatial scales. This lack of significant cage effects indicates that this experimental design was effective. Such information concerning the effects of cages on predator and prey densities is important when evaluating the impact of predators on prey populations. Interpretation of the results of predator-removal (or addition) experiments and the extrapolation of these results to natural conditions is difficult unless the magnitude of cage effects in known. The use of the three caging treatments (closed cage, open cage, and uncaged plots) allowed clear evaluation of both cage effects and the effects of predator removal.

The removal of fish resulted in increased density of Cladocera at all three spatial scales (Figure 1). This effect, however, was statistically significant only at the largest spatial scale (t-test, $p < 0.05$). The response of Cladocera to fish removal and spatial scale was analyzed further at the family level (Chydoridae) and at the species level (Daphnidae: *Simocephalus vetulus*).

SPATIAL-SCALE CONSIDERATIONS IN PREDATOR-PREY EXPERIMENTS

Bruce K. Orr and Vincent H. Resh

Division of Entomology and Parasitology
University of California
Berkeley, California 94720

ABSTRACT

A field study conducted at Coyote Hills Marsh (Alameda County, California) demonstrated that spatial scale can influence the outcome of predator-removal experiments. The densities of several taxa of Cladocera (the daphnid *Simocephalus vetulus*, Chydoridae, and all taxa of Cladocera combined), which are important alternative prey for mosquito predators (e.g. *Gambusia affinis* and *Gasterosteus aculeatus*), increased when fish were removed. However, statistically significant increases in the density of these prey taxa could only be demonstrated at the largest of the spatial scales examined. Edge effects (e.g. high rates of prey movement across the boundaries of exclosures) may mask the effects of predator removal at smaller spatial scales. These results illustrate that spatial scale may be as important as temporal scale (i.e. the duration of the experiment) in the design of predator-prey experiments.

INTRODUCTION.-The use of general predators, especially the mosquitofish *Gambusia affinis*, can be an important component of mosquito control programs (Bay 1976, Gall et al. 1980, Legner and Sjogren 1984). The degree of control provided by general predators is affected by a variety of factors, including the type and abundance of alternative prey available. Thus, knowledge of the interactions between mosquito predators and alternative prey under field conditions is often essential in the design of a successful control program. Field experiments are generally the best means of acquiring this type of information.

Given the need to conduct such field experiments, how does one determine the appropriate scale of study? Both spatial and temporal scales may affect the outcome of the study and our ability to perceive the relevant processes involved (Wiens et al. 1986). To date, most emphasis has been placed on the influence of temporal scale on experimental design of predator-prey studies. However, the size and location of experimental plots is also of critical importance in designing these studies.

This paper focuses on the influence of spatial scale in assessing the outcome of predator-removal experiments. In particular, we discuss preliminary results from an experiment designed to assess (1) the impact of an assemblage of fish species on the abundance of invertebrates that are alternative prey for mosquito predators and (2) the influence that the spatial scale of experimental manipulation may have on the outcome of such predator-removal experiments.

STUDY SITE.-The experiment was conducted at Coyote Hills Marsh (Fremont, Alameda County, California), a man-made freshwater marsh located along the southwestern margin of San Francisco Bay. The interior of the marsh is dominated by sago pondweed (*Potamogeton pectinatus*), which forms a dense floating canopy from late spring to early autumn (Balling and Resh 1984). This canopy of pondweed provides habitat for a variety of invertebrates, including *Anopheles* and *Culex* mosquitoes (Collins et al. 1983, Lamberti and Resh 1984). In addition, five species of fish (*Cottus asper, Cyprinus carpio, Gambusia affinis, Gasterosteus aculeatus*, and *Lepomis microlophus*) occur in the pondweed community at the study site. Microcrustacea, especially Cladocera, and midge larvae (Diptera: Chironomidae) are the dominant invertebrates in the pondweed community. These invertebrates are important prey in the diets of fish at Coyote Hills Marsh (Fleming and Schooley 1984; B.K. Orr, unpublished data). Additional information on the physical and biological characteristics of Coyote Hills Marsh can be found in Collins et al. (1983), Balling and Resh (1984), Lamberti and Resh (1984), Collins and Resh (1985), Collins et al. (1985), and Feminella and Resh (1986).

METHODS.-The impact of fish predators on selected taxa of alternative prey (Chydoridae, the daphnid *Simocephalus vetulus*, and total numbers of Cladocera) in the pondweed community was assessed by comparing fish and invertebrate densities in caged plots (hereafter referred to as "closed cage treatment" or "exclusion cages") that excluded fish with those in open cage plots in which fish were present. Exclusion cages consisted of 1 m high panels of fiberglass window screen (1 mm mesh size) suspended from a floating framework of 1" PVC pipe. The bottom edge of each screen wall was weighted with iron reinforcing bar and embedded at least 10 cm into the sediment. Retaining corner posts kept the floating framework in position. The screen walls of the closed cages excluded fish but allowed water, young cladocerans, and other small prey to move into or out of the exclosures. Minnow traps were used to remove fish from exclusion cages.
exclusion cages except that fish could enter each cage through 25 cm wide vertical openings that were cut in each side of the cage at the beginning of the experiment (the number of openings per wall varied with size of cage so that the total amount of screen removed was equal to about 25% of the total surface area of each wall). The use of cages in field experiments may result in unwanted changes in environmental conditions (i.e.

Mosq. and Vector Contr. Assoc. 52:140.
Dean, J. L. 1969. Biology of the crayfish *Orconectes causeyi* and its use for control of aquatic weeds in trout lakes. U.S. Bur. Sportfish. Wildlife Tech. Pap. 24:3-15.
Farley, D. G. 1980. Prey selection by the mosquitofish *Gambusia affinis* in Fresno County rice fields. Proc. Calif. Mosq. and Vector Contr. Assoc. 48:51-55.
Hess, A. D. and J. F. Hall. 1943. The intersection line as a factor in anopheline ecology. J. Nat. Malaria Soc. 2:93-98.
Huner, J. V. and J. E. Barr. 1984. Red swamp crayfish biology and exploitation. Publ. Louisiana Sea Grant Coll. Prog.: Louisiana State University, Baton Rouge, Louisiana.
Lodge, D. M. 1984. The role of macrophyte herbivory and predation by crayfish in determining benthic community structure in lakes. 32nd North Amer. Benthol. Soc. Meet., Raleigh, N.C. (abstract).
Miura, T., R. M. Takahashi, and F. S. Mulligan, III. 1978. Evaluation of the effectiveness of predaceous insects as a mosquito control agent. Proc. Calif. Mosq. and Vector Contr. Assoc. 46:80-81.
Penn, G. H., Jr. 1943. A study of life history of the Louisiana red crawfish, *Cambarus clarkii* Girard. Ecology 24:1-18.
Rickett, J. D. 1974. Trophic relationships involving crayfish of the genus *Orconectes* in experimental ponds. Prog. Fish Cult. 36:207-211.
Rozeboom, L. E. and A. D. Hess. 1944. The relation of the intersection line to the production of *Anopheles quadrimaculatis*. J. Nat. Malaria Soc. 3:169-179.

Figure 2.-Photograph of submerged crayfish exclosure (and two other exclosures in background) set within 1 m of the marsh margin, where the "halo" zone was observed in 1983 and 1984. Note dense surface canopy of pondweed within the exclosure, compared with the absence of surface canopy in adjacent open-water areas where crayfish could graze freely. The series of ropes set to one side of the exclosures were used to guide a small boat through the study area so that physical disturbance of the rooted plants and surface canopy could be minimized. (Photo: J. N. Collins, July 1985).

fication of *P. clarkii*. Support for these studies was provided by the University of California Mosquito Research Funds and the Coastal Region Mosquito Abatement Districts.

REFERENCES

Abrahamsson, S. A. A. 1966. Dynamics of an isolated population of the crayfish *Astacus astacus* Linn. Oikos 17:96-107.

Balling, S. S. and V. H. Resh. 1984. Seasonal patterns of pondweed standing crop and *Anopheles occidentalis* densities in Coyote Hills Marsh. Proc. Calif. Mosq. and Vector Contr. Assoc. 52:122-125.

Bence, J. R. and W. M. Murdoch. 1982. Ecological studies of insect predators and *Gambusia* in rice fields: a preliminary report. Proc. Calif. Mosq. and Vector Contr. Assoc. 50:48-53.

Collins, J. N., S. S. Balling, and V. H. Resh. 1983. The Coyote Hills Marsh Model: calibration of interactions among floating vegetation, waterfowl, invertebrate predators, alternate prey, and *Anopheles* mosquitoes. Proc. Calif. Mosq. and Vector Contr. Assoc. 51:69-73.

Collins, J. N. and V. H. Resh. 1984. Do waterfowl affect mosquitoes in Coyote Hills Marsh? Proc. Calif. Mosq. and Vector Contr. Assoc. 52:129-133.

Curtin, S.L., T.W. Young, and J.K. Schooley. 1984. Effects of pondweed density on predator efficiency: *Gambusia affinis* and *Gasterosteus aculeatus* as predators on *Anopheles occidentalis* larvae. Proc. Calif.

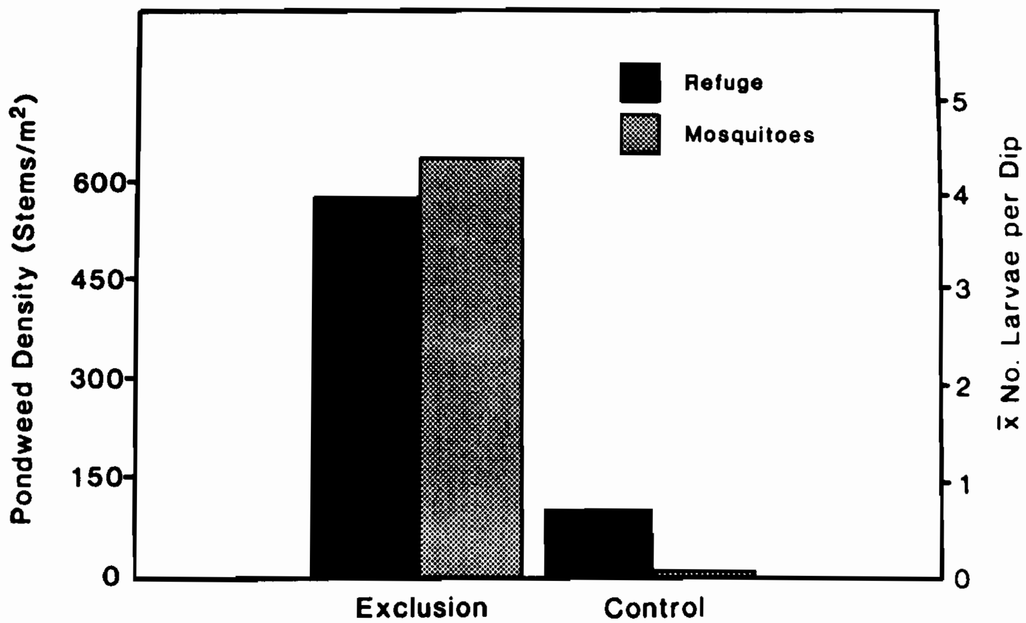


Figure 1.-Effects of crayfish exclusion on amount of mosquito refuge (pondweed) and mosquito larval abundance, 9 - 17 July 1985. Exclusion = plots where crayfish were prevented from grazing; Control = plots where crayfish had free access to pondweed. Stem density and mosquito abundance in each plot represent $\bar{x}$'s of five subsamples (N = 6). Differences between exclusion and control plots for both pondweed and mosquito density were significant ($p < 0.05$, Mann-Whitney U Test).

higher densities of *An. occidentalis* (4.4 ± 1.4 larvae/dip, $\bar{x}$ ± SE) than the open control plots (0.03 ± 0.03 larvae/dip, $\bar{x}$ ± SE) ($p < 0.05$, Mann-Whitney U Test; Figure 1). Mosquito densities in the crayfish exclosures were similar to those found in the dense pondweed beds of the marsh interior.

DISCUSSION.-Red swamp crayfish at Coyote Hills Marsh reduce pondweed density and, most likely, are responsible for the halo zone that forms at the marsh periphery. The extent of pondweed reduction and width of this zone may vary with crayfish density. For example, during August 1984, the halo zone composed 10% of the total marsh surface, whereas in August 1985 when crayfish density was 40% higher (J. Feminella, unpublished data), 70% of the marsh was cleared of pondweed. Reductions in aquatic vegetation are frequently associated with high crayfish densities (Abrahamsson 1966, Rickett 1974, Lodge 1984) and in some cases crayfish may completely clear macrophytes from littoral zones of lakes (Dean 1969).

Mosquito populations were essentially eliminated in areas lacking surface canopies of pondweed. Intense predation by *G. affinis* and *G. aculeatus* was largely responsible for these reductions in larval densities. In laboratory predation trials, Curtin et al. (1984) found an inverse relationship between pondweed abundance and *G. affinis* and *G. aculeatus* predation rates on *An. occidentalis*. Similar associations between aquatic vegetation and anopheline mosquitoes have been reported (Hess and Hall 1943, Rozeboom and Hess 1944, Balling and Resh 1984).

Other large herbivores have been shown to be important in regulating mosquito refuge at Coyote Hills Marsh. Dabbling ducks (e.g. mallards, gadwalls, pintails) can also disrupt the surface canopy and accelerate pondweed senescence, dramatically reducing mosquito habitat. However, because these herbivores migrate, their ability to regulate this habitat will depend upon the degree of synchronization between migratory patterns and pondweed phenology (Collins and Resh 1984). In contrast, *P. clarkii* (and other benthic herbivores), which are year-round residents of aquatic systems, are perhaps more effective in long-term management programs designed to control macrophytes that shelter mosquito populations. Furthermore, *P. clarkii* grazes a wide variety of submersed aquatic plants, has a high reproductive output, and can tolerate extreme environmental fluctuations (Penn 1943, Huner and Barr 1984). Thus, this species has high potential for use in vector-control programs in a variety of freshwater marshes.

ACKNOWLEDGMENTS.-This study is the result of a cooperative effort among personnel of the University of California at Berkeley, the East Bay Regional Park District, and the Alameda County Mosquito Abatement District. We thank Dr. Horton H. Hobbs, Jr. (Smithsonian Institution, Washington D.C.) for confirming the identi-

EFFECTS OF CRAYFISH GRAZING ON MOSQUITO HABITAT AT COYOTE HILLS MARSH

Jack W. Feminella and Vincent H. Resh

Division of Entomology and Parasitology
University of California
Berkeley, California 94720

ABSTRACT

The dense surface canopy of sago pondweed (*Potamogeton pectinatus*) is an important habitat for larval *Anopheles occidentalis* at Coyote Hills Marsh (Alameda County, CA). To determine if herbivorous crayfish (*Procambarus clarkii*) can regulate biomass of pondweed, crayfish were eliminated from selected quadrats along marsh margins, using 3 m^2 exclosures. The exclusion of crayfish resulted in a six-fold increase in pondweed stem density compared with that of adjacent control areas where crayfish grazed freely. In addition, pondweed in exclusion plots had mosquito densities that were significantly higher ($p < 0.05$) than in open control areas (4.4 larvae/dip cf. 0.03 larvae/dip). These results suggest that *P. clarkii* is potentially useful in the management of mosquito habitat in freshwater marshes.

INTRODUCTION.-In freshwater systems, reductions in mosquito numbers may result from direct (e.g. predation) or indirect sources (e.g. loss of habitat from herbivory). The importance of predators in mosquito control has been well studied (Miura et al. 1978, Farley 1980, Bence and Murdoch 1982), whereas the influence that aquatic herbivores may have in reducing mosquito habitat has received considerably less attention. At Coyote hills Marsh (Coyote Hills Regional Park, Alameda County, California) the introduced red swamp crayfish (*Procambarus clarkii*) occurs in high densities and readily consumes sago pondweed (*Potamogeton pectinatus*), a submersed macrophyte that forms a dense floating canopy and functions as an important habitat for larval *Anopheles occidentalis*. This report summarizes experimental studies conducted during 1985 that were designed to 1) determine the effects of crayfish grazing on mosquito habitat (pondweed), and 2) assess the effects of loss of this habitat on densities of mosquito populations.

STUDY AREA.-Coyote Hills Marsh, located near Fremont, California, is a man-made freshwater basin, which was originally a tidal salt marsh prior to construction of dikes and diversion sion of local streamwater (Collins et al. 1983). Although saline soils and some salt-tolerant vegetation persist, the primary aquatic plants are typical freshwater species, including emergent cattails (*Typha spp.*), bulrushes (*Scirpus sp.*), and spike-rushes (*Eleocharis sp.*) along the shallow marsh edges, and sago pondweed in the deeper (> 1 m), interior portion of the basin. Beginning in May, the pondweed surface canopy covers most of the water surface and persists until October (Balling and Resh 1984). During this period, pondweed does not occur within 1-3 m of the marsh margin; this open-water perimeter, which we have termed the "halo" zone, composed 10% (by area) of the total marsh surface in August of 1983 and 1984.

MATERIALS AND METHODS.-To determine if crayfish grazing was responsible for the creation of this peripheral halo zone, we began an exclusion experiment in spring 1985, before pondweed germination. Six plywood-sided exclosures (dimensions 2.4 x 1.2 x 1.2 m; enclosed area = 3 m^2) were used to eliminate crayfish from selected areas where the halo zone normally formed, approximately 1 m from the *Typha* zone. Any crayfish that remained in the exclosures after installation were removed with D-frame nets or with baited, cylindrical crayfish traps (Gee's® minnow traps, modified by widening the entrance). The exclosures had open tops and bottoms, which when completely submerged allowed colonization by various invertebrates and insectivorous fish (*Gambusia affinis, Gasterosteus aculeatus*) but prevented crayfish entry. Control plots of equal area (3 m^2), delineated with wooden cornerposts, were also established immediately adjacent to the exclosures; crayfish had free access to the pondweed in these plots. Pondweed density (stems/m^2) and mosquito abundance (using a standard one-pint dipper) were measured in exclusion and control plots in mid-July, 1985, a time when pondweed normally reaches its highest biomass in the marsh (Balling and Resh 1984).

RESULTS.-Beginning in mid-May, a conspicuous halo zone formed along the marsh periphery surrounding the exclosures; this open-water area gradually expanded toward the marsh interior, isolating the dense, central pondweed beds from the *Typha* zone. Areas where crayfish were excluded had significantly higher pondweed biomass (as indicated by stem density) than areas where crayfish could graze freely ($p < 0.01$, Mann-Whitney U Test; Figure 1). Eventually, the pondweed mats within exclusion plots became completely isolated from the large, interior pondweed beds (Figure 2). Crayfish damage to pondweed, as indicated by fresh detritus and cut stems, could be observed at the margins of the receding pondweed bed. These pondweed "islands" within the exclosures persisted until October, the time when pondweed senescence normally occurs.

The crayfish exclusion plots not only had denser pondweed, they also had significantly

Murray, W. D. 1971. Panel: The current status and future of the pasture mosquito control problem in California. The Lower San Joaquin Valley. Proc. Calif. Mosq. Cont. Assoc. 39:12-13.

Silveira, S. M. 1971. Panel: The current status and future of the pasture mosquito control problem in California. The Northern San Joaquin Valley Region. Proc. Calif. Mosq. Cont. Assoc. 39:11-12.

Smith, G. F., A. F. Geib, and L. W. Isaak. 1956. Investigations of a recurrent flight pattern of flood water *Aedes* mosquitoes in Kern County, California. Mosq. News 16:251-256.

Womeldorf, D. J., P. A. Gillies, and K. E. White. 1971. Insecticide susceptibility of mosquitoes in California: status of resistance and interpretations through 1970. Proc. Calif. Mosq. Cont. Assoc. 39:56-61.

only certain traps for analysis needs to be done in further studies. The correlation between the trends of *Ae. nigromaculis* abundance in these 2 districts over 32 years was highly significant. We expect the same will be true for most of the Central Valley.

In California, *Ae. nigromaculis* was first found in 1937 in Shasta County (Aitken 1940). By 1939, specimens had been collected throughout the Central Valley, as far south as Greenfield in Kern County. It was postulated that this new species would take over breeding sites usually associated with *Ae. melanimon*. Indeed, the 2 species are frequently found together, although studies (Kliewer et al. 1964) showed that *Ae. nigromaculis* were more tolerant of high temperatures but were less common than *Ae. melanimon* in water of high salinity.

The seasonal average New Jersey light trap indices for *Ae. melanimon* are shown in Figure 2. Except for the unusually high population in 1966 (for which the operation of 5 new traps was responsible), the period of maximum *Ae. melanimon* abundance in Kern County was in the mid 1970's, about 4 years after the *Ae. nigromaculis* peak. We plan to look at temperature data to determine if the preference of *Ae. melanimon* for lower temperatures will explain this lag. The early 1970's also saw high populations of *Ae. melanimon* in the Sutter-Yuba area, although the decline in abundance since then has not been so dramatic as in Kern County.

By 1970, insecticide resistance was widespread in both *Cx. tarsalis* and *Ae. nigromaculis* throughout the Central Valley (Womeldorf et al. 1971). Pasture mosquitoes seemed to be out of control, and were the subject of a panel at the 1971 conference of the C.M.V.C.A. One participant blamed insecticide resistance (Kauffman 1971), another blamed a combination of insecticide resistance and poorly managed pastures (Silveira 1971), and a third blamed the climate or environment, by which he meant the attitudes of the landowners (Murray 1971).

We examined data on permanent pasture acreage in Kern and Sutter-Yuba Counties, to see if there had been a decrease in recent years which might account for the decrease in *Aedes* abundance (Figure 3). There has been a decrease in pasture acreage in both areas. The correlation between pasture acreage and *Aedes* population levels is not statistically significant, and is insufficient to explain the decline in mosquito populations. It seems that despite the doomsayers of 15 years ago, effective control of *Aedes* mosquitoes is being achieved in these 2 districts.

Population trends of *Cx. tarsalis* (Figure 4) have had a very different pattern from the 2 *Aedes* species, or perhaps no pattern at all. In Kern County, there were peaks in 1958 and 1969, both following very wet winters. The 1975 peak is difficult to explain, as rainfall in the 1974-75 winter was only slightly above normal. However, one interesting associated phenomenon is that there was virtually no WEE or SLE virus activity in Kern County in the early 1970's, but a resurgence was seen in the late 1970's, following the high population peak in 1975. As the *Cx. tarsalis* populations have fallen off again, so has virus activity. There have been no isolations of WEE or SLE viruses from mosquitoes in Kern County in 1984 or 1985. Only 1 sentinel chicken out of 8 flocks developed WEE antibodies very late in the 1984 season, and there were no seroconversions in 1985.

Perhaps we could blame the passage of Proposition 13 for the 1978-1980 peak in *Cx. tarsalis* populations in the Sutter-Yuba district. Again, there is a highly significant correlation between the relative abundance of *Cx. tarsalis* in the Kern and Sutter-Yuba districts over this time span. There has been no evidence of virus activity in the Sutter-Yuba district since 1981, despite the presence of *Cx. tarsalis* population levels which historically supported transmission of viruses.

These analyses are not complete. In addition to adding records from other districts and eliminating data from traps which operated for only a short period, we plan to consider additional factors such as insecticide usage, agricultural practices, water management, and weather data.

ACKNOWLEDGMENTS.-This research was funded by Research Grant AI-3028D from the National Institute of Allergy and Infectious Diseases, Biomedical Research Support Grant 5-S07-RR-05441 from the National Institutes of Health and by special funds for mosquito research allocated annually through the Division of Agriculture and Natural Resources, University of California. We are especially grateful to the Kern and Sutter-Yuba M.A.D.'s for their continuing support of our research.

REFERENCES

Aitken, T. H. G. 1940. Notes on *Aedes nigromaculis* (Ludlow), *A. increpitus* Dyar and *Culex territans* Walker in California (Diptera, Culicidae). Proc. Ent. Soc. Wash. 42:142-147.

Bidlingmeyer, W. L. 1985. The measurement of adult mosquito population changes -- some considerations. J. Am. Mosq. Cont. Assoc. 1:328-348.

Husbands, R. C., and D. E. Reed. 1969. A comparison of larval mosquito species occurrence and light trap data. Proc. Calif. Mosq. Cont. Assoc. 37:101-108.

Kauffman, E. E. 1971. Panel: The current status and future of the pasture mosquito control problem in California. The Sacramento Valley Region. Proc. Calif. Mosq. Cont. Assoc. 39:10-11.

Kliewer, J. W., T. Miura, and K. E. White. 1964. Temperature and salinity relationships of *Aedes nigromaculis* and *A. melanimon*. Proc. Calif. Mosq. Cont. Assoc. 32:42-45.

Miura, T., R. C. Husbands, and W. H. Wilder. 1968. Observations on the hatching of *Aedes nigromaculis* (Ludlow) eggs (Diptera: Culicidae). Proc. Calif. Mosq. Cont. Assoc. 36:42-43.

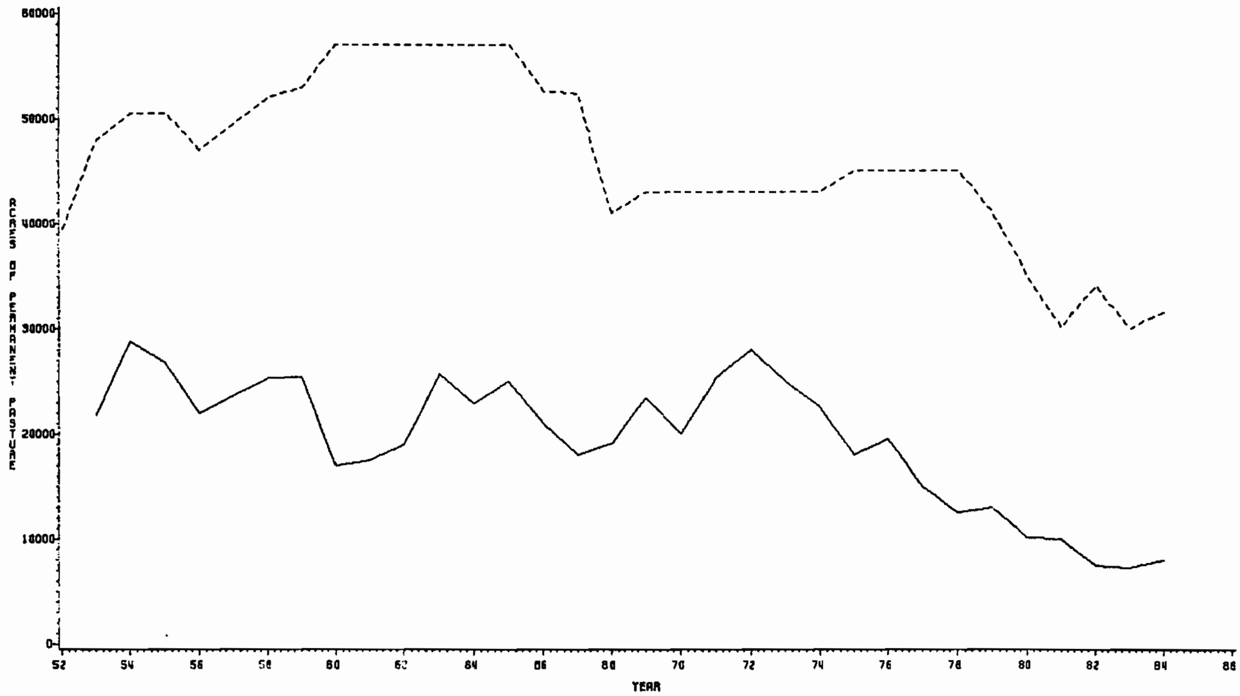


Figure 3. Acres of permanent pasture, 1952-1984.
——— Kern County; - - - - Sutter and Yuba Counties.

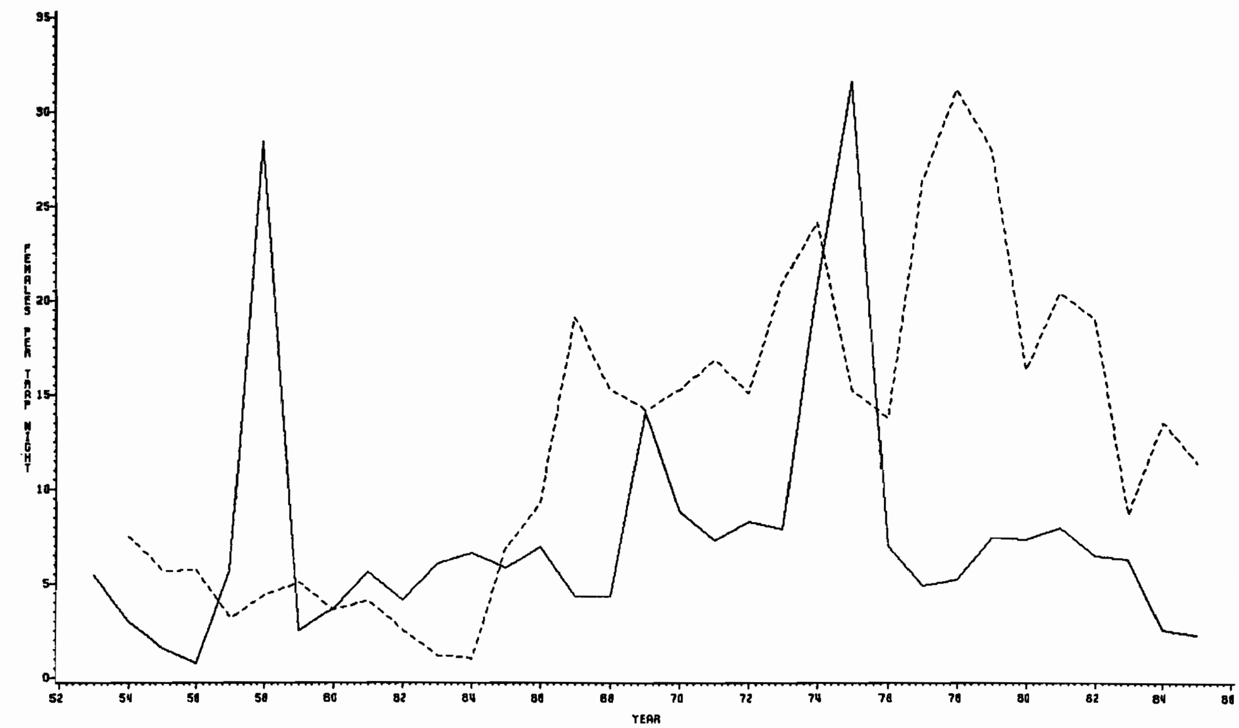


Figure 4. Seasonal average female *Culex tarsalis* per trap night, 1953-1985.
——— Kern M.A.D.; - - - - Sutter-Yuba M.A.D.

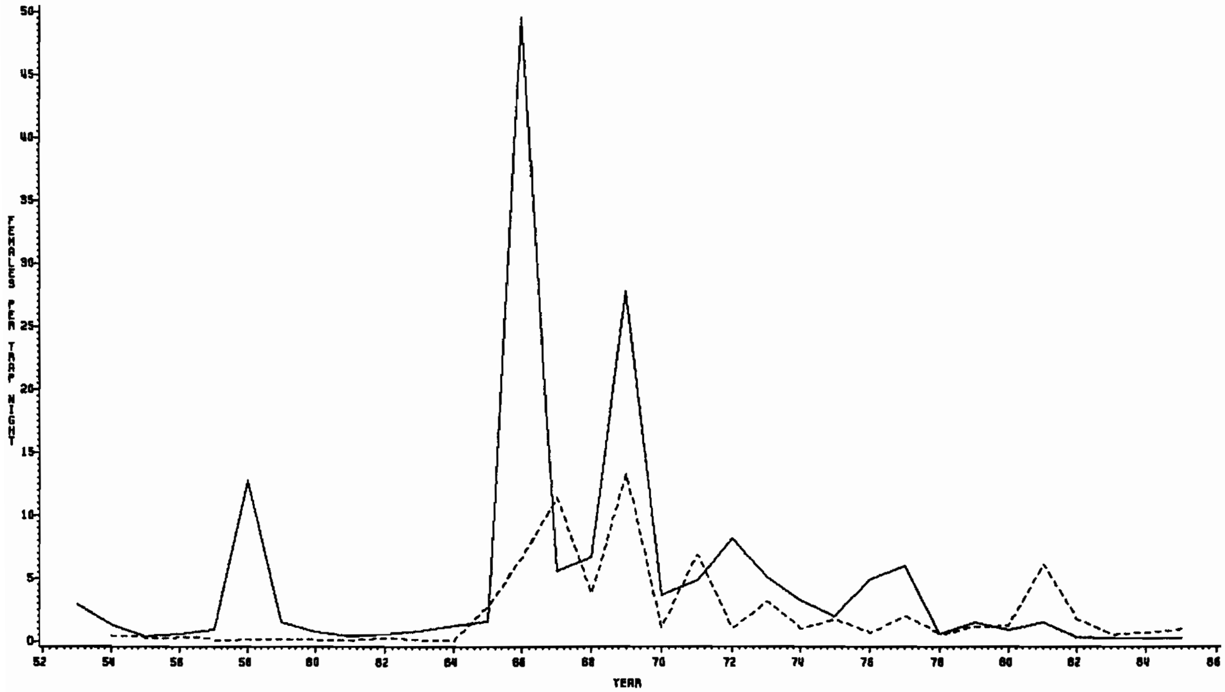


Figure 1. Seasonal average female *Aedes nigromaculis* per trap night, 1953-1985.
——— Kern M.A.D.; - - - - Sutter-Yuba M.A.D.

