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held jointly with the

Nevada Mosquito Control Association

January 28 thru January 31, 1990

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California Mosquito and Vector Control Association, Inc.

Volume 58 **January 28 - 31, 1990**

SURVEILLANCE FOR ARTHROPOD-BORNE VIRAL ACTIVITY AND DISEASE IN CALIFORNIA DURING 1989

Richard W. Emmons[1], Dale V. Dondero[1], Marilyn M. Milby[2], Lucia T. Hui[3], Robert A. Murray[4], John Tueller[4], Franklin Ennik[3], Barbara A. Wilson[3], Lenore Pitstick[1], James L. Hardy[2], Sally B. Presser[2], William C. Reeves[2], Larry Barrett[5], Michael S. Ascher[1]

This is a report on arbovirus surveillance activities during 1989. It is the 20th report to the California Mosquito and Vector Control Association (CMVCA) since 1969. The arbovirus surveillance program reflects extensive cooperative efforts and contributions by staff from local mosquito control agencies, the Arbovirus Research Program at the University of California at Berkeley, county and local health departments, the California Department of Food and Agriculture, the Viral and Rickettsial Disease Laboratory (VRDL), the Infectious Disease Branch and the Environmental Management Branch of the California Department of Health Services, and physicians and veterinarians throughout the state.

Announcements were sent out in early May that the program was in operation, and then 21 weekly reports of surveillance findings were disseminated widely from May 27 through December 22. There is clearly need, however, for greater efforts next season to assure better, quicker and even wider distribution of the information. Improved inter- and intra-agency sharing and discussion of the data also are indicated, since some of the findings this year caught some groups by surprise. Further efforts must be made to develop electronic distribution of test results on mosquito pools and chicken sera.

As usual, clinical and laboratory surveillance for human and equine encephalitis and/or meningoencephalitis cases was conducted throughout the state. This effort eventually yielded 29 confirmed or presumptive-positive cases of St. Louis encephalitis (SLE). The initial case occurred

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Table 1. Human cases of St. Louis encephalitis, California, 1989

Case Number	Age	Sex	County of residence/ place of probable contraction	Date of onset
1	65	M	Los Angeles County	8/11/89
2	14	F	Kings County	8/17/89
3	36	M	Kings County	8/18/89
4	29	F	Kings County	8/21/89
5	50	M	Kern County	8/22/89
6	85	M	Kings County	8/23/89
7	32	M	Kings County	8/24/89
8	7	M	Kern County	8/28/89
9	52	M	Kern County	8/28/89
10	69	M	Kings County	8/29/89
11	40	M	Kern County	9/1/89
12	16	F	Kern County	9/9/89
13	50	M	Tulare County	9/11/89
14	28	M	Kings County	9/12/89
15	17	M	Kern County	9/12/89
16	12	F	Kern County	9/15/89
17	9	F	Tulare County	9/17/89
18	61	F	Kern County	9/17/89
19	60	F	Kern County	9/17/89
20	26	F	Kern County	9/19/89
21	7	F	Kings County	9/20/89
22	57	F	Kern County	9/20/89
23	63	F	Kern County	9/20/89
24	28	M	Kern County	9/23/89
25	61	M	Tulare County	9/27/89
26	52	M	Kern County	9/27/89
27	25	M	Tulare County	10/2/89
28	28	M	Tulare County	10/7/89
29	37	M	Kern County	10/8/89

Table 2. Number of mosquitoes and pools tested during 1989.

	Cx. tarsalis		*Cx. pipiens* complex		*Cx. stigmatosoma*		*Ae. melanimon*		Other spp.		Total	
County	Mosq.	Pools	Mosq.	Pools	Mosq.	Pools	Mosq.	Pools	Mosq.	Pools	Mosq.	Pools
Butte	4800	96	.	.	.	.	1250	25	.	.	6050	121
Impr.	16361	361	3324	78	.	.	.	.	311	7	19996	446
Impr.*	27532	651	2123	57	.	.	.	.	7993	198	37648	906
Inyo	208	6	.	.	.	.	835	17	.	.	1043	23
Kern	13709	299	2046	42	58	3	2915	75	.	.	18728	419
Kern*	1136	31	1082	27	6	2	907	23	.	.	3131	83
Lake	924	19	.	.	.	.	160	4	.	.	1084	23
Los A.	6623	181	15355	366	976	39	.	.	.	.	22954	586
Los A.*	331	18	4507	167	68	4	.	.	1514	41	6420	230
Marin	165	5	.	.	.	.	.	.	.	.	165	5
Merced	871	18	.	.	50	1	350	7	.	.	1271	26
Orange	3615	88	4850	130	77	4	.	.	.	.	8542	222
Orange*	1555	57	3150	126	436	18	.	.	268	18	5409	219
River.	40755	884	9892	237	1172	41	.	.	.	.	51819	1162
River.*	23164	497	10175	232	429	15	.	.	8363	231	42131	975
Sacram.	5606	129	568	14	.	.	92	2	.	.	6266	145
San D.	193	5	5	1	16	1	.	.	.	.	214	7
S. Bar.	2552	52	19	1	.	.	.	.	.	.	2571	53
San B.	10046	221	1430	35	961	31	.	.	.	.	12437	287
San B.*	11855	259	282	9	.	.	.	.	2692	67	14829	335
Shasta	379	8	.	.	.	.	.	.	.	.	379	8
Sonoma	195	6	.	.	.	.	.	.	.	.	195	6
Stanis.	219	5	673	14	.	.	576	12	.	.	1468	31
Sutter	971	20	.	.	.	.	86	2	.	.	1057	22
Ventura	671	18	540	12	20	1	.	.	.	.	1231	31
Yolo	10312	214	39	2	.	.	200	4	.	.	10551	220
Yuba	85	2	.	.	.	.	.	.	.	.	85	2
Total	119260	2637	38741	932	3330	121	6464	148	311	7	168106	3845
Total*	65573	1513	21319	618	939	39	907	23	20830	555	109568	2748
Both Labs	184833	4150	60060	1550	4269	160	7371	171	21141	562	277674	6593

* Tested at UC Berkeley; all others tested at VRDL.