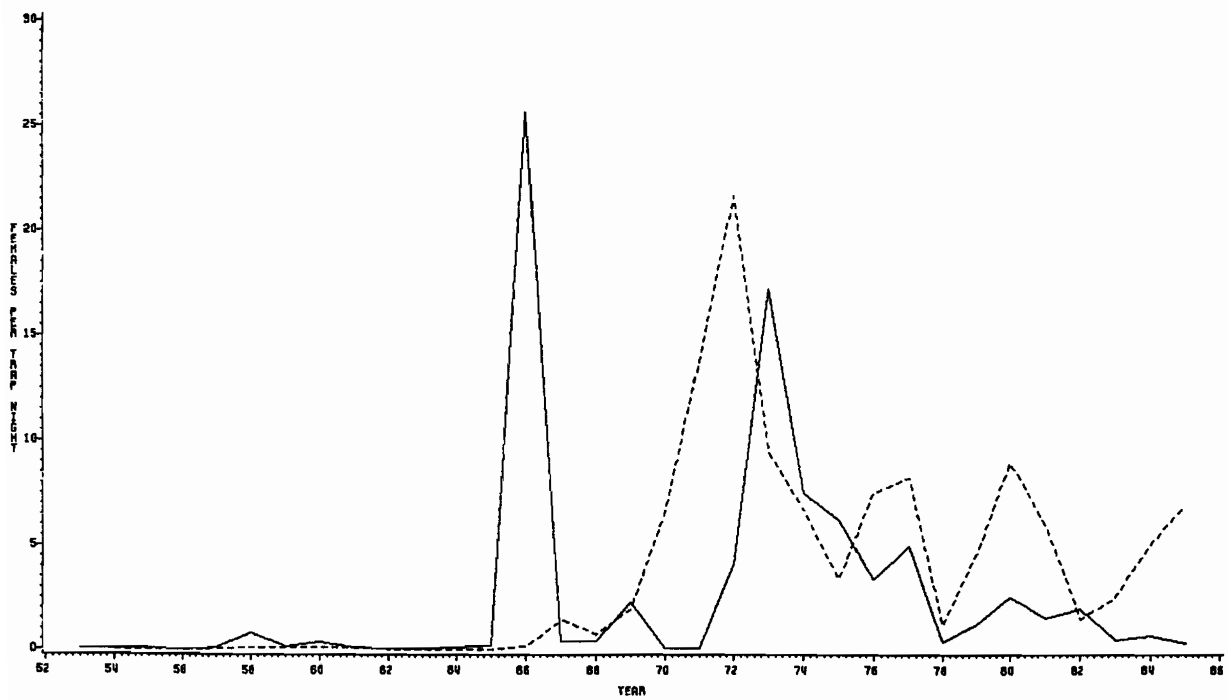


Figure 2. Seasonal average female *Aedes melanimon* per trap night, 1953-1985.
——— Kern M.A.D.; - - - - Sutter-Yuba M.A.D.

CHANGES IN THE RELATIVE ABUNDANCE OF *AEDES NIGROMACULIS*, *AEDES MELANIMON* AND *CULEX TARSALIS* IN THE CENTRAL VALLEY OF CALIFORNIA

M. M. Milby and W. C. Reeves

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

ABSTRACT

Seasonal average relative abundance of *Aedes nigromaculis* and *Aedes melanimon* females in the Kern and Sutter-Yuba M.A.D.'s, as measured by New Jersey light traps, peaked in the late 1960's and early 1970's and has declined since. The annual relative abundance of *Culex tarsalis* in these 2 districts had no consistent trend. The correlation between acres of permanent pasture and *Aedes* abundance was not statistically significant. Other factors need to be examined to explain mosquito abundance patterns.

In a recent review of our files on mosquito abundance in the Central Valley, we noted that collections of *Aedes nigromaculis*, which formerly were quite high throughout most of the Valley, seemed to have declined to relatively low levels in recent years. In 1985, yearly average *Ae. nigromaculis* light trap counts exceeded 10 per trap night at only 2 of 35 sites reviewed in the Central Valley: Grey Lodge (17.7) and an uncontrolled area near Williams (15.3).

A review of the Proceedings of the C.M.V.C.A. for the past 20 years seemed to indicate a general decrease in concern regarding *Ae. nigromaculis*. The number of papers that discussed *Ae. nigromaculis* dropped from 27 in 1973 to only 2 in 1984. And yet, Miura and coworkers (1968) called this mosquito "one of the most abundant and noxious mosquitoes in Central California". We initiated an analysis of the detailed light trap records from several districts to see if we could detect long-term trends in the abundance of this and other mosquito species. If such trends were found, we would attempt to correlate the changes in abundance with other factors.

Our analyses have been based on New Jersey light trap records from the Kern and Sutter-Yuba M.A.D.'s which are the only districts for which we had immediate on-line computer access to all collection data since the early 1950's. We plan to expand this study to include at least 2 additional districts: Delta, from which we have received operational reports dating back to 1951, and another district from the Sacramento Valley, probably Butte because we already have their light trap data for the same time period on IBM cards or tape. Light trap collections may not be the best measure of abundance for some species (Husbands & Reed 1969, Bidlingmeyer 1985), but we feel they provide the most reliable indication of trends in a particular district over a period of time. This is especially true when collections are averaged over a 6-month mosquito season, from April through September.

We have limited our examination so far to 3 species. *Aedes nigromaculis* provided the impetus for the study and continues to be an important pest in some areas. *Culex tarsalis* is the primary vector of western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) in California, and the species on which most of our research has concentrated. *Aedes melanimon* is the primary vector of California Encephalitis (CE) virus, the only human disease virus that is known to be transovarially transmitted by mosquitoes in the field in California. *Aedes melanimon* also is a competent vector of WEE virus in the laboratory. Numerous strains of WEE virus have been isolated from field collected adults, and *Ae. melanimon* seems to be associated with a transmission cycle of WEE virus in jackrabbits.

The annual relative abundance of these 3 species in New Jersey light traps operated by the Kern and Sutter-Yuba M.A.D.'s is shown in Figures 1,2 and 4. An average of 50 *Ae. nigromaculis* females per trap night (Figure 1, 1966) is very high, especially when you consider that it's the average of all traps in the district over a 6-month period. In fact, it's nearly twice as high as any other annual index for the other 2 species in either M.A.D. In 1966, the Kern M.A.D. operated traps at 5 new sites. One of these traps had collections of *Ae. nigromaculis* that often exceeded 1,000 females per night. These 5 trap sites were discontinued the following year.

The adding and dropping of traps complicates the task of analyzing data collected over a long time period. We plan to refine these figures by basing our analyses only on traps that have remained in about the same place for long periods of time. Even then, a trap's surroundings can change and alter its effectiveness. However, *Ae. nigromaculis* were recovered as far as 28 miles east of their release location in a 1955 mark-release-recapture study in Kern County (Smith et al. 1956). It certainly does not seem to be necessary to have traps near breeding sites to determine if *Ae. nigromaculis* are in an area.

The largest collections of *Ae. nigromaculis* in the Kern M.A.D. were in the late 1960's and early 1970's, and populations have diminished since then. The same is true, to a lesser extent, for the Sutter-Yuba district. The number of traps was reduced in 1981. This would artificially inflate the annual district-wide index if the traps were in locations which normally collected few *Ae. nigromaculis*. Again, the selection of changes in sediment and water supplies. Such adjustments will be mainly limited to the sedimentation of small headward channels and mosquito control ditches.

Biological responses to ditching in the long-term may involve dramatic changes in the distribution and abundance of species that are sensitive to changes in tidal regimen. The greatest percent change in regimen occurs at the upper elevational threshold of direct tidal influence, which corresponds to the margins of low-gradient marsh plains. Slight changes in tidal height at these plains cause large changes in the extent of inundation and thus can affect large areas of surface vegetation.

Some geomorphic effects of ditching relate to ditch arrangement and design, rather than ditch function. For example, elevated lines of spoil alongside ditches that traverse across natural drainage patterns can inhibit surface runoff (Resh and Balling 1983a). Water trapped on the marsh surface behind these spoil lines promotes the death and decay of surface vegetation. In some cases the lentic environments that are produced in this way support salt march mosquitoes (Miller and Egler 1950). More commonly these features are probably too large and unprotected from wind and prolonged desiccation to serve as mosquito habitat. They resemble the early developmental stages of "turf pans" (Collins et al. in press), which are large compartments for water storage that evolve naturally on drainage divides. These features are functionally independent of tidal drainage systems.

Preliminary analysis of the geomorphic effects of ditching suggests that many of these effects can be mitigated by modifications of ditching techniques. Both the amount of ditching and the associated increase in drainage system capacity can be reduced if ditching is limited to the excavation of ancient channel courses that link together channel pans. The ancient waterways can be readily identified on low-altitude aerial photographs. Where ditches are necessarily numerous, the dimensions of tidal sources should be increased, such that flows are sufficient to retard the process of channel retrogression. In large marshes with tidal sources that are widely separated, tidal schedules can differ substantially among drainage systems. Ditches that are added along the hydraulic gradients produced by differences in tidal schedule can be maintained by natural erosion and sediment transport. If ditches that cross drainage divides parallel the natural patterns of surface runoff, then the effects of spoil lines can be eliminated. This may be less expensive than spoil removal or grading. Both the economic and the environmental costs of ditching to control salt marsh mosquitoes can be minimized by practices that reflect an understanding of the functional relationship between ditching and tidal marsh geomorphology.

ACKNOWLEDGMENTS.-This paper is the result of cooperative efforts among personnel of the University of California at Berkeley in the Division of Entomology and Parasitology and the Department of Geology and Geophysics, and the California Department of Fish and Game. The project has been supported by the University of California Mosquito Research Funds, the University of California Water Resources Center, the Coastal Region Mosquito Abatement Districts, the United States Geological Survey, and the Conservation Associates.

REFERENCES

Balling, S.S., T. Stoehr and V.H. Resh. 1980. The influence of mosquito control recirculation ditches on the fish community of a San Francisco Bay salt marsh. Calif. Fish Game. 66:25-34.

Balling, S. S. and V. H. Resh. 1982. Arthropod community response to mosquito control recirculation ditches in San Francisco Bay salt marshes. Environ. Entomol. 11:801-808.

Balling, S. S. and V. H. Resh. 1983a. Mosquito control and salt marsh management: factors influencing the presence of *Aedes* larvae. Mosq. News 42:212-218.

Balling, S. S. and V. H. Resh. 1983b. The influence of mosquito control recirculation ditches on plant biomass, production, and composition in two San Francisco Bay salt marshes. Estuar. Coastal Shelf Sci. 16:151-161.

Barnby, M. A. and V. H. Resh. 1984. Distribution and seasonal abundance of brine flies (Diptera: Ephydridae) in a San Francisco Bay salt marsh. Pan-Pac. Entomol. 60:37-46.

Barnby, M. A., J. N. Collins and V. H. Resh. 1985. Aquatic macroinvertebrate communities of natural and ditched potholes in a San Francisco Bay salt marsh. Estuar. Coastal Shelf Sci. 20:331-347.

Collins, J. N. and V. H. Resh. 1985. Utilization of natural and man-made habitat by the salt marsh song sparrow, *Melospiza melodia samuelis* Baird. Calif. Fish Game 71:40-52.

Collins, L. M., J. N. Collins and L. B. Leopold. in press. Geomorphic processes of an estuarine marsh: preliminary results and hypotheses. Proc. First Internat. Conf. Geomorph. Manchester, U.K. 1985.

Ferrigno, F. and D. M. Jobbins. 1968. Open water management. Proc. N.J. Mosq. Exterm. Assoc. 55:105-115.

Garcia, R. and E. I. Schlinger. 1972. Studies of spider predation on *Aedes dorsalis* (Meigne) in a salt marsh. Proc. Calif. Mosq. and Vector Contr. Assoc. 40:117-118.

Resh, V. H. and S. S. Balling. 1983. Tidal circulation alteration for salt marsh mosquito control. Environ. Manage. 7:79-84.

Smith, J. B. 1904. The common mosquitoes of New Jersey. N. J. Agric. Ex. Sta. Bull. 171. 40pp.

Yapp, R. H., D. John and O. T. Jones. 1917. The salt marshes of the Dovey Estuary, Part II, the salt marshes. J. Ecol. 5:65-103.

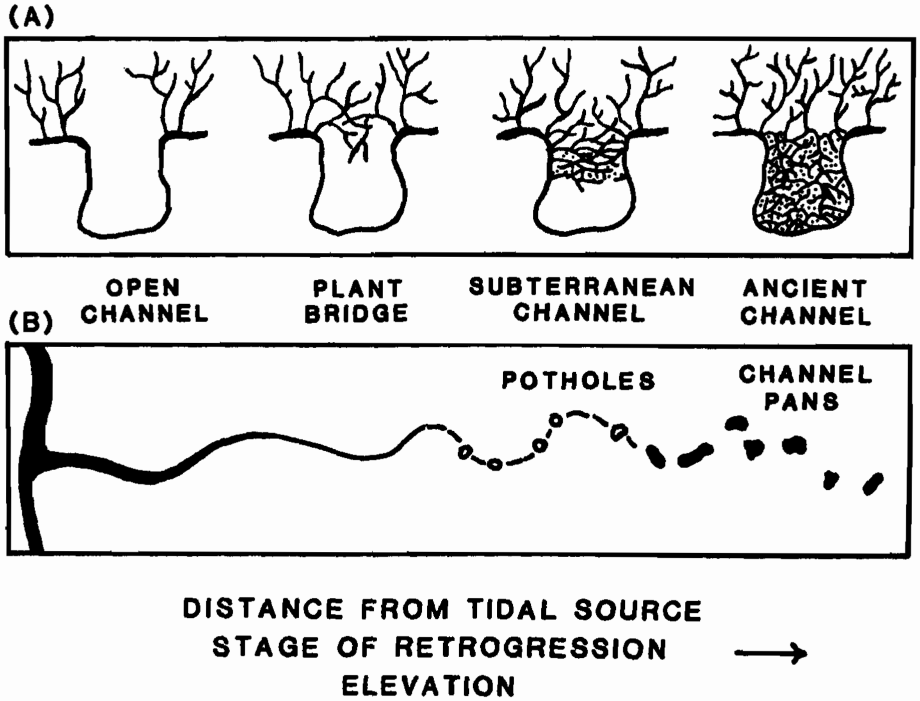


Figure 2.-Schematic diagram of channel retrogression showing (A) formation of plant bridges and subterranean channels in crossection and (B) distribution of retrogression stages in plan form.

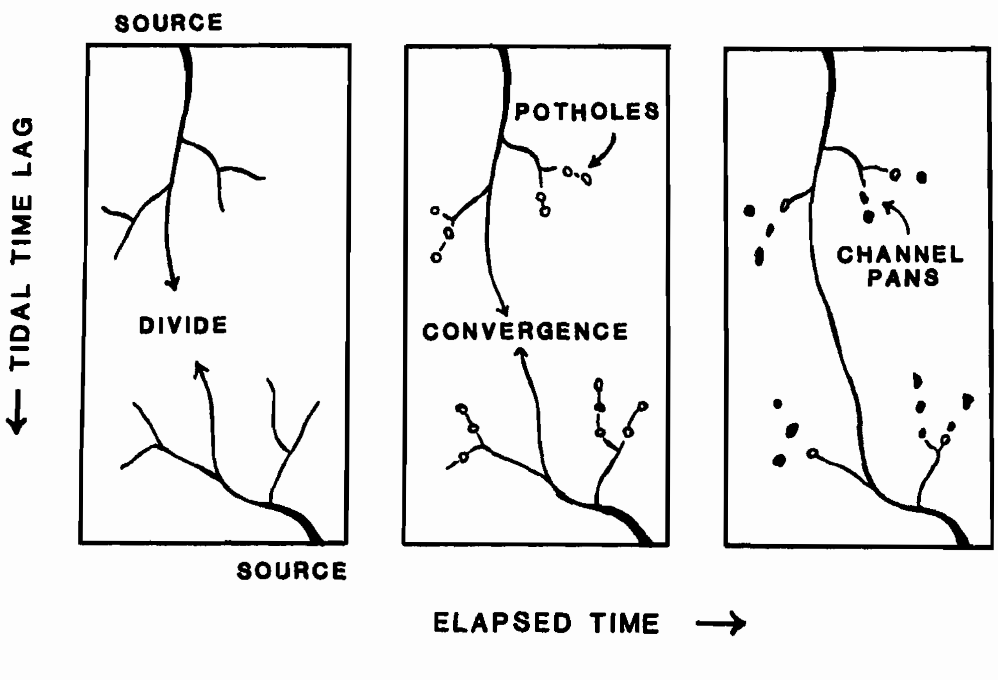


Figure 3.-Schematic diagram of looped slough and channel pan formation.

additions that resist erosion. The increased structure is provided by plant growth that is not directly affected by the tides.

Along the immediate margins of natural channels (and mosquito control ditches), where tidal flushing reduces soil salinities and increases aeration, tall forms of vascular vegetation grow. Wherever channel width is less than twice plant height, the vegetation that collapses into the channel from opposite banks tends to produce fibrous bridges. As tidal water flows through the channel, the bridges collect suspended sediment and detritus, thereby increasing in mass, dimension, and resistance to erosion. Gradually the channel fills from the top downward (Figure 2). This natural process of degradation is termed "channel retrogression" (Collins et al. in press). It begins in the headward reaches of small natural channels and mosquito control ditches, and tends to proceed slowly down the channel gradient.

Channel retrogression is the principal cause of salt marsh mosquito habitats. Sections of retrogressing channels that are too wide for the formation of plant bridges exist for some time as exposed "potholes" (Collins et al. in press), which have subterranean channel connections to tidal flows. Eventually, the channels become completely occluded with plants and sediment. The resulting features are isolated lentic environments called "channel pans" (Yapp et al. 1917), which compose nearly all the salt marsh mosquito habitats in Bay Area tidal marshlands.

The various successional stages of channel retrogression correspond to discrete positions along an elevational gradient. The oldest stages are channel pans that occur at the highest elevations. Most of the mosquito habitats are therefore restricted to the headward reaches of drainage systems, between exposed channels and drainage divides. Many channel pans are arranged in chains that indicate ancient channel courses (Figure 2). In some places eroding channels parallel chains of pans. This suggests that renewed erosion in an area of historical retrogression does not necessarily result in the exhumation of the ancient channel.

Although mosquito control ditches are subject to retrogression, they do not evolve into mosquito habitats. Apparently, ditches are narrow enough throughout their lengths to be entirely bridged by vegetation. No sections of ditches are wide enough to remain exposed as potholes or channel pans. Also, the maintenance program for ditches includes occasional excavation that prevents the ditches from becoming completely occluded.

Within a single tidal drainage system, the erosion and retrogression of channels may be simultaneous and compensatory processes. For example, unless the volume of tidal flow through the system is reduced at its source, the system must somehow accommodate the tidal volume displaced by retrogression. Preliminary investigations suggest that the flows displaced from retrogressed channels are accommodated by other channels that erode. This would explain the elongation of some small channels in headward reaches of drainage systems where retrogression also is observed.

Channels in adjacent drainage systems that erode headward can capture each other at their common drainage divide. If the tidal schedules (i.e., the predicted times of slack high tide) for the two systems differ, then the flood tides that flow through the two connected channels will converge away from the place of original channel capture. The zone of tidal convergence will shift in the direction of the time lag. Eventually, sedimentation may produce a new divide at the new convergence zone, but in the interim the two connected channels are continuous between two tidal sources. Such channels are termed "looped sloughs" (Collins et al. in press) and represent a successional stage in the reconfiguration of entire drainage networks (Figure 3). These large-scale changes in marsh form help to explain the abundance of mosquito habitats throughout mature tidal marshes.

GEOMORPHIC EFFECTS OF MOSQUITO CONTROL DITCHES.-The primary physical change that mosquito control ditches cause in Bay Area tidal marshes is an immediate and substantial increase in the tidal capacity of headward drainage systems. Unlike marshland reclamation, which typically reduces drainage capacity and causes remaining channels to be shallow throughout their lengths, ditching tends to increase drainage capacity without changing downstream channel dimensions. Although the same tidal flows move through a drainage system before and after ditching, these flows are dispersed through a larger system after ditches are added.

Tidal dispersal can profoundly affect the physical nature of marshes, including the evolution of salt marsh mosquito habitats. If the capacity of a tidal drainage system is increased artificially (i.e., without an increase in tidal flows), then the heights of the tides in the headward reaches of the system will be reduced. Thus the amount of tidal energy conveyed by small channels and the frequency of tidal inundation at interior marsh plains will also be reduced. Less tidal energy in headward channels will increase the influence of non-tidal processes on channel morphology. This suggests that ditching can accelerate channel retrogression and the rate of evolution of mosquito habitats. Under relatively natural conditions, numerous channels in Petaluma Marsh have achieved advanced stages of retrogression, including channel pan formation, during the last 100 years. Continued monitoring is necessary to measure any acceleration of the process that can be attributed to ditching. Mosquito abatement programs will need to contend, however, with future habitat evolution.

Reductions in tidal height and inundation frequency will influence small channel dynamics, especially headward erosion and channel capture. Thus, the compensatory relationship between channel retrogression and erosion of marsh plains will be disrupted. Marsh surfaces will largely be exempt from geomorphic adjustments to regional

ASSESSMENT OF ADULT MOSQUITO POPULATIONS IN A FRESHWATER MARSH IN SOUTHERN CALIFORNIA BY VARIOUS TRAPPING METHODS

Michael J. Bangs, A. Ralph Barr, Stanton E. Cope,

Amy C. Morrison, and Pensri Guptavanij

School of Public Health
University of California
Los Angeles, California 90024

During the summer and fall months of 1984, Southern California experienced an outbreak of St. Louis encephalitis that resulted in 26 confirmed cases, one of which was fatal. This epidemic was unusual in that cases occurred in urban rather than rural areas.

It has been well documented that *Culex tarsalis* is an important vector of SLE virus in California but this mosquito is much more abundant in rural than in urban areas although it is found in urban localities as well. The general impression is that *Cx. p. quinquefasciatus*, an important vector of SLE virus in the Central United States, is much more abundant than *tarsalis* in the urban areas although all of our current trapping methods seem to be biased in favor of one or the other species. In particular, light traps, the usual method of surveillance of mosquito populations in California, seem to be much more attractive to *Cx. tarsalis* than to *Cx. quinquefasciatus*.

Our objectives, therefore, were (1) to sample adult mosquito populations by four different methods in an attempt to assess the relative abundance of the various species, and (2) to assess the relative man-biting behavior of the various species present. Details concerning the first objective will be presented here.

The area chosen for the study was the San Joaquin freshwater marsh in Irvine, California, part of the U.C. Reserve System. This site was specifically selected because (1), SLE virus activity was present there in 1984, (2) its limited access to the public would help to minimize possible vandalism or disturbance of trapping equipment, and (3) the concurrent monitoring of bird and mosquito populations for arboviral activity by the Orange County Vector Control District. In addition, a sentinel chicken flock was already present in the area.

Weekly collecting trips were made to the marsh from June 3 to September 30. On each trip a team of two workers set up and operated the trapping methods for approximately 12 hours on that given night. The methods employed were as follows: bare-leg landing collections by both workers sitting approximately 10 meters apart, 3 lard-can traps baited with one fledgling chicken apiece, 12 one foot cubed red boxes serving as artificial resting sites, and 3 CO_2 traps, operated without light. The various trapping methods were placed at fixed stations throughout the marsh with easy access along roads or footpaths. The mosquitoes collected were placed on ice and taken to UCLA where they were identified and in some cases dissected for parity.

RESULTS.-Table 1 gives total numbers of mosquitoes collected by each method. Some 172,000 mosquitoes were collected during 18 nights in the marsh; overall, 94.2% were *Cx. erythrothorax*, with the next highest percentage being 3.5% for *Cx. tarsalis*. The most productive method used was the CO_2 trap from which 90% of the mosquitoes were collected. Landing catches accounted for about 9% of the total, the bait-traps for 1%, and resting boxes for a negligible proportion. The *Anopheles* species, we are calling the 'southern form' of *An. occidentalis* at this time.

The bar graph compares the various trapping methods using *Cx. tarsalis* as a standard and expressing the abundance of each species in relation to the number of *Cx. tarsalis* captured by that method. For example, comparing the *Cx. erythrothorax* with that of *Cx. tarsalis* we see the carbon dioxide trap captured 29.2 times as many *Cx. erythrothorax* as *Cx. tarsalis*, the bait trap 2.8 times as many, while the red boxes only .08 times as many. In the landing collections there were 45.6 *Cx. erythrothorax* for each *Cx. tarsalis* captured. The CO_2 trap and landing collections, therefore, seem to be especially useful for this species while baited lard-can traps and resting boxes were unproductive in this marsh habitat.

Cx. quinquefasciatus was considerably less abundant than *Cx. tarsalis* for all trapping methods except landing collections, indicated as .67 *Cx. quinquefasciatus* for each *Cx. tarsalis* captured. This suggests that *Cx. quinquefasciatus* was nearly as attracted to humans as was *Cx. tarsalis*. Even though we lack estimates of the absolute size of the populations sampled, it is evident that landing collections were more useful for *Cx. quinquefasciatus* surveillance than were the other methods.

In Table 2, if we look at the proportion of each species collected among the different trapping methods, we observe that the CO_2 traps accounted for 80 to 90% of the *Anopheles*, *Cx. erythrothorax*, *Cx. tarsalis*, and *Cs. inornata* but only 45% of the *Cx. quinquefasciatus*. The bait traps collected 10% of the *Cx. tarsalis* but only 0 to 5% of the other species encountered. Resting boxes yielded 13% of the *Cs. inornata*, which were primarily males, but only 0 to 1% of the other species.

Landing collections included 48% of the *Cx. quinquefasciatus* but no more than 17% of each of the other species. From this, it would appear

Table 1.-Collection summary: total numbers of mosquitoes collected by method.

Species	CO_2	Bait	R.B.	Landing	Total
Anopheles sp.	2654	1	38	562	3255
Cx. erythrothorax	146432	1614	5	13811	161862
Cx. tarsalis	5020	571	60	303	5954
Cx. quinquefasciatus	191	21	6	202	420
Cs. inornata	242	0	41	21	304
TOTAL	154539	2207	150	14899	171795

Table 2.-Proportions of each species collected by method.

Species	CO_2	Bait	R.B.	Landing
Anopheles sp.	.82	.00	.01	.17
Cx. erythrothorax	.90	.01	.00	.09
Cx. tarsalis	.84	.10	.01	.05
Cx. quinquefasciatus	.45	.05	.00	.48
Cs. inornata	.80	-	.13	.07

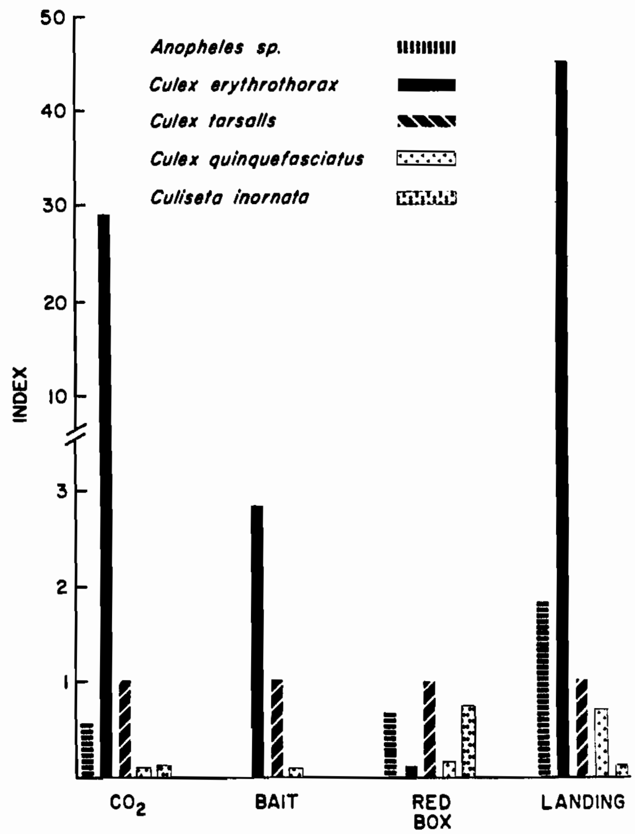
that landing collections are especially useful for surveillance of populations of *Cx. quinquefasciatus* in comparison with the other methods tested. Although artificial resting sites have proved useful in other studies, the poor overall catches is probably a reflection of the ample availability of natural resting places (tules, cat-tails, etc.) in the marsh as a whole.

SEASONALITY.-With the information that was collected, it would be interesting to briefly look at the seasonality of each species. When the total number of *Cx. erythrothorax* collected by all methods is plotted over time (Graph 1), a steady increase in numbers is seen during June and July with a dramatic peak on week 8 (July 22). Over 30,000 *Cx. ervthrothorax* were collected in this week, one week after the peak average night time temperature was recorded. For the second half of the study period a rapid drop in numbers collected was observed.

In Graph 2, *Cx. tarsalis* produced low numbers during the first seven weeks of between 50 to 300 per collection night; but then had a precipitous rise within a single week to peak on week 8, with over 1,600 collected. Afterwards, there was a sharp drop in collected numbers until week 18. The *Anopheles* presented a bimodal distribution during the first half of the study then a gradual decline to near zero in the second half.

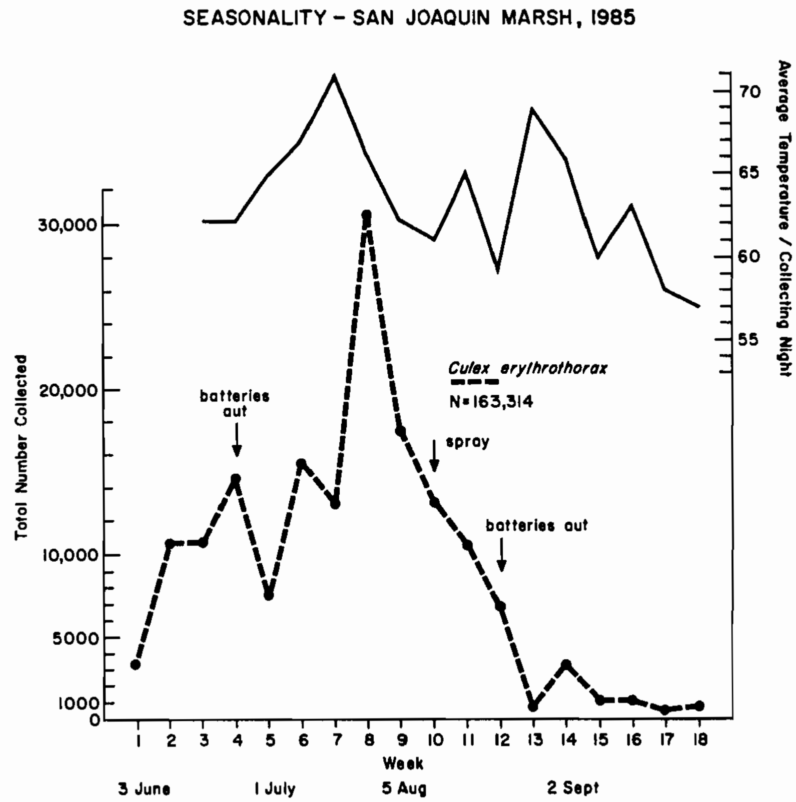
In Graph 3, *Cs. inornata* had peaked early in the study on June 10 and from then on out continued a steady decline until completely absent from collections from mid-August to mid-September. These results reflect the fact that *Cs. inornata* is a cooler weather mosquito and present only in low numbers during the warmer months. *Cx. quinquefasciatus* was also present in relatively low numbers throughout the study. It likewise had a steady decline in numbers as the summer progressed.

NUMBER IN RELATION TO *Culex tarsalis*

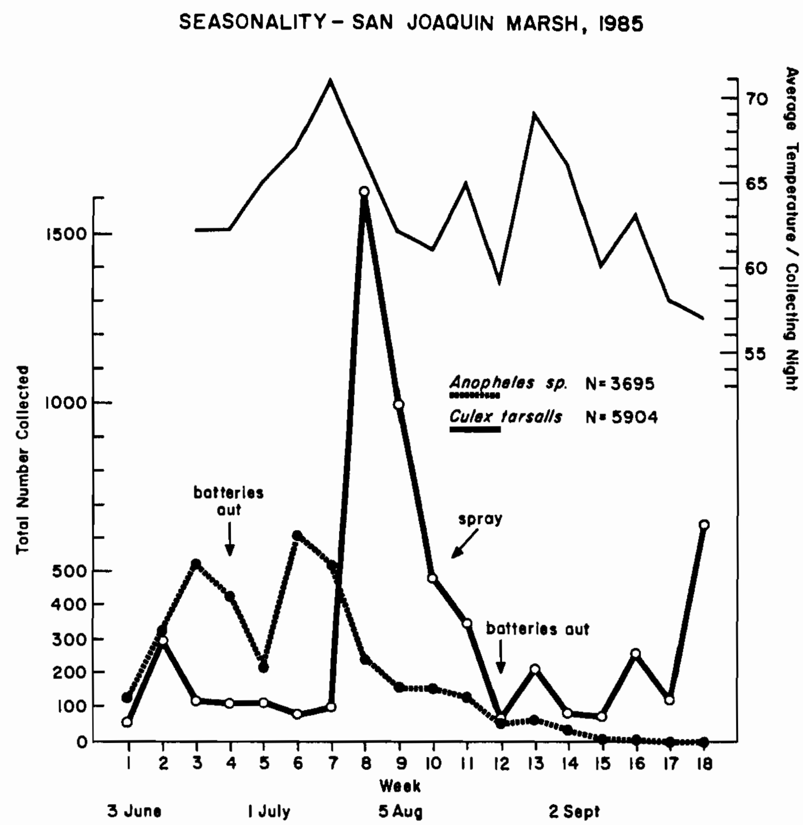


Bar Graph

The seasonality of mosquito populations is influenced by a number of interrelated environmental factors; none the least, temperature, Graph 1.

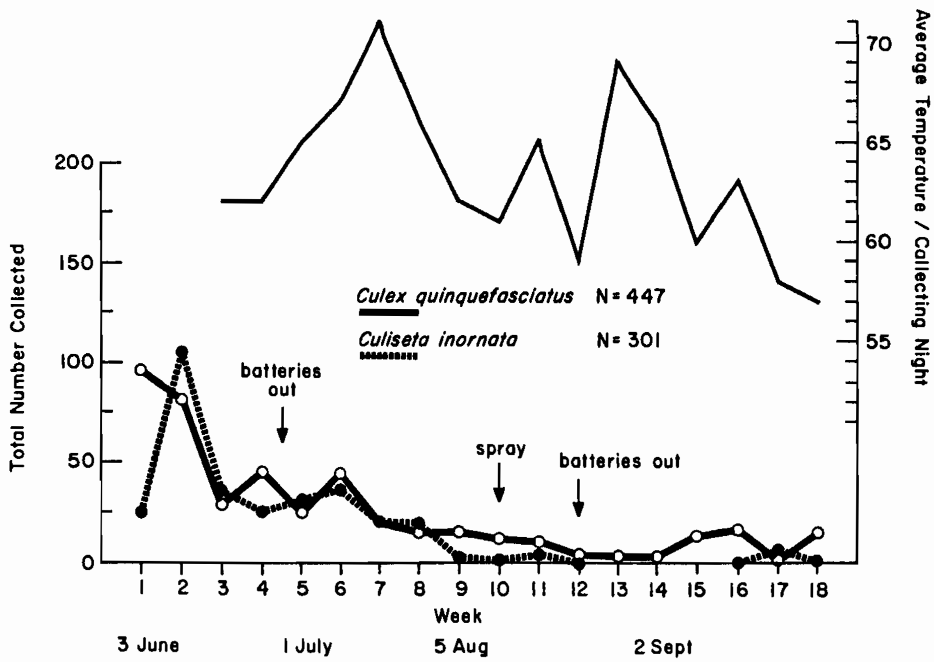


Graph 2.



SEASONALITY - SAN JOAQUIN MARSH, 1985

Graph 3.



humidity, rainfall, photoperiod, number of suitable breeding sites, the natural biologic cycle of given species, inter- and intracompetition between species, and man-made changes.