Table 3. Viral isolates from mosquitoes during 1989

Mosquito species	County	Viruses isolated						
		WEE	SLE	CEV	HP	TUR	Unid.	Total
Cx. tarsalis	Riverside	.	28	.	3	1	.	32
	Imperial	13	10	.	1	.	4	28
	Kern	.	70	.	.	.	1	71
	Los Angeles	.	1	.	.	.	.	1
	Orange	.	.	.	.	.	1	1
Cx. pipiens complex	Imperial	1	.	.	.	.	.	1
	Kern	.	14	.	.	.	.	14
	Los Angeles	.	2	.	.	.	.	2
Cx. stigmatosoma	Kern	.	1	.	.	.	.	1
	Los Angeles	.	1	.	.	.	.	1
Cx. erythrothorax	Imperial	1	.	.	.	.	.	1
Ae. melanimon	Inyo	.	.	1	.	.	.	1
	Kern	.	.	1	.	.	.	1
Total		15	127	2	4	1	6	155

Table 4. Positive mosquito pools, 1989

Virus	Species	Date	Location	Pool no.	Laboratory
SLE	*Cx. tarsalis*	01-23	Riverside, Mecca, Adohr	CHLV-62	UCB
SLE	*Cx. tarsalis*	06-19	Riverside, Mecca, Adohr	CHLV-638	VRDL
SLE	*Cx. tarsalis*	06-20	Riverside, Mecca, DexOTex	CHLV-662	VRDL
SLE	*Cx. tarsalis*	06-20	Riverside, Mecca, DexOTex	CHLV-665	VRDL
SLE	*Cx. tarsalis*	06-20	Riverside, Mecca Duck Club	CHLV-668	VRDL
SLE	*Cx. tarsalis*	06-20	Riverside, Mecca Duck Club	CHLV-669	VRDL
SLE	*Cx. tarsalis*	06-20	Riverside, Mecca Duck Club	CHLV-670	VRDL
SLE	*Cx. tarsalis*	06-20	Riverside, North Shore	CHLV-680	VRDL
SLE	*Cx. tarsalis*	06-20	Riverside, North Shore	CHLV-681	VRDL
SLE	*Cx. tarsalis*	06-26	Riverside, Mecca, Adohr	CHLV-692	VRDL
SLE	*Cx. tarsalis*	06-26	Riverside, Mecca, Adohr	CHLV-693	VRDL
SLE	*Cx. tarsalis*	07-06	Riverside, Mecca, Adohr	CHLV-710	VRDL
SLE	*Cx. tarsalis*	07-06	Riverside, Mecca, Adohr	CHLV-712	VRDL
SLE	*Cx. tarsalis*	07-10	Imperial, Seeley	IMPR-729	VRDL
SLE	*Cx. tarsalis*	07-10	Imperial, Seeley	IMPR-732	VRDL
SLE	*Cx. tarsalis*	07-10	Riverside, Mecca, Adohr	CHLV-733	VRDL
SLE	*Cx. tarsalis*	07-12	Imperial, Seeley	IMPR-747	VRDL
SLE	*Cx. tarsalis*	07-18	Riverside, Mecca Duck Club	CHLV-748	VRDL
SLE	*Cx. tarsalis*	07-18	Riverside, Mecca Duck Club	CHLV-751	VRDL
SLE	*Cx. tarsalis*	07-18	Riverside, Mecca Duck Club	CHLV-754	VRDL
SLE	*Cx. tarsalis*	07-18	Riverside, North Shore	CHLV-758	VRDL
SLE	*Cx. tarsalis*	07-19	Kern, Cole's Levee	KERN-179	VRDL
SLE	*Cx. tarsalis*	07-20	Imperial, Seeley	IMPR-751	VRDL
SLE	*Cx. tarsalis*	07-20	Imperial, Seeley	IMPR-753	VRDL
SLE	*Cx. tarsalis*	07-20	Imperial, Seeley	IMPR-754	VRDL
SLE	*Cx. tarsalis*	07-20	Kern, Cole's Levee	KERN-196	VRDL
SLE	*Cx. tarsalis*	07-20	Kern, Cole's Levee	KERN-197	VRDL
SLE	*Cx. tarsalis*	07-26	LA, City of Industry	LAHD-048	VRDL
SLE	*Cx. quinquefasciatus*	07-26	LA, City of Industry	LAHD-049	VRDL
SLE	*Cx. tarsalis*	07-31	Riverside, Mecca, Adohr	CHLV-772	VRDL
SLE	*Cx. tarsalis*	08-01	Kern, Cole's Levee	KERN-212	VRDL
SLE	*Cx. tarsalis*	08-01	Kern, Cole's Levee	KERN-213	VRDL
SLE	*Cx. tarsalis*	08-01	Kern, Cole's Levee	KERN-214	VRDL
SLE	*Cx. tarsalis*	08-01	Kern, Cole's Levee	KERN-216	VRDL
SLE	*Cx. tarsalis*	08-01	Kern, Cole's Levee	KERN-217	VRDL
SLE	*Cx. tarsalis*	08-01	Kern, Cole's Levee	KERN-218	VRDL
SLE	*Cx. tarsalis*	08-01	Kern, Cole's Levee	KERN-219	VRDL
SLE	*Cx. tarsalis*	08-01	Kern, River Bottom	KERN-221	VRDL
SLE	*Cx. tarsalis*	08-01	Kern, River Bottom	KERN-224	VRDL
SLE	*Cx. stigmatosoma*	08-01	LA, Whittier	LAHD-052	VRDL
SLE	*Cx. tarsalis*	08-14	Kern, Kern Refuge	KERN-237	VRDL
SLE	*Cx. tarsalis*	08-14	Kern, Eureka Duck Club	KERN-250	VRDL
SLE	*Cx. tarsalis*	08-14	Riverside, Thermal	CHLV-794	VRDL
SLE	*Cx. tarsalis*	08-15	Kern, Bakersfield	KERN-253	VRDL
SLE	*Cx. tarsalis*	08-15	Kern, John Dale	KERN-260	VRDL
SLE	*Cx. tarsalis*	08-15	Kern, John Dale	KERN-261	VRDL
SLE	*Cx. tarsalis*	08-15	Kern, John Dale	KERN-262	VRDL
SLE	*Cx. tarsalis*	08-15	Kern, John Dale	KERN-263	VRDL
SLE	*Cx. tarsalis*	08-15	Riverside, Mecca, DexOTex	CHLV-822	VRDL
SLE	*Cx. tarsalis*	08-15	Riverside, Mecca Duck Club	CHLV-830	VRDL
SLE	*Cx. tarsalis*	08-15	Riverside, Mecca Duck Club	CHLV-832	VRDL
SLE	*Cx. tarsalis*	08-15	Riverside, Mecca Duck Club	CHLV-833	VRDL
SLE	*Cx. tarsalis*	08-15	Riverside, North Shore	CHLV-840	VRDL
SLE	*Cx. tarsalis*	08-16	Kern, Cole's Levee	KERN-264	VRDL
SLE	*Cx. tarsalis*	08-16	Kern, Cole's Levee	KERN-266	VRDL
SLE	*Cx. tarsalis*	08-16	Kern, Cole's Levee	KERN-268	VRDL
SLE	*Cx. tarsalis*	08-16	Kern, Cole's Levee	KERN-269	VRDL

continued-

Table 4.-continued.