With this in mind, it should be noted that spray coverage involving both aerial adulticiding, using a synthetic pyrethoid, and BTI applications commenced on week 10 and were repeated on a weekly basis thereafter. In addition, no appreciable amount of water was added to the marsh either deliberately or by precipitation, so the site was in a near continual state of evaporation throughout the study.

The general decline in all mosquito species numbers has been attributed to the combined effects of the gradual seasonal temperature decline, the selective drying up of available breeding sites and the weekly spray and BTI applications.

PARITY RATES OF MOSQUITOES COLLECTED IN THE SAN JOAQUIN MARSH

A. Ralph Barr, A. C. Morrison, Pensri Guptavanij,

Michael J. Bangs and Stanton E. Cope

School of Public Health
University of California
Los Angeles, California 90024

The adult mosquitoes collected in the San Joaquin Marsh Study described in the preceding papers by Stanton Cope and Michael Bangs were examined by the ovarian tracheation method for evidence of having laid eggs (Detinova 1962). The ovaries were dissected in distilled water (not saline) and placed on glass slides to dry, after which they were examined by direct light microscopy at a magnification of 10 or 40 diameters. The parous rate is defined as the proportion parous of all females examined. The overall parous rates of adult mosquitoes collected by all methods are shown in Table 1. The figures indicate that the *Culex tarsalis* and the *Cx. p. quinquefasciatus* collected, were, on the average, older than the specimens of the other mosquitoes collected. In the table, specimens from all of the collecting techniques used have been added together. The finding that *Cx. tarsalis* and *Cx. p. quinquefasciatus* had higher parous rates than the other species suggests that these species live longer in the field than do the others. This may be one reason that these species are important vectors of encephalitis viruses. Assuming an ovarian cycle of 5 days and a steady state population, the 0.34 parous rate of *Culex tarsalis* suggests a daily survival rate of about 81% ($0.806^5 = 0.340$), or a daily mortality rate of 19%.

Parous rates of the different species collected by different methods are shown in Table 2. The starred totals indicate those groups in which the parous rates of specimens collected by the three collection methods differed significantly. The parous rates for mosquitoes taken landing on people were greater than those of mosquitoes taken in carbon dioxide traps or bait traps for *Culex erythrothorax*, *Anopheles*, and *Culex tarsalis*, but not for *Culex p. quinquefasciatus* or *Culiseta inornata*. These results suggest that the mosquitoes taken attacking people were older on the average than those taken in traps in which carbon dioxide or birds were the attractants, at least for the three species indicated. There was no significant difference in parity rate for mosquitoes taken in carbon dioxide traps as contrasted with those in bait traps.

The parous rate of each species as a function of date is shown in Table 3. Parous rates were high in most of the species during the first five or six weeks of the study and then low for weeks 7 through 10 and high once again from weeks 11 onward. All of the species tabulated overwinter in the adult stage. The data suggest that during the first six weeks of the study, through the first of July, the population was older on the average, perhaps because of more overwintered females or cooler ambient temperatures at that time. Parous rates were lower for most of the species from weeks 7 through 10, indicating increased mosquito breeding or perhaps higher ambient temperatures, which presumably would shorten longevity. At the end of the season, from weeks 11 on, most of the species again showed higher parous rates. After week 10 the marsh was treated with BTI and Scourge®, a synthetic pyrethrin insecticide, because of the large number of mosquitoes present. The parity data indicate an increase in parous rate at that time, which suggests that the larvicide was effective but not the adulticide. There also was

Table 1.-Proportions of adult mosquitoes parous (all collecting methods).

Species	No.	Prop. parous	Signif.*
Culex tarsalis	1574	.34	A
Culex p. quinquefasciatus	149	.32	A
Culex erythrothorax	2422	.18	B
Anopheles sp.	594	.11	C
Culiseta inornata	105	.09	C

*A>B>C; $p<.05$.

Table 2.-Parous rate by trapping method.

	Species**				
Trapping method	CE	AN	CT	CQ	CI
Dry ice	.15	.08	.35	.33	.09
Bait trap	.17	.00	.27	.58	.00
Landing	.25	.15	.40	.29	.09
Total	.18*	.11*	.34*	.32	.09

*Heterogeneous.

**CE = *Cx. erythrothorax*, AN = *Anopheles sp.*, CT = *Cx. tarsalis*, CQ = *Cx. pipiens quinquefasciatus*, CI = *Cu. incidens*.

Table 3.-Parous rate by week of collection.

Species	Week																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total
Cx.eryth	.45	.51	.32	.22	.33	.08	.10	.07	.11	.10	.21	.12	.32	.11	.16	.14	.13	.28	.18
Anoph.	-	.08	.13	.04	.18	.14	.02	.00	.20	.02	.16	.31	.03	.20	.10	.29	-	#	.11
Cx. tar.	#	.47	.28	#	.51	.50	#	.06	.17	.19	.29	.51	.54	.59	.82	.73	.41	.38	.34
Cx.quin	.48	.30	.27	.14	.38	.05	.14	#	-	-	-	#	#	-	.22	.60	#	.22	.32
Cu.inorn.	-	.00	.08	.13	.09	.07	.00	.08	#	-	#	-	-	-	-	-	#	-	.09

- = none dissected; # = fewer than 5 dissected.

extensive drying of the marsh at that time, which may have caused the increase in parous rates, at least in part.

In summary, the ascertainment of whether or not collected mosquitoes had laid eggs previously gave an additional dimension to data on the numbers of mosquitoes collected.

REFERENCES

Detinova, T. S. 1962. Age-Grouping Methods in Diptera of Medical Importance With Special Reference to Vectors of Malaria. Wld. Hlth. Organ., Mon. Ser. No. 47, 216 pp.

MOSQUITO BREEDING IN A CATTAIL-TULE MARSH MANAGED FOR CLEAN-UP OF SECONDARY SEWAGE EFFLUENT

C. H. Schaefer and T. Miura

Mosquito Control Research Laboratory
University of California
9240 S. Riverbend Avenue
Parlier, California 93648

INTRODUCTION.-EPA funding for sewage treatment plants for small communities with less than 1 million gallons per day effluent discharge has been greatly reduced in recent years. There is a need to identify novel means of sewage treatment which can be accomplished more cheaply, than with sophisticated treatment plants, for use by smaller communities. One possibility is to utilize plant root systems to "polish" effluent from secondary discharge ponds. An experiment at the Gustine sewage treatment plant demonstrated that cattail rhizomes effectively reduced suspended solids and algae in secondary effluent to the degree required by the California Water Quality Control Board. Unfortunately, this dense cattail stand irrigated with secondary effluent resulted in high breeding of *Culex tarsalis*; larval sampling by personnel of the Merced Mosquito Abatement District during 1983, revealed populations which averaged ca. 6 larvae/dip. This population density is regarded as well above the acceptable threshold.

A series of meetings were held, including personnel of the State Water Quality Control Board, the Regional Water Quality Control Board, the California Department of Health Services, the Merced County Health Department, the Merced County Mosquito Abatement District, the City of Gustine and the University of California. The Mosquito Control Research Laboratory evaluated mosquito breeding in an experimental plot at the Gustine sewage plant. The primary objective was to attempt to slow the cattail stand redevelopment after harvest so that the interval between renovations could be lengthened as much as possible.

METHODS.-The experimental plot was rectangular (50 x 850 feet) and was composed of a mature stand of common cattails, *Typha latifolia* (ca. 95% of stand) and tules, *Scirpus acutus* (ca. 5% of stand). On 7/22/84 the stand was burned, the stubble was cut to a height of ca. 2 inches and the debris was removed. A central strip (lengthwise through plot) was ripped with heavy equipment to a depth of 18 inches to remove most of the cattail rhizomes. The physical changes were completed on 8/9/84 and beginning on 8/13/84 the plot was continuously flooded with secondary sewage effluent. The growth and development of the new vegetative stand was monitored bi-weekly by measurements and by color photography during the fall and spring and monthly during the winter. Mosquito immatures were sampled by dipping at the same intervals.

On 9/28/84, the plot was stocked with 2 pounds of mosquitofish. *Gambusia affinis*, and this population was sampled with minnow traps on 10/9/84.

RESULTS. Vegetation.-The cattails quickly redeveloped; within 2 weeks after the irrigation was initiated they had attained 2-3 feet in height and after 3 months they were 6-7 feet. However, the cattails were slow to reoccupy the center strip that was ripped. During the winter months (Dec-Mar), there was considerable die-back and some lodging (ca. 5-10% of total area) occurred. In April the dead vegetation was mostly 2 feet high and bent over but in some areas the lodged material covered the water surface completely. The cattail-tule stand reached a height of 5-6 feet in April and 7-8 feet in May. During the latter period the lodging extended over larger areas; the central strip was now densely covered. In June and July the percentage of the plot covered by flat, dense, lodged (dead and green) vegetation increased to ca. 50%.

Insect Predators.-Numerous insect predators developed in the central strip, that was ripped, after the initiation of flooding. Those organisms found are shown in Table 1. Numerous beetle larvae developed during the summer and fall of 1984 but then disappeared during the winter months. Beetle larvae reappeared in small numbers in April 1985 and these populations increased slowly until May when the plot dried up, due to failure of a pump. After the plot was reflooded, the populations of predator organisms did not redevelop, except in very small numbers. The open water areas that seemed to favor development of aquatic insect predators earlier were minimal by this time.

Mosquitoes.-Mosquito larvae (see Table 1) were of found in the experimental plot in low numbers (0.06/dip or less in the summer and fall of 1984. They disappeared during the winter months (Dec-Mar) as is normal, and reappeared in April, but in low numbers (0.08/dip or less in April and 0.04/dip or less in May). After the plot dried out, they disappeared, but soon after the water was restarted a hatch of *Aedes melanimon* occurred (their numbers were highly variable but up to 44/dip were found - average 6.7/dip). *Culex spp.* then reappeared with the largest numbers occurring beneath the dense, lodged mats of vegetation (average 5.2 dip 6/3/85 with all larval stages and pupae present).

Other Insect Pests.-Large numbers of chironomid midges were present in the fall of 1984. Moderate numbers of *Culicoides varipennis* (the vector of bluetongue in sheep) were also present.

Mosquitofish.-The mosquitofish planted on

Table 1.-Aquatic insects identified from collections from the Gustine experimental plot.

Odonata:	*Anax junius* - nymphs
Hemiptera:	*Corisella spp.* - nymphs
Coleoptera:	Dytiscidae - *Hygrotus lutescens* - adults, *Laccophilus spp.* - adults and larvae, *Thermonectus basillaris* - adults and larvae
	Hydrophilidae - *Berosus spp.* - adults *Thropisternus lateralis* - adults and larvae *Tropisternis ellipticus* - adults and larvae *Helophorus spp.* - adults *Enochrus spp.* - adults
Diptera:	Culicidae - *Culex tarsalis* - larvae, *Culex quinquefasciatus* - larvae, *Culex erythrothorax* - larvae, *Aedes melanimon* - larvae
	Ceratopogonidae - *Culicoides varipennis* - larvae
	Chironomidae - *Chironomis spp.* - larvae
	Syrphidae - not further identified
	Tabanidae - not further identified
	Tipulidae - not further identified
Collembola:	Isotomidae - not further identified

9/28/84 were never observed again in any of the samplings (including fish traps) and presumably died. On 6/17/85 the dissolved oxygen content of the water showed only 0.6 ppm in shaded areas and 0.7-0.8 ppm in water exposed to sunlight (measurements were made at 10:00 a.m., water temperature 24.5°C). Fish will not survive if the dissolved oxygen level falls below ca. 1.0 ppm. (Note: the dissolved oxygen content is lowest just prior to sunrise and should have achieved a much higher level by 10:00 a.m.) Due to the low dissolved oxygen content, no further plantings of mosquitofish were made.

DISCUSSION.-After harvesting the cattail-tule stand recovered quickly except where the rhizomes had been ripped-out. In the latter area, open water persisted for an extended period (Figure 1) which seemed to favor the development of aquatic insect predators, especially beetles. The numbers of mosquitoes remained low during the period when large numbers of insect predators occurred. (Note: maximum mosquito numbers were 0.06/dip or less whereas in 1983 they averaged ca. 6.0/dip). Die-back and lodging of the stand during the winter months initiated a vegetative mat over parts of the water surface. This mat increased during the spring and early summer and by July 1985 the plot was breeding unacceptable numbers of mosquitoes. Control measures were not possible because the dense mat would even prevent the penetration of insecticide granules (note: the vegetative mat was so thick in some areas that one could walk on it without contacting the water beneath). The occurrence of mosquitoes breeding beneath the mats of vegetation is in agreement with the report of Bogaert et al. (1985) who state, "Larvae were collected only in habitats with emergent vegetation. Sampling sites in the interior of ponds A-1 and B, which were characterized by extensive mats of cattail, had the highest populations."

It is apparent that should stands of cattail-tules be used for "polishing" sewage effluent at the Gustine sewage facility, it will be necessary to harvest the vegetation and remove the debris every six to nine months in order to prevent unacceptable levels of mosquito breeding.

It appears advantageous to seek alternate plant species for "polishing" effluent that would have less tendency for lodging and the development of dense mats above the water surface. Efforts to identify such species are now in progress.

Other possibilities include abandonment of the marsh-type of reclamation system for a management scheme that might be more expensive initially but may be cheaper to maintain and have lesser vector breeding potential, e.g. and overland-flow system.

Figure 1.-Central strip where rhizomes were removed remained open for several months after the renovated plot was continuously irrigated.

Figure 2.-Cattail-tules heavily lodged, July 1985.

COLLABORATORS AND ACKNOWLEDGMENTS.-Douglas White, Merced County Mosquito Abatement District, David Witter and John Galante, City of Gustine and Earl Mortenson, California Department of Health Services. This study was funded, in part, by the California Water Quality Control Board and from a special California State appropriation for mosquito control research. The suggestions of Cecil Martin and Tom Inouye of the State Water Quality Control Board, Lonnie Wass of Central Valley Regional Water Quality Control Board and Dr. Russell Fontaine, U. C. Mosquito Research Coordinator, are gratefully acknowledged.

REFERENCES

Bogaert, R., C. Beesley, and R. Yescott. 1985. Mosquito occurrence in a man-made wetland habitat maintained by treated wastewater. 18 pp. Unpublished report, available from California Department of Health Services, Vector Biology and Control Branch.

ABUNDANCE AND DISTRIBUTION OF IMMATURE *CULEX TARSALIS* AND *ANOPHELES FREEBORNI* IN RICE FIELDS OF THE SUTTER-YUBA M.A.D.: II. FOLLOW-UP SAMPLING TO DETECT SIMILARITIES IN LARVAL DISTRIBUTION, 1984 VS. 1985.

Debra Case Lemenager and Eugene E. Kauffman

Sutter-Yuba Mosquito Abatement District
Post Office Box 726, Yuba City, California 95992

INTRODUCTION.-During the mosquito breeding seasons of 1984 and 1985, large areas of rice were sampled in order to detect similarities in larval distribution and abundance. The main objective of the on-going study was to determine if the same fields produced high numbers of larvae from one season to the next (Lemenager et al. 1985). *Culex tarsalis* and *Anopheles freeborni* are the primary rice field mosquitoes within the District. Both species are responsible for the transmission of diseases of public health importance and can be the major nuisance mosquitoes in the area. Control efforts could be greatly enhanced if major breeding locations among area rice fields could be more clearly defined. Earlier investigations have shown that larval densities can differ dramatically from one rice field to the next, with only a few fields being major larval producers (Miura et al. 1983 and Case and Washino 1979). During the 1984 rice growing season, this was found to be true (Lemenager et al. 1985) in District rice fields. The same fields were resampled during the 1985 growing season to determine similarities, if any, in mosquito production.

MATERIALS AND METHODS.-The same 2 study areas sampled in 1984 were sampled again during the 1985 rice growing season. In 1985, the Yuba County study area was located ½ mile NE of Marysville, CA, and contained 2,078 acres of rice with 186 larval sampling sites (9 sites/100 acres of rice). In 1984, there were 3,283 acres of rice with 208 sampling sites or 6 sites/100 acres of rice. The Sutter County study area was located about 5 miles west of Yuba City, CA, and had 2,872 acres of rice with 298 sampling sites or 10.4 sites/100 acres of rice in 1985. In 1984, there were 3,937 acres of rice with 298 sampling sites or 8 sites/100 acres of rice. Sampling was, therefore, more intensive in 1985 than in 1984, having approximately the same number of sample sites but considerably less rice acreage (approximately 32% less).

Larval sampling sites were located at the edges of fields and were usually accessible by road. The sampling method was the same as that used in 1984 (Lemenager et al. 1985), except in the Sutter County study area. At each sample site, 3 dipper samples were taken at each of 8 stations, resulting in a total of 24 dips/site. The genus and stage of each larvae present/dipper sample was recorded and the sample returned. Water temperature and depth and rice height and type of stand (open, sparse, average or dense) was recorded at each site. The Sutter County study area was dipped using a semi-circular pattern (Stewart et al. 1983) having 1 dip/station with a total of 25 stations.

Data were analysed to obtain mean values of mosquito larvae/dip/field. As the Sutter County study area was sampled by another group, sampling in each area was allowed to progress evenly throughout the season. Data in each study area were compared to that of 1984 in order to detect any similarities or patterns in larval distribution and abundance among fields sampled. As in the 1984 study, a threshold level of .08 larvae/dip was used as a cut off point to separate fields into those that produced large numbers of mosquitoes and those that produced relatively low numbers of mosquitoes.

RESULTS AND DISCUSSION.-When data collected during the 1984 season were compared to that collected in 1985, it was found that it would be difficult to predict that a certain field would produce large numbers of mosquito larvae from one year to the next. The following data are shown in Table 1. In the Yuba County study area, a total of 27 fields were compared, 1984 to 1985. For *Cx. tarsalis* and *An. freeborni* larvae combined (all stages combined also), 13 fields (or 48%) of the 27 fields had similar numbers of larvae in 1984 and 1985. Ten of these 13 fields (77%) had numbers of larvae averaging < .08/dip in both seasons. The remaining 3 fields (23%) had averages ≥ .08/dip in both seasons. Fourteen fields (52%) of the 27 fields compared had different numbers of larvae in 1984 and 1985, the difference being that in one season, by field, numbers of larvae averaged < .08/dip, while in the other season, numbers averaged ≥ .08/dip. The remaining percentages shown in Table 1 for 1984=1985 represent fields that had numbers of larvae averaging ≥ .08/dip for each individual year. For *Cx. tarsalis*, 16 fields (60%) of the 27 fields had similar numbers of larvae in 1984 and 1985. Fifteen of the 16 fields (94%) had numbers of larvae averaging < .08/dip for both seasons. Only 1 field out of the 16 (6%) had averages ≥ .08/dip in 1984 and 1985. Eleven fields (40%) of the 27 fields compared had different averages for 1984 and 1985. For immature *An. freeborni*, 18 fields out of 27 (67%) had similar numbers of larvae in 1984 and 1985. Seventeen of these 18 fields (94%) had numbers averaging < .08/dip for both seasons, with only 1 field (6%) having

Table 1.-Comparison of larval abundance by fields sampled, 1984 vs. 1985.

Study Area	*Cx. tarsalis* & *An. freeborni* % Fields[1] 1984 = 1985	% Fields[2] 1984 ≠ 1985	*Cx. tarsalis* % Fields[1] 1984 = 1985	% Fields[2] 1984 ≠ 1985	*An. freeborni* % Fields[1] 1984 = 1985	% Fields[2] 1984 ≠ 1985
Yuba	77% < .08 48% 23% ≥ .08	57% ≥ .08 in '84 52% 43% ≥ .08 in '85	94% < .08 60% 6% ≥ .08	36% ≥ .08 in '84 40% 64% ≥ .08 in '85	94% < .08 67% 6% ≥ .08	100% ≥ .08 in '84 33% 0% ≥ .08 in '85
Sutter	93% < .08 56% 7% ≥ .08	100% ≥ .08 in '84 44% 0% ≥ .08 in '85	100% < .08 84% 0% ≥ .08	100% ≥ .08 in '84 16% 0% ≥ .08 in '85	94% < .08 64% 6% ≥ .08	100% ≥ .08 in '84 36% 0% ≥ .08 in '85

[1] Percentage of fields having similar numbers of larvae, 1984 vs. 1985.

[2] Percentage of fields having different numbers of larvae, 1984 vs. 1985.

Table 2.-Comparison of average numbers of immature *Cx. tarsalis* and *An. freeborni*, 1984 vs. 1985, for each study area.

Study Area	*Cx. tarsalis* & *An. freeborni* 1984	1985	*Cx. tarsalis* 1984	1985	*An. freeborni* 1984	1985
Yuba	.13	.09	.04	.08	.09	.01*
Sutter	.12	.03*	.03	.01*	.09	.02*

* Indicates significant differences between average numbers of larvae, 1984 vs. 1985 (t-test, $\alpha = .05$).

numbers of larvae averaging ≥ .08/dip in 1984 and 1985. Nine fields (33%) out of the 27 compared had different averages for 1984 and 1985. After comparing the data in 1984 and 1985, only 3 fields out of 27 (11%) proved to have numbers of larvae averaging ≥ .08/dip in both years. When converting this from "by field" data to acreage, only 285 acres (represented by the 3 fields) out of the 2,078 acres sampled (or 14% of the study area) had larvae occurring in numbers averaging ≥ .08/dip during both years.

In the Sutter County study area, a total of 25 fields were compared. For immature *Cx. tarsalis* and *An. freeborni* combined, 14 fields (56%) of the 25 fields sampled had similar numbers of larvae in 1984 and 1985. Thirteen of the 14 fields (93%) had numbers of larvae averaging < .08/dip for both seasons. The remaining field (7%) had larvae occurring in numbers ≥ .08/dip in both years. Eleven fields (44%) out of the 25 compared had different averages in 1984 and 1985. For immature *Cx. tarsalis*, 21 fields out of the 25 sampled (84%) had similar numbers of larvae in 1984 and 1985. All of the 21 fields sampled had numbers of larvae averaging < .08/dip in 1984 and 1985. None of the fields proved to have larvae occurring in numbers ≥ .08/dip in 1984 and 1985 for *Cx. tarsalis*. Four fields out of the 25 fields compared (16%) had different averages in 1984 and 1985. For *An. freeborni* larvae sampled, 16 fields (64%) out of the 25 sampled had similar numbers of larvae in 1984 and 1985. Fifteen out of the 16 fields (94%) had numbers of larvae averaging < .08/dip in 1984 and 1985. Only 1 field out of the 16 (6%) had numbers of larvae averaging ≥ .08/dip in 1984 and 1985. Nine fields (36%) out of the 25 sampled had different averages in 1984 and 1985. In the Sutter County study area, only 1 out of 25 fields (4%) proved to have numbers of larvae averaging ≥ .08/dip in 1984 and 1985. When converting from "by field" numbers to acreage, only 75 acres (represented by the 1 field) out of the 1,820 acres sampled in the Sutter County study area (or 4% of the study area) had larvae occurring in numbers averaging ≥ .08/dip for both years. Figures 1-6 show the preceding data in bar graph form, by field for each study area and mosquito species, 1984 vs. 1985.

Table 2 shows significant differences in average numbers of larvae/dip by study area. In the Yuba County study area, for *Cx. tarsalis* and *An. freeborni* combined, no difference was seen between averages found in 1984 and 1985. The same was true for *Cx. tarsalis* alone but *An. freeborni* proved to have significantly lower numbers in 1985. In the Sutter County study area, significantly lower averages were found overall in 1985 when compared to 1984 data for *Cx. tarsalis* and or *An. freeborni*.

Other observations made during the 1984 season were found to occur in 1985 as well. In 1984, it was observed that immature *Cx. tarsalis* were the most abundant at the beginning of the growing season, dropping significantly in numbers by mid-August. Immature *An. freeborni* were significantly lower at the beginning of the summer and were the most abundant by mid-August. These trends were also observed in 1985.

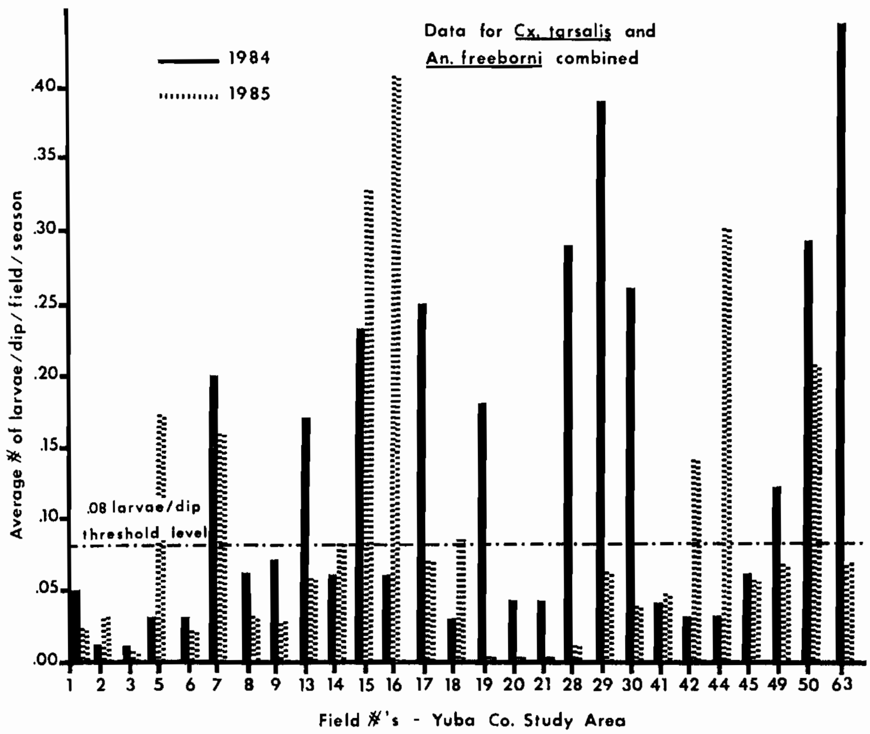


Figure 1.-Histogram showing differences between fields for average numbers of larvae/dip/season, *Cx. tarsalis* and *An. freeborni* data, combined, Yuba County study area, 1984 vs. 1985.

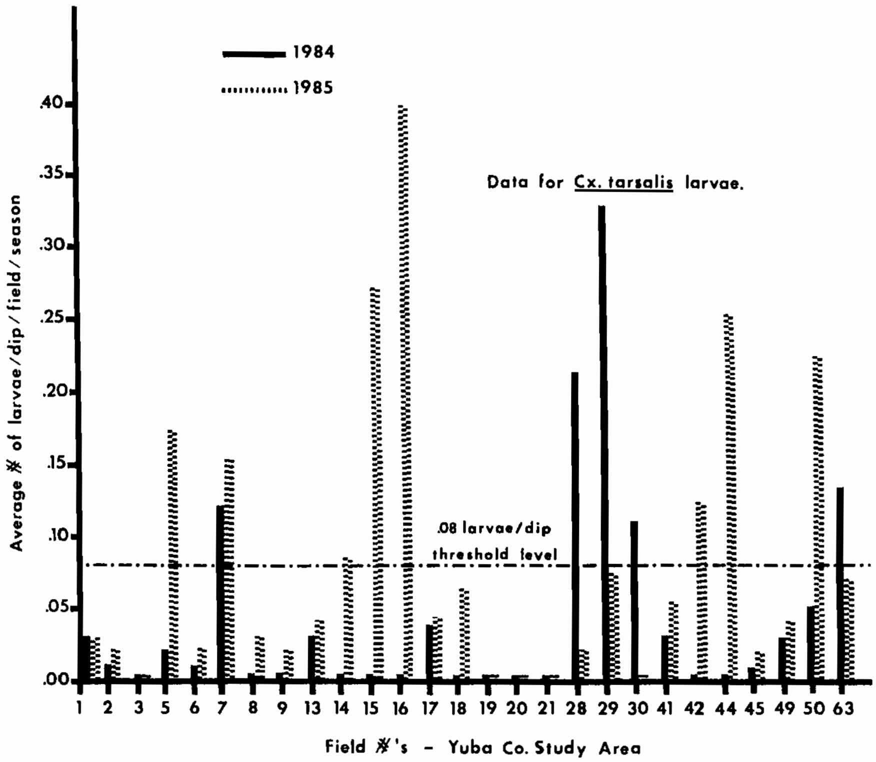


Figure 2.-Histogram showing differences between fields for average numbers of larvae/dip/season, *Cx. tarsalis* data, Yuba County study area, 1984 vs. 1985.

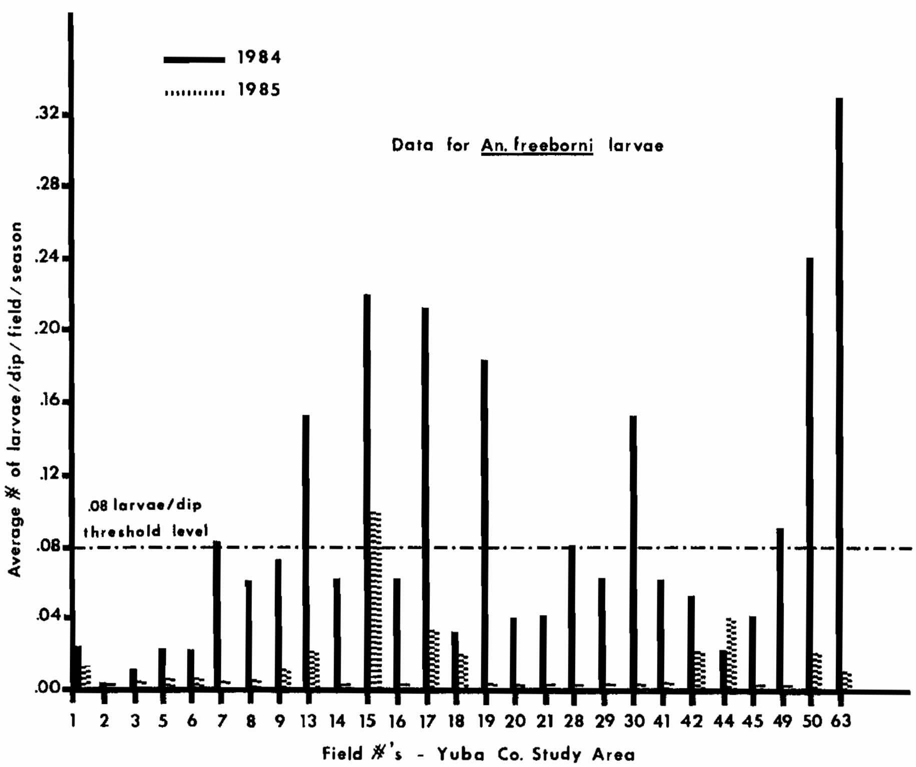


Figure 3.-Histogram showing differences between fields for average numbers of larvae/dip/season, *An. freeborni* data, Yuba County study area, 1984 vs. 1985.

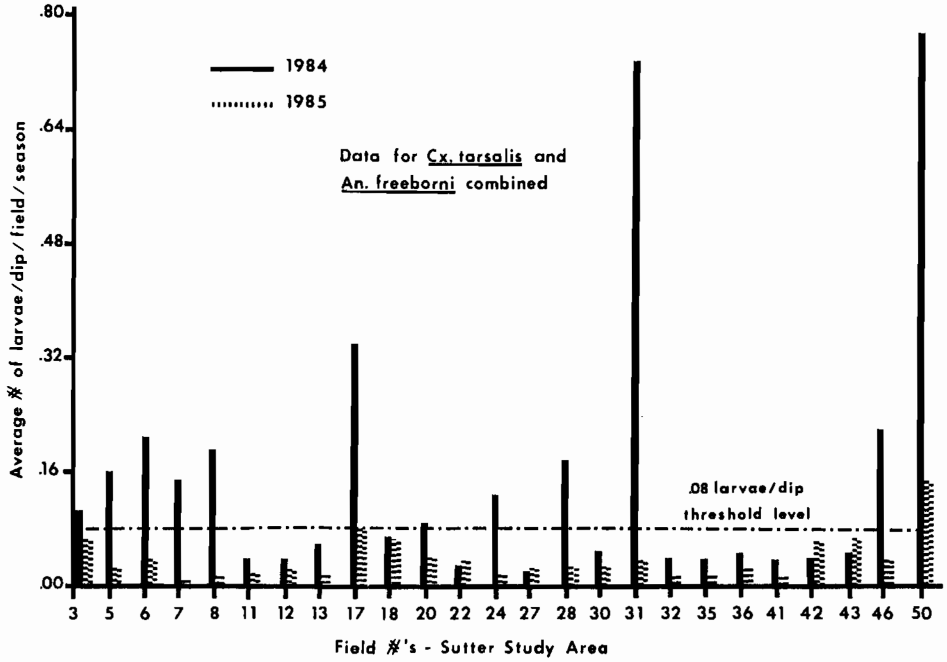


Figure 4.-Hisotgram showing differences between fields for average numbers of larvae/dip/season, *Cx. tarsalis* and *An. freeborni* data combined, Sutter County study area, 1984 vs. 1985.

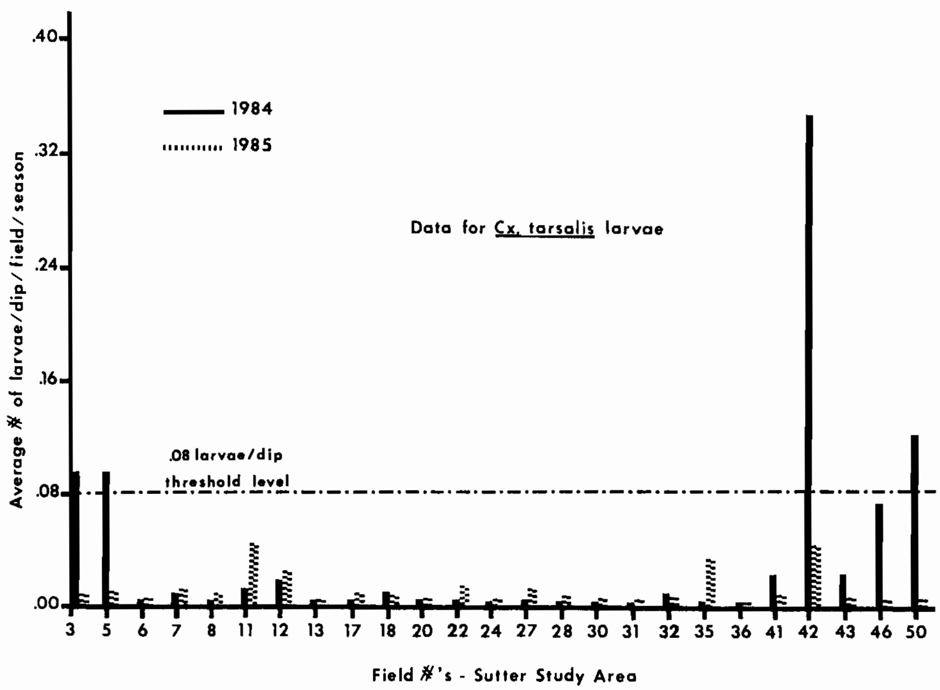


Figure 5.-Histogram showing differences between fields for average numbers of larvae/dip/season, *Cx. tarsalis* data, Sutter County study area, 1984 vs. 1985.