Virus	Species	Date	Location	Pool no.	Laboratory
SLE	*Cx. tarsalis*	08-16	Kern, Cole's Levee	KERN-270	VRDL
SLE	*Cx. tarsalis*	08-16	Kern, Cole's Levee	KERN-271	VRDL
SLE	*Cx. tarsalis*	08-16	Kern, Cole's Levee	KERN-272	VRDL
SLE	*Cx. tarsalis*	08-16	Kern, Cole's Levee	KERN-273	VRDL
SLE	*Cx. tarsalis*	08-16	Kern, River Bottom	KERN-274	VRDL
SLE	*Cx. tarsalis*	08-16	Kern, River Bottom	KERN-275	VRDL
SLE	*Cx. tarsalis*	08-16	Kern, River Bottom	KERN-276	VRDL
SLE	*Cx. tarsalis*	08-16	Kern, River Bottom	KERN-277	VRDL
SLE	*Cx. tarsalis*	08-16	Kern, River Bottom	KERN-278	VRDL
SLE	*Cx. tarsalis*	08-16	Kern, River Bottom	KERN-279	VRDL
SLE	*Cx. tarsalis*	08-16	Kern, River Bottom	KERN-281	VRDL
SLE	*Cx. tarsalis*	08-16	Kern, River Bottom	KERN-282	VRDL
SLE	*Cx. tarsalis*	08-16	Kern, River Bottom	KERN-283	VRDL
SLE	*Cx. tarsalis*	08-21	Riverside, Thermal	CHLV-841	VRDL
SLE	*Cx. tarsalis*	08-21	Riverside, Thermal	CHLV-842	VRDL
SLE	*Cx. tarsalis*	08-22	Imperial, Seeley	IMPR-777	VRDL
SLE	*Cx. tarsalis*	08-22	Imperial, Seeley	IMPR-779	VRDL
SLE	*Cx. tarsalis*	08-23	Imperial, Bard	COLO-135	VRDL
SLE	*Cx. tarsalis*	08-28	Kern, Cole's Levee	KERN-303	VRDL
SLE	*Cx. tarsalis*	08-28	Kern, Cole's Levee	KERN-305	VRDL
SLE	*Cx. tarsalis*	08-28	Kern, Cole's Levee	KERN-306	VRDL
SLE	*Cx. tarsalis*	08-28	Kern, Cole's Levee	KERN-309	VRDL
SLE	*Cx. tarsalis*	08-28	Kern, Cole's Levee	KERN-311	VRDL
SLE	*Cx. tarsalis*	08-28	Kern, John Dale	KERN-315	VRDL
SLE	*Cx. quinquefasciatus*	08-28	Kern, John Dale	KERN-318	VRDL
SLE	*Cx. quinquefasciatus*	08-28	Kern, John Dale	KERN-319	VRDL
SLE	*Cx. quinquefasciatus*	08-29	Kern, Buena Vista	KERN-327	VRDL
SLE	*Cx. quinquefasciatus*	08-29	Kern, Buena Vista	KERN-331	VRDL
SLE	*Cx. tarsalis*	08-29	Kern, Eureka Duck Club	KERN-339	VRDL
SLE	*Cx. tarsalis*	08-30	Kern, River Bottom	KERN-342	VRDL
SLE	*Cx. tarsalis*	08-30	Kern, River Bottom	KERN-343	VRDL
SLE	*Cx. tarsalis*	08-30	Kern, River Bottom	KERN-344	VRDL
SLE	*Cx. tarsalis*	08-30	Kern, River Bottom	KERN-345	VRDL
SLE	*Cx. tarsalis*	08-30	Kern, River Bottom	KERN-346	VRDL
SLE	*Cx. tarsalis*	08-30	Kern, River Bottom	KERN-348	VRDL
SLE	*Cx. tarsalis*	08-30	Kern, River Bottom	KERN-351	VRDL
SLE	*Cx. quinquefasciatus*	08-30	Kern, River Bottom	KERN-352	VRDL
SLE	*Cx. quinquefasciatus*	08-30	Kern, River Bottom	KERN-356	VRDL
SLE	*Cx. quinquefasciatus*	08-30	Kern, River Bottom	KERN-357	VRDL
SLE	*Cx. quinquefasciatus*	08-30	Kern, River Bottom	KERN-358	VRDL
SLE	*Cx. quinquefasciatus*	08-30	Kern, River Bottom	KERN-360	VRDL
SLE	*Cx. stigmatosoma*	08-30	Kern, River Bottom	KERN-362	VRDL
SLE	*Cx. quinquefasciatus*	08-30	Kern, Bakersfield	KERN-363	VRDL
SLE	*Cx. quinquefasciatus*	08-30	Kern, Bakersfield	KERN-368	VRDL
SLE	*Cx. quinquefasciatus*	08-30	Kern, Bakersfield	KERN-369	VRDL
SLE	*Cx. quinquefasciatus*	08-30	Kern, Bakersfield	KERN-372	VRDL
SLE	*Cx. tarsalis*	08-30	Kern, Bakersfield	KERN-373	VRDL
SLE	*Cx. tarsalis*	08-30	Kern, Bakersfield	KERN-374	VRDL
SLE	*Cx. quinquefasciatus*	09-06	Kern, Bakersfield	KERN-378	VRDL
SLE	*Cx. tarsalis*	09-09	Imperial, Seeley	IMPR-790	VRDL
SLE	*Cx. tarsalis*	09-12	Kern, Eureka Duck Club	KERN-385	VRDL
SLE	*Cx. tarsalis*	09-12	Kern, Wildlife Refuge	KERN-388	VRDL
SLE	*Cx. tarsalis*	09-12	Kern, Wildlife Refuge	KERN-390	VRDL
SLE	*Cx. tarsalis*	09-12	Kern, River Bottom	KERN-399	VRDL
SLE	*Cx. tarsalis*	09-12	Kern, River Bottom	KERN-400	VRDL
SLE	*Cx. tarsalis*	09-12	Kern, River Bottom	KERN-401	VRDL
SLE	*Cx. tarsalis*	09-12	Kern, River Bottom	KERN-403	VRDL
SLE	*Cx. tarsalis*	09-12	Kern, River Bottom	KERN-405	VRDL
SLE	*Cx. tarsalis*	09-13	Kern, John Dale	KERN-411	VRDL

continued-

Table 4.-continued.