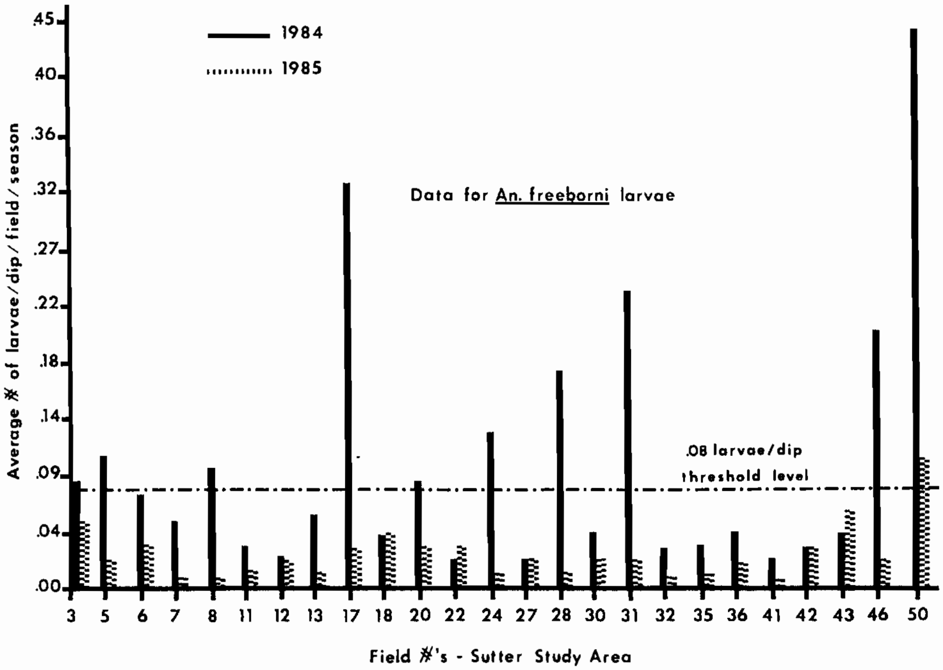


Figure 6.-Histogram showing differences between fields for average numbers of larvae/dip/season, *An. freeborni* data, Sutter County area, 1984 vs. 1985.

CONCLUSIONS.-In both the Yuba and Sutter County study areas, only a small percentage of fields (11% in Yuba, 4% in Sutter) proved to have numbers of larvae averaging ≥ .08/dip in both 1984 and 1985. When disregarding the .08/dip threshold level, it was found that there was roughly a 50-50 chance that a field would have the same numbers of larvae from one season to the next. When using the .08 larvae/dip threshold level, numbers of fields that produced large numbers of mosquito larvae from one season to the next were very low. Several of the fields that were ≥ .08/dip in 1984 were, unfortunately, fallow in 1985. It is possible that some of these fields might have had high numbers of larvae in 1985 if they had been in rice.

These findings would make it very difficult, if not impossible, to predict that certain fields would be producers of large numbers of mosquitoes, season after season. These data represent only 2 years of comparisons, but possibly reflect the actual situation in the field. It would be beneficial, however, to check the 4 fields that proved to have numbers of larvae averaging ≥ .08/dip during both seasons again in 1986. If these fields proved to have ≥ .08 larvae/dip 3 seasons consecutively, it would put a more positive light on the hypothesis that only a few fields consistently produce large numbers of mosquito larvae.

REFERENCES

Case Lemenager, D., S. D. Bauer and E. E. Kauffman. 1985. Abundance and distribution of immature *Culex tarsalis* and *Anopheles freeborni* in rice fields of the Sutter-Yuba M.A.D.: I. initial sampling to detect major mosquito producing rice fields, augmented by adult light trapping. Calif. Mosq. and Vector Contr. Assoc. 53:101-104.

Case, T. J., and R. K. Washino. 1979. Flatworm control of mosquito larvae in rice fields. Science 206:1412-1414.

Miura, T., R. M. Takahashi and W. H. Wilder. 1983. Spatial distribution of *Culex tarsalis* larvae within rice paddies. Calif. Mosq. and Vector Contr. Assoc. 51:58-60.

Stewart, R. J., T. Miura and R. B. Parman. 1983. Comparison of sample patterns for *Culex tarsalis* in rice fields. Calif. Mosq. and Vector Contr. Assoc. 51:54-58.

DEVELOPMENTAL RATES OF MOSQUITO LARVAE IN A WATER MANAGEMENT PROGRAM

Susan Palchick and Robert K. Washino

University of California
Department of Entomology
Davis, California

Over the years our laboratory has been involved in many projects investigating mosquito larval mortality in northern California rice fields. We have examined commercial fields for a gamut of potential factors influencing larval survival including predators, parasites, water temperature, water depth, water source, and field preparation. A major problem with conducting experiments in commercial rice fields is that the extreme variations in cultural practices introduce a number of complicating factors.

This year field research was done in cooperation with the University of California Rice Integrated Pest Management program which provided excellent logistical support that limited variation in experimental parameters involving cultural practices and allowed us to concentrate specifically on the effects of variable water management and weed control strategies on mosquito larval populations.

MATERIALS AND METHODS.-The primary IPM study site was located in Sutter County 3/4 mile west of highway 99, south of East Nicolaus. The field, which was previously planted in wild rice, was divided into 36 plots either 100 ft wide x 305 ft long (nonweed control) or 100 ft wide x 410 ft long (weed controlled). One set of 18 plots was treated with herbicide following the same procedures as would be used in commercial production. No weed control measures were taken on the other set. Of 6 water management regimes planned, 4 were utilized for this study. Those studied included 3 continuous flood: (a) shallow (water level 1-2 in. during first 60 days and then raised to 6-8 inches), (b) medium (3-5 in. for first 60 days and then raised to 6-8 inches) and (c) deep (6-8 in all season). The other water regime was a (d) discontinuous flood, i.e. the water was drained 5 days after sowing, raised to 3-5 inches until 60 days and then raised to 6-8 inches on 8/1/85 for the remainder of the season. Three replicates of the water management regimes were studied for both the nonweed and weed controlled areas.

Dip samples to monitor native larval abundance were taken on 2 occasions (7/10/85 and 8/6/85) with 3 people taking 30 dips apiece with a standard mosquito dipper in each plot. Dips were taken on a transect perpendicular to the levees and parallel to the canal. Numbers of larvae and number per instar were recorded for each dip sample.

Relative larval developmental rates were evaluated by placing first instar mosquito larvae in predator exclusion buckets. The buckets have mesh bottoms and sides which allow for exchange of water but exclude the entry of predators from the rice fields. Cages were collected at several day intervals and larvae were placed in 95% EtOH until their developmental stage (instar) could be determined at a later date.

Predator exclusion sentinel buckets were set up in the Sutter County field with *An. freeborni* on 7/12/85 (collected 7/18/85) and with *Cx. tarsalis* on 7/24/85 (collected 8/5/85).

To assess the effect of water management treatments on the invertebrate and vertebrate faunas of the rice fields, minnow traps were placed in the fields for 24 hour periods (9/3/85-9/4/85 and 9/10/85-9/11/85). When traps were collected the contents were placed in 95% EtOH until the contents could be catalogued in the laboratory.

Colusa county IPM project.-A similar study was conducted in Colusa county. The plots were designed as in 1984. Variations a, b, c, and d (shallow, moderate, deep, and discontinuous flood, respectively) were dipped on July 1 to ascertain the native *Cx. tarsalis* and *An. freeborni* larval populations. Sentinel buckets were set out to monitor relative larval development on June 25. Each bucket contained 20 first instar *Cx. tarsalis*. These were placed in variations a, b, c, and d in rep I and in variations a, b, and c in rep II. Buckets were collected 2, 3, 6, 7, 8, and 10 days after placement.

Another sentinel trial was set up on July 12 with *Cx. tarsalis* and *An. freeborni* larvae in each bucket. This trial was aborted when parathion was inadvertently applied at the inlet boxes on July 19 resulting in 100% mortality.

Diversity was evaluated using the macroinvertebrates collected in dip samples.

RESULTS AND DISCUSSION.-In the Sutter field there was no significant difference in the number of *Cx. tarsalis* (Table 1) or *An. freeborni* (Table 2) collected in the dip samples between treatments, replicates or comparing weed controlled and nonweed basins.

There was less diversity of organisms collected in the minnow traps from treatment "a" than from other treatments on September 4. There was a slight difference in diversity from traps in treatment "a" collected September 10 (Table 3). It was interesting that while there were either *Gambusia affinis, Carrassius auratus*, or *Cyprinus carpie*, in all other treatments on this date, no fish were found in treatment "a", which was held at the shallowest water depth.

For the *An. freeborni* (Figure 1), on the last collection date there was a higher percentage of treatment "a" larvae still in the second instar and the percentage of those attaining the fourth stage was smallest for this treatment.

The same trend was evident of the *Cx. tarsalis*. On all collection dates (days 5, 7, and 13 after being placed in the fields) the development of *Cx. tarsalis* was slower in treatment "a"

Table 1.-Dip sample evaluation of native *Culex tarsalis* larval abundance in Sutter county water management fields, compared for weed treatment, replicates and water depth variations.

Mean Number of Larvae (± s.d.)*			
weed		weed controlled	
0.83 (± 1.2)		1.53 (± 2.6)	
rep I	rep II	rep III	
0.88 (± 1.2)	1.2 (± 1.7)	1.5 (± 2.8)	
a	b	c	d
2.2 (± 3.4)	0.61 (± 1.1)	0.67 (± 0.9)	1.2 (± 1.2)

* per 45 dips

Table 2.-Dip sample evaluation of native *Anopheles freeborni* larval abundance in Sutter county water management fields, compared for weed treatment, replicates and water depth variations.

Mean Number of Larvae (± s.d.)*			
weed		weed controlled	
3.1 (± 3.1)		5.5 (± 6.6)	
rep I	rep II	rep III	
6.4 (± 6.4)	5.1 (± 5.3)	1.3 (± 1.0)	
a	b	c	d
2.9 (± 3.2)	5.5 (± 5.5)	5.9 (± 7.5)	3.6 (± 3.2)

* per 45 dips

Table 3.-Predators collected from minnow traps, Sutter county water management fields at regulated water depths (shallow, medium and deep with drain).

9/3/85 - 9/4/85

	Water level			
	a	b	c	d
individuals	20	14	21	24
kinds	4	9	10	8
traps	8	8	6	8

9/10/85 - 9/11/85

	Water level			
	a	b	c	d
individuals	27	29	55	42
kinds	7	9	10	11

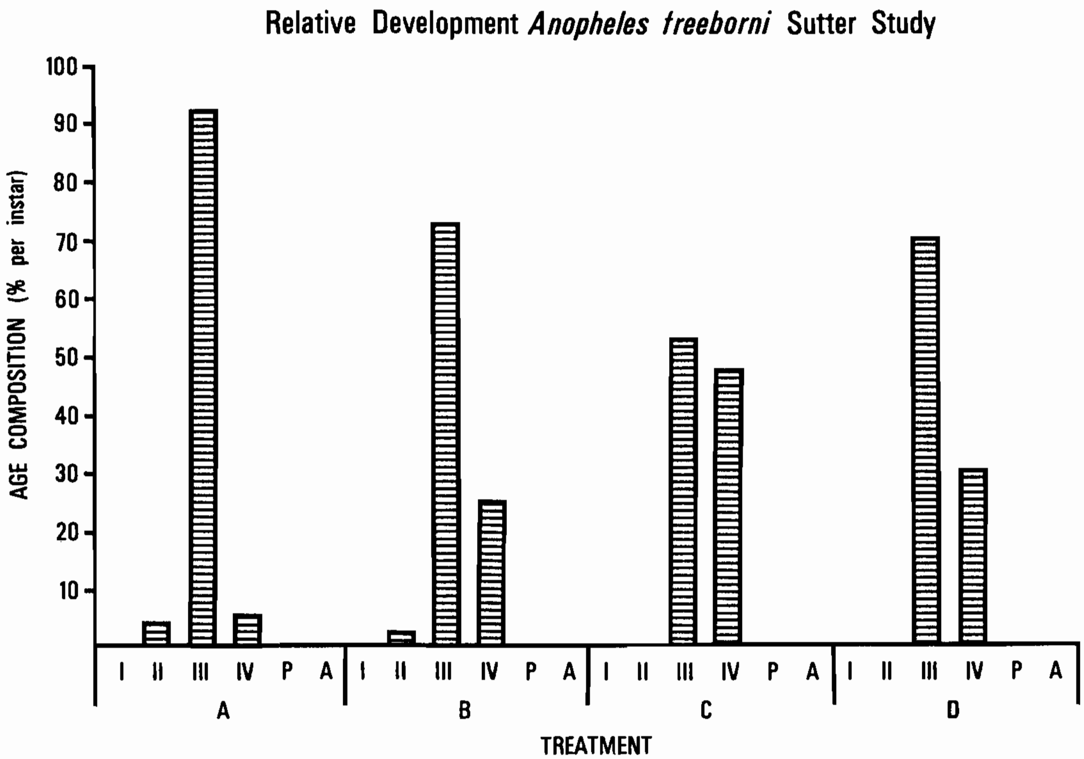


Figure 1.-Age composition of sentinel *Anopheles freeborni* larvae collected 6 days after being placed in the fields as first instars.

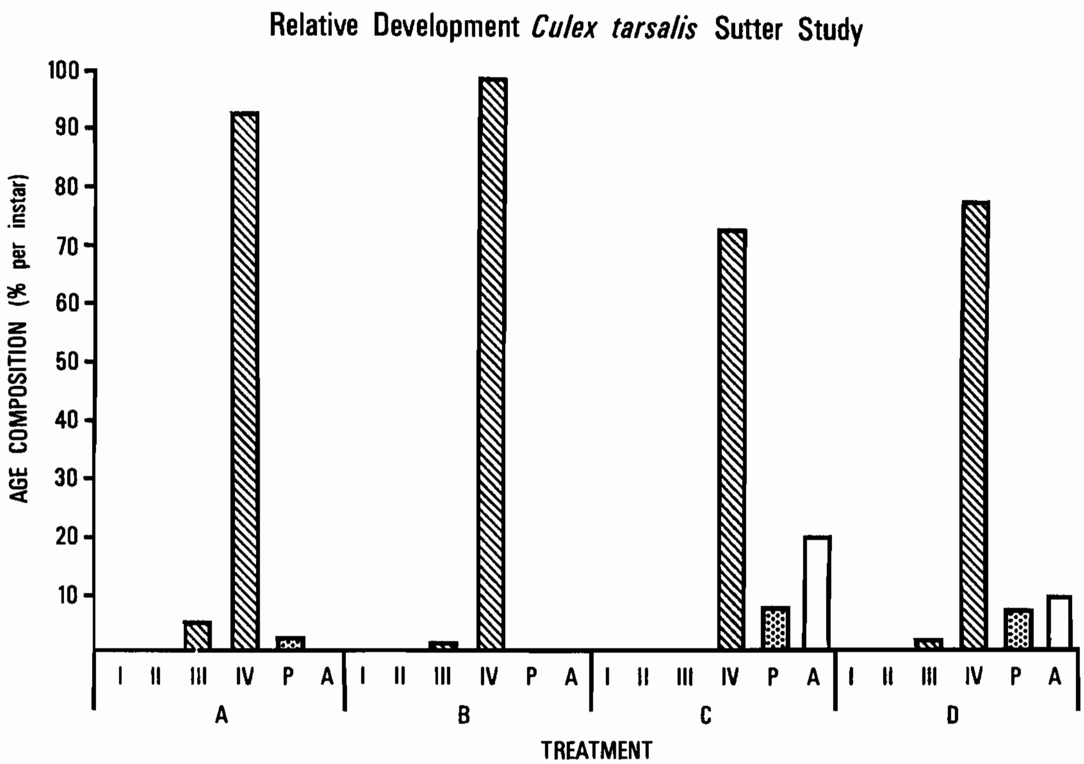


Figure 2.-Age composition of sentinel *Culex tarsalis* larvae collected 12 days after being placed in the fields as first instars.

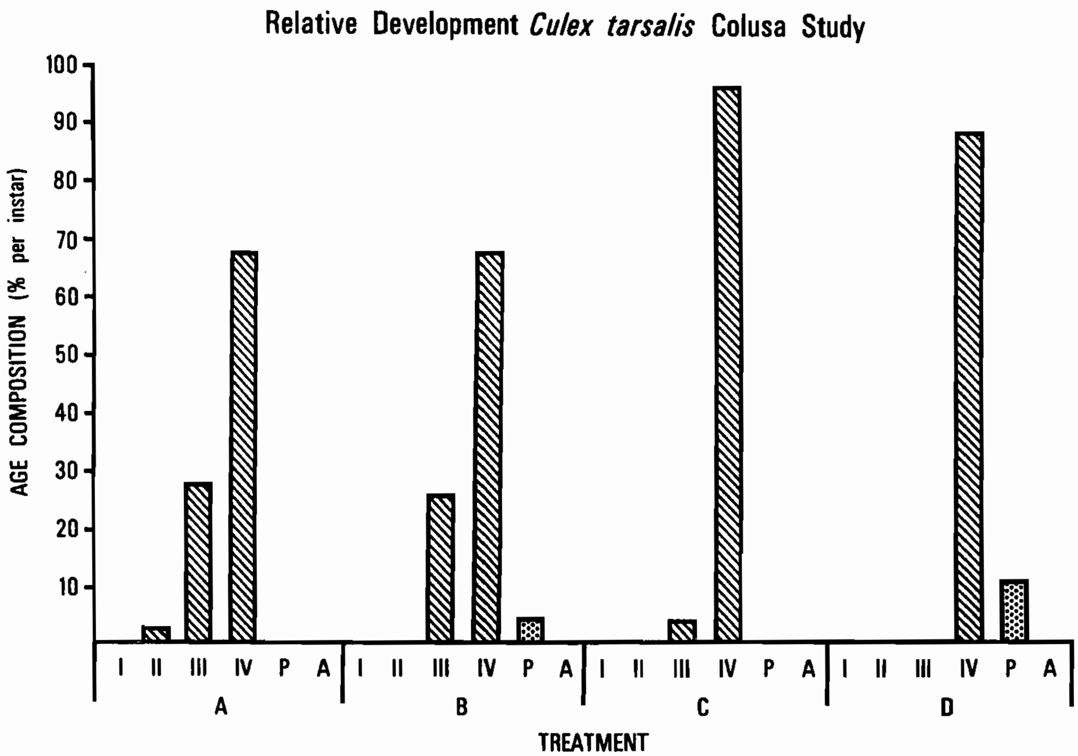


Figure 3.-Age composition of sentinel *Culex tarsalis* larvae collected 10 days after being placed in the fields as first instars.

both in the weed and weed controlled plots (Figure 2). On the last collection date treatment "a" contained the smallest percentage of those developing to pupae and was the only one without *Cx. tarsalis* development to the adult stage. This treatment also had the greatest percentage still in the third larval stage.

In the Colusa county field, all rice checks were negative for both mosquito species when dipped on July 1. The macroinvertebrates from the dip samples were tabulated and there appeared to be a greater diversity of organisms in treatment "d" -- the discontinuous flood treatment.

On all dates that the sentinel buckets were collected (days 2, 3, 6, 7, 8, and 10 days after being placed in the basins), survivorship in the different treatments were similar. Relative development was compared by looking at the proportion of those mosquitoes surviving that reached a particular instar. Larvae in treatment "d" developed faster than in other treatments with only pupae and adults present on the last collection date (Figure 3). Treatment "a" still had second instar larvae and no adults.

This year's sentinel results in both counties support last year's findings in the Colusa field where development was slowest in treatment "a" or the lowest water level. The findings that the development was fastest in the discontinuous flood treatments suggest that some negative factor may be being flushed out when the fields are drained. The slower development in the lowest water depth suggests that some negative factor is more concentrated or more prevalent in this lower water level or that there is simply a lower nutrient level in rice checks with less water.

These differences should be taken into account when implementing water management policies. Elucidation of factors enhancing and detrimental to larval development would be of great interest. It is also necessary to evaluate whether these differences extend through the adult mosquito stage with influence on fecundity and adult longevity.

SURVIVORSHIP AND GONOCYCLE LENGTH OF *ANOPHELES FREEBORNI* AND *CULEX TARSALIS* IN THE SACRAMENTO VALLEY OF CALIFORNIA

C. P. McHugh and R. K. Washino

Department of Entomology
University of California
Davis, California 95616

Preliminary results of a 1984 study of an adult population of *Anopheles freeborni* were previously reported (McHugh and Washino 1986). In essence, the 1984 study consisted of 23 days (13 August through 7 September except the mornings of 1-3 September) of sampling to document the abundance, trophic status, parity, and host selection of *An. freeborni* in a small valley at the edge of the Sierra foothills near Sheridan, California.

Of particular interest in the 1984 study was the use of a mathematical analysis technique described by Birley and Rajagopalan (1981). The Birley-Rajagopalan technique defines 4 estimators: u, the length of the gonotrophic cycle; P(u), the estimated survivorship per gonotrophic cycle; S.E. P(u), the standard error of the estimate of P(u); and R(u), and index of the correlation between the total number and the number of parous mosquitoes collected. In the original paper their method of estimating survivorship, P(u), is only a modification of the parous/total ratio which has been used by many other workers to describe mosquito survivorship. The main distinction of Birley and Rajagopalan (1981) from the other studies is that comparing the number parous and the total number collected at time "t" is not accepted as completely correct. In their version, the parous mosquitoes at time "t" represent survivors of the total from some earlier time, "t-u" where "u" is the length of the gonotrophic cycle.

A preliminary analysis of the 1984 parity data (Table 1) indicated that the parity rate of bloodfed mosquitoes was significantly greater than the parity rate for empty mosquitoes. For this reason, we considered the data for bloodfed and empty mosquitoes separately in subsequent analyses.

Values for the various estimators, P(u), S.E. P(u), and R(u) were computed for values of "u" from 0 through 6 for bloodfed mosquitoes and 0 through 7 for empty mosquitoes (i.e. until a peak in R(u) was demonstrated). The best estimate for P(u) and, therefore, survivorship is that value at which R(u), the correlation index, is highest (Table 2). For empty *An. freeborni* this occurs when "u", the estimate of the length of the gonotrophic cycle, is 6 days. The estimated survivorship over the cycle is 0.14, giving a daily survivorship of 0.72. For bloodfed mosquitoes, the estimated gonocycle length is 4 days with a daily survivorship of 0.75. The additional 2 days required for empty mosquitoes to complete the gonotrophic cycle as well as the lower parity

Table 1.-Summary of age grading data for female *Anopheles freeborni* collected near Sheridan, CA. 19 July - 13 September 1984.

Parity	Trophic Status: Empty Frequency (Column %)	Trophic Status: Bloodfed Frequency (Column %)
Nulliparous	3647 (90.1)	1416 (63.5)
1-Parous	393 (9.7)	764 (34.2)
2-Parous	7 (0.2)	44 (2.0)
3-Parous	2 (0.05)	7 (0.3)
Total	4049	2231

Significant χ^2, $\alpha = .05$.

Table 2.-Parameter estimates for female *Anopheles freeborni* collected near Sheridan, CA, 19 July - 13 September 1984.

Estimator	Empty	Bloodfed
u	6	4
P(u)	0.14	0.31
S.E. (P(u))	0.0071	0.011
R(u)	0.63	0.65
Daily Survivorship	0.72	0.75

Table 3.-Summary of age grading data for female *Culex tarsalis* collected near Sheridan, CA 9-26 July 1985.

Parity	Trophic Status: Empty Frequency (Column %)	Trophic Status: Bloodfed Frequency (Column %)
Nulliparous	760 (62.5)	346 (56.6)
Parous	457 (37.5)	265 (43.4)
Total	1217	611

Significant χ^2, $\alpha = .05$.

rate reflect the time necessary for newly emerged females to mature and mate prior to their first bloodmeal.

While these estimates of gonocycle length and survivorship seem biologically plausible, we attempt to corroborate them using a mark-release-recapture (MMR) and an in-tent release. Unfortunately, neither the MRR nor the in-tent release gave satisfactory results. We are planning additional studies in 1986 to confirm the estimates derived from our 1984 study.

In 1985, we conducted a similar study at the Sheridan study site, this time focusing on *Culex tarsalis*. We sampled for 18 days from 9-26 July. Initial analysis of parity data again showed a significant difference in parity between the empty of bloodfed mosquitoes (Table 3). The mosquitoes were graded only as nulliparous or parous; no attempt was made to determine multiparity.

Parity data were analyzed for "u" = 0 through 9 for empty mosquitoes and "u" = 0 through 8 for bloodfed *Cx. tarsalis*. Results (Table 4) indicate a best estimate of 7 days for the gonotrophic cycle and a daily survivorship of 0.86 for empty mosquitoes. Estimates for bloodfed *Cx. tarsalis* were 5 days for the gonotrophic cycle and a daily survivorship of 0.84. We did not attempt any additional studies in 1985 to corroborate survivorship or gonocycle length estimates for *Cx. tarsalis*. Studies in Kern county by Reisen et al. (1983) and Nelson et al. (1978) using a variety of experimental and analysis techniques have provided estimates which are in reasonable agreement with those of the present study.

In both the *An. freeborni* and the *Cx. tarsalis* there is a 2 day difference in gonotrophic cycle length between empty and bloodfed mosquitoes. It is interesting to note that in their original study with *Culex quinquefasciatus*, Birley and Rajagopalan (1981) did not analyze data for these trophic states separately. Their data indicated a gonotrophic cycle length estimate of 4 and 6 days. They explained away this "two peak" estimate and apparently did not realize the significance of the difference.

A few possible problems exist with the Birley-Rajagopalan technique. It is not clear how

Table 4.-Parameter estimates for female *Culex tarsalis* collected near Sheridan, CA 9-26 July 1985.

Estimator	Empty	Bloodfed
u	7	5
P(u)	0.35	0.42
S.E. (P(u))	0.018	0.024
R(u)	0.65	0.64
Daily Survivorship	0.86	0.84

sensitive the estimation technique is to changes in sampling efficiency. For example, how will estimates be influenced by a red box (or other sampling device) which collects "X" percentage of the population today and 1/2 or 2 "X" % tomorrow? And, in the case of autogenous mosquitoes such as *Cx. tarsalis*, what impact will autogeny have on gonocycle or survivorship estimates? Quantitative answers to these questions will augment the basic information provided here. The analysis technique appears to work, however, and we encourage other field biologists to test it with their data sets.

We would like to acknowledge the help of the Sutter-Yuba M.A.D. in both the 1984 and 1985 studies.

REFERENCES

Birley, M. H. and P. K. Rajagopalan. 1981. Estimation of the survival and biting rates of *Culex quinquefasciatus* (Diptera: Culicidae). J. Med. Entomol. 18: 181-6.

McHugh, C. P. and R. K. Washino. 1986. A study of a semi-isolated population of *Anopheles freeborni* near Sheridan, California. Proc. Calif. Mosq. and Vector Contr. Assoc. 53: 119.

Nelson, R. L., M. M. Milby, W. C.Reeves, and P. E. M. Fine. 1978. Estimates of survival, population size and autogeny in *Culex tarsalis* (Diptera: Culicidae). Entomol. Exp Appl. 9: 327-31.

Reisen, W. K., M. M. Milby, W. C. Reeves, R. P. Meyer and M. E. Bock. 1983. Population ecology of *Culex tarsalis* (Diptera: Culicidae) in a foothill environment of Kern county, California: Temporal changes in female relative abundance, reproductive status, and survivorship. Annals Entomol. Soc. Amer. 76: 800-8.

THE NIGHTLY HOST-SEEKING RHYTHMS OF SEVERAL CULICINE MOSQUITOES (DIPTERA: CULICIDAE) IN THE SOUTHERN SAN JOAQUIN VALLEY OF CALIFORNIA[1]

R. P. Meyer[2], W. K. Reisen[2], M. E. Eberle, M. M. Milby
and W. C. Reeves

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

ABSTRACT

The endogenous host-seeking rhythms of *Aedes melanimon*, *Culex tarsalis*, and *Culex quinquefasciatus* were measured at 3 rural locations in Kern County, California, during 1983 and 1984. Host-seeking females were collected from 1900 to 0700 h at each location by a time segregated sampler (TSS) that partitioned collections into 12 one hour components. The TSS operated on either AC or DC current, and bottled CO_2 gas released at a rate of 1 liter per minute was used as an attractant.

Host-seeking females of all 3 species were most active from 1-4 hours after sunset. During the late spring (May-June) and early fall (September-October), host-seeking activity was most prominent from 1-2 hours after sunset. However, during mid-summer (July-August) when evening air temperatures were warmer, peak host-seeking activity was delayed by 1-4 hours.

Host-seeking activity patterns were not entirely uniform among species or locations. The observed variability may have been related to a combination of factors including interspecific differences in the expression of the endogenous host-seeking rhythm, TSS placement relative to mosquito settlement and breeding sites, mosquito relative abundance, mosquito control activities, and local climatic conditions.

The host-seeking pattern of female *Cx. tarsalis* measured at a location in the Sierra Nevada foothills differed from the pattern measured at 2 other trap sites located on the floor of San Joaquin Valley. At the foothill location, the level of host-seeking activity remained relatively constant throughout the night. However, simultaneous sampling at the 2 valley locations indicated that host-seeking activity was most prominent from 1-4 hours after sunset. Differences in the host-seeking patterns measured at foothill versus valley locations were most likely the result of density dependent factors that affect the success of females being able to successfully blood feed. TSS trap indices at the foothill location frequently exceeded 750 females per trap night while those at valley locations averaged only 30-430 females per trap night. Therefore, we speculate that the persistence in host-seeking activity observed at the foothill location was a direct result of the inability of the abundant resident population to successfully blood feed during the 1-4 hour post sunset period. Either too few hosts were available as blood meal sources or those hosts that were present exhibited some form of avoidance behavior in response to presumed high mosquito attack rates.

In 1965, the nocturnal flight activity of *Cx. tarsalis* was measured at one valley location in Kern County by truck trap. Hourly sampling indicated that flight activity and host-seeking activity are distinctly different. Sampling by truck trap revealed that flight activity of adult *Cx. tarsalis* increased abruptly less than 1 hour after sunset and continued thereafter for 1-3 hours, subsided, and increased abruptly again less than one hour before sunrise. Intensive sampling with TSS samplers at all locations failed to show the existence of a major predawn peak in host-seeking activity. The predawn peak in flight activity probably represents generalized population movements associated with settlement into diurnal resting sites.

Based upon the results of this investigation, infective females of *Ae. melanimon* or *Cx. tarsalis* would most likely transmit arboviruses to humans from 1-4 hours after sunset in the southern San Joaquin Valley. Therefore, adulticiding of host-seeking and potentially infective females would be most effective between 2000 h and 2400 h. Truck trapping indicated that overall population suppression could be achieved by either predawn or post sunset applications of aerosols.

---

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

[2]Arbovirus Field Station, P.O. Box 1564, Bakersfield, California 93302.

POPULATION DYNAMICS OF IMMATURE *CULEX TARSALIS*

William K. Reisen and Richard P. Meyer

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

ABSTRACT

Temporal changes in the population ecology of immature *Cx. tarsalis* were studied weekly from March to October 1985 at a relatively stable and semi-isolated foothill breeding habitat created by oil field effluent (Poso West, Kern County). The relative abundance of adult females collected at CO_2 baited traps was not correlated with larval ($r = 0.322$) or pupal ($r = 0.009$) abundance estimated by dipper sampling at 3 transects, even when lag periods of 1 and 2 weeks were considered. In addition, the number of females with saculate ovaries collected per trap night (an index of recruitment) was not well correlated over time with the number of first ($r = 0.279$) or first + second ($r = 0.238$) instar larvae collected per dip. Lack of time series correlation between adult and immature abundance was attributed to female immigration and difficulty in adequately sampling first instar larvae.

The developmental rates for fed and unfed cohorts of 100 first instar larvae were estimated in replicated predator and oviposition exclusion cages at monthly intervals. Developmental time from first instar through pupation varied inversely as a function of water temperature and the addition of supplemental food. Data on adult wing length and autogeny rate indicated that adults emerging from wild-caught pupae were intermediate in quality between fed and unfed caged cohorts. The quantity and/or quality of food in field water at Poso West was unsuited for optimal immature development. Estimates of instarspecific developmental times indicated that over 50% of immature life was spent in fourth instar, the stage during which maximal growth occurs.

The abundance of each immature life stage was averaged over transects and weekly samples to produce a monthly composite of immature age structure. Abundance was corrected by instar duration, an estimate of sampling probability, and then was used to calculate vertical life tables to estimate survivorship from eclosion to emergence. By comparing the horizontal survivorship of fed and unfed cohorts in predator exclusion cages with vertical estimates of survivorship based on immature age structure, it was possible to estimate the impact of abiotic factors, competition for food, and predation on immature population losses (Table 1). These procedures indicated that predation probably was the most important biotic cause of immature mortality. Disasters such as decreased discharge and thermal pollution had a

Table 1.-Mortality of preimaginal *Culex tarsalis* attributable to abiotic and experimental factors, lack of food, and predation.

	Time periods (1985)			
	Mar/Apr	May/Jun	Jul/Aug	Sep/Oct
First instar to adult survivorship (%):				
1. Caged fed cohorts	ND	91.5	87.3	90.3
2. Caged unfed cohorts	87.7	78.7	73.0	79.3
3. Vertical life tables*	3.2	2.5	1.7	17.7
Possible cause of mortaltiy (%):				
Abiotic factors (100%) - 1)	ND	8.5	12.7	9.7
Lack of food (1 -2)	ND	12.8	14.3	11.0
Predation (2 -3)	84.5	76.2	71.3	61.6

* Mean of first instar to adult survivorship estimated by vertical life table methods for larvae collected at transects during each estimation period.
ND = not done.

marked effect on mosquito abundance and the predator community.

The relative abundance of the associated macroinvertebrates was estimated concurrently by dipping. Damselfly naiads (mostly *Enallagma* and *Ischnura*) were the most abundant predator taxa (0.726 naiads/dip) that successfully tracked *Cx. tarsalis* through time ($r = 0.45$ and 0.61 for third and fourth instar larvae, respectively).

Survivorship data estimated indicated that few *Cx. tarsalis* immatures successfully emerge. Since predation accounted for 60 to 75% of immature population losses, the application of mosquito-specific control agents would be more successful in exerting long term control than broad-spectrum agents which would eliminate the predator community and thus expedite mosquito population recovery. Estimates of developmental time from eclosion to pupation indicated that an insecticide application cycle of 10 day intervals would prevent adult emergence. Since parameter estimates may have been habitat-specific, future research will attempt to provide comparative values for representative breeding sites on the floor of the San Joaquin Valley during 1986.

ACKNOWLEDGMENT.-B.R. Hill,V.M. Martinez and C. Arbolante assisted with specimen collection and processing. This research was funded by research grant AI-3028d from the National Institute of Allergy and Infectious Diseases and by special funds for mosquito research allocated through the Division of Agriculture and Natural Resources, University of California.

LARVAL SURVIVAL ADAPTATIONS OF SOME *AEDES* SPECIES

Robert F. Schoeppner

San Mateo County Mosquito Abatement District
1351 Rollins Road, Burlingame, California 94010

Three larviciding oils, GB 1356, GB 1111, and Flit MLO, were noted to exhibit field and laboratory failures when used against *Aedes squamiger* Coquillett and *Ae. increpitus* Dyar. Surviving larvae in the samples appeared to be in a state of quiescence, resting motionless in a supine position on the bottom of the container. Often larvae could be encouraged to move only after being nudged several times with a probe, but when activated the larvae wiggled to the surface for a brief contact with the oil, then settled to the bottom once again. It was speculated that if the larvae could remain submerged long enough, then the unfavorable contamination or irritant at the surface may be destroyed by the action of the sun or blown away by the wind.

Several questions were raised. How long could the larvae survive when deprived surface contact? Were there alternate means for the larvae to obtain oxygen? If so, do some species show a greater adaptation to withstand such deprivation? What role do the anal papillae serve in respiration?

A search of literature related some early work by Wigglesworth (1933) where he studied alternate forms of respiration using *Ae. aegypti* L. He managed to plug the air siphons of *Ae. aegypti* larvae with paraffin oil and thus demonstrated, with the aid of *Polytoma* flagelates, that oxygen can be absorbed cutaneously throughout the body wall and to a lesser degree across the anal papillae. Only in species that exhibit densely tracheated anal papillae, as *Ae. argenteopunctatus* (Theobald), do workers find any significant amount of oxygen absorption through the anal papillae.

Wigglesworth (1933a), in his work, demonstrated a primary function of the anal papillae was to maintain the osmotic-ionic balance of body fluids. As in the case of brackish water mosquitoes, *Ae. squamiger, Ae. taeniorhynchus* Giles, and *Ae. detritus* (Holiday), the anal papillae are mere nubbins relative to the size of anal papillae of species in habitats where there is an absence of dissolved salts, such as *Ae. aegypti* and *Ae. sierrensis* Ludlow. Wigglesworth utilized *Culex pipiens* larvae to demonstrate the influence of distilled water, tap water, and salt water upon the development of the anal papillae in different media. In the distilled water medium, where salts are lacking, the anal papillae exhibited the greatest development, while the anal papillae of larvae in the salt water medium were atrophied relative to larvae in distilled and tap water.

Lewis (1949) observed larvae of *Ae. argenteopunctatus* rested in an inverted position, balanced on their thoracic hairs and air siphon. Movement of the labral brushes, by the larvae, produced a current that circulated water across their body and densely tracheated papillae. In the laboratory, I observed *Aedes* larvae quiescent on the bottom and assuming a similar supine position as *Ae. argenteopunctatus*.

Macfie (1917) introduced a single *Ae. aegypti* larva into a closed system that permitted aerated tap water to circulate. He found that the larvae grew and molted, as normal but development stopped short of pupation. He concluded that without the addition of supplementary food, the larvae apparently found sufficient food in the circulating water to sustain its survival.

In the laboratory, we attempted to determine the survival time of *Aedes* larvae when subjected to prolonged total submersion, that is submersion without contact to the water surface and maintained in their ambient water, without aeration or supplementary food. The larvae were kept at three temperatures, 72° F, 52° F, and 42° F. The field water temperature, at the time of greatest larval development of *Ae. squamiger*, *Ae. increpitus* and *Ae. sierrensis*, was approximately 52° F. Twenty larvae, 3rd and 4th instars, were introduced into 700 mls. of ambient water. Each sample was replicated three times.

A plastic insert, shaped to fit snuggly into the 11.5 cm. inside diameter of the container, was covered with a fine mesh nylon organdy. The organdy was utilized to permit the escape of trapped air bubbles that formed as the insert contacted the water surface, and too, provided greater ease for introduction of the insert into the container. Different colored inserts were utilized for each species in order to avoid possible confusion.

Activation of the larvae, for daily survival counts, often required light tapping of the container. However, prolonged hard tapping often elicited a negative activation response from the larvae.

Culex pipiens L. exhibited only a slight ability to obtain oxygen by means of cutaneous respiration. At 72° F, 85% larval mortality occurred within two hours of submersion. Total mortality, at each temperature, appeared before three days (Table 1).

Ae. squamiger survived 83 days at 52° F. This represented the longest duration of the six species tested. While the survival of *Ae. squamiger* appeared the highest, in number of days, at 52° F, the other species exhibited their greatest survival at 42° F.

Macfie (1917) noted that younger larvae of *Ae. aegypti* appeared more resistant to submersion than older larvae of the same species. We considered that if early instar larvae had an ability to adapt to longer periods of submersion than late instar larvae, then it would seem that

Table 1.-Days of survival of six mosquito species[1] denied surface contact and maintained at three temperatures. Percent mortality expressed at three levels.

	72°F			52°F			42°F		
	Percent Mortality								
Species	50	95	100	50	95	100	50	95	100
Ae. sierrensis	2.5	5.0	6.0	6.2	10.8	12.0	9.6	17.0	18.0
Ae. squamiger	2.5	17.8	20.0	16.8	52.0	83.0	19.0	27.8	36.0
Ae. nigromaculis	2.5	8.0	14.0	3.3	8.0	10.0	4.8	11.8	15.0
Ae. increpitus	3.0	6.3	7.0	6.5	12.3	13.0	9.8	24.3	26.0
Cs. incidens	1.4	2.6	3.0	5.4	17.0	28.0	8.6	29.5	33.0
Cx. pipiens	0.06	1.0	2.8	0.48	1.4	2.8	0.46	0.8	3.0

[1] Twenty 3rd and 4th instar larvae per sample. Each sample replicated three times.

Table 2.-Comparison of two larval instars of two mosquito[1] species denied surface contact and maintained at three temperatures. Percent mortality expressed at three levels.

	72°F			52°F			42°F		
	Percent Mortality								
Species and Instar	50	95	100	50	95	100	50	95	100
Ae. increpitus 2nd instar	5.0	28.0	32.0	39.0	65.0	94.0	15.6	57.0	67.0
Ae. increpitus 4th instar	1.5	2.6	3.0	13.0	35.0	40.0	3.0	10.0	11.0
Cs. incidens 2nd instars	2.2	4.0	5.0	7.4	27.0	57.0	10.6	18.0	20.0
Cs. incidens 3rd & 4th instars	1.4	2.6	3.0	5.4	17.0	28.0	8.6	29.5	33.0

[1] Twenty larvae per sample. Each sample replicated three times.

larviciding oils would be even less effective against a young population than a mature population.

Tests were initiated using 2nd and 4th instar *Ae. increpitus* and *Culiseta incidens* Thomson larvae. Twenty larvae were introduced into each sample, replicated three times, and exposed to the similar prolonged submersion tests (Table 2) described earlier. The 2nd instar larvae of *Ae. increpitus* exhibited a markedly greater ability to survive prolonged submersion than 4th instar larvae of the same species. Likewise, 2nd instar larvae of *Cs. incidens* exhibited a longer survival period than 4th instar larvae, but not nearly as long as survival shown by *Ae. increpitus*.

From these tests, it appears, as noted by Wigglesworth (1933b), that the body wall of late instar larvae becomes increasingly less permeable to the passage of oxygen and carbon dioxide.

Pupae of each species, when subjected to total submersion tests, as the larvae, died within a short time after being denied surface contact. I suspect that because of their well developed chitinous body wall, pupae no longer could respire cutaneously, thus would die when subjected to total submersion.

In summary, this study of larval survival adaptations to total submersion, provides answers to questions and solutions to problems this District encountered with field failures to control *Ae. squamiger* and *Ae. increpitus* larvae with GB 1356.

REFERENCES

Lewis, D. J. 1949. Tracheal gills in some African culicine mosquito larvae. Proc. R. Ent. Soc. Lond. A 24:60-66.

Macfie, J. W. S. 1917. The limitations of kerosene as a larvicide, with some observations on the cutaneous respiration of mosquito larvae. Bul. Ent. Res. 7:277-295.

Wigglesworth, V. B. 1933a. The effect of salts on the anal gills of mosquito larvae. J. Exp. Biol. 10:1-15.

Wigglesworth, V. B. 1933b. The function of anal gills of mosquito larvae. J. Exp. Biol. 10:16-26.

OBSERVATIONS ON THE DEVELOPMENT OF *CULEX PEUS* SPEISER IN SOUTHERN CALIFORNIA DAIRY WASTEWATER PONDS

Allan R. Pfuntner[1]

ABSTRACT

The immature developmental cycle of *Culex peus* Speiser in dairy wastewater ponds varied from 7 days to 12 days during the observation months of April to September, 1977. Oviposition was initiated in April and terminated in early January of the following year. The mean developmental time during the summer was 10.2 days at an average water temperature range of 69.8 - 81.5° F. The mean developmental period during the spring was 15.2 days at a mean water temperature range of 64.3 - 75.6° F. The mean number of eggs per raft was 269. Chemical analyses of the dairy waste water revealed an ammonia content of up to 150 ppm and a dissolved oxygen content approaching zero.

INTRODUCTION.-The agricultural area of the Chino Valley is located in both Riverside and San Bernardino Counties. Nearly 500 dairies operate within about four miles or less of suburban housing developments. Inevitably, the residents adjacent to the dairy area experience problems with various insects, particularly mosquitoes. It is not uncommon to collect over 800 male and female mosquitoes in one week in a single New Jersey light trap located in the rural/suburban interface. The most frequently trapped species are *Culex peus* Speiser and *Culex quinquefasciatus* Say, with the former predominating. Though not a common biter of humans, *Cx. peus* has been shown to harbor both western equine and St. Louis encephalitis viruses (Hammon and Reeves, 1943a, b; Emmons, Grodhous, and Bayer, 1974). The sources wherein these species breed are ponds used to impound water resulting from the washing of dairy cows. In many, if not most instances, the ponds contain varying amounts of floating organic materials that clump together forming moving islands. In addition, the banks of the ponds support weed growth which frequently overhangs the water's edge. The result of the above is a multitude of protected habitats optimum for the aquatic portion of the mosquito life cycle.

In 1977, the following study was initiated to ascertain specific information regarding the life cycle of *Cx. peus* in an effort to increase the effectiveness of control measures applied by the Northwest Mosquito Abatement District.

MATERIALS AND METHODS.-Two dairy waste-water ponds were selected in the western agricultural area of the District. These sources had a history of producing high numbers of mosquitoes if left untreated. The ponds were approximately one-half and three-quarters of an acre in size, with moderate floatage (less than one-fifth the pond surface area). Oviposition devices and rearing chambers, fabricated from one inch sheet styrofoam and one quart plastic cups (Figure 1.), were placed in each pond. Water and air temperatures were recorded using maximum/minimum thermometers. Chemical tests, other than pH, were performed by a commercial laboratory.

Each observation day, any egg rafts laid during the previous 24-hour period were removed. The number of eggs per raft and the raft dimensions were documented in the laboratory using a stereo scope and ocular micrometer. Randomly selected egg rafts were placed in each rearing chamber in the dairy pond and allowed to progress through the life cycle. On a daily basis, a sample of 30 immatures were removed from the chamber. The life cycle stage of each specimen was noted and each was returned to the chamber. Species other than *Cx. peus* were discarded. When pupae occurred, they were removed, counted, and transferred to identical chambers for continued development in the pond environment. As adults emerged, they were removed by aspiration, counted, and sexed.

From March through November, 1977, observations were made daily or bi-daily as deemed necessary. During the months of December, 1977, through February, 1978, observations were performed on a three day cycle.

RESULTS AND DISCUSSION.-Chemical analyses of the ponded waste water showed dissolved oxygen to be in the range of 0.0 to 0.6 ppm. The pH ranged from 7.0 to 7.8, and the ammonia (NH_3) was between 100 and 150 ppm. By way of comparison, typical water supporting mosquito fish (*Gambusia affinnis*) usually has a dissolved oxygen content range of 7 to 15 ppm, with the minimum being 1.0 ppm. The usual pH range is 7.5 to 10.0, and the ammonia ranges from 0.1 to 1.0 ppm, with a maximum of over 5 ppm (Coykendall, 1980).

The larvae develop readily in these polluted conditions in concentrations greater than 1500 per dip (personal observation). During this study, the immature densities ranged from zero in February and March to nearly 1000 per dip in August and September.

The number of eggs present in the rafts sampled ranged from 152 to 374 with the mean being 269. The large standard deviation (58) indicates a wide variability in the number of eggs laid by each female in each raft (Table 1.). The range of the raft width values is small (SD=0.3)

---

[1]Study was conducted while in the employ of the Northwest Mosquito Abatement District, 6851 Granite Hill Drive, Riverside, California 92509.

Table 1.-Egg Raft Characteristics, *Culex peus* Speiser.

	Range	Mean	Std. Dev.	N
Num.eggs/raft	152 - 374	269	58	30
Raft length	8.1 - 11.0 mm	9.4 mm	0.8	30
Raft width (maximum)	3.5 - 4.8 mm	4.3 mm	0.3	30

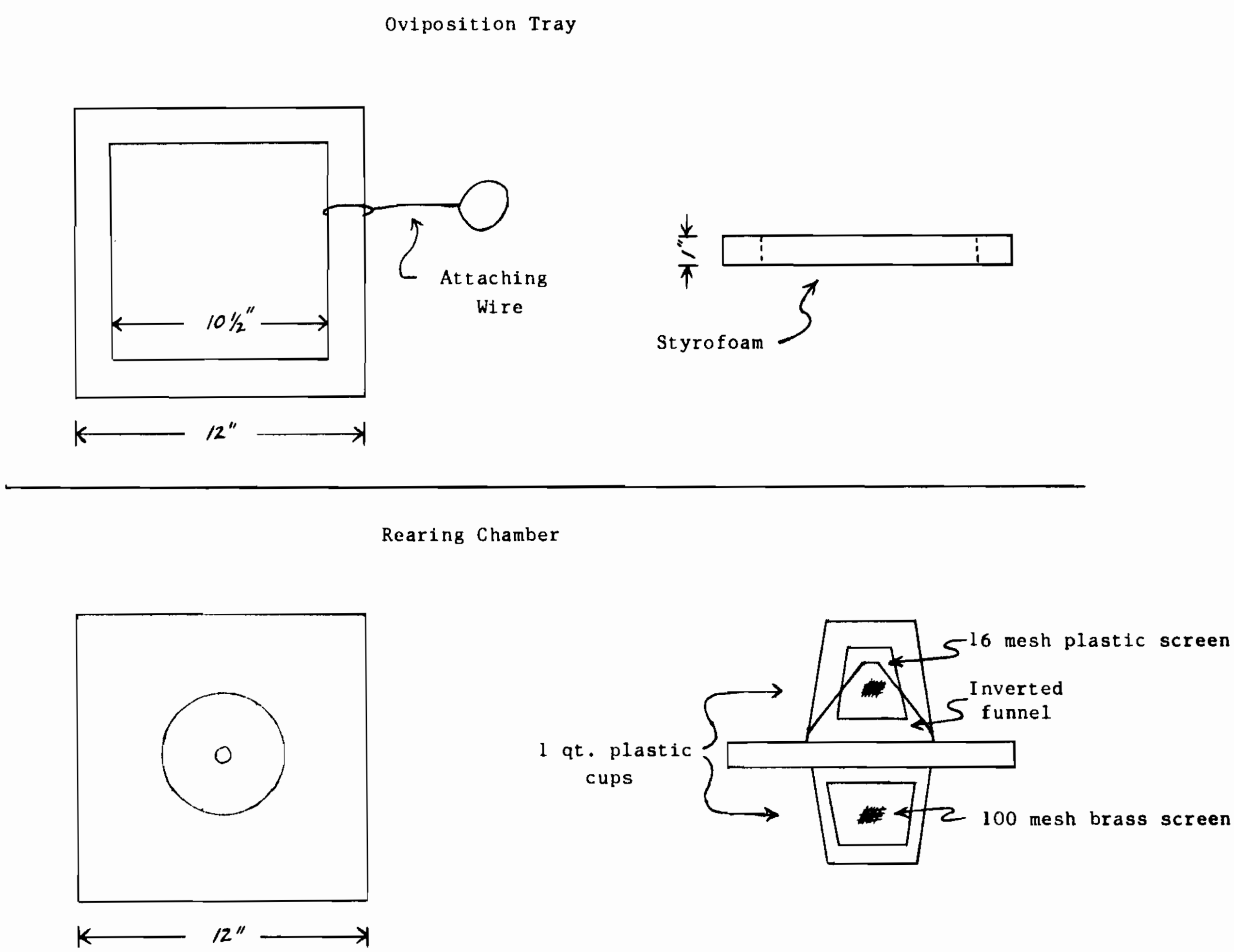


Figure 1.-Oviposition Tray and Rearing Chamber, *Culex peus* Speiser.

whereas the range of the raft length values is larger (SD=0.8).

The first egg rafts laid in the oviposition trays were observed on April 7. The water temperature ranged between 64 and 74° F (mean = 69°F), with an ambient temperature of 66° F at 9:00 AM. The range of the air temperature was 42 to 88° F, with an ambient reading of also 66° F. Though the ambient water and air temperatures were the same in the above instance, the ambient air temperature does not appear to be a satisfactory parameter by which to judge the probability or possibility of egg deposition as readings varied greatly during a given time frame. Ambient water temperatures tended to be more restricted in their ranges. Oviposition was sporadic through the months of April and May. In June, the number of rafts deposited in the trays increased dramatically. When rafts were observed, the average number present was 137. The maximum rafts counted in one tray was 630. During July, the rafts found per tray decreased to an average of 27 - the maximum noted was 175. The mean for August was 38, with a maximum deposition of 75 rafts. After August, oviposition was reduced to an average of less than 10 rafts per observation, excluding a one day peak of 223 rafts on October 24. Sporadic oviposition again occurred during November and December. The last observed raft deposition (a single raft of 97 eggs) was on January 9, 1978, at 9:30 AM with a water temperature range of 49 to 55° F and an ambient water temperature of 50° F (Figure 2).

Of the initial volume of 1897 eggs, 1571 individuals reached the fourth instar stage, indicating a survival rate in the rearing chambers of 82.8% (Table 2). The number of adults produced from the aforementioned larvae totalled 1442 for a 91.8% survival rate. The overall survival rate of the chamber reared specimens, based upon the initial egg count and ending adult tally, was 76.0%. In the natural state, the number of surviving adults would be much less. In general, mosquito species exhibit overall survival rates of less than 5% (Reisen and Siddiqui, 1979; Reisen et al, 1982; Reisen et al, 1986).

The observed sex ratio of *Cx. peus* followed the expected norm of 1:1 (Table 3). On the first day of emergence, male mosquitoes were predominant with a ratio of about 2:1. The situation was reversed on day two. The final day of emergence produced females in the ratio of nearly 3:1. The majority of adults were produced

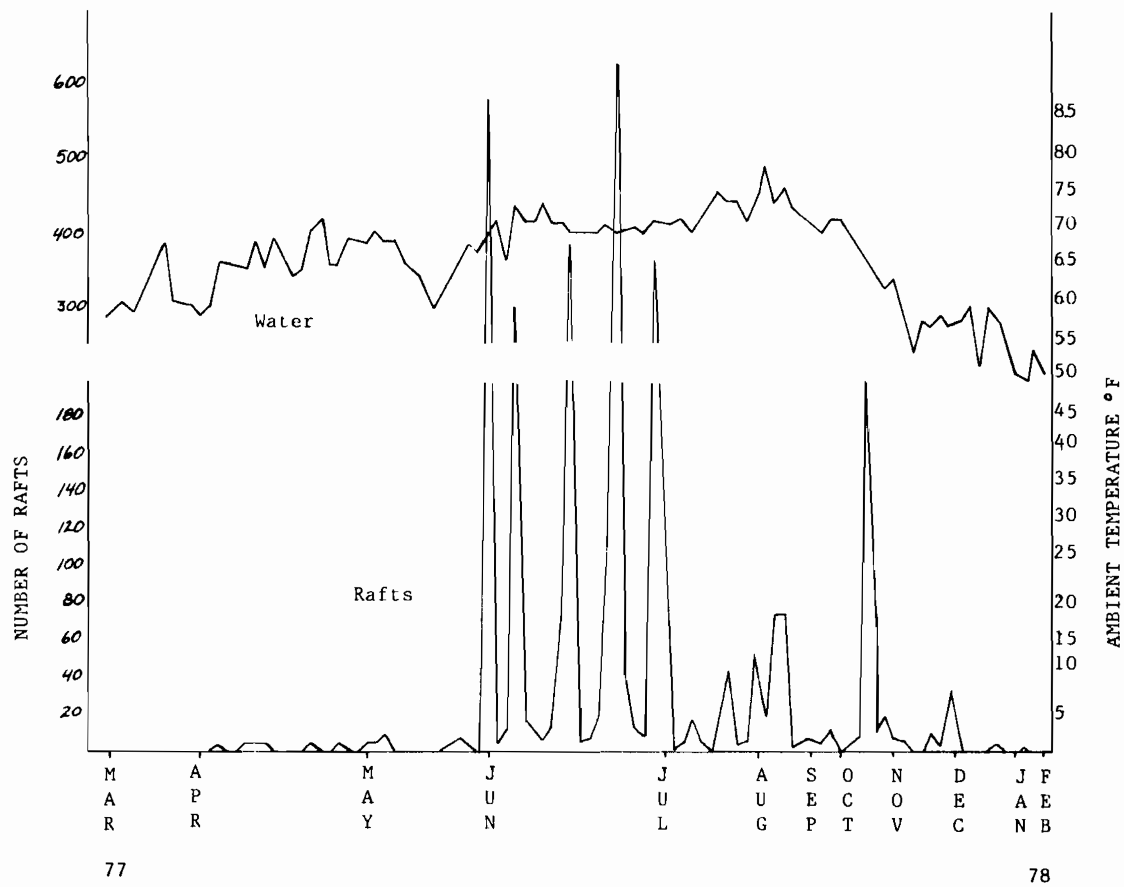


Figure 2.-Seasonal Egg Raft Deposition, *Culex peus* Speiser.

on days one and two (93%), while day three yielded the lowest volume (7%).

The developmental time spans of successive stages, during the months of June through August, were significantly shorter than those noted for the months of april and May (Table 4). Note that the maturation of the first and second instars during the time periods cited in Table 4 are very similar, even though statistically different. Greater time differentials occurred from the third instar through the adult stage. The later developmental stages also exhibited greater ranges of values. Similar developmental time spans were noted in urban catch basins (Pfuntner, 1978) and under laboratory conditions (Bohart and Washino, 1978). Ball and Chao (1956) observed that the cycle from egg to adult required 18 to 26 days at 72° F.

As the Northwest Mosquito Abatement District uses oils such as Golden Bear 1356 to achieve control, treatments must be completed on a cycle that produces mortality of the immature stages prior to adult emergence. During the summer months, the cycle should be less than ten calendar days as the adult emergence begins on developmental day ten. Based upon the observed oviposition in the first week of April, initiation of control activities in the spring should begin by April 15. Mosquito activity could begin either before or after the above date, of course, depending upon the water and air temperatures, diurnal photoperiod, and the presently unknown habits of *Cx. peus*. It appears, however, that periodic checks of sources for ambient water temperatures approaching 66° F, between 9:00 and 10:00 AM, could forewarn field personnel of impending oviposition activity.

ACKNOWLEDGMENTS.-This study was authorized by Mr. L. Lino Luna, Manager, Northwest Mosquito Abatement District. Invaluable field assistance was afforded by Mr. R. R. (Doc) Coplen, Field Supervisor, Northwest M.A.D. Thanks must also be extended to Mr. Jon Bos and Mr. Neil DeVries for allowing the use of their dairy waste water ponds.

Table 2.-Survivorship of *Culex peus* Speiser in Rearing Chambers Suspended within Dairy Wastewater Ponds.

Raft Number	Number of Eggs	Number of 4th Instars	Number of Adults Produced
1	152	129	120
2	218	174	161
3	374	292	273
4	305	256	230
5	276	237	215
6	252	214	196
7	320	269	247
Total	1897	1571	1442

Percent immatures surviving: 82.8
Percent adults hatching: 91.8
Percent overall survival: 76.0

Table 3.-Comparison of Adult Emergence by Sex, *Culex peus* Speiser.

Day	Males	Females	Combined	Percent	Ratio (male to female)
1	214	106	320	58	2.02 : 1
2	62	133	195	35	1 : 2.15
3	8	30	38	7	1 : 3.75
Total	284	269	553		

Table 4.-Development Duration by Stage, *Culex peus* Speiser.*

Date Range	Avg Days to 1st Instar	Avg Days to 2nd Instar	Avg Days to 3rd Instar	Avg Days to 4th Instar	Avg Days to Pupa	Avg Days to Adult	Avg Water Temp min	Avg Water Temp max	Avg Water Temp ambient
4/22 - 5/10 **	1.9 SD = 0.7 R = 1 - 3	3.0 SD = 0.4 R = 2 - 4	5.4 SD = 1.0 R = 4 - 6	7.9 SD = 1.7 R = 6 - 10	10.2 SD = 1.8 R = 8 - 12	15.2 SD = 2.2 R = 13 - 18	64.3 ~ SD = 2.4 R = 60 - 68	75.6 ~ SD = 4.2 R = 69 - 82	67.2 ~ SD = 3.1 R = 62 - 74
6/14 - 9/3 +	1.3 SD = 0.5 R = 1 - 2	2.4 SD = 0.6 R = 2 - 3	4.5 SD = 0.6 R = 4 - 5	6.4 SD = 1.3 R = 5 - 8	8.3 SD = 1.2 R = 7 - 9	10.2 SD = 1.6 R = 9 - 11	69.8 ~~ SD = 2.3 R = 62 - 74	81.5 ~~ SD = 2.9 R = 76 - 90	72.2 ~~ SD = 2.0 R = 70 - 76
Statistical Comparison of Above ++	t = 2.7 (sig dif)	t = 3.3 (sig dif)	t = 3.1 (sig dif)	t = 2.8 (sig dif)	t = 3.5 (sig dif)	t = 4.6 (sig dif)	t = 34.6 (sig dif)	t = 26.8 (sig dif)	t = 29.4 (sig dif)

* - stage designation based upon 50% Of specimens exhibiting stage characteristics
** - N=4
\+ - N=5
\++ - Student's t at 95% CL
~ - N=19
~~ - N=30

REFERENCES

Ball, G. H. and J. Chao. 1956. Laboratory Colonization of *Culex stigmatosoma*. Mosq. News 16:306.

Bohart, R. M. and R. K. Washino. 1978. Mosquitoes of California. University of California, Berkeley, CA. 153 pp.

Coykendall, R. L. (ed.). 1980. Fishes in California Mosquito Control. Calif. Mosq. and Vector Contr. Assoc. Sacramento, CA. 63 pp.

Emmons, R. W., G. Grodhaus, and E. V. Bayer. 1974. Surveillance for Arthropod-borne Viruses and Disease by the California State Department of Health, 1973. Proc. Calif. Mosq. and Vector Contr. Assoc. 42:193-203.

Hammon, W. McD. and W. C. Reeves. 1943a. Laboratory Transmission of St. Louis Encephalitis Virus by Three Genera of Mosquitoes. J. Exp. Med. 78:241-53.

(Ibid). 1943b. Laboratory Transmission of Western Equine Encephalomyelitis Virus by Mosquitoes of the Genera *Culex* and *Culiseta*. J. Exp. Med. 78:425-34.

Pfuntner, A. R. 1978. The Development and Control of *Culex quinquefasciatus* Say and *Culex peus* Speiser in Urban Catch Basins. Proc. Calif. Mosq. and Vector Contr. Assoc. 46:126-29.

Reisen, William K. and T. Siddiqui. 1979. Horizontal and Vertical Estimates of Immature Survivorship for *Culex tritaeniorhynchus* (Diptera: Culicidae) in Pakistan. J. Med. Entomol. Vol. 16, no. 3:207-218.

Reisen, William K., K. Azra and F. Mahmood. 1982. *Anopheles culicifacies* (Diptera: Culicidae): Horizontal and Vertical Estimates of Immature Development and Survivorship in Rural Punjab Province, Pakistan. J. Med. Entomol. Vol. 19, no. 4:413-422.

Reisen, William K. 1986. Observations on the Ecology of Immature *Culex tarsalis*. Longitudinal studies in a foothill habitat. Proc. Calif. Mosq. and Vector Contr. Assoc. (in press).

PARASITISM OF *AEDES SIERRENSIS* BY *OCTOMYOMERMIS TROGLODYTIS* (NEMATODA: MERMITHIDAE) IN CALIFORNIA TREEHOLES

J. O. Washburn, J. R. Anderson, and D. E. Egerter

Division of Entomology and Parasitology
University of California
Berkeley, CA 94720

ABSTRACT

Octomyomermis troglodytis is a recently described natural enemy of the western treehole mosquito, *Aedes sierrensis*. Previously, this parasite was known from only a single treehole in Marin county, California. From field investigations of natural enemies affecting mosquito populations, we have 384 records of mermithid-infected mosquitoes from 24 treeholes. This paper presents new findings on the distribution, biology, dispersal, and habits of this little known mermithid.

IMPROVING FIELD COMPETITIVENESS OF LABORATORY-REARED *CULEX TARSALIS* MALES

S. M. Asman and R. H. Dadd

Department of Entological Sciences
University of California, Berkeley

ABSTRACT

In pursuing research relevant to the use of the sterile-male technique as part of integrated programs for controlling *Cx. tarsalis*, mass-produced release males did not adequately compete against the field males for field females. Since it was known that the essential fatty acid, eicosapentaenoic acid, was necessary in the completely-defined sterile organic diet for newly emerged adults to fly and survive, and data demonstrated high levels of fatty acids in field material compared to low levels in laboratory-reared, the possibility of a nutrient deficiency in the rearing diet became highly suspect.

Various modifications of the routine laboratory diet by the incorporation of fish-based materials known to have high contents of the long-chained fatty acids that give rise particularly to eicosapentaenoic acid in mosquito tissues, gave promising results. Adding codliver oil as a uniform deposit via ether solution directly onto the routine dietary mixture to fourth instars proved to be the best method of diet supplementation.

Relative to growth and vigor the adults reared on the codoil supplement had weight increases, parallel increases in adult size and increased adult longevity. A newly-obtained flightmill system which by automated processing through an IBM XT computer measures flight capability in terms of frequency, duration, velocity and distance, is currently testing possible flight behavior improvements in laboratory-reared material.

THE INFLUENCE OF CHILLING ON LARVAL DIAPAUSE IN *AEDES SIERRENSIS*

Truls Jensen and G. A. H. McClelland

Department of Entomology
University of California, Davis

ABSTRACT

Studies done in our laboratory have shown that *Aedes sierrensis* larvae will enter and remain in a 4th instar diapause if the photoperiod to which they are exposed does not exceed 12 hours at 20 degrees C or less. This was found to be the case in larvae collected from the field in October, November and early December as well. Larvae collected in January and early February will however pupate at a photoperiod of 11 hours. This indicates a shift in the critical photoperiod necessary to break diapause occurring in the overwintered larvae.

Previous experiments performed in our laboratory indicated that larvae exposed to low temperatures (below 5 degrees C) for at least 30 days pupated earlier and with greater synchrony than larvae exposed to less than 30 days of chilling. From these results we postulated that the natural exposure of larvae to low temperatures during the coldest part of the year shifts their sensitivity to photoperiod allowing them to pupate at a shorter photoperiod.

To test the effect of chilling on larvae under natural conditions we placed larvae in artificial plastic treeholes outdoors where they would be exposed to the naturally changing photoperiod and temperatures. To modulate the amount of chilling for different treatments we used heating cables under the plastic containers. Four treatments were established based on the length of heating cable in contact with the artificial treeholes: Ambient (with no extra heat), Low (contact with 1 length of heating cable), Moderate (contact with 2 lengths of heating cable) and High (contact with 3 lengths of heating cable). The heat output of the cable was controlled by a variable resistor.

The temperatures in each of the treatments differed significantly with each of the treatments following the daily and seasonal fluctuations in ambient temperature but remaining approximately 5, 7.5, and 10 degrees C. above the ambient temperature in the low, moderate and high heat treatments respectively. The mean pupation time (days ± St. Dev.) in each of the treatments were as follows: high heat 93.6 ± 12.5, moderate heat 99.2 ± 7.9, low heat 95.6 ± 8.8 and ambient 100.3 ± 6.4. The differences between the ambient and high, ambient and low, and the moderate and high treatments were significant ($P < .05$). There was no significant differences in pupation times between the ambient and moderate and the low and high treatments inspite of significant temperature differences in the treatments. We interpret this as follows:

Temperatures in the high treatment were so high that they overode the slowing effect of no chilling. Temperatures in the ambient treatment, due to the natural below average temperatures in the winter of 1985 caused slower than normal development. The chilling effect alone is demonstrated by the moderate heat treatment pupating 3.6 days lower than the low heat treatment that was 2.5 degrees C cooler.

THE BIOLOGY AND BIOLOGICAL CONTROL POTENTIAL OF *LAMBORNELLA CLARKI* (CILIOPHORA: TETRAHYMENIDAE), AN ENDOPARASITE OF THE WESTERN TREEHOLE MOSQUITO, *AEDES SIERRENSIS*

J. R. Anderson, D. E. Egerter, and J. O. Washburn

Division of Entomology/Parasitology
University of California
Berkeley, California 94720

Ciliatosis in mosquitoes has been described from such diverse areas as Africa (Muspratt, 1945, 1947), Europe (Grasse and de Brissezon, 1929), Korea (Yu et al., 1978), Southeast Asia (Corliss, 1961), California (Kellen et al., 1961; Sanders, 1972; Clark and Brandl, 1976; Egerter and Anderson, 1985; Washburn and Anderson, 1986) and Oregon (Hawley, 1985) in the U.S., and the U.S.S.R. (Dzerzhinsky et al., 1976). Given their widespread occurrence, it is somewhat surprising that so little research has focused on these ciliate-mosquito interactions. On the basis of past limited knowledge, some reviewers have concluded that ciliates have low potential for controlling mosquito populations (McLaughlin, 1971; Henry, 1981), while others have cited a need for more research before the biological control potential of ciliates can be accurately assessed (Corliss and Coats, 1976; Annon., 1981; Canning, 1982; Clark, 1985).