Virus	Species	Date	Location	Pool no.	Laboratory
SLE	*Cx. tarsalis*	09-13	Kern, John Dale	KERN-413	VRDL
SLE	*Cx. tarsalis*	09-13	Kern, John Dale	KERN-414	VRDL
SLE	*Cx. tarsalis*	09-13	Kern, John Dale	KERN-415	VRDL
SLE	*Cx. quinquefasciatus*	09-17	Long Beach, El Dorado Park	SOUE-310	UCB
SLE	*Cx. tarsalis*	09-26	Kern, Eureka DC	KERN-430	UCB
SLE	*Cx. tarsalis*	09-26	Kern, Eureka DC	KERN-431	UCB
SLE	*Cx. tarsalis*	09-26	Kern, Eureka DC	KERN-433	UCB
SLE	*Cx. tarsalis*	09-26	Kern, Eureka DC	KERN-434	UCB
SLE	*Cx. tarsalis*	09-28	Kern, River Bottom	KERN-441	UCB
SLE	*Cx. tarsalis*	10-10	Kern, Eureka DC	KERN-456	UCB
SLE	*Cx. tarsalis*	10-10	Riverside, Thermal	CHLV-1060	UCB
			Total = 127		
WEE	*Cx. tarsalis*	06-27	Imperial, Holtville	IMPR-696	UCB
WEE	*Cx. tarsalis*	06-27	Imperial, Holtville	IMPR-705	VRDL
WEE	*Cx. quinquefasciatus*	06-27	Imperial, Holtville	IMPR-707	VRDL
WEE	*Cx. erythrothorax*	07-07	Imperial, Holtville	IMPR-717	UCB
WEE	*Cx. tarsalis*	07-07	Imperial, Holtville	IMPR-723	VRDL
WEE	*Cx. tarsalis*	07-10	Imperial, Seeley	IMPR-731	VRDL
WEE	*Cx. tarsalis*	07-10	Imperial, Seeley	IMPR-733	VRDL
WEE	*Cx. tarsalis*	07-12	Imperial, Holtville	IMPR-736	VRDL
WEE	*Cx. tarsalis*	07-12	Imperial, Holtville	IMPR-737	VRDL
WEE	*Cx. tarsalis*	07-12	Imperial, Seeley	IMPR-744	VRDL
WEE	*Cx. tarsalis*	07-12	Imperial, Seeley	IMPR-746	VRDL
WEE	*Cx. tarsalis*	07-20	Imperial, Seeley	IMPR-749	VRDL
WEE	*Cx. tarsalis*	07-23	Imperial, Holtville	IMPR-757	VRDL
WEE	*Cx. tarsalis*	08-01	Imperial, Seeley	IMPR-764	VRDL
WEE	*Cx. tarsalis*	09-09	Imperial, Seeley	IMPR-792	VRDL
			Total = 15		
HP	*Cx. tarsalis*	01-23	Imperial, Seeley	IMPR-35	UCB
HP	*Cx. tarsalis*	02-16	Riverside, Mecca Duck Club	CHLV-123	UCB
HP	*Cx. tarsalis*	02-20	Riverside, Mecca, Adohr	CHLV-149	UCB
HP	*Cx. tarsalis*	03-22	Riverside, Desert Beach	CHLV-221	UCB
			Total = 4		
CEV	*Ae. melanimon*	08-01	Kern, Kern Refuge	KERN-207	VRDL
CEV	*Ae. melanimon*	08-02	Inyo, Bishop	INYO-005	VRDL
			Total = 2		
TUR	*Cx. tarsalis*	11-27	Riverside, Mecca, Adohr	CHLV-1298	UCB
			Total = 1		
Unid.	*Cx. tarsalis*	03-29	Imperial, Calapatria	IMPR-243	UCB
Unid.	*Cx. tarsalis*	03-29	Imperial, Calapatria	IMPR-251	UCB
Unid.	*Cx. tarsalis*	04-26	Imperial, Seeley	IMPR-406	UCB
Unid.	*Cx. tarsalis*	04-26	Imperial, Calapatria	IMPR-417	UCB
Unid.	*Cx. tarsalis*	10-16	Orange, 20 Ranch Duck Club	ORCO-406	UCB
Unid.	*Cx. tarsalis*	10-24	Kern, Kern Refuge	KERN-485	UCB
			Total = 6		

Table 5. SLE seropositive chickens/number tested (percent positive), northern California, 1989.

Flock location	Number SLE positive/number tested (percent positive)						
	[a]Jun 12-Jun 16	Jul 10-Jul 14	Aug 7-Aug 11	Sep 4-Sep 8	Oct 2-Oct 6	Oct 30-Nov 3	Nov 27-Nov 28
				Northern California			
Shasta, Cottonwood	0/20	0/20	0/20	0/20	0/20	NS	
Shasta, Pine Grove MAD	0/19	0/19	0/19	0/17	0/14	NS	
Tehama, MAD office	0/20	0/20	0/20	0/20	0/20	NS	
Butte, Chico	0/19	0/20	0/20	0/20	NS	NS	
Butte, Honcut	0/20	0/20	0/20	0/19	NS	NS	
Butte, Gray Lodge	0/19	0/20	0/20	0/19	NS	NS	
Glenn, Willows	0/19	0/18	0/18	0/16	0/17	NS	
S-Yuba, P. V. Ranch	0/13	0/13	0/13	0/13	0/13	NS	
S-Yuba, Dean's	0/19	0/19	0/19	0/19	0/19	NS	
S-Yuba, Barker	0/20	0/20	0/20	0/20	0/20	NS	
Sac-Yolo, Merritt	0/20	0/20	0/20	0/20	0/20	0/19	NS
Sac-Yolo, Natomas	0/20	0/20	0/20	0/20	0/20	0/20	NS
Sac-Yolo, Elk Grove	0/20	0/20	0/20	0/20	0/20	0/20	NS
Lake, MAD Office	0/20	0/20	0/20	0/20	0/20	NS	
Marin-Sonoma, W. Santa Rosa	0/19	0/19	0/19	0/19	0/18	NS	
Solano, Dixon	0/20	0/20	0/20	0/20	0/20	NS	
Santa Clara, San Martin	0/24	0/24	0/22	0/21	0/20	NS	
No. Calif. total	**0/331**	**0/332**	**0/330**	**0/323**	**0/261**	**0/59**	
Fallon, Nevada	.	.	.	.	1/19(5)		
				San Joaquin Valley			
San Joaquin, Thornton	0/18	0/20	0/18	0/20	0/20	NS	
Eastside, Valley Home	0/20	0/20	0/18	0/18	0/19	NS	
Turlock, Vitoria	0/19	0/18	0/18	0/18	0/18	NS	
Merced, Gustine	0/20	0/20	0/20	0/19	0/20	NS	
Merced, Veldhaus	0/19	0/17	0/17	0/17	0/14	NS	
Fresno W'side, Mendota Ref.	0/19	0/19	0/19	0/18	0/19	0/20	NS
Consolidated, Friant Rd.	0/16	0/16	0/16	2/13(15)	3/16(19)	3/16(19)	1/12(8)
Kings, MAD Office, Hanford	0/19	0/20	2/19(11)	11/20(55)	10/12(83)	18/19(95)	NS
Delta, Kingsburg GC	0/19	0/19	0/19	0/19	0/19	3/19(16)	4/16(25)
Tulare, MAD office	0/20	0/20	0/20	5/20(25)	8/20(40)	14/20(70)	NS
West Side, Belridge	0/20	6/20(30)	14/19(74)	17/18(94)	18/18(100)	NS	
West Side, Maricopa	0/20	0/20	2/20(10)	19/20(95)	13/13(100)	NS	
Delano, Teviston	0/19	0/18	0/17	16/19(84)	19/19(100)	18/18(100)	NS
[b]Kern, Wasco	0/19	0/19	0/19	8/19(42)	19/19(100)	19/19(100)	19/19(100)
Kern, F.C.Tracy	0/19	0/19	0/19	15/19(79)	19/19(100)	19/19(100)	NS
Kern, Buttonwillow	0/19	0/18	0/18	17/18(94)	17/17(100)	17/17(100)	NS
Kern, Wildlife Refuge	0/17	0/17	0/17	0/16	2/17(12)	8/17(47)	12/17(71)
Kern, Oildale	0/20	0/20	0/20	8/20(40)	11/20(55)	15/20(75)	15/20(75)
Kern, John Dale	0/20	0/20	0/20	10/20(50)	18/20(90)	20/20(100)	20/20(100)
Kern, River Bottom	0/20	0/20	0/20	12/20(60)	19/20(95)	19/20(95)	19/20(95)
San Joaquin total	**0/382**	**6/380(2)**	**18/373(5)**	**140/371(38)**	**176/359(49)**	**173/244(71)**	**90/124(73)**

NS = not sampled

a. All flocks negative May 15-19.

b. Kern flocks bled on same schedule as southern Calif. flocks (2 weeks **before** northern Calif. flocks)

Table 6. SLE and WEE seropositive chickens/number tested (percent positive), southern California, 1989.

Flock location	SLE positive/number tested (percent positive)								
	May 29-Jun 2[a]	Jun 26-Jun 30	Jul 24-Jul 28	Aug 21-Aug 25	Sep 18-Sep 22	Oct 16-Oct 20	Nov 13-Nov 17	Dec 11-Dec 15	Jan 8-Jan 12
Goleta, Gray's Ranch	0/19	0/19	0/20	0/20	0/20	0/19	NS	-	-
Ventura, Pt. Mugu	0/20	0/20	0/20	0/20	0/20	0/20	dead	-	-
Ventura, Simi Valley	0/20	0/20	0/20	0/19	0/19	0/18	dead	-	-
Los Angeles, La Brea	0/24	0/24	0/24	0/24	0/24	0/22	0/22	0/22	0/22
Los Angeles, Cal Poly*	0/25	0/25	0/24	0/24	0/26	0/26	0/15	0/15	0/15
Southeast, Harbor Lake	0/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24
Long Beach, El Dorado	0/20	0/20	0/19	0/19	3/19(16)[e]	4/19(21)	4/19(21)	4/19(21)	-
Southeast, Sepulveda	0/22[b]	0/26	0/25	0/25	0/25	0/25	0/25	0/24	0/24
Southeast, Encino*	0/24	0/25	0/25	1/25(4)	1/25(4)	1/25(4)	1/25(4)	1/25(4)	1/25(4)
Southeast, Norwalk*	0/25	0/25	0/25	0/25	0/25	0/25	0/25	0/25	0/25
Orange, Fullerton*	0/24	0/25	0/24	1/25(4)[f]	1/25(4)	1/25(4)	3/25(15)[g]	3/25(15)	3/25(15)
Orange, Duck Club	0/25	0/25	0/25	0/25	0/25	0/25	0/24	0/24	0/23
San Bernardino, 5th Street	0/19	0/18	0/17	0/17	0/16	0/18	NS	-	-
San Bernardino, Flood Control	0/19	0/15	0/15	0/14	0/10	0/11	NS	-	-
West Valley, Chino, Smith	0/19	.[c]	0/14	0/18	0/20	0/19	NS	-	-
Northwest, Corona	0/20	0/19	0/19	0/12	0/11	0/11	0/12	0/12	0/12
Coachella Valley, Palm Desert[d]	0/17	0/17	0/14	0/14[d]	0/23	0/20	0/20	0/18	0/15
Coachella Valley, Indio	0/23	0/23	0/22	0/21	0/21	0/21	2/21(10)	2/21(10)	2/21(10)
Coachella Valley, Mecca[d]	0/23	5/23(22)	16/21(76)	16/21(76)[d]	0/23	0/20	0/20	0/20	0/20
Coachella Valley, Thermal	0/23	0/23	0/23	5/23(22)	6/23(26)	6/22(27)	6/22(27)	6/22(27)	6/22(27)
Imperial, Finney/Ramer[d]	0/23	0/21	0/19[d]	dead	0/18	1/17(6)	1/17(6)	2/17(12)	2/17(12)
Imperial, Keffer Rd., Holtville[d]	0/23	0/23	0/23	0/22[d]	dead	-	-	-	0/25
Imperial, Drew Rd., Seeley[d]	0/24	0/24	9/24(38)	19/24(79)[d]	1/21(5)	3/21(14)	5/21(24)	5/21(24)	7/21(33)
Imperial, Palo Verde	0/22	0/22	0/20	0/19	0/19	0/19	0/19	0/19	0/19
Imperial, Bard	0/23	0/21	0/21	0/20	0/20	0/20	0/18	0/18	0/18
San Diego, San Ysidro	0/20	0/18	0/17	0/18	0/16	0/18	dead	-	-
San Diego, Lakeside	0/19	0/19	0/19	0/19	0/19	0/19	dead	-	-
San Diego, Vista	0/20	0/20	0/20	0/19	0/20	0/20	dead	-	-
Colorado River, Needles	0/22	0/16	0/14	0/15	0/14	0/13	0/12	0/13	0/11
Colorado River, Havasu Refuge	0/24	0/24	0/20	0/20	0/20	0/20	0/20	0/20	0/20
Colorado River, Blythe	0/22	0/22	0/20	0/20	0/16	0/14	0/14	0/14	0/14
So. Calif. total	**0/677**	**5/646(1)**	**25/637(4)**	**42/611(7)**	**12/607(2)**	**16/596(3)**	**22/420(5)**	**23/418(6)**	**21/418(5)**
Flock location	WEE positive/number tested (percent positive)								
Imperial, Finney/Ramer[d]	0/23	0/21	1/19(5)[d]	dead	0/18	0/17	0/17	0/17	0/17
Imperial, Keffer Rd., Holtville[d]	0/23	0/23	10/23(43)	11/22(50)[d]	dead	-	-	-	0/25
Imperial, Drew Rd., Seeley[d]	0/24	0/24	12/24(50)	22/24(92)[d]	0/21	0/21	0/21	0/21	0/21
Colorado River, Needles	0/22	0/16	1/14(7)	1/15(7)	1/14(7)	1/13(8)	1/12(8)	1/13(8)	1/11(9)
So. Calif. total	**0/677**	**0/646**	**24/637(4)**	**34/611(6)**	**1/607(<1)**	**1/596(<1)**	**1/420(<1)**	**1/418(<1)**	**1/418(<1)**

* 5 mini-flocks.

a. All birds negative May 1-5.

b. All killed after May 31 bleeding; birds were replaced.

c. Blood samples spoiled.

d. Chickens killed; replaced on Aug 28.

e. Two sera positive in interim samples taken Sep 5.

f. Serum positive in interim sample taken Aug 7.

g. One serum positive in interim sample taken Oct 30.

in the Antelope Valley in northern Los Angeles County, and 28 cases were in Kern, Kings and Tulare Counties (Table 1). The Antelope Valley case is the first evidence of SLE virus activity in this locality since the surveillance program began. Several non-ill family or household members associated with cases in the San Joaquin Valley were found to have antibody levels indicative of recent infection, but were not counted as clinical cases. Most cases came to the attention of public health officials long after their illness occurred, because serum samples for diagnosis had not been submitted initially to public health laboratories. An intensive retrospective search for cases was made. The unusual features of this episode will be presented in detail at this conference by Dr. John Tueller and will be published separately.