For many years, ciliates were classified as members of the Phylum Protozoa, but in 1980 the Society of Protozoologists (Levine et al., 1980) elevated Protozoa to Subkingdom rank and split the old Phylum Protozoa into six separate Phyla. Ciliates now are classified in the Phylum Ciliophora (Lee et al., 1985).

We currently are studying the biology of *Lambornella clarki*, a ciliate which is an endoparasite of the western treehole mosquito, *Aedes sierrensis*. The primary goal of our research is to evaluate the potential of *L. clarki* as a biological control agent of *Ae. sierrensis* and other container-breeding mosquitoes.

The life-cycle of *L. clarki* is closely synchronized to that of its natural host. *Aedes sierrensis* survives the dry season in desiccation-resistant eggs, while *L. clarki* is maintained in dry treeholes in desiccation-resistant cysts. First-instar larvae of *Ae. sierrensis* and motile ciliates of *L. clarki* can be found shortly after winter rains begin to fill the treeholes. The ciliates start their endoparasitic existence by adhering to the cuticle of their mosquito larval hosts in an unique invasion cyst first described by Clark and Brandl (1976). Through these cuticular cysts, the ciliates enter the hemocoel where they multiply slowly. In nature parasitized larvae are overwhelmed in approximately three weeks by the multiplying ciliates. Moribund or dead larvae appear opaque from the hundreds of ciliates which occupy the hemocoel of all major body regions from the head capsule to the anal papillae, and such larvae tend to float at the water surface. As these cadavers decompose they can release hundreds of ciliates which may horizontally infect other larvae or form desiccation-resistant cysts. Since late instar *Ae. sierrensis* larvae indiscriminately filter-feed on microorganisms of all kinds, including *L. clarki* ciliates, Washburn and Anderson (1986) postulated that endoparasitism may represent an effectively evolved strategy to avoid predation.

In addition to parasitizing all immature stages, *L. clarki* is also commonly found infecting *Ae. sierrensis* adults (Egerter and Anderson, 1985; unpubl. data). Although they only survive about half as long as normal adults, infected adults have been implicated as dispersal agents of this parasite (Egerter et al., 1986). Two methods of dispersal have been observed. First, *L. clarki* is actively dispersed by infected females which have been parasitically castrated by ciliate invasion and occupation of the reproductive tract. Such females exhibit oviposition behavior, but instead of laying eggs as a normal, gravid female would, the parasitized females deposit ciliates into water containers. Secondly, adults of both sexes may also passively disperse ciliates into treeholes by dying and decomposing on water surfaces. Since infected adults tended to die in water containers in the laboratory, it seems likely that wild *Ae. sierrensis* adults resting and dying in treeholes may also serve as an important natural means of inoculating treeholes with this parasite.

At our principle study site in Mendocino County, approximately 50% of the treeholes are positive for *L. clarki*. This widespread distribution indicates that dispersal by infected adult mosquitoes is indeed effective. In addition, we have found persistance within treeholes to be excellent with 90% of the treeholes sampled over three years remaining positive for *L. clarki* (Washburn and Anderson, 1986).

Surveys of treehole mosquito populations throughout the coast range and in the Sierra Nevada during the past several years revealed the presence of *L. clarki* in all geographic areas sampled so far in California. The parasite is also known to occur in Oregon (Hawley, 1985). A total of 37 out of 142 treeholes sampled in our surveys have been found positive for the ciliate representing sites as far south as San Diego and as far north in California as Mendocino county (University of California Hopland Field Station). However, our studies indicate that *L. clarki* may be more prevalent in northern California. In general, the ciliate was absent from treeholes having water with extreme pH and electrical conductivity (salinity) (Egerter and Anderson, 1985; Washburn and Anderson, 1986). We have found *L. clarki* in treeholes near sea level and at elevations of 1300 m where ice had to be chipped off the treehole water surface to obtain a sample. In all cases, *L. clarki* was found to be pathogenic to *Ae. sierrensis*.

Our most recent studies indicate that *L. clarki* is a major cause of mortality in developing larval populations of *Ae. sierrensis*. This parasite therefore appears to have much promise as a manipulated biological control agent of *Ae. sierrensis*, and possibly other container-breeding mosquito species.

REFERENCES

Annon. 1981. Data sheet on the biological control agents-*Lambornella clarki* Corliss and Coats, 1976, and *Lambornella stegomyiae* Keilin, 1921. World Hlth. Orgn./VBC 81.803 Mimeo Document, Feb. 1981.

Canning, E. U. 1982. An evaluation of protozoal characteristics in relation to biological control of pests. Parasitology 84:119-149.

Clark, T. B. 1985. *Tetrahymena* and *Lambornella* (Protozoa). In Biological control of mosquitoes, Eds. Chapman, H. C., A. R. Barr, M. Laird, and E. E. Weidhaas. American Mosquito Control Association, Bull. #6. Bookcrafters, Chelsea, Michigan. pp. 56-58.

Clark, T. B. and D. G. Brandl. 1976. Observations on the infection of *Aedes sierrensis* by a tetrahymenine ciliate. J. Invert. Path. 23:341-349.

Corliss, J. O. 1961. Natural infection of tropical mosquitoes by ciliated protozoa of the genus *Tetrahymena*. Trans. Roy. Soc. Trop. Med. Hyg. 55:149-152.

Corliss, J. O. and D. W. Coats. 1976. A new cuticular cyst-producing tetrahymenid ciliate, *Lambornella clarki* n. sp., and the current status of ciliatosis in culicine mosquitoes. Trans. Amer. Micros. Soc. 95:725-739.

Dzerzhinsky, V. A., E. A. Nam, and A. M. Dubitsky. 1976. The finding of *Lankesteria culicis* and *Tetrahymena stegomyiae* in larvae of *Aedes aegypti*. Parasitologija 10:381-382 (in Russian with English summary).

Egerter, D. E. and J. R. Anderson. 1985. Infection of the western treehole mosquito, *Aedes sierrensis* (Diptera: Culicidae) with *Lambornella clarki* (Ciliophora: Tetrahymenidae). J. Invert. Path. 46:296-304.

Egerter, D. E., J. R. Anderson, and J. O. Washburn. 1986. Dispersal of the parasitic ciliate, Lambornella clarki: Implications for ciliates in the biological control of mosquitoes. Proc. Natl. Acad. Sci. USA 83:7335-7339.

Grasse, P. P. and P. de Boissezon. 1929. *Turchinella culicis* n.g., n.sp. infusoire parasite de l'hemocoele d'un *Culex* adulte. Bull. Soc. Zool. Fr. 54:187-191.

Hawley, W. A. 1985. Population dynamics of *Aedes sierrensis*, In: Ecology of Mosquitoes: Proceedings of a Workshop. L. P. Lounibos, J. R. Rey, and J. H. Frank, Eds., Ent. Soc. Amer. pp. 167-184.

Henry, J. E. 1981. Natural and applied control of insects by Protozoa. Ann Rev. Entomol. 26:49-73.

Kellen, W. R., W. Wills, and J. E. Lindegren. 1961. Ciliatosis in *Aedes sierrensis* (Ludlow). J. Insect Path. 3:335-338.

Lee, J. J., S. H. Hunter, and E. C. Bovee. 1985. An illustrated guide to the Protozoa. Society of Protozoologists, Allen Press, Inc. Lawrence, Kansas U.S.A. 629 pp.

Levine, N. D., J. O. Corliss, F. E. G. Cox, G. Deroux, J. Grain, B. M. Honigberg, G.F. Leedale, A. R. Loeblich, J. Lom, D. Lynn, E. G. Merinfield, F. C. Page, G. Poljansky, V. Sprague, J. Vavra, F. G. Wallace. 1980. A newly revised classification of the Protozoa. J. Protozool. 27:37-59.

McLaughlin, R. E. 1971. Use of protozoans for microbial control of insects. In Microbial Control of Insects and Mites (ed. H. D. Burges and N. W. Hussey), Academic Press, New York. pp. 151-172.

Muspratt, J. 1945. Observation on the larvae of treehole breeding Culicini (Diptera: Culicidae) and two of their parasites. J. Ent. Soc. S. Africa, 8:13-20.

Muspratt, J. 1947. Notes on a ciliate protozoan, probably *Glaucoma pyriformis*, parasitic in culicine mosquito larvae. Parasitology 38:107-110.

Sanders, R. D. 1972. Microbial mortality factors in *Aedes sierrensis* populations. Proc. Calif. Mosq. and Vector Contr. Assoc. 40:66-68.

Washburn, J. O., and J. R. Anderson. 1986. Distribution of *Lambornella clarki* (Ciliophora: Tetrahymenidae) and other mosquito parasites in California treeholes. J. Invert. Path. 48:296-309.

Yu, H. S., H. W. Cho, and J. S. Pillai. 1978. Field survey of mosquito pathogens in South Korea and infection of *Anopheles, Aedes,* and *Culex* larvae by protozoan parasites *Tetrahymena* sp. and *Lankesteria culicis* (Ross). Rpt. Natl. Inst. Hlth. Korea 15:283-290.

EVALUATION OF SEVERAL EMERGENT MOSQUITO SAMPLING ATTRACTANTS WITH NEW MICROCOSM ENVIRONMENTS FOR USE IN MOSQUITO CONTROL RESEARCH

Daniel T. Castleberry

Department of Wildlife and Fisheries Biology
University of California
Davis, California 95616

ABSTRACT

As part of a study to evaluate the mosquito control efficacy of fishes in waste water environments, a series of microcosms were developed to be used as replicated, controlled test sites. This paper describes these microcosms, presents results of a test of their efficiency for trapping emerged mosquitoes and evaluates use of economical attractants to improve this efficiency.

Results indicate that lighted, striped, and clear (non-attractant) traps are equally efficient, capturing greater than 60 percent of emerged mosquitoes in enclosed microcosms. Sugar-baited and dark traps were less efficient. Overall, results show the percentage of emerged mosquitoes captured is relatively high and the variation reasonably low. This suggests that these microcosms should provide statistically significant evaluations of the mosquito control efficacy of selected larvivorous fishes in waste water environments.

INTRODUCTION.-I am presently participating in a study to evaluate the mosquito control efficacy of selected larvivorous fish species in waste water environments. As part of this evaluation, a series of microcosm ponds were developed to be used as replicated, controlled tests sites. Such sites allow rigorous statistical comparisons of mosquito control measures under carefully controlled environmental conditions. This paper describes a newly designed microcosm system, presents the results of a test of the efficiency of this system for trapping emerged mosquitoes and evaluates the use of economical attractants to improve this efficiency.

Microcosms are easily controlled, observed and replicated experimental systems. These features make microcosms valuable for testing hypotheses and establishing the effects of environmental manipulations, such as mosquito control measures. As a result, microcosm environments are often used to evaluate the effects of various treatments on the ecology of complex systems(Giesy 1980, Dickerson and Robinson 1985, Uhlmann 1985), including evaluating mosquito control measures in rice fields (Blaustein and Karban 1984).

The microcosm evaluated in this study used passive mosquito collection traps. To take advantage of the benefits of a microcosm design, it is important that these traps provide representative samples of emerged mosquitoes. Samples should be large relative to their variability to increase their statistical tractability. Traps should also capture mosquitoes soon after emergence to minimize effects of natural adult mortality and possible autogenous reproduction in enclosed microcosms, both of which increase variability between replicates. The most efficient traps, those that capture the largest percentage of emerged mosquitoes as soon after emergence as possible, are desired.

Attractants are known to improve the efficiency of a wide variety of mosquito traps (Service 1976, Bidlingmayer 1985). Attractants commonly used with traps fall into these categories: animal-baited traps, carbon dioxide baited traps, sound traps, light traps, and visual attraction traps (Service 1976). The first three types proved economically unfeasible as the cost in money and time was too great to permit their long term, continuous use. Light traps and visual attraction traps were time economical and inexpensive, and therefore fit the criteria for inclusion in the study. I also included another category, food-baited traps. This was plausible as the structure of the microcosms did not allow emerged mosquitoes access to food. Overall, efficiencies of one light trap, two visual attraction traps (contrasting stripes and dark body), one food-baited trap, and a control, non-attractant trap were compared.

MATERIALS AND METHODS.-The microcosm design consisted of placing large funnels of fiberglass window screen over 210 liter fiberglass tanks (Figure 1). The window screen funnel was supported by four hardwood dowels. These extended form a ring of polyethylene pipe, which rested on the outside edge of the tank, to a piece of plywood. The window screen funnel opened through a round hole in the center of the plywood. Another similarly cut piece of plywood was fixed on top of the other piece and a wide-mouthed canning jar ring lid was glued in its opening. This allowed a collection jar (0.95 L wide-mouth canning jar) to be screwed over the opening atop the window screen. A clear plastic funnel sat base down inside the opening of the collection jar and prevented captured mosquitoes from easily escaping. The entire top structure was fixed to the tank by a drawstring which ran

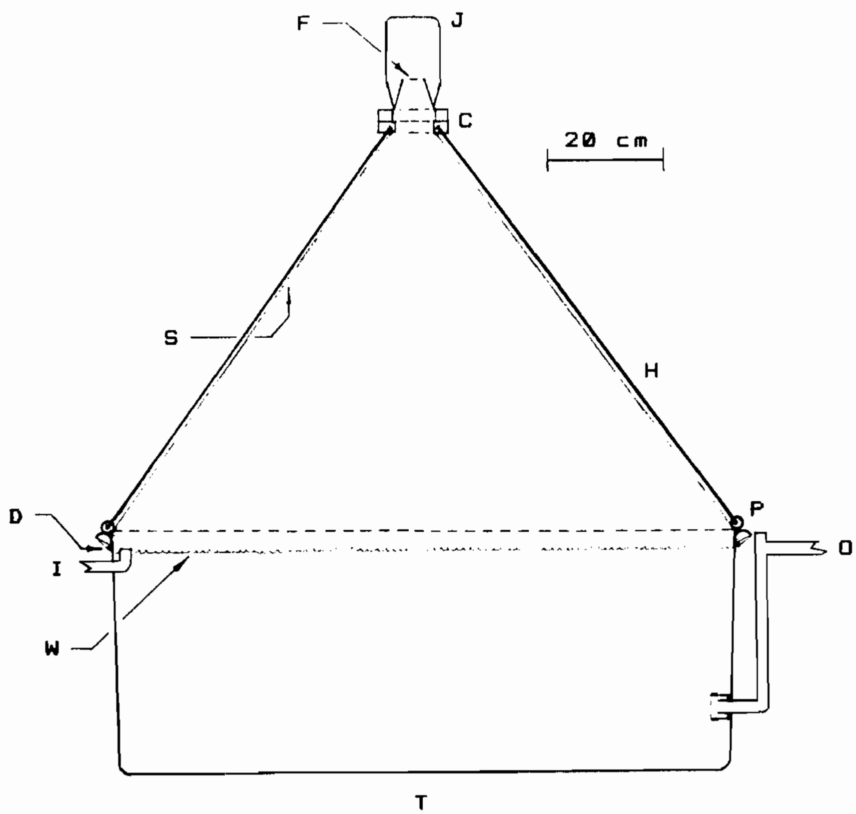


Figure 1.-Cut-away, scale diagram of a microcosm (J = 0.95 L glass collection jar, F = clear plastic funnel, C = plywood collection jar support, S = fiberglass window screen, H = hardwood dowels supporting window screen and collection jar support, P = polyethylene tubing to which dowels attach, D = drawstring holding top structure to lower tank, I = tank inflow, W = water level in tank, O = screened tank outflow, T = 210 L fiberglass tank).

through a strip of nylon material sewn to the bottom of the window screen. The drawstring allowed access to the microcosm enclosures. The window screen and plastic funnels completely enclosed the microcosm, preventing mosquitoes from escaping or entering, except for escape into the collection jars. Inflow and outflow PVC pipe provided water circulation through each tank. Twenty of these microcosms are located at the University of California, Davis, Waste Water Treatment Facility where the following experiment was conducted on 25 June through 7 July 1985.

I tested four attractants with the collection jars. These were lighting the jars (lighted), covering them with black and white striped material (striped), baiting them with raisins (sugar-baited), and covering them with opaque material (dark). One set of jars were left unaltered (clear) as a control. The light source on the lighted jars was an Archer 25 mA, 1.5 volt bulb powered by an Eveready Energizer Alkaline 1.5 volt, size D battery. The material on the striped jars had alternating 20 mm wide, verticle, black and white stripes. Eight raisins were situated inside the jar where the plastic funnel and jar met and provided the sugar-bait in the sugar-baited jars. The material on the dark jars was black cloth covered with a 15 cm x 10 cm x 6.5 cm brown paper bag. There were four replicates of the five treatments distributed in a randomized block design throughout a ten by two microcosm grid.

Fifty *Culex tarsalis* pupae, taken from a laboratory population at the University of California, Davis, were placed in water contained in 207 ml cups that were then floated in each of the microcosm ponds. Twice daily counts were made of the number of pupae emerged and number and sex of the mosquitoes trapped in each jar at around 0800 and 1900 hours. These counts were continued until all pupae had emerged and all adults were trapped or had died in the microcosm enclosures. After each count, captured mosquitoes were removed from the microcosm so that the next count would consist only of those mosquitoes captured since the last count. The two counts for each day were summed to give daily counts.

Differences in the total numbers of mosquitoes captured in each of the jars with the different attractants were evaluated by taking the ratios of numbers of adults trapped to numbers of pupae emerged (to remove variation in numbers of pupae emerged from variation in numbers of adults trapped), applying a logarithmic transformation to these data (to normalize the ratios) and a two-way ANOVA (Snedecor and Cochran 1980) followed by Duncan's new multiple range test (Steel and Torrie 1960). Differences in numbers of female and male mosquitoes captured in jars with different attractants were analyzed using t-tests (Snedecor and Cochran 1980). Since numbers of males and females emerged were unknown, these data cannot be corrected for differences in numbers emerged between sexes.

RESULTS.-Lighted, striped, and clear jars were similar in their mosquito capture efficiencies (Figure 2). Sugar-baited jars had lower capture efficiencies early in the trial and caught up with the other attractants by the seventh day, while dark jars had much lower capture rates throughout the trial.

Lighted jars were the only jars to capture significantly more males than females, although all treatments showed a tendency in this direction (Figure 3).

DISCUSSION.-Results indicate that lighted, striped, and clear jars are equally efficient mosquito attractants under the experimental conditions, capturing about 60 percent of emerged mosquitoes (Figure 2). Some of those that were not captured died immediately after emergence, probably before or during their initial flight, and were never available for capture. A better representation of trap efficiency would include only mosquitoes that successfully complete their first flight. This would suggest that the traps were more efficient than my analysis would indicate. Other untrapped mosquitoes survived the initial flight period but died one or two days after emergence, probably due to starvation as mosquitoes denied access to food under similar conditions in the laboratory die within three days (Personal observation). This suggests that, although the capture efficiency was probably greater that 60 percent, it was less than 100 percent. Given the relatively small variation in these measurements, capture efficiencies of this magnitude should yield statistically significant results within reasonable levels of replication. As clear jars are easiest and least expensive to build and maintain, these will be used for future microcosm experiments.

Sugar-baited jars had lower capture rates early in the experiment (Figure 2). In addition, mosquitoes in microcosms with sugar-baited jars lived longer than those in other microcosms. All microcosms with sugar-baited jars had first and second instar larvae in the microcosm following the death of adults. These observations indicate that mosquitoes in these microcosms were feeding on the sugar source in the jars, allowing them to maintain high levels of activity in the jars, which increased their probability of escape. Those that escaped could not be counted as captured. With energy acquired from raisins, females were presumably able to produce autogenous egg rafts.

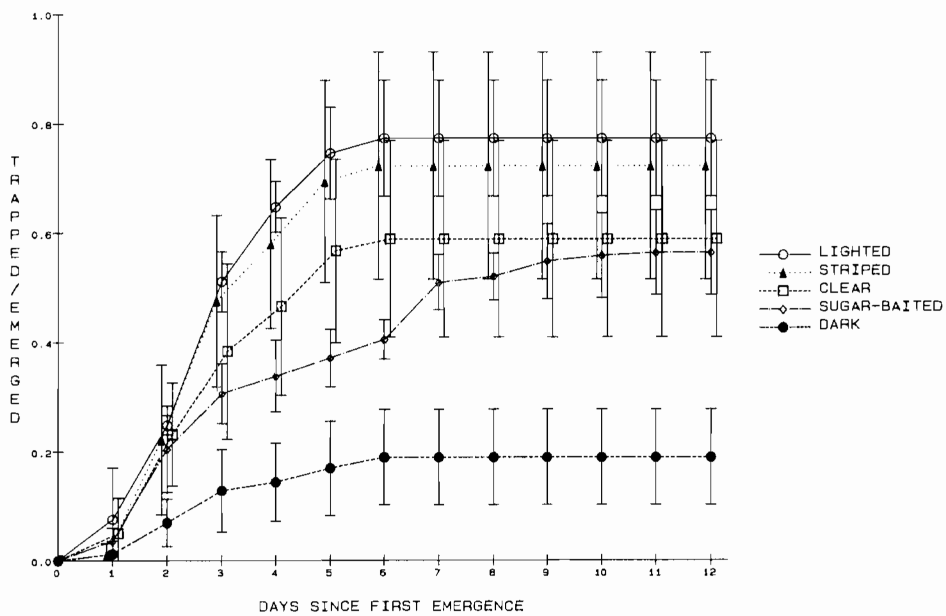


Figure 2.-Mean cumulative number of mosquitoes trapped per number emerged (± 2 S.E., n = 5) of 50 *Culex tarsalis* pupae placed in microcosms. Overlap of standard error bars correspond to a lack of significant difference between means, except between lighted and sugar-baited, which are not significantly different after day seven despite a lack of overlap (two-way ANOVA, $P < 0.05$). Differences in capture efficiency are attributed to the attractants (see figure key) used with the collection jars.

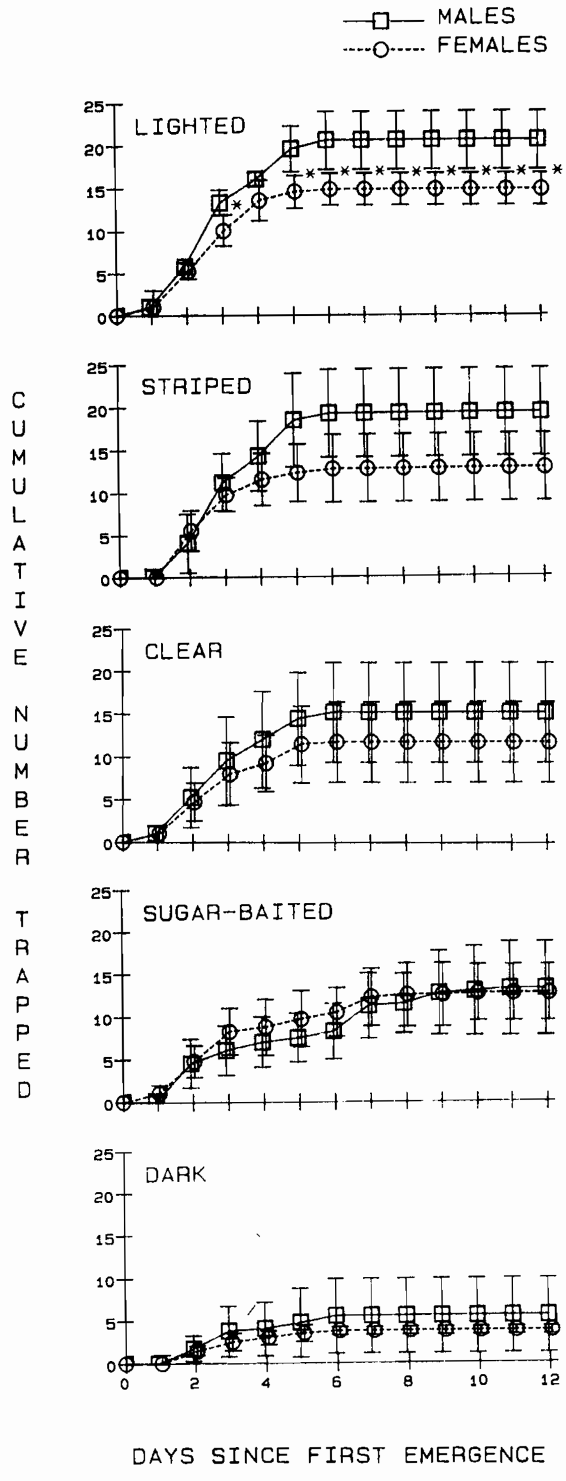


Figure 3.-Mean numbers of male and female *Culex tarsalis* adults trapped for each of the attractants tested with the collection jars. *: indicates a significant difference between the numbers of males and females captured by the indicated trap types (P < 0.05).

This would account for reproduction in these microcosms and not others. Since, upon reentry into capture jars, mosquitoes that had escaped earlier would again have only a probability of escape, sooner or later it would be expected that these mosquitoes would be counted as captured. This escape followed by an eventual capture accounts for the time lag difference between sugar-baited microcosms and all other microcosms. As the time lag effect and reproductive capability of mosquitoes in microcosms with sugar-baited capture jars were considered detrimental to microcosm results, this type of attractant will be avoided.

Dark jars suppressed numbers of mosquitoes captured and are therefore to be avoided in microcosm experiments of this type as higher capture efficiencies are more statistically tractable (Figure 2). Large numbers of mosquitoes in these microcosms died, presumably of starvation, within two days of emerging without being captured.

Lighted jars captured significantly more males than females (Figure 3). Other trap types showed a trend in this directions, but none of these was statistically significant. This suggests that males and females were differentially attracted to lighted collection jars, or that light effected the behavior of males and females differentially (Bidlingmayer 1985, Robinson 1952). If this difference represents a true sexual bias, then lighted jars could not be considered representative samples of emerged mosquitoes. A complete interpretation of these results requires a measure of numbers of male and female mosquitoes emerged in each microcosm. I was unable to quantify these numbers.

Previous comparisons of trap efficiencies have generally looked at traps sampling wild populations of mosquitoes (Bidlingmayer 1985, Service 1976). This investigation differs from previous investigations in that it sampled from a known number of captured mosquitoes. A similar experiment was conducted by Kloter et al. (1983), but mosquitoes in their experiment were allowed access to six different traps simultaneously. The reason underlying the differences between previous investigations and my own is that those investigations attempted to test the efficiency of traps sampling natural populations of mosquitoes while my experiment was confined to sampling mosquitoes over specifically designed microcosms. This could explain why I found no improvement in capture efficiencies with addition of attractants while previous studies had found attractants to increase capture efficiencies.

In conclusion, these results indicate that the percentage of emerged mosquitoes captured by collection jars is relatively high and the variation reasonably low. This suggests that these microcosm environments should provide statistically significant evaluations of the mosquito control efficacy of selected larvivorous fishes in waste water environments.

ACKNOWLEDGMENTS.-I thank J. J. Cech, Jr., R. K. Washino, and R. E. Fontaine for their advice and J. P. Riordan, L. J. Castleberry, S. M. Castleberry, A. E. Castleberry, and M. A. Castleberry for their help. This study was supported by University of California Mosquito Research Funds to J. J. Cech, Jr.

REFERENCES

Bidlingmayer, W. L. 1985. The measurement of adult mosquito population changes - some considerations. Journal of the American Mosquito Contr. Assoc. 1 (3) :328-348.

Blaustein, L., and R. Karban. 1984. The mosquitofish, green sunfish, and southern naiad and their interactive effects on mosquito abundance in rice fields. Pages 61-64 in Mosquito Control Research Annual Report 1984. Entomology Extension, University of California, Davis, California, U.S.A.

Dickerson, J. E., Jr., and J. V. Robinson. 1985. Microcosms as islands: a test of the MacArthur-Wilson equilibrium theory. Ecology 66(3):966-980.

Geisy, J. P. (Editor). 1980. Microcosms in Ecological Research. Technical Information Center, U.S. Department of Energy, Washington, D.C., U.S.A.

Kloter, K. O., J. R. Kaltenbach, G. T. Carmichael, and D. D. Bowman. 1983. An experimental evaluation of six different suction traps for attracting and capturing *Aedes aegypti*. Mosquito News 43(3):297-301.

Robinson, H. S. 1952. On the behavior of night flying insects in the neighborhood of a bright source of light. Proceedings of the Royal Entomological Society of London 27: 13-21.

Service, M. W. 1976. Mosquito ecology: Field sampling methods. Applied Science Publishers Ltd., London, England.

Snedecor, G. W., and W. G. Cochran. 1980. Statistical Methods. Seventh edition. The Iowa State University Press, Ames, Iowa, U.S.A.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York, New York, U.S.A.

Uhlmann, D. 1985. Scaling of microcosms and the dimensional analysis of lakes. Int. Review ges. Hydrobiol. 70(1):47-62.

BREEDING SUPERIOR PARASITOIDS OF DIPTERA USING A NOVEL EXTRANUCLEAR INHERITANCE MECHANISM

E. F. Legner

University of California
Division of Biological Control
Department of Entomology
Riverside, California 92521

ABSTRACT

Superior strains of synanthropic fly parasitoids of the genus *Muscidifurax* can be produced in the laboratory using conventional breeding techniques. The pathway to inheritance of special traits (e.g., gregarious and solitary oviposition, thelytoky) is novel, whereby the mated female parasitoid acquires the traits directly from the male with whom she mates, and demonstrates them shortly after. These traits are then fixed into the genome of her offspring which demonstrate them in the virgin state. The term "accretive inheritance" is tentatively assigned to this inheritance. The kind of extranuclear phenomenon involved is unknown, but may involve microorganisms or DNA present is hymentopteran sperm.

The discovery that hybrids of different strains of synanthropic fly parasitoids demonstrated heterotic behavior (Hoy 1975, Legner 1972), widens possibilities for biological control through inoculative releases. The direct effects of increased host mortality wrought by the individuals released may be compounded when their heterotic offspring show enhanced killing power, greater longevity and superior reproductive capacity.

Current research has attempted to direct heterosis to favor certain desirable behavioral traits. The process entails locating two compatible strains of a species, each with certain desirable characteristics that would be advantageous to have in a single "super strain". An example is found in *Muscidifurax raptorellus* Kogan & Legner from South America. One strain from central Chile is gregarious, but possesses a relatively low host searching capacity. Although its oviposition rate is comparable to another strain from coastal Peru, the Chilean form distributes its eggs among fewer hosts (Legner 1967). Adults of the Chilean strain are also comparatively smaller and lethargic (Kogan & Legner 1970, Legner 1969, Legner 1983). Although the Peruvian form, demonstrates higher mobility and aggressiveness, it is largely solitary in its ovipositional behavior.

MATERIALS AND METHODS.-To study fecundity, host searching and destruction, cohorts of 10 1-day-old female parasitoids that had been given honey but were not exposed to hosts, were isolated in screened, 46-cm^3 polystyrene vials, with a basal area of 7 cm^2. Each female was supplied daily with 20 24-to 30-h-old pupae of *Musca domestica* L., 6.4 ± 0.5 mm x 2.8 ± 0.2 mm, distributed randomly over the vial base, and which were reared until pupation using commercial CSMA® medium. Parasitization efficiency at this host density and in this environment at 25.5°C was near optimum (Legner 1967, 1979).

Host pupae that were exposed to parasitoids for 24-h, were incubated at 25.5 ± 1°C, 55% RH and 13L:11D photoperiod supplied by fluorescent lamps, giving a table-level intensity of ca. 25 ft-c (269 lux), until adult flies ceased to emerge and died (ca. 9 days). The remaining pupae were then incubated separately in gelatin capsules (10 x 25 mm) for the emergence of F_1 parasite progeny. Unemerged puparia were dissected for aborted parasitism.

Parasite longevity, total progeny and sex ratio were recorded for each female for 16 days, which is ca. the one-half life expectancy of a population of females. The importance of viewing behavior over an extended time period is becoming recognized for other parasitoids (Hey & Gargiulo 1985) and *Drosophila* (Templeton 1982). The net reproductive rate (R_o) and intrinsic rate of natural increase (r_m) were derived according to Birch (1948) from an initial cohort of 10 ovipositing females. A close approximation of r_m described by Birch (1948) was made using trial and error substitutes in the formula

$$Z_x\ e^{-rx} 1_x m_x = 1. \quad (Z = \text{summation sign})$$

Additionally, a net parasitization rate (total number hosts parasitized) (R_p) was calculated as

$$R_p = Z_x 1_x p_x \text{ and}$$

the intrinsic rate of parasitization (r_p) was derived using Birch's (1948) formula, modified as follows:

$$Z_x\ e^{-r_p x} 1_x p_x = 1$$

where p_x = mean number of hosts producing parasitoids per female at age $\underline{x}$. These computations are useful for viewing female parasitoid host searching ability weighted equally with her intrinsic rate of natural increase.

A net total fecundity rate (males + females) (R_t) was calculated as $R_t = Z_x 1_x t_x$ and the intrinsic total fecundity rate (r_t) was derived using Birch's (1948) formula, but modified as follows:

$$\sum_x e^{-r_t x} l_x t_x = 1$$

where t_x = mean number of total male and female progeny per parent at age x. These computations are useful for appraising a female's total oviposition activity.

Finally, a net host destruction rate (number of hosts killed as corrected for natural mortality by Abbott's formula (Abbott 1925)) was calculated as $R_d = \sum_x l_x d_x$ and the intrinsic total destruction rate was derived using Birch's (1948) formula, but modified as follows:

$$\sum_x e^{-r_d x} l_x d_x = 1$$

where d_x = mean number of hosts destroyed per female parasitoid at age x, as corrected by Abbott's formula. These computations enabled a precise measurement of host destruction.

In determining R_o and r_m values, the pivitol age (Birch 1948) was estimated as the mean length of development of females at 25.5 ± 1°C, 55% RH. Because females were 1 day old (post ecolsion) when an exposure began, their mean pivitol age was 21.5 days. An estimated 90% survival rate of immature females from oviposition was used to calculate l_x. The statistic m_x measured the "effective" number of female offspring per female in the age interval x, as only emerged offspring were counted.

The use of such modified reproductive potential statistics for laboratory studies involving parasitiods was discussed previously (Legner 1985). They are especially valuable in comparisons of virgin with mated females in Hymenoptera, which continue to lay viable eggs, albeit only of the male sex. Thus, direct effects of mating on the mothers is often possible.

Three series of identical experiments with replicate modifications were conducted over a two year period, but the results reported herein consider the more simple basic matings of the last experiment, which did not differ appreciably from the previous two.

RESULTS.-Cross matings of the two *M. raptorellus* strains produced offspring which were heterozygous for what appears as a partially dominant trait for solitary oviposition, considering that the gregarious trait was more weakly expressed in the F_1 generation and, therefore, seems more recessive than dominant, if conventional ideology of inheritance is considered (Table 1). Resultant F_1 hybrids were heterotic, being expressed here by greater parasitization rates, fecundity, superior host kill, and a greater longevity (Table 1). Backcrosses to either original parent (data not shown) resulted in cultures which demonstrated continued heterosis, indicating the complexity of genetic processes involved. The 50:50 ratio of gregarious/solitary individuals expected in the backcross to Chilean males was not obtained. Rather, heterotic individuals were produced which apparently had fixed the gregarious trait along with increased vigor of host attack (data not shown).