No cases of western equine encephalomyelitis (WEE) were detected in humans or in the 29 equines tested. One interesting case of non-fatal encephalitis-like illness was found in an 8-year old mare from Kern County, with onset on September 16. This horse had high stationary antibody titers to SLE, suggesting but not proving an etiological association. SLE antibody has been found in 35 of 633 equines tested in the VRDL from 1971 through 1988, but rising antibody titers and serological proof of an etiologic association have been very rare.

There were 3,845 mosquito pools, containing 168,106 mosquitoes, tested during the year by the VRDL (Table 2). The majority (75%) of the pools were collected in and submitted from Imperial, Los Angeles, Riverside, San Bernardino and Kern Counties. *Culex tarsalis* Coquillett made up 69% of the pools, *Culex pipiens* L. complex 24% and *Culex stigmatosoma* Dyar, *Aedes melanimon* Dyar and miscellaneous other species the remainder. A total of 133 viral isolates were made by the VRDL (Tables 3 and 4). These included 118 SLE, 13 WEE and two California (CE) serogroup viruses. All isolates of SLE and WEE were from *Culex tarsalis* except one WEE and 15 SLE from *Culex quinquefasciatus* Say and two SLE from *Cx. stigmatosoma*. The two CE isolates, as usual, were from *Ae. melanimon*.

An additional 2,748 mosquito pools from southern California and Kern County, comprising 109,568 mosquitoes, were tested by the University of California at Berkeley Arbovirus Laboratory (Table 2). Many of these were odd species not usually accepted for testing at VRDL. There were eight isolates of SLE virus from *Cx. tarsalis* and one from *Cx. quinquefasciatus*, two of WEE (one each from *Cx. tarsalis* and *Culex erythrothorax* Dyar), four of Hart Park (HP) virus and one of Turlock (TUR) virus from *Cx. tarsalis*, and six from *Cx. tarsalis* that are not yet identified (Tables 3 and 4). A complete listing of all viral isolates is given in Table 4.

Sentinel chicken flocks were located at 68 selected sites throughout endemic areas of the state. Serum samples were tested for WEE and SLE viral antibody monthly from May through October by the VRDL and during the winter for some flocks by the Arbovirus Laboratory (Tables 5 and 6). SLE viral seroconversions occurred from June through November in 210 chickens from the southern San Joaquin Valley. The seroconversions in Kern, Tulare and Kings Counties preceded or coincided with the occurrence of human cases in these counties. Six flocks in Kern County and one in Tulare County were 100% seropositive for SLE virus by the end of November. In addition, a single SLE positive chicken was detected in a flock from Fallon, Nevada, that was bled and tested in October. WEE seroconversions were found in 35 chickens in flocks from the Imperial Valley and Needles.

It is gratifying that the surveillance program was effective in that it detected virus activity in mosquitoes or sentinel chickens in nine counties (Imperial, San Bernardino, Riverside, Los Angeles, Orange, Inyo, Kern, Kings and Tulare). The only human case that occurred outside an area covered by the surveillance program was the initial SLE case in a resident of the Antelope Valley. The three counties where significant numbers of SLE cases occurred had early evidence of virus activity in mosquitoes or chickens. Seroconversions in sentinel chickens in the southern San Joaquin Valley defined the area where the search for cases should be focused.

The University of California at Berkeley Arbovirus Research Program's staff is working with local MAD's to maintain surveillance for continued SLE activity this winter in Kern and Tulare Counties and the Coachella Valley. During the 1990 season, attempts will be made to maintain or extend the broad surveillance network, since it is difficult to predict and focus on specific areas where SLE or WEE will flare up. Many areas of the state still have a minimal participation in the surveillance program. If agencies in these areas utilized the testing program, it would make the system more sensitive. We could save a lot of money if we knew more specifically how to predict the occurrence of cases. A major challenge continues to be obtaining sufficient funds to support this effort and to demonstrate to our funding sources that surveillance is cost-effective in detecting virus activity, predicting case occurrence, and helping in the prevention of

epidemics of mosquito-borne encephalitis.

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AN OUTBREAK OF ILLNESS DUE TO ST. LOUIS ENCEPHALITIS VIRUS IN THE SOUTHERN SAN JOAQUIN VALLEY, CALIFORNIA, 1989

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ABSTRACT

In the Summer and Fall of 1989, surveillance data showed increased St. Louis encephalitis (SLE) virus activity in mosquitoes and sentinel chickens located in Kern, Kings, and Tulare Counties in the Southern San Joaquin Valley in California. At this time there was also an unexplained increase in human cases of viral central nervous system (CNS) disease in these counties. After several of these cases were found to have antibody evidence of acute SLE infection, all 77 human cases of viral CNS disease with onsets in August through October, 1989, reported in Kern, Kings and Tulare Counties were retrospectively surveyed to determine the proportion with an arboviral etiology, to identify risk factors, and to compare illness onsets with mosquito and sentinel chicken surveillance data. Preliminary results show that of the 65 people participating in the study, 28 (43%) had antibody evidence of acute SLE virus infection, three (5%) had antibody evidence of past SLE infection, and 34 (52%) were negative for SLE antibody. All of the 65 participants were negative for antibody to western equine encephalomyelitis (WEE) virus. Initial human case onsets roughly coincided with collection of initial SLE-seropositive blood from sentinel chickens in the same locales. Collection of SLE virus-positive mosquito pools preceded initial human case onsets by approximately four weeks in Kern County.

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OBSERVATIONS ON ST. LOUIS ENCEPHALITIS VIRUS IN KERN COUNTY, 1989[1]

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Introduction.

The present research represents a collaborative effort by the School of Public Health, U.C. Berkeley, the Kern MAD and the Viral and Rickettsial Disease Laboratory of the California Department of Health Services and will describe the surveillance, ecology and control of St. Louis encephalitis (SLE) virus in Kern County during 1989. Earlier papers at this meeting have described the epidemiology of the 29 laboratory confirmed human SLE cases in California (Tueller 1990) and listed the SLE positive pools from mosquitoes and chicken seroconversions (Emmons et al. 1990). The specific objectives will be to 1) describe seasonal and spatial changes in the activity of SLE virus in Kern County during 1989, 2) attempt to identify ecological factors which may have led to the initiation and amplification of SLE virus, and 3) briefly evaluate special control efforts by the Kern MAD in response to elevated mosquito counts and SLE viral activity at housing areas near the Kern River.