Table 1 gives population statistics derived from the initial crosses. The parameters calculated for these experimental populations express heterosis as increases in parasitization rates (R_p, r_p), longevity and host destruction (R_d, r_d).

The expressions of gregarious behavior were remarkably uniform among replicate females from the second through 10th days of oviposition, their daily values approximating the averages shown in Table 1.

Mode of Inheritance.-Inheritance in the present study appears to involve extranuclear phenomena because of the behavioral changes noted in parental females shortly after mating. Table 1 gives details of the pathways to inheritance of gregarious or solitary oviposition behavior. The average daily % gregarious ovipositions for the Chilean strain of *M. raptorellus* ranged from 80.9% to 66.6% for mated and virgin females, respectively; while in the Peruvian strain only 0.7% gregarious oviposition was observed (Table 1). Mating Peruvian males with Chilean females reduced the latter's gregarious oviposition significantly to 56.1%. However, mating Chilean males with Peruvian females caused the latter to increase their gregarious oviposition rate to 8.5% (Table 1). There was a significant (<0.01) positive correlation between the % of gregarious ovipositions and the number of individuals oviposited per host (r = 0.940, 9df).

The virgin hybrids of these crosses, possessing inheritance from both Chilean and Peruvian parents, showed gregarious oviposition capabilities that were ca. 2/3rds reduced. However, as in the case of their parents, matings with Chilean males increased their gregarious abilities, while Peruvian males reduced them (Table 1). These differences were all significant at <0.05%, as tested by Wilcoxon's signed rank test. An ANOVA and Duncan's New Multiple range test also established significant differences at the same minimum probability level when analyses were performed on data transformed to the arcsin times the square-root of the %.

Thus, although a power of "gregariousness" might be ascribed to Chilean males, a contrary partially dominant "solitariness" befits the Peruvian males. For want of a better term, this unusual phenomenon is herewith termed "accretive inheritance."

Recently, while transmitting thelytoky to an experimental *Muscidifurax raptor* Girault & Sanders population by mating hybrid females of interhemispheric strains with males of *Muscidifurax uniraptor* Kogan & Legner, a change in ovipositional behavior was also observed in the mated hybrid females which subsequently produced thelytokous F_1 offspring (Legner, in press). It was thought that the transmission of thelytoky also involved extranuclear factors.

Extranuclear heredity is a well documented

Table 1.-Gregarious oviposition, longevity, sex-ratios, and net and intrinsic rates of natural increase, parasitization, total fecundity, and host destruction in P_1 and hybrid cultures of *Muscidifurax raptorellus* Kogan & Legner, where 10 females oviposit continuously at 25.5° ± 1°C., 55% RH on 20 *Musca domestica* L. puparia daily for 16 days.

Parasitoid Strain and Cross	AVERAGE NO.					POPULATION VALUES			
	Parasitoid longevity (days)	% Daily Gregarious Oviposition	Total Progeny	% ♀♀	Parasitoids per Gregarious Oviposition	R_o/r_m	R_t/r_t	R_p/r_p	R_d/r_d
CHILE ♀ - virgin	11.0	66.6	113.3	0	3.13	0	102.0 / 0.1650	38.7 / 0.1299	141.5 / 0.1713
CHILE ♀ X CHILE ♂	11.3	80.9*	165.4	63.9	3.33	95.1 / 0.1648	148.8 / 0.1813	47.7 / 0.1423	153.5 / 0.1810
CHILE ♀ X PERU ♂	13.4	56.1*	132.4	63.4	2.56	75.6 / 0.1486	119.2 / 0.1660	64.6 / 0.1430	197.9 / 0.1849
PERU ♀ - virgin	14.2	0	84.7	0	--	0	76.2 / 0.1540	76.2 / 0.1540	209.5 / 0.1893
PERU ♀ X PERU ♂	9.8	0.7	58.8	74.8	2.0	39.6 / 0.1355	52.9 / 0.1469	52.7 / 0.1468	139.4 / 0.1858
PERU ♀ X CHILE ♂	11.7	8.5	86.3	77.1	1.95	59.8 / 0.1464	77.7 / 0.1569	71.5 / 0.1544	170.4 / 0.1867
F_1 Hybrids									
(CHILE ♀ X PERU ♂) ♀ - virgin	15.6	21.1	134.6	0	2.11	0	121.1 / 0.1677	98.1 / 0.1604	269.4 / 0.1932
(CHILE ♀ X PERU ♂) ♀ X PERU ♂	15.9	9.2*	123.3	72.3	2.10	80.3 / 0.1526	110.9 / 0.1644	101.8 / 0.1616	273.2 / 0.1935
(CHILE ♀ X PERU ♂) ♀ X CHILE ♂	15.0	41.4*	165.4	75.1	2.37*	111.8 / 0.1647	148.9 / 0.1753	94.5 / 0.1595	251.1 / 0.1922
(PERU ♀ X CHILE ♂) ♀ - virgin	15.8	19.4	123.9	0	2.07	0	111.5 / 0.1638	92.4 / 0.1585	269.1 / 0.1927
(PERU ♀ X CHILE ♂) ♀ X PERU ♂	16.0	10.4*	118.8	68.2	2.03	72.9 / 0.1520	106.9 / 0.1623	95.6 / 0.1597	275.4 / 0.1927
(PERU ♀ X CHILE ♂) ♀ X CHILE ♂	15.5	48.5*	155.8	83.6	2.35*	117.2 / 0.1658	140.2 / 0.1735	85.7 / 0.1570	255.6 / 0.1918

*Significantly different from preceding virgin test at 0.05 probability.

phenomenon among primitive organisms (Beale & Knowles 1978), but has not been involved directly in such highly evolved groups as the Hymenoptera. Although extranuclear factors in the form of microorganisms are known to alter behavior in parasitoids (Krell & Stoltz 1979, Stoltz & Vinson 1977, Stoltz et al. 1976, Vinson & Stoltz 1986, Werren et al. 1986), and other Metazoa (Bull 1983), their direct effects on the genome have never been shown.

Experiments are continuing to measure vigor of succeeding generations of these and other crosses among parasitoids of synanthropic Diptera, and to study the genetic mechanisms governing the expressed effects. Although microorganisms may be involved as previously stated, a more direct role of hymenopteran sperm DNA might also be involved. The field liberation of male parasitoids with desired superior characteristics might improve the biological control capacity of resident strains in the first generation as well as in the resultant hybrids.

REFERENCES

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18:265-267.

Beale, G. & J. Knowles. 1978. Extranuclear Genetics. Edward Arnold, London. 142 p.

Birch, L. C. 1948. The intrinsic rate of natural increase of an insect population. J. Anim. Ecol. 17:15-26.

Bull, J. J. 1983. Evolution of Sex Determining Mechanisms. The Benjamin/Cummings Publ. Co., Inc., Menlo Park, CA. 316 p.

Hey, J. & M. K. Gargiulo. 1985. Sex-ratio changes in *Leptopilina heterotoma* in response to breeding. The J. of Heredity 76:209-211.

Hoy, M. A. 1975. Forest and laboratory evaluations of hybridized *Apanteles melanoscelus* (Hym.: Braconidae), a parasitoid of *Porthetria dispar* (Lep.: Lymantriidae). Entomophaga 20:261-268.

Kogan, M. & E. F. Legner. 1970. A biosysematic revision of the genus *Muscidifurax* (Hymenoptera: Pteromalidae) with descriptions of four new species. Canad. Entomol. 102: 1268-2190.

Krell, P. J. & D. B. Stoltz. 1979. Unusual baculovirus of the parasitoid wasp, *Apanteles melanoscelus:* isolation and preliminary characterization. J. Virol 29:1118-1130.

Legner, E. F. 1967. Behavior changes the reproduction of *Spalangia cameroni, S. endius, Muscidifurax raptor*, and *Nasonia vitripennis* (Hymenoptera: Pteromalidae) at increasing fly host densities. Ann. Entomol. Soc. Amer. 60:819-826.

Legner, E. F. 1969. Reproductive isolation and size variation in the *Muscidifurax raptor* complex. Ann. Entomol. Soc. Amer. 62: 382-385.

Legner, E. F. 1972. Observations on hybridization and heterosis in parasitoids of synanthropic flies. Ann. Entomol. Soc. Amer. 65:254-263.

Legner, E. F. 1983. Broadened view of *Muscidifurax* parasites associated with endophilous synanthropic flies and sibling species in the *Spalangia endius* complex. Proc. Calif. Mosq. & Vector Contr. Assoc. 51:47-48.

Legner, E. F. 1985. Natural and induced sex ratio changes in populations of thelytokous *Muscidifurax uniraptor* (Hymenoptera: Pteromalidae). Ann. Entomol. Soc. Amer. 78:398-402.

Legner, E. F. (1987). Transfer of thelytoky to arrhenotokous *Muscidifurax raptor* (Hymenoptera: Pteromalidae). Canad. Entomol. 119 (in press).

Stoltz, D. B. & S. B. Vinson. 1977. Baculovirus-like particles in the reproductive tracts of female parasitoid wasps II: The genus *Apanteles*. Canad. J. Microbiol. 23:28-37.

Stoltz, D. B., S. B. Vinson & E. A. Mackinnon. 1976. Baculovirus-like particles in the reproductive tracts of female parasitoid wasps. Canad. J. Microbiol. 22:1013-1023.

Templeton, A. R. 1982. The prophecies of parthenogenesis. In: H. Dingle & I. P. Hagmann pp 75-101, Evolution and Genetics of Life Histories. Springer-Verlag, N.Y./ Berlin.

Vinson, S. B. & D. B. Stoltz. 1986. Cross-protection experiments with two parasitoid (Hymenoptera: Ichneumonidae) viruses. Ann. Entomol. Soc. Amer. 79:216-218.

Werren, J. H., S. W. Skinner & A. M. Huger. 1986. Male-killing bacteria in a parasitic wasp. Science 231:990-992.

TRAPPING EFFICIENCY OF EXOPHILOUS SYNANTHROPIC DIPTERA INCREASED THROUGH CONSIDERATIONS OF BEHAVIOR

E. F. Legner

Department of Entomology
University of California
Riverside, California 92521

ABSTRACT

Reductions of Australian bush fly, *Musca vetustissima* Walker to non annoyance levels were sustained for periods of ca. 2-weeks from single 24-h trapping periods with poison baits on Kwajalein Island, Marshall Islands. Placement of traps in preferred fly haunts was essential for maximum trapping efficiency. The method might be applicable for periodic containment of bush flies in continental areas.

The achievement in Denmark of sustained house fly reductions by selective applications of insecticides to restricted portions of the breeding habitat (Keiding 1965, Keiding & ben Hannine 1965, 1966), demonstrated the possibility to greatly reduce insecticide volume in synanthropic fly management. This fortunate discovery was followed by detailed studies into interacting biological and physiological factors that govern pesticide resistance (Georghiou 1972, Legner & Olton 1968). Contemporary approaches emphasize trapping with poison baits for a number of dipterous species (Hwang et al. 1976, Mulla & Ridsdill-Smith 1986, Mulla et al. 1984).

Management practices which stress that natural enemy complexes be left intact and undisturbed as much as possible, utilize bait-trapping as a practical means for reducing populations of the more domesticated (endophilous) synanthropic flies (Legner et al. 1974a, Povolny 1971). However, for wilder (exophilous) fly species, eg. *Musca sorbens* Wiedemann and *M. vetustissima* Walker, effective trapping requires knowledge of local fly behavior (Legner et al. 1974b). The bush fly, *M. vetustissima* in particular affects the well-being of inhabitants over large portions of the southern Pacific. Little practical reductions have been realized thus far from the principal mode of attack, biological control (Legner 1986). Chemical control for widely scattered bush fly immatures is impractical. However, periodic bait-trapping may offer one expedient means of reducing annoyance.

The infestation of *M. vetustissima* in Micronesia emanates principally from the wastes of domestic pets and livestock, namely dogs and pigs (Legner et al. 1974b). In the continuous high humidity and general evenly distributed rainfall of this area, *M. vetustissima* flourishes, producing several generations from the same habitat source, where it acts as a direct primary decomposer (Legner et al. 1974b). Natural enemies and scavengers do not effectively influence the fly population under current conditions. Apart from eliminating the mammalian species that produce the breeding habitat, and in the absence of known effective biological controls, another form of control seemed imperative.

METHODS.-Experiments were conducted on Kwajalein Atoll in the Marshall Islands during the 1970's, to develop an effective control for the *M. sorbens* group of flies, ie., predominantly *M. vetustissima* (Legner 1976). Among various baits tested, it was soon determined that a mixture of rotting fresh eggs with equal parts of water was most effective for luring large number of flies to selected areas. Surveys conducted in 1971 revealed that the *M. sorbens* group was most strongly attracted to the bait (Table 1). The number of flies attracted, varied considerably with the location of the baited area, however.

Table 1.-Relative abundance of synanthropic flies trapped with rotting-egg baits on Kwajalein Island in the 1970's.[1]

FLY SPECIES OR TYPE	PERCENT Total Flies Trapped	♀♀
Musca sorbens	59.7	46.5
Musca domestica	18.2	49.4
Calliphoridae spp.	20.8	----
Sarcophagidae spp.	1.3	----

[1]Bait consisted of 6-day old whole rotting egg mixture with an equal volume of water.

In order to characterize fly movements and their preferred haunts, a study of *M. sorbens* behavior was conducted on Kwajalein Island (ca. 4.8 x 0.8 km). The attraction of this fly to 6-day old rotting egg baits that contained 0.5 ppm Dichlorvos (2.2-dichlorovinyl dimethyl phosphate) was studied at the northeastern portion of Kwajalein Island, an area known for its heavy concentrations of flies.

Poisoned baits mixed with beach sand, were offered on paper plates that were variously positioned outdoors or beneath a 15 x 30 m roofed rain shelter extending from a completely enclosed cooking shed. Outdoor baits were suspended from trees at 2, 1 and ½ m above the ground and placed at ground level, 10 replicates at each position. Beneath the shelter, 10 replicates each were placed against the wall of the shed at ground level and at one and two meters; and in the center and perifery of the roofed area at table height (0.75 m), and on the concrete floor. The poisoned baits were renewed each morning and dead flies removed and counted over a 2-week period in April 1971.

Fly control experiments were conducted over the entirety of Kwajalein Island in May 1971, and November 1972 and 1973. Twenty poisoned bait stations were located in the primary fly gathering sites as determined in the above experiment. Adult fly population densities were monitored several times each day at varying intervals beginning with bait placement, by calculating the number of flies alighting on the face of human subjects walking randomly about the island.

RESULTS.-Behavioral studies of *M. vetustissima* indicated a strong affinity for baits located at or near the surface of the ground both outdoors and within the sheltered area (Table 2). These flies showed a notable wariness of confinement, so that baits placed against buildings or in the center of the roofed shelter attracted few individuals (Table 2).

With the knowledge that ground level baits were favored, it was further determined that areas shaded by natural vegetation were superior to unshaded. As wind velocities exceeding 8 km./hr caused flies to become quite inactive at ground level, the added protection of vegetation against wind may have accounted for some of the greater attraction to such areas.

Fly densities dropped noticeably within 2-hr of bait placement and were reduced to non annoyance levels in 1971 after 24 hr (Table 3). Repititions of the experiment in 1972 and 1973 showed a similar trend. Control duration depended on local fly reproduction and the persistence of effective baits. But generally, fly densities remained at low levels for ca. two weeks, following a single 24-hr baiting period. This apparently was long enough to remove more than 99% of the adult flies from the 4.8 km-long island, so that recolonization of breeding sites was significantly slowed.

The reduction of densities of the *M. sorbens* group of flies to non annoyance levels for two-week intervals on Kwajalein points to the possible application of baiting for practical fly reductions in small towns and urban neighborhoods in Australia and elsewhere. However, effectiveness of the technique on continents would depend especially on the rate of immigration from the wilderness and location of the primary adult foraging sites. These would be expected to vary according to wind patterns and geographical and vegetative features. The potential for resistance to baits seems minimal in situations where the majority of breeding occurs in the wilderness, as is apparently the case in Australia.

Table 2.-Positioning effect of poisoned rotting egg baits on attraction of *Musca vetustissima* Walker.[1]

Bait Position	Percent Total Flies Trapped in 24-h
Outdoors	
2 meters above ground	0
1 meter above ground	0
½ meter above ground	5
ground level	95
Roofed Shelter	
against wall of shed	0
table height, center	6.8
floor, center	10.2
table height, perifery	14.8
floor, perifery	68.2

[1]Bait = 6-day old whole rotting egg with equal volume H_2O and 0.5 ppm Dichlorvos.

Table 3.-Reductions of *Musca vetustissima* on Kwajalein Island by poisoned rotting-egg bait.[1]

Date	Time after bait placement (hr)	No. adult flies attracted to human face per minute
2 May 1971	0	10.7
	2	8.2
	12	3.5
	24	0.5
13 Nov 1972	0	8.5
	4	6.4
	12	1.5
	24	0.25
7 Nov 1973	0	5.8
	4	2.3
	12	0.5
	24	(less than) 0.1

[1]Bait = 6-day old whole rotting egg with equal volume H_2O and 0.5 ppm Dichlorvos.

REFERENCES

Georghiou, G. P. 1972. The evolution of resistance to pesticides. Ann. Rev. Ecol. & Systematics 3:133-168.

Hwang, Y.-S., M. S. Mulla & H. Axelrod. 1976. Attractants for synanthropic flies: Identification of attractants and coattractants for *Hippelates* eye gnats (Diptera: Chloropidae). J. Agric. Food Chem. 24:164-169.

Keiding, J. 1965. Observations on the behavior of the housefly in relation to its control. Riv. Parasitol. 26:45-60.

Keiding, J., & S. ben Hannine. 1965. Fly control by selective spraying of woodwork in piggeries. Ann. Rept. 1964, Govt. Pest Infest. Lab., Lyngby, Denmark. 36 pp.

Keiding, J. & S. ben Hannine. 1966. Strategic fly control. Ann. Rept. 1965. Govt. Pest Infest. Lab., Lyngby, Denmark. 39 pp.

Legner, E.F. 1976. The *Musca sorbens* Wiedemann complex in Kwajalein Atoll, Marshall Islands Ent. News 87:39-48.

Legner, E.F. 1986. The requirement for reassessment of interactions among dung beetles, symbovine flies and natural enemies. In: Entomol. Soc. Amer. Misc. Publ. "Biological Control of Muscoid Flies". 61:120-131.

Legner, E.F. & G.S. Olton. 1968. The biological method and integrated control of house and stable flies in California. Calif. Agric 22(6): 1-4.

Legner, E.F., R.D. Sjogren, & I. M. Hall. 1974a. The biological control of medically important arthropods. Crit. Rev. Environ. Control 4:85-113.

Legner, E.F., B.B. Sugerman, H.-S. Yu and H. Lum. 1974b. Biological and integrated control of the bush fly, *Musca sorbens* Wiedemann and other filth breeding Diptera in Kwajalein Atoll, Marshall Islands. Bull. Soc. Vect. Ecol. 1:1-14.

Mulla, M. S., H. Axelrod & Y.-S. Hwang. 1984. Field evaluation of chemical attractants against the fly *Fannia femoralis* (Diptera: Muscidae). J. Chem. Ecol. 10:349-360.

Povolny, D. 1971. Synanthropy: definition, evolution and classification. In: "Flies and Disease," Ecology, Classification and Biotic Associations, Vol. I, pp 17-54, B.Greenberg (ed.). Princeton Univ. Press, Princeton, N.J. 856 pp.

PESTIFEROUS DIPTERANS AND TWO RECENTLY INTRODUCED AQUATIC SPECIES AT CLEAR LAKE

Norman L. Anderson, David L. Woodward, and Arthur E. Colwell

Lake County Mosquito Abatement District
P.O. Box 310, Lakeport, California 95453

ABSTRACT

During 1985, high overwintering larval densities of several species of midges (*Chironomidae*) and Clear Lake gnats (*Chaoborus astictopus*) occurred at Clear Lake, California. The density of the Inland silverside (*Menidia beryllina*), a possible agent for the control of these dipterans, was low. Also in 1985, a clupeid fish (*Dorosoma petenense*) and a large cladoceran (*Leptodora kindtii*) were collected for the first time in Clear Lake.

INTRODUCTION.-The nuisance and economic importance of chaoborid and chironomid midges emanating from Clear Lake has been well documented (Herms 1937, Cook 1964, 1965, 1967, Jacobs et al. 1979). Chemical (Apperson et al. 1978, Colwell and Schaefer 1981, Hazeltine 1963) and biological (Brown and Washino 1977, Cook 1981, Li et al. 1979, Turlington et al. 1985) methods for controlling these pestiferous dipterans have been studied. In order to assess the efficacy of control stratagies and to monitor the population densities of the midge larvae, plankton and fish in Clear Lake ongoing sampling routines have been established.

MATERIALS AND METHODS.-Chironomid and chaoborid larvae and pupae are monitored monthly by taking 2 Ekman dredge samples from each of 29 stations on Clear Lake. Each 232 cm^2 sample of bottom sediment is sieved through a brass strainer screen which has 19.6 meshes per cm and apertures of ca. 0.6 mm. Samples are then returned to the laboratory for identification and enumeration of the organisms.

Since the Clear Lake gnat larvae inhabit the water column (Anderson et al. 1980) as well as the benthos, a plankton trap (Schindler 1969) is used periodically on the lake to sample planktonic larvae and to monitor plankton population densities. The plankton samples are preserved in a 4% formaldehyde solution which contains rose bengal dye. Samples are brought back to the laboratory and identified and enumerated at 30x magnification. Plankton samples are taken from 10 stations on the Upper Arm of Clear Lake 4 times per year. In 1985, plankton samples were also taken from 10 stations on the Oaks and Lower Arms of Clear Lake.

The population density of Inland silversides in Clear Lake is assessed with the use of a surface trawl that is pulled through the lake behind a boat. Four times per year a transect (a series of 10 samples) of the Upper Arm is performed with the trawl. In 1985, littoral and limnetic trawl hauls were also taken in the Oaks and Lower Arms of Clear Lake. Fish are identified and counted in the field. A representative sample is returned to the laboratory for measurement of standard lengths and for gut content analysis.

RESULTS AND DISCUSSION.-During May of 1985, the density of chironomids in Clear Lake reached 80.66 larvae per Ekman dredge sample (Figure 1). The 1985 average of 21.44 chironomids per dredge was the highest yearly average since the Lake County Mosquito Abatement District started its monitoring program more than 30 years ago. There are several species of chironomids in Clear Lake. These species are primarily in two subfamilies, the Tanypodinae and the Chironominae. The Tanypodinae (mostly *Procladius bellus* and other *Procladius sp.*) have predaceous larvae and are a small percentage of the total chironomid population. The Chironominae (mostly *Chironomus plumosus, C. frommeri* and *C. decorus*) have larvae that are bottom-dwelling filter feeders. The adult Chironominae are large and form huge swarms that create nuisances to humans near the shoreline of Clear Lake. Unfortunately, the recent increase in chironomid density is primarily in this group.

The 1985-86 overwintering population of Clear Lake gnat larvae peaked in November with an average of 33.57 larvae per dredge. The 1985 average of 10.94 *C. astictopus* per dredge (Figure 2) was the highest annual density in Clear Lake during the last 23 years. Gnats were controlled from 1962 to 1975 with treatments of methyl parathion. When the gnat larvae developed resistance (Apperson 1976), these treatments were discontinued. In 1967 the Inland silverside, a planktivorous fish, was introduced into Clear Lake in hopes that it would help to control the Clear Lake gnat (Cook 1981). Surface trawl data has been collected each year from 1979 through 1986 to monitor silverside population density in Clear Lake (Figure 3). Silverside density peaked in 1981-83 which corresponds to the three lowest years for choaborid density. Also low silverside years have corresponded to high chaoborid years during this time period. A regression analysis was done on data from these years which produced a significant ($P<0.02$) negative correlation (Figure 4). The cause of this negative correlation is under study. Analysis of silverside gut contents has shown some direct predation on chaoborid larvae, pupae and adults but not enough to explain the high magnitude of

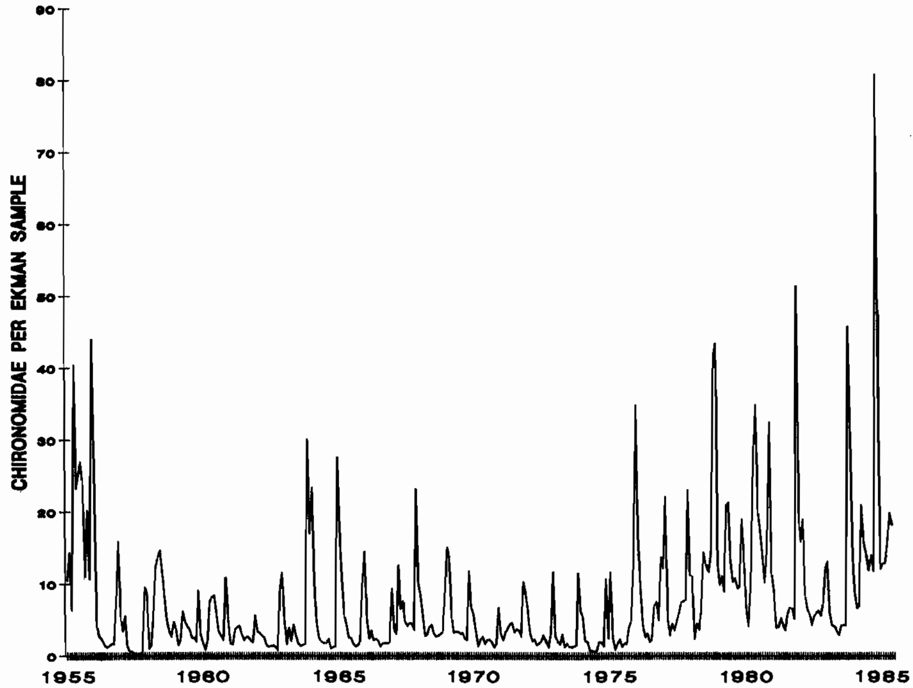


Figure 1.-Mean number of larval Chironomidae per Ekman dredge sample collected from Clear Lake.

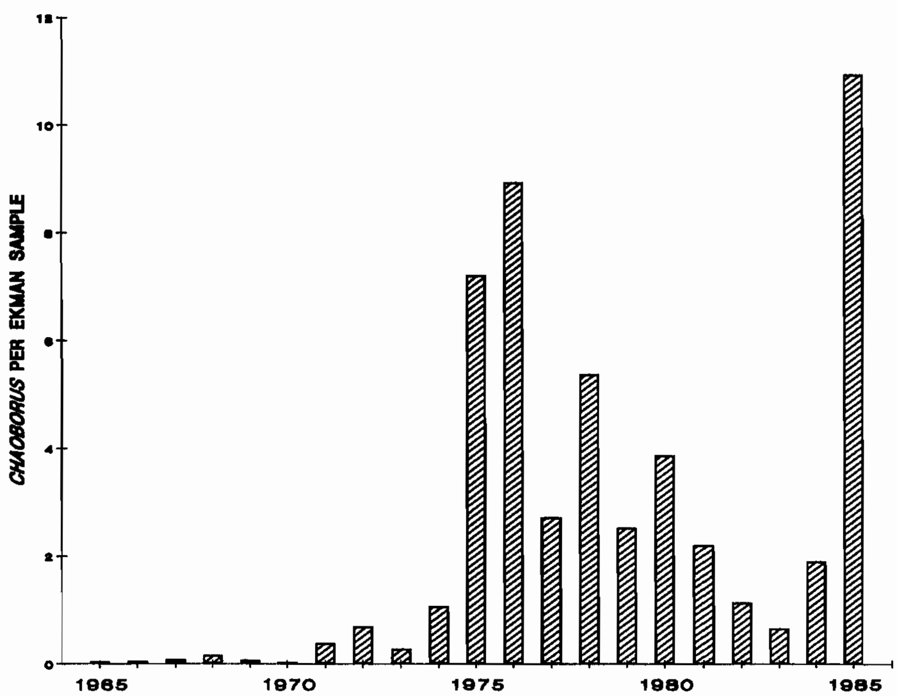


Figure 2.-Mean number of *Chaoborus astictopus* larvae per Ekman dredge sample collected from Clear Lake.

chaoborid population declines during high silverside years. Comparison of chaoborid crop contents (Colwell 1980) to silverside diets shows some overlap, particularly between late instar chaoborids and silversides. Both feed extensively on copepods and cladocerans indicating competition for food may be a significant interaction between Clear Lake gnat larvae and silversides.

Further evidence of the influence of silverside density on chaoborid density is provided by the distribution of each in the three arms of Clear Lake. Before silversides were introduced to Clear Lake, the Upper Arm had the greatest concentration of Clear Lake gnat larvae. However, sampling during November 1985 indicated that the Upper Arm had the lowest gnat density and the highest silverside density in Clear Lake. Also a comparison of the three arms of Clear Lake showed that density of the cladoceran *Bosmina longirostris* was positively correlated with *Chaoborus* density. *Bosmina* is an important constituent of the diet of *Chaoborus* larvae and is also eaten by silversides. In the winter of 1985-86, silverside density was high in the Upper Arm while *Chaoborus* and *Bosmina* densities were low compared to the Oaks and Lower Arms.

The trophic relationships in Clear Lake might be further complicated by the recent discovery of two species new to the lake. During September of 1985, Threadfin shad (*Dorosoma petenense*) were collected for the first time from Clear Lake in a surface trawl haul. Threadfin shad were once proposed as a possible biological control agent for the Clear Lake gnat (Cook 1981). In many lakes these fish are open-water planktivores (Moyle 1976). However, a transect of the Upper Arm with the surface trawl showed the highest concentrations of shad were in the near-shore areas. These fish were juveniles, the largest being 44 mm standard length. Perhaps mature adults will have a more limnetic distribution. Gut content analyses of the juvenile shad revealed no chaoborid larvae. Copepods and cladocerans were the main dietary constituents. If prey items such as *Bosmina* are limiting the abundance of *Chaoborus*, a large population of Threadfin shad feeding on zooplankton might be beneficial in competing with gnat larvae for food.

On November 13, 1985, *Leptodora kindtii*, a large predatory and nearly transparent cladoceran, was collected for the first time from Clear Lake during periodic plankton monitoring. The *Leptodora* were distributed throughout the water column vertically but were more concentrated offshore than near the shore. The winter population averaged 0.01 to 0.02 *Leptodora* per liter. Upon dissecting fish from Clear Lake, numerous *Leptodora* were found. Calculations of Electivity Indices (Windell 1971) indicated that *Leptodora* had quickly become the most preferred food for the silversides. Chaoborid densities might increase if some fish stopped feeding on chaoborids or on the food of chaoborids and switched to feeding on *Leptodora*. Alternatively, chaoborid densities might decrease if large num-

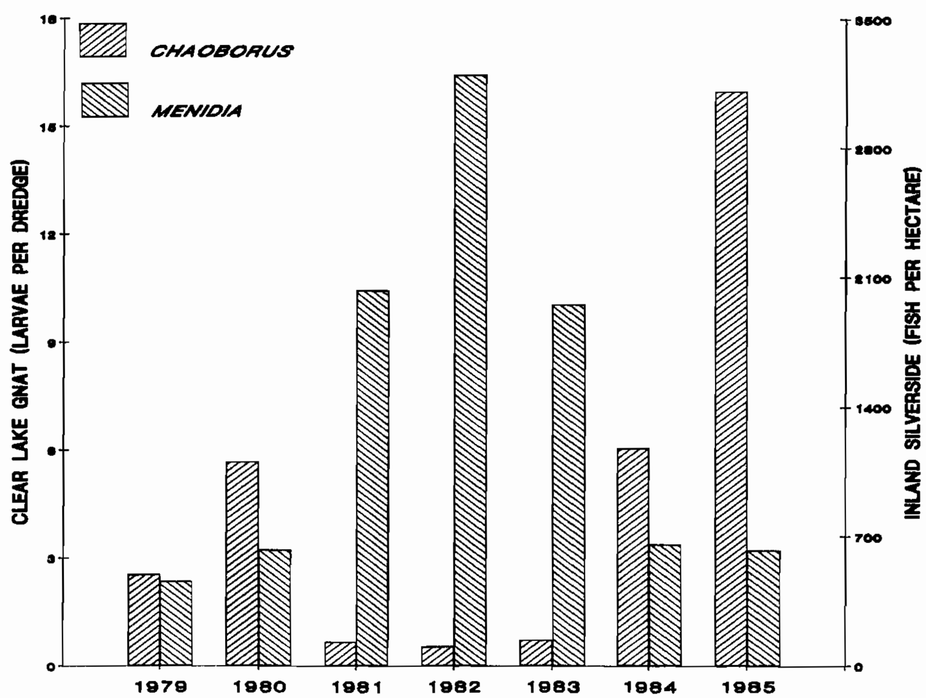


Figure 3.-Mean numbers of *Chaoborus astictopus* (larvae per Ekman dredge sample) and *Menidia beryllina* (fish per hectare) in the Upper Arm of Clear Lake.

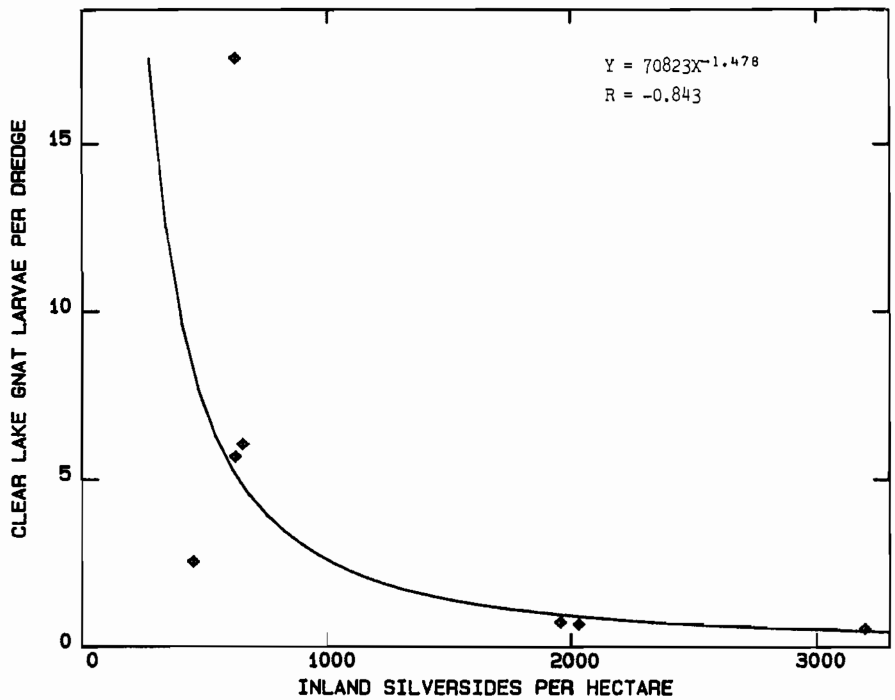


Figure 4.-Regression line of density of *Menidia beryllina* against density of *Chaoborus astictopus* in Clear Lake. Each point is the mean value for one year.

bers of *Leptodora* fed on early instar gnat larvae or competed with chaoborids for the same prey items since *Leptodora* is known to feed on cladocerans and copepods (Cummins et al. 1969, Havel 1985). *Leptodora* or Threadfin shad might kill invertebrate predators of *C. astictopus*. If the Threadfin shad and the *Leptodora* become established they could possibly alter the existing trophic relationships in the Clear Lake ecosystem.