Although the southern San Joaquin Valley was historically endemic for SLE virus (Reeves and Hammon 1962), transmission to sentinel chickens has not been detected during most years since 1965, regardless of water availability. Thus, the extraordinary increase in SLE activity during 1989, as indicated by the seroconversion of 71% of 244 sentinel chickens (92% of 181 in Kern County), was totally unexpected and followed six years of unproductive study by the Arbovirus Research Program during which SLE virus was detected only sporadically by the isolation of virus from mosquitoes (8 isolates in 1983, 3 in 1986) and by the seroconversion of sentinel chickens (3 in 1986, 3 in 1988).

Methods and Results.

Surveillance. Sentinel chickens were positioned at 10 localities throughout Kern County and bled monthly from May to October 1989. Flocks at Belridge and Maricopa were maintained and bled by the West Side Mosquito Abatement District (MAD) and at Teviston by the Delano MAD. Chickens at these three locations were bled two weeks after the remaining seven flocks which were maintained and bled by the Kern MAD and the Arbovirus Field Station. Sera were tested by indirect enzyme immunoassay (EIA) with positives confirmed by an indirect fluorescent antibody test (IFA).

Mosquito abundance and virus infection was monitored biweekly at three to six permanent CO_2 trap stations at each of five localities. Up to 10 pools of 50 *Culex* females of each species from each site per sample were tested for virus by an *in situ* EIA using Vero cells.

SLE virus was detected initially in Kern County by the seroconversion of six chickens at Belridge, bled during the week of July 10. By the week of August 7, 74% of the chickens in this flock were seropositive, and seroconversions also were detected at Maricopa on the west side of the valley. Two weeks later, seroconversions were detected at six of seven remaining flocks; only chickens at the Kern National Wildlife Refuge remained negative until the next bleeding in

[1]We especially thank B.R. Hill, V.M. Martinez and M. Phair of the Arbovirus Field Station, D. Dondero and the staff of the Viral and Rickettsial Disease Laboratory, and the staff of the Kern Mosquito Abatement District for technical support. This research was funded, in part, by Research Grant 5-R22-AI-03028 from the National Institute of Allergy and Infectious Diseases and Biomedical Research Support Grant 8-S07-RR-05441 from the National Institutes of Health. A complete presentation of this data will be submitted for publication in the Journal of Medical Entomology.

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September. By the final bleeding in October, positive chickens were present in all flocks, and overall 92% of the 181 surviving chickens were seropositive.

From July 22 to September 30, SLE virus antigen was detected by EIA in 85 pools from three species of *Culex*. Virus was detected first and most frequently from *Culex tarsalis* Coquillet (70 EIA positive pools/14,846 females tested in 329 pools, minimum infection rate (MIR) =4.7/1,000 tested). The MIR was similar for *Culex quinquefasciatus* Say (14 positive pools/2,978 females in 65 pools, MIR =4.7) and higher for *Culex stigmatosoma* Dyar (1/59 in 4 pools, MIR =17.2) than for *Cx. tarsalis*; however, most females of these two species were collected along the Kern River drainage system after the onset of virus activity, whereas *Cx. tarsalis* was collected throughout Kern County during the entire summer.

Patterns of virus activity and mosquito abundance varied temporally and spatially among sampling sites. Mosquito abundance and virus infection did not increase until late August at both the Kern Refuge and Eureka Duck Club, even though these sites were situated on the west side of the valley and were relatively near Belridge, Buttonwillow and Tracy Ranch where sentinel chicken seroconversions were detected in late July-early August.

At water recharge ponds at Cole's Levee near the Kern River, *Cx. tarsalis* abundance peaked during the week of July 22 when SLE virus was detected initially in three pools. Although *Cx. quinquefasciatus* abundance remained low at this site, SLE virus was detected twice in females collected at the Lake Buena Vista recreation area on September 2. Mosquito and virus activity peaked concurrently along a transect of traps operated at the Kern River to the NE of Cole's Levee. *Culex tarsalis* abundance and virus infection peaked during July 22 - September 16, prior to *Cx. quinquefasciatus* which peaked during September 2-16. Mosquitoes trapped at this site were produced in adjacent agricultural breeding sources, since the Kern River bed had been completely dry since 1986. In agreement, the abundance of both species decreased markedly by September 30 in conjunction with cotton defoliation and expanded mosquito control.

After virus was detected at Kern River, surveillance activities were initiated on August 12 at housing tracts recently constructed north of the river levee. Although the SLE virus was detected initially in *Cx. tarsalis, Cx. quinquefasciatus* was more abundant and more frequently found to be infected with SLE virus in this residential environment.

Although *Cx. tarsalis* abundance at John Dale Ranch increased during June and July (100-200 females/trap-night), SLE virus was not detected until August 19. *Culex quinquefasciatus* were abundant from July to September (50-100 females/trap-night), but only a single pool was SLE virus positive during the week of September 2.

Ecological factors. For SLE virus to be amplified, sufficient numbers of competent vector mosquitoes must blood feed on viremic avian hosts, survive long enough under favorable weather conditions to complete virus extrinsic incubation, and then feed on one or more susceptible hosts. By examining selected components of this transmission cycle, it may be possible to determine which factor or combination of factors may have been responsible for the increased SLE activity experienced during 1989.

The Kern MAD operates 32 New Jersey Light Traps (NJLT) two days/week from April to November to monitor *Cx. tarsalis* abundance. Trap counts during 1989 increased unseasonably early, but then decreased again in June and were similar to 1987 and 1988 during August-September. Mosquito abundance increased during August-September, paralleling cotton irrigation, but then decreased during defoliation and drying in mid-September before harvest. Although the seasonal NJLT index of 1.8 females/trap- night/season was higher than in 1987 (1.1) or 1988 (1.0), weekly means remained less than half the threshold of 10 females/trap-night generally considered necessary for widespread virus activity (Olson et al. 1979).

Mean monthly *Cx. tarsalis* abundance at CO_2 traps provides an index of host-vector contact. Abundance at the Kern Refuge did not peak until September 1989 which was similar to the previous two years. In agreement, SLE virus activity was not detected at this site in either sentinel chickens or mosquito pools until September 1989. In contrast, *Cx. tarsalis* abundance at John Dale Ranch was almost 50 females per trap-night greater during June-August 1989 than during the same period in 1987 and 1988. However, early vector abundance did not result in early virus activity. Similar to John Dale, *Cx. tarsalis* abundance at the Kern River transect increased one month earlier during 1989 than during 1987 or 1988, although maximal abundance during late summer was similar during all three years. Thus, although *Cx. tarsalis* abundance was elevated during the vernal

amplification period at several sites during 1989 when compared to the previous years of low or no SLE activity, abundance during the late summer transmission period was comparable to previous years.