ACKNOWLEDGMENTS.-The authors would like to express their appreciation to J. Haberthur, L. W. Davidson and Dr. W.A. Wurtsbaugh for their technical assistance and help during this study.

REFERENCES

Anderson, N. L., A. E. Colwell and R. K. Washino. 1980. Equipment for studying the aquatic stages of the Clear Lake Gnat. Proc. Calif. Mosq. and Vector Contr. Assoc. 48:127-130.

Apperson, C. S., R. Elston and W. Castle. 1976. Biological effects and persistence of menthyl parathion in Clear Lake, California. Environ. Entomol. 5:1116-1120.

Apperson, C. S., C. H. Schaefer, A.E. Colwell, G. H. Werner, N. L. Anderson, E. F. Dupras, and D. R. Longanecker. 1978. Effects of diflubenzuron on *Chaoborus astictopus* and nontarget organisms and persistence of diflubenzuron in lentic habitats. J. Econ. Entomol. 71:521-527.

Brown, J. K. and R. K. Washino. 1977. Developments in research with the Clear Lake gnat *Chaoborus astictopus* in relation to the fungus *Lagenidium giganteum*. Proc. Calif. Mosq. and Vector Contr. Assoc. 48:106.

Colwell, A. E. 1980. Spatial and temporal distribution and diet of larvae of the Clear Lake Gnat. Proc. California Mosquito Vector Control Assoc.48:127.

Colwell, A. E. and C. H. Schaefer. 1981. Effects of the insect growth regulator BAY SIR 8514 on pest diptera and nontarget aquatic organisms. Can. Ent. 113:185-191.

Cook, S. F. 1964. The potential of two native California fishes in the biological control of chironomid midges (Diptera: Chironomidae). Mosq. News 24:332-333.

Cook, S. F. 1965. The Clear Lake gnat: Its control, past , present, and future. Calif. Vector Views 12:43-50.

Cook, S. F. 1967. The increasing chaoborid midge problem in California. Calif. Vector Views 14:39-44.

Cook, S. F. 1981. The Clear Lake example: An ecological approach to pest management. Environment 23:25-30.

Cummins, K. W., R. R. Costra, R. E. Rowe, G. A. Moshiri, R. M. Scanlon and R. K. Zajdel. 1969 Ecological energetics of a natural population of the predaceous zooplankter *Leptodora kindtii* Focke (Cladocera). Oikos 20:189-223.

Havel, J. E. 1985. Predation of common invertebrate predators on long- and short-featured *Daphnia retrocurva*. Hydrobiologia 24 (2):141-149.

Hazeltine, W. E. 1963. The development of a new concept for control of the Clear Lake Gnat. J. Econ. Entomol. 56:621-626.

Herms, W. B. 1937. The Clear Lake gnat. Univ. Calif. Expt. Sta. Bull. 607:1-22.

Jacobs, S. E., A. E. Colwell and W. A. Wurtsbaugh. 1979. Predation by the Mississippi Silverside (*Menidia audens)* in Clear Lake, California. Proc. Calif. Mosq. Vector Contr. Assoc. 47:38-41.

Li, J. L., S. E. Jacobs and A. E. Colwell. 1979. Cyclopoid copepod predation on *Chaoborus astictopus*. Proc. Calif. Mosq. and Vector Contr. Assoc. 47:41.

Moyle, P. B. 1976. Inland Fishes of California. University of Calif. Press. pp. 101-103.

Schindler, D. W. 1969. Two useful devices for vertical plankton and water sampling. J. Fish. Res. Bd. Canada. 26:1948-1955.

Turlington, L. W., D. L. Woodward and A. E. Colwell. 1985. Susceptibility of the Clear Lake gnat, *Chaoborus astictopus*, to the hyphomycete fungus *Tolypocladium cylindrosporum*. Proc. Calif. Mosq. and Vector Contr. Assoc. 53:98-100.

Windell, J. T. 1971. Food analysis and rate of digestion, In: Methods for assessment of fish production in fresh waters, edited by W. E. Ricker, Blackwell Scientific Publications, Oxford, U. K., pp. 215-226.

AN EXPERIMENTAL YELLOWJACKET CONTROL PROGRAM

Dennis J. Jewell

San Mateo County Mosquito Abatement District
1351 Rollins Road
Burlingame, California 94010

INTRODUCTION.-The San Mateo County Mosquito Abatement District provides yellowjacket control on a limited basis to the residents of this District. The standard method of control consists of colony treatment with the chemical 2.2 Dichlorvinyl Dimethyl Phosphate (DDVP). An application of 1/8 oz. of 2% material in one gallon of water introduced into the hive opening by means of a hand can has proved to be a quick and reliable method of nest destruction. This material is not available for use by the general public, and commercial products that are available are often of marginal value. The yellowjackets that occasionally warrant control are *Vespula vulgaris, V. pensylvanica* and *Dolichovespula arenaria*. Colony control is affected by District personnel (if it is within District boundaries) and nest location is known. If the location is outside of the District or situated within a structure, it is recommended that a professional exterminator be contacted for control.

Dr. John McDonald (1985) suggested a control method used by Richard Keyel of Cornell University. This method employs the use of DDVP strips and is used successfully in controlling yellowjackets at Purdue University.

The object of my study was to determine if DDVP strips will control the ground and aerial nesting yellowjacket colonies in San Mateo county and thus have another option for residents outside of the District.

METHODS AND MATERIALS.-Two Texize® No-Pest insecticide strips 19.2% DDVP were purchased at a local store on sale for $2.99 per strip. After cutting the strips to various sizes, it was found that a 1/8 inch by 2 inch strip was optimal for control of the ground nesting species. I had no *D. arenaria* nests with which to work either season of the study, July-September 1984 and June-December 1985. During the first season of the study, it was found that the strips could be reused a number of times if desired. But for this study, they were not reused. Approximately twenty-four hours post-treatment, the colonies were excavated, taken to the District laboratory and examined for control and numbers.

The strips were introduced into the nest openings by means of a small lid from an aerosol can attached to the end of a broom handle in which a strip was placed. The lid was pinched to create an oval shaped container to hold the strip. All of the colonies were treated during the daytime when the yellowjackets are most active. After experiencing some attacks from the foragers, it was found that a rapid approach, deposit of the strip, and withdrawal from the site, was the best method of treatment. It appeared that for the first few seconds, the nest defense was oriented towards the strip rather than the applicator. After inserting the strip in or near the entrance (in some cases it was not possible to insert into the entrance due to obstructions or multiple openings) of the hive, it was left until the following day.

Excavation of the sites were done as near twenty-four hours as possible to determine efficacy of strips on various sizes and location of colonies from the entrance tunnels.

RESULTS AND DISCUSSION.-A total of seventeen nests were treated in 1984 using this technique. During this same period, five colonies were treated using our standard control method. This was due to the strip method not being practical at some sites. The second season, June-December 1985, a total of thirty-seven nests were treated with strips and twenty treated with our standard control method. The DDVP strips readily kill the adults, but have little effect on the larval or pupal stages of yellowjackets at twenty-four hours and no effect on the ants that attack the treated hives. If ants were present in the area of the hive during treatment, as occurred on twenty-three occasions, there was total hive mortality upon excavation.

The size of the colonies from a low of 442 to a high of 2,460 adults was probably limited due to early detection in this largely urban environment. The strips were equally effective on large or small colonies. I observed that in some instances where workers had removed the strips from the entrance tunnels, control was still achieved.

CONCLUSIONS.-This method of yellowjacket control is simple, clean, inexpensive, fast acting and if applied during a time of low hive activity, should be relatively safe for home owners to use. The strips are marketed by a number of companies under different names and are available at nurseries, grocery, hardware and drug stores.

REFERENCES

McDonald, J. F. 1985. Personal communication. Purdue University, West Lafayette, Ind. 47957.

ADAPTING THE LOTUS-123 COMPUTER PROGRAM TO VECTOR CONTROL OPERATIONS

Allan R. Pfuntner and Lyle M. Stotelmyre

West Valley Vector Control District
5050 Schaefer Avenue
Chino, California 91710

ABSTRACT

Many of the routine activities of a vector control district can be made less time-consuming when processed by a spreadsheet computer program such as Lotus-123. Such items as budget, surveillance data, source treatment records, and personnel data can be tracked electronically saving both time and storage space.

INTRODUCTION.-When the West Valley Vector Control District began field operations in May, 1984, every effort was made to promote efficiency in all phases of operation. Record-keeping in vector and mosquito control can be very labor intensive. The retrieval of source treatment information or past surveillance trap results can be a time consuming and frustrating endeavor. Electronic storage and retrieval was considered to be a viable alternative to the usual stuffed file cabinets.

METHODS AND MATERIALS.-To minimize the time spent on record-keeping activities, a computer tracking system was initiated in July, 1985. An IBM PC/XT computer system was purchased and the Lotus-123 spreadsheet program installed. The following items were targeted for inclusion in the program:

1. Budget Balance
2. New Jersey Traps
3. CO^2 Traps
4. Source List
5. Source Treatment Records
6. Personnel Records
7. Inventory
8. Vehicle Maintenance
9. Service Requests

The Lotus-123 program was chosen as it incorporates a large, easily manipulated spreadsheet with a database and graphics. A wealth of information on the use of the program is available from many publishers. Though, of course, nothing is specifically directed to vector control, the general procedures employed in most any Lotus spreadsheet can be adapted for use in vector control.

One area of prime concern was the tracking of source treatment activities. If an operator relies solely upon his or her memory when performing routine source inspections and control activities, some sources will inevitably be overlooked. The use of records in the form of sheet paper or cards becomes cumbersome to continuously update in the field. Moreover, the operator is entering data twice, as a daily report denoting each source checked and/or treated is also prepared.

To lessen the paperwork burden and enhance source tracking, a daily report form was prepared that requires a minimum of operator input with a maximum of source information (Figure 1). The operator completes the daily report each working day and forwards the report to the office secretary to be entered into the computer program. Once entered, the data can be automatically manipulated by a program subroutine designed to eliminate the need for extensive keyboard work by the operator when source tracking is required. On Monday morning, an operator can ascertain the sources that should be inspected and/or treated that week by merely punching two keys to obtain the source list for the desired vector (Figures 2 & 3). The list produced divides the mosquito sources, for example, into four categories - weekly, bi-weekly, tri-weekly, and monthly. The category into which a source is placed is dependent upon the cycle of treatment desired by the operator and the last date the source was inspected. Thus the list is a dynamic accounting rather than a static one. The program will not, of course, output a given source if that source is consistently overlooked by the operator. The list is a tool to augment a "thinking" operator, not a substitute for an "unthinking" person. The subroutine yielding the automatic source output is somewhat complicated, but the symbols designate various command sequences that are no different than those entered via the keyboard (Figure 4).

Should an inquiry be made regarding the treatment of a certain source, all information for a given time period can be quickly retrieved for perusal utilizing the database system (Figure 5). The usual shuffling of innumerable papers is eliminated.

Another example of the usefulness of the program is graph production. The weekly trap data are entered into the program and the resulting graph produced on the screen. A printout of the graph can be provided upon request (Figure 6).

The remaining items on the previously mentioned list (budget, inventory, etc.) have been successfully entered into the program also.

DAILY REPORT - WEST VALLEY VECTOR CONTROL DISTRICT

Date: Technician: Zone:

Location			Pest						Source					Area			Treatment											Time		
SR#	Site Code	Map Code	Vector Code	Imm. Den	Imm. Stage	Adult Den	Pupae Present	Rodent Evid	Source Desig.	Source Code	Source Cycle	Source Access	Source Status	Sq. Ft. Trtd	Cu. Ft. Trtd	Acres Trtd	O-gals.	DG-lbs.	DEC-fl.ozs.	KO-fl.ozs.	TS-each	PY-fl. ozs.	RUP-fl.ozs.	M-gals.	RDO-gals.	SP-lbs.	Fish-lbs.	Insp.	Treat.	Misc.

Codes: Vector - M(osquitoes), F(lies), R(odents), W(eeds), O(ther)
Imm. Stage - 1, 2, 3, 4, P
Rodent Evidence - S(tains), F(eces), G(gnawing), N(ests)
Source - Mosquitoes - D(airy), S(treet Drainage), P(onds),
R(ivers, streams), I(rrigation), M(isc.)
Flies - P(oultry), D(airy), G(arbage), F(eces)
Rats - E(xterior), I(nterior)

Cycle - W(eekly), B(i-weekly), T(ri-weekly), M(onthly)
Access - V(ehicle), F(oot)
Status - C(lean), W(eedy)

Chemicals: O=G.B. Oil DG=Dursban Granules DEC=Dursban Liquid
BG=Baytex Granules TS=Tossits PY=Pyrethrin
RP=Roundup M=Montar Rd=Rodeo Sp=Spike

Rev. 8/85

Figure 1.-Daily Report of Field Operator Activities.

```
A857: (D2) @DATE(86,8,26)                                                  MENU
Mosquitoes  Flies  Rodents  Weeds  Other  Quit
Press first letter of desired vector
        A    B C  D   E   F   G   H I   J  K  L   M  N   O   P   Q   R   S
1       Daily Report 1986  -  Zone I
2
3
4
5    Date       Tech SR #     Site Cd Map Cd    Vec Cd Im Den Im Stg  Ad Den
6    ---------- ---- -------  ------- --------- ------ ------ ------- -------
857    26-Aug    KT            T003              M        0
858    26-Aug    KT            K003              M       15      4
859    26-Aug    KT            V024              M        0
860    26-Aug    KT     536                      M        0
861    26-Aug    KT            D004              M        0
862    26-Aug    KT            MISC
863    27-Aug    KT            K004              M        0
864    27-Aug    KT            K004                       0
865    27-Aug    KT            J001              M       50      4
866    27-Aug    KT            B004              M      100      4
867    27-Aug    KT            L002              D
868    27-Aug    KT            MISC
869    27-Aug    RW            MISC
870
04-Sep-86   03:03 PM SQZ!                    CMD
```

Figure 2.-Menu of Lotus-123 for Slection of Vector Source Information.

11-Nov-85
Zone I
Mosquitoes

Date	Site Cd	Vec Cd	Src Des
Weekly Sources			
01-Nov	H007	M	1
01-Nov	H007	M	2
01-Nov	H007	M	3
01-Nov	H007	M	4
01-Nov	L002	M	1
01-Nov	L002	M	2
01-Nov	K004	M	1
Bi-weekly Sources			
24-Oct	J010	M	3
25-Oct	V045	M	1
25-Oct	V045	M	2
25-Oct	V045	M	3
25-Oct	V045	M	4
25-Oct	E001	M	1
25-Oct	S013	M	1
25-Oct	D011	M	1
25-Oct	S006	M	1
25-Oct	D013	M	1
25-Oct	D013	M	2
25-Oct	S004	M	1
25-Oct	V002	M	1
25-Oct	S002	M	1
25-Oct	S002	M	2
25-Oct	S002	M	3
25-Oct	S002	M	4
25-Oct	S002	M	5
25-Oct	S002	M	6
25-Oct	S002	M	7
25-Oct	S002	M	8
25-Oct	P009	M	1
25-Oct	D004	M	1
25-Oct	W004	M	1
25-Oct	R001	M	1
25-Oct	R001	M	2

Figure 3.-Output Denoting Sources to be Inpsected and/or Treated (partial printout).

```
{goto}bs512~+a7=@int(@now-10){right 4}^M~{right 4}
^W~/dqia5..ab2000~cbs510..ca512~obs514..by514~eq{goto}cc510~@now~
/ppcrr~agcrrgl..il~grcf509~gllrcd510~grbs514..by514~gq{goto}bs516~
/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-7)~{query}/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-6)~{query}/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-5)~{query}/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-4)~{query}/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-3)~{query}/xibs516>0~/xcPa~
/xcPc~
{goto}bs512~/ppllrcd512~grbs514..by514~gq+a7=@int(@now-17)~
{goto}ca512~^B~
{query}/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-14)~{query}/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-13)~{query}/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-12)~{query}/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-11)~{query}/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-10)~{query}/xibs516>0~/xcPa~
/xcPd~
{goto}bs512~/ppllrcd513~grbs514..by514~gq+a7=@int(@now-24)~
{goto}ca512~^T~{query}/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-21)~{query}/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-20)~{query}/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-19)~{query}/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-18)~{query}/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-17)~{query}/xibs516>0~/xcPa~
/xcPe~
/ppllrcd514~grbs514..by514~gq+a7=@int(@now-31)~{goto}ca512~^M~{query}
/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-28)~{query}/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-27)~{query}/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-26)~{query}/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-25)~{query}/xibs516>0~/xcPa~
{goto}bs512~+a7=@int(@now-24)~{query}/xibs516>0~/xgPb~
/pppq/xmcj512~
```

Figure 4.-Subroutine to Extract Cyclic Source Activity (Mosq.).

Daily Report 1985 - Zone I

Date	Tech	SR #	Site Cd	Map Cd	Vec Cd	Im Den	Im Stg	Ad Den
30-Sep	BO		A015		M	20	4	
30-Sep	BO		A015		M			
30-Sep	BO		A015		M			
30-Sep	BO		A015		M			
08-Nov	BO		A015		M	200	4	
08-Nov	BO		A015		M	200	4	
08-Nov	BO		A015		M			
08-Nov	BO		A015		M	200	4	
19-Nov	BO		A015		M			
19-Nov	BO		A015		M			
19-Nov	BO		A015		M			
19-Nov	BO		A015		M			

Pup Prs	Rod Evid	Src Des	Src Cd	Src Cyc	Src Acc	Src Sta	Sq Ft
		4	D	B	F	C	900
		3	D	B	F	C	
		2	D	B	F	W	
		1	D	B	F	W	
Y		4	D	B	F	W	
Y		2	D	B	F	W	
		1	D	B	F	C	
Y		3	D	B	F	W	
		1	I	B	F	C	
		4	D	B	F	C	
		3	D	B	F	C	
		2	D	B	F	C	

Cu Ft	Acres	GO gal	DG lbs	DEC oz	BG lbs	TS ea	Py oz	RP gal	M gal
		0.10							

RD gal	SP lbs	Fish lbs	Insp	Trt	Misc
			15	20	
			15		
			20		
			20		
			15		
			15		
			15		
			15		
			15		
			15		
			15		
			15		

Figure 5.-Summation of Activity at a Given Site.

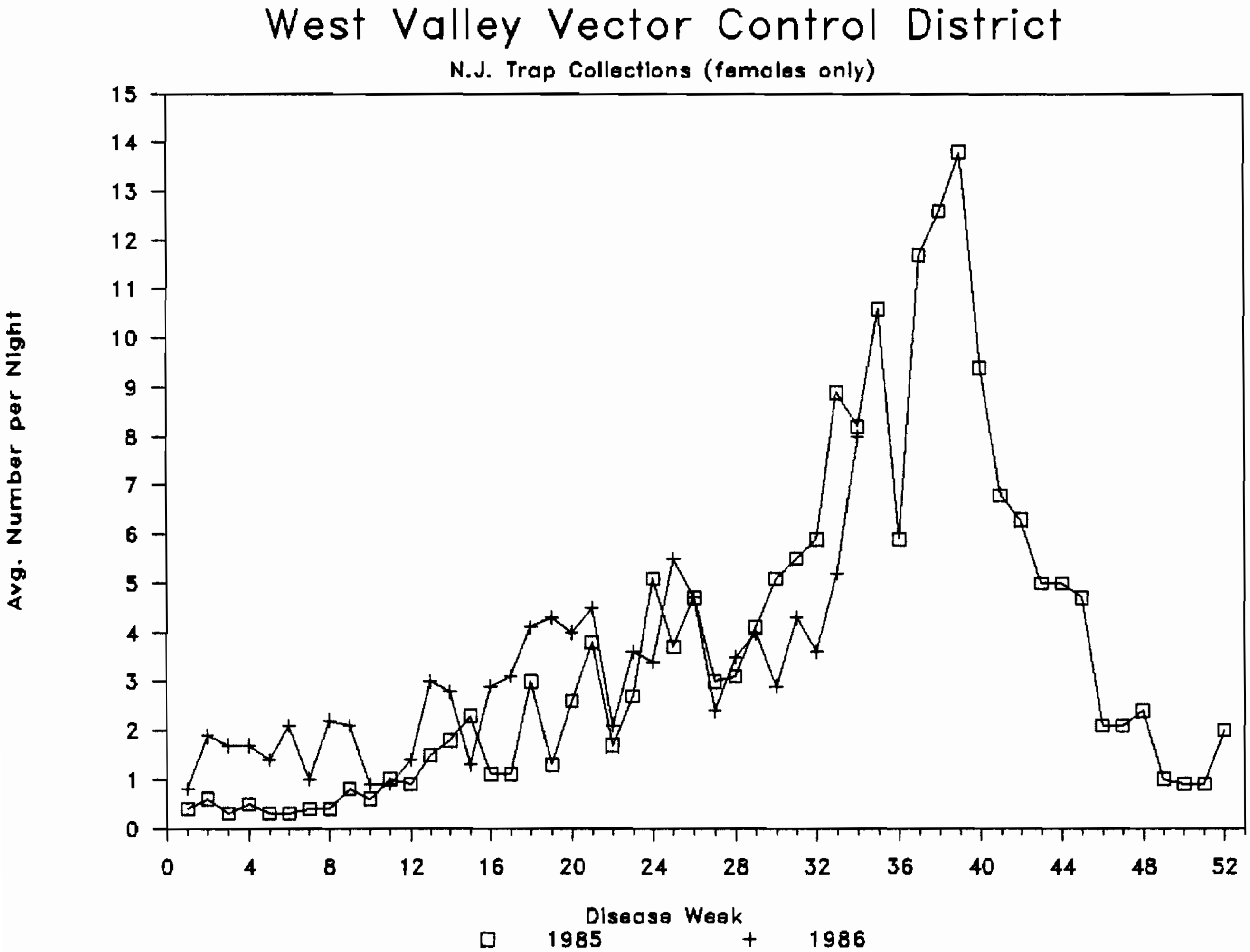


Figure 6.-Graph Produced by Lotus-123.

CONCLUSIONS.-Though the Lotus-123 program is an extensive one with many commands, it can be manipulated to yield information in a quick and concise manner. Record-keeping becomes a manageable task as compared to an overbearing burden.

ACKNOWLEDGMENTS.-Sincere thanks must be extended to the Board of Trustees of the West Valley Vector Control District without whose ongoing support endeavors such as this one would not be possible.

USES OF A VIDEO CAMERA IN A MOSQUITO ABATEMENT DISTRICT

Ronald L. McBride and Eugene E. Kauffman

Sutter-Yuba Mosquito Abatement District
Post Office Box 726
Yuba City, California 95992

INTRODUCTION.-During the summer of 1985, the Sutter-Yuba Mosquito Abatement District began investigating the relative merits of video tape recording systems in our mosquito control program. Throughout the course of a typical mosquito control season, we have often taken hundreds of still photographs to document various aspects of mosquito control operations and research projects. We felt by using video equipment we could improve upon our still photographic techniques and modernize our documentations of District activities. Following are descriptions of the new equipment, including the camera, video recorder, television monitor, required accessories and the many diversified ways we used our system this past year.

DESCRIPTION OF EQUIPMENT.-After many weeks of comparing the different types of video cameras available, we decided to purchase the Panasonic PK-958 Color Video Camera. The primary features of this camera are its 8:1 zoom lens with automatic/manual iris control providing a minimum light sensitivity on optical images of 7 lux. Most other currently manufactured cameras offer only a 6:1 zoom lens and a minimum sensitivity of 10 lux. To put this in meaningful terms, a 7-lux system requires 300% less light than a 10-lux system. Other useful features include an electronic viewfinder, built-in stop watch, date/time display, a macro zoom lens which can be used for time lapse photography, an omnidirectional stereo microphone and a typewriter keyboard control panel which allows you to compose and display your own titles. The basic cost for this camera was $899. The companion recording unit for this particular camera is the Panasonic PV-8500 Portable Video Cassett Recorder. The most important features offered by this unit is its 4-head video system and 2-head audio for stereo sound. The 4-head video system is a must for slow-motion and stop-action when reviewing what you have taped. The cost for this unit was $899. The television monitor was a basic 19-inch color television. We feel the 19-inch screen size gives a sharper image than larger ones. The cost of the monitor was $249. To complete the system we purchased a camera tripod for $99 and carrying cases for the camera and portable recorder for $110.

USES OF THE VIDEO CAMERA.-Our most important use of the video camera was to document major activities we were involved in this past year. We made recordings of rice dipping techniques, aerial application of Bti on rice and wildlife refuges and wide-area adulticiding with ULV foggers. We also documented mosquito sources for trustee meetings and legal abatement hearings and taped the seining of mosquitofish in oxidation ponds. Each of these District activities was titled using the character keyboard entry with the date and time displayed. With this we will have historical documentation of District activities for each year.

Training and education at the district benefited by our use of video equipment. We used the video camera to produce a film on pesticide safety which showed the correct way to pour and mix chemicals, the type of protective clothing to wear and proper clean-up in a pesticide storage and mixing area. In the near future, we intend to make safety films on defensive driving, proper handling of tools in the shops, forklift use and operation of spray equipment. These films will be used to indoctrinate new employees and to refresh or update our experienced personnel. We discovered the video camera was useful for the recording of training sessions featuring guest speakers and to film continuing education courses at either district facilities or colleges. As specific examples, we taped an officer from the California Highway Patrol as he spoke to our employees on seat belt safety and defensive driving. We also taped a toxic waste seminar at Butte County Mosquito Abatement District and a Northern California Region Continuing education session at Yuba College in Marysville, California. These films may be shown to our employees in the future. As an added benefit, we discovered that this camera was sensitive enough to be used for taping of projected 16mm motion pictures and 35mm slides. These films and slides were projected onto a screen in a darkened room and we were able to capture the images directly from the screen with the 7-lux video camera with very little loss of detail. It is our objective to transpose all the slides in our library onto tape for easy future reference.

CONCLUSION.-As stated previously, we feel it is imperative to have a camera with a minimum light sensitivity of 7 lux in order to produce quality reproduction under most lighting conditions. Since purchasing our video camera last summer, development of a new compact and much lighter camera called a camrecorder has made our heavier unit somewhat obsolete; however, the disadvantages of these new cameras are that they currently offer low light minimum sensitivies of only 10 lux and 6:1 zoom ratios. When foreseeable technological advances provide us with a camrecorder with a low light sensitivity of 7 lux combined with an 8:1 zoom lens, we plan to obtain a newer camera.

We feel videotaping technology will substantially improve our district's mosquito control capabilities, while providing historical and possibly legalistic documentation. As time passes, we find more and more beneficial uses for our video recording equipment and feel it has been a very worthwhile and reasonably-priced tool for the Sutter-Yuba Mosquito Abatement District.

EFFECTIVE USE OF ATV'S IN A MOSQUITO CONTROL PROGRAM IN UTAH

J. Lawrence Nielsen, VCS, District Manager

Box Elder County Mosquito Abatement District
P.O. Box 566
Brigham City, Utah 84302

Box Elder County is located 60 miles north of Salt Lake City and is bordered by Idaho on the north, Nevada on the west, Cache county to the east, (The other side of the mountain), and Weber county to the south. The county is 5627 square miles in size and the mosquito and vector control program serves 22 cities and towns, working in a 3500 square mile area. We have 75 private duck clubs, 6 State Waterfowl Areas, a 64,000 acre Federal Bird Refuge which is, at the present time, under three to six feet of the Great Salt Lake, and not a mosquito problem. We also have to contend with over 120,000 acres of irrigated pastureland and hundreds of smaller potential sources. Many of these areas are inaccessible by two or even four wheel drive vehicles, and district crews spent a lot of time walking into these potential mosquito sites. Good coverage was maintained, however, crews were simply not getting around fast enough in these areas to suppress mosquito activity. In 1982, the district leased a three-wheeler. This unit was tested in several areas, under all types of conditions in the county and was found to be satisfactory in performance and overall traction. However, it was found to be unstable when moving around swampy lake edges, rat runs, and other holes that could very easily cause the unit to tip and throw the operator, causing injury even at low speeds. This unit had no reverse gear and its ability to carry the required chemical and equipment was somewhat of a problem. The only advantage I could see with the three-wheeler is the turning radius (they do turn sharp).

In 1983, the District purchased one four-wheel Suzuki LT 125 Quad Runner. A 283# rubber-tired vehicle with excellent performance and the ability to carry up to 200# of chemical and light equipment, either front or rear. This unit also has a reverse gear, a forward power gear and four forward speed transmission for all types of terrain. With four wheels the LT 125 had excellent stability and safety for the operator. It also has excellent traction and maneuverability under most field conditions. It was light enough that the operator could lift the wheels out of the mud if they became stuck. After one season of testing in all types of conditions, this vehicle proved very satisfactory. Three more were purchased in 1983. All together, they were driven over 15,000 miles before we sold them this past fall. They were used three full seasons and proved to be dependable, for the most part. However, we did have some lubrication problems with the chains and the two-wheel brake system. Water, rust and the heavy alkali in some of our soils were hard on the chains. The District decided to purchase new Yamaha 200's with a lubricated shaft drive and a fully enclosed four-wheel brake system. These units are presently being outfitted for the 1986 season. All of our employees have to be responsible individuals, complete a safety course and a service maintenance course, all before they can operate these time-saving units. I'm now a State Certified Instructor and can present these courses right at our District Headquarters in Brigham City. And finally, the advantages that the Box Elder Mosquito Control Program has realized, from the use of the four-wheeler.

The District maintains 20 radio-equipped trucks in its fleet, and up to a few years ago, 12 of them were four-wheel drives. We have now reduced the number of four-wheel drive units down to four; simply because we don't need them any longer. We've save hundreds of dollars on each unit on purchase price and maintenance because of this reduction. We are now purchasing two-wheel drive pickups with positraction and automatic transmissions. Field inspection time has also been reduced by 30% in some areas. We're getting around more efficiently and doing a better job. Spraying time has also been reduced accordingly.

In 1984 and 1985, we reduced our Air Spray costs by 15% simply because we're doing a better, more thorough job of inspecting and ground spraying with these machines. Each operator carries a 5-Watt portable radio while operating these machines and has a radio contact with other mobile units on the base, depending on his or her location. In conclusion, each four-wheeler pays for itself the very first season with the time saved. The morale of our employees is at a all-time high. They're enjoying their work, and we're doing a better job at less cost and saving the taxpayers some hard earned dollars.

Several other districts in Utah are also utilizing this type of equipment with good results. I understand districts here in California have been operating this type of equipment for some time.

PUBLICATION POLICIES AND INFORMATION FOR CONTRIBUTORS

"THE PROCEEDINGS" is the Proceedings and Papers of the California Mosquito and Vector Control Association, Inc. One volume is published each year. Intended coverage by content includes papers and presentations of the Association's Annual Conference, contributions and meritorious reports submitted for the conference year, and a synopsis of actions and achievements by the Association at large during the preceding year.

CONTRIBUTIONS: Articles are original contributions in the field of mosquito and related vector control providing information and benefit to the diverse interests in technical development, operations and programs, and management documentation. Papers on controversial points of view are accepted only as constructive expositions and are otherwise generally dissuaded, as is the case with an excessive number of papers on one subject or by one author where imbalance might ensue. Although preference is given to papers of the conference program, acceptability for publication rests on merit determined on review by the editors and the Publications Committee.

MANUSCRIPTS: The diversity of interests and fields of endeavor represented by contributors and readership of the Proceedings precludes strict conformance as to style. Authors should refer to recent issues of Mosquito News for general guidance. Authors of technical papers should follow the basic recommendations as presented in the Council of Biology Editors Style Manual. Authors should submit an original on white bond paper, with one additional copy. All parts of manuscripts (text, tables, references and legends) must be typed, double-spaced with ample margins. Avoid footnotes in text. Author should indicate with pencil, in the margins, the approximate positions desired for illustrations and tables.

The complete scientific name of an organism must be given the first time it is used. Terms commonly abbreviated in specific fields should be given in full the first time used, followed with the abbreviation. The abbreviation alone is acceptable in further usage in the paper. Common latin abbreviations (et al., e.g., i.e., etc.) are not italicized. Use of the metric system is encouraged. The bi-letter system of generic abbreviations is used for Culicidae.

All manuscripts will be edited to improve communications, if needed. Editors are biased against verbosity or needless complexity or jargon. Grammar will be corrected if necessary. Articles needing extensive editing or not conforming to style and instructions will be returned to the author for correction.

Manuscripts should be submitted to the editor within 45 days after the Annual Conference to ensure publication. Mail all material to the CMVCA PRESS, 197 Otto Circle, Sacramento, California 95822.

ABSTRACTS: Only technical papers need be accompanied by an abstract, which should not exceed 3% of the length of the article. When an abstract is submitted for publication in lieu of a paper, the abstract length may be extended. If possible, the journal where the paper is to be published in full should be stated.

TABLES: Tables are typed on separate sheets placed in correct sequence in the text and should be limited to those strictly necessary. Tables made up by the author in the form of line drawings for photocopy are acceptable. Graphs and line drawings should be prepared with regard to the ultimate printed size of one column (3¼") or page width of seven inches, as applies to columns of table data.

Submitted figures as maps and charts should not exceed 8½ X 11" (22 X 28 cm), with labels and line weight adapted to the published size.Total page space for tables and figures must necessarily be limited by the editors.

ILLUSTRATIONS: Illustrative material must be mailed flat. A copy for use of reviewers is desirable. Figures should be numbered consecutively. Illustrations prepared for printing as line drawings are preferred but those requiring half tones are acceptable. Titles, legends or other headings should be grouped according to the arrangement of the figures and are typed double-spaced, on a separate sheet at the end of the paper. As with tables, the illustrations should be planned to fit reasonably the width of one column (3¼") or one page (7"). Figure numbers, as well as author's name and paper title should be written in blue pencil on the back of each illustration.

PROOF AND REPRINTS: Authors will receive page proof, as well as an order blank for reprints with a schedule of charges. Authors should not make major revisions of their work at this stage. Proofs with corrections, if any, should be returned within ten days to the printer (CMVCA PRESS, 197 Otto Circle, Sacramento, California 95822).