Temperature affects the duration of extrinsic incubation of the virus in the mosquito, mosquito population generation time and survivorship. Although slightly warmer during March and April, temperatures recorded at Bakersfield airport during 1989 did not differ markedly from the 30 year normals. Warmer temperatures during early spring may have enhanced vector abundance and viral amplification during 1989.

Similarly, rainfall approached or was less than the 30 year normals during each month except May and September. Since the Kern River has been dry since 1986, mosquito production was presumed to be related solely to water mismanagement, especially cotton irrigation during late summer.

Mosquito abatement. After elevated *Cx. tarsalis* abundance was detected at housing tracts adjacent to the Kern River, the Kern MAD initiated special control activities aimed at protecting residents of these housing areas north of the Kern River. Adult control included 1) cold fogging with Scourge® on four evenings and 2) late afternoon aerial applications of Baygon suspended in oil. In addition, all fields were inspected on foot and treated with Golden Bear Oil or *Bti* by ground or aerial equipment for larval control.

Cold fogging with Scourge, aerial applications of Baygon in oil or both did not markedly affect *Culex* abundance or eliminate infected females from the study area. In fact, *Cx. quinquefasciatus* abundance peaked, and 27 of 31 SLE virus positive pools were detected at the Kern River after special control efforts were initiated. Similar results were obtained at housing areas sampled along the Kern River. Concurrent with control activities, the abundance of *Cx. tarsalis* decreased; however, *Cx. quinquefasciatus* populations increased and infected mosquitoes were collected into September.

Conclusions.

SLE virus surveillance and control activities in Kern County during 1989 allowed us to draw several conclusions. Virus activity began on the west side of the San Joaquin Valley and then spread up the Kern River drainage and to other areas of the county. Low level virus activity also was detected here during 1988 by the seroconversion of three sentinel chickens. Thus, surveillance on the west side of the Valley should be enhanced to more accurately forecast SLE activity in the Bakersfield area.

Culex tarsalis was the primary vector mosquito. *Culex quinquefasciatus* became involved during late summer and may have been important in residential areas near the Kern River. However, it should be pointed out that not all infected mosquitoes are capable of transmission and that *Cx. quinquefasciatus* from Kern County historically is a poor laboratory vector of local strains of SLE virus (Meyer et al. 1983).

Culex tarsalis abundance at NJLT's throughout Kern County and at two of three virus monitoring sites was greater and peaked earlier during 1989 than either 1987 or 1988. Perhaps this early summer increase in vector abundance facilitated virus amplification. However, the reasons for this increase in population abundance remained cryptic, since temperatures were only slightly above normal and rainfall was markedly less than the 30 year normal.

Special control efforts north of the Kern River by the Kern MAD, unfortunately, were insufficient to interrupt virus transmission. *Culex* abundance increased and most virus positive pools were recovered after control efforts were initiated. Population decreases in late-September were attributed to the termination of cotton irrigation and defoliation, and secondarily to special mosquito control efforts. These results underscore the importance of improving our technology to control adult mosquito populations to interrupt virus transmission. The compounds and methods employed by the Kern MAD utilized most of the "arsenal" currently available. Although the adult control problems were described for Kern County, the results also may be applicable to the major valleys of California where housing tracts are rapidly encroaching on both agricultural lands and the remaining wild habitats which consistently or sporadically support arbovirus transmission.

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RECENT STUDIES OF CALIFORNIA AND BUNYAMWERA SEROGROUP VIRUSES IN CALIFORNIA[1]

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Introduction.

Most arboviruses in California fall into two large categories: 1) the viruses now classified in the families Alphaviridae and Flaviviridae, i.e. western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE), respectively, and 2) the viruses classified in the family Bunyaviridae. Because of their known public health importance, viruses of the former category have been studied much more in California. The ecology and public health importance of viruses in the latter category, i.e. those in the family Bunyaviridae, are poorly understood in California. This paper is a summary of recent results of studies of this interesting group of mosquito- and gnat-borne pathogens.

The family Bunyaviridae.

Classification and relationship to other arboviruses: Two groups of viruses in the family Bunyaviridae occur in California: those in the California (CAL) serogroup (2 viruses) and those in the Bunyamwera (BUN) serogroup (4 viruses). There may be others in the state, but six are presumed to be present on the basis of virus isolations from arthropods or evidence of antibodies in various vertebrate animals. Because both serogroups contain a number of closely related viruses in North America it is not always possible to identify antibodies from animal sera with absolute certainty. Even with a relatively sensitive test, such as the serum dilution plaque reduction neutralization test (SDN) using a battery of known antigens, the degree of cross reactivity among closely related viruses makes interpretation of these tests difficult. The same problem applies to identification of viral isolates from arthropods or vertebrates in nature. Here, identification relies on the availability of known specific antibodies produced in laboratory animals. Again, cross reactions are common.

Public health importance: One member of the CAL serogroup, LaCrosse (LAC) virus, is known to be the cause of considerable human disease in the midwestern United States. During the 1980's, there were more human cases of central nervous system disease caused by LAC virus reported in the United States, than for any other arbovirus except SLE (Anonymous 1986, 1987, 1988). Other viruses which have been associated with human disease elsewhere in North America include Jamestown Canyon (JC), snowshoe hare, and Trivittatus virus (Campbell 1990).

Historical background: The first CAL serogroup virus strains were isolated in California in 1943 and 1944 by Hammon and Reeves from *Aedes melanimon* Dyar and Knab mosquitoes collected in Kern County (Hammon et al. 1952). Three human cases of encephalitis from Kern County were later associated with these newly discovered viruses, and the name California encephalitis (CE) virus was applied to them (Hammon and Reeves 1952). No human cases of disease have been associated with this virus since that time, but numerous isolates of CE virus have been obtained from *Aedes melanimon* mosquitoes in the Central Valley of California, and jackrabbits have been incriminated as an important vertebrate host based on serological evidence (Hardy et al. 1977).

In 1962, the first BUN serogroup virus, Lokern (LOK), was isolated from *Culex tarsalis* and *Culicoides variipennis* (Coquillett) collected in Kern County, California (Karabatsos 1985). In January 1963, a second CAL serogroup virus, Jerry Slough (JS), was isolated from *Culiseta inornata* Williston

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collected in Kern County, California (Karabatsos 1985). In 1964, a second BUN serogroup virus, Main Drain (MD), was isolated from *C. variipennis* collected in Kern County (Karabatsos 1985).

Recent studies: California serogroup viruses.

California encephalitis virus (CE): There have been no recent studies of this virus in California. Based on isolations from *Aedes melanimon* and serosurveys in vertebrates it appears to be most active in the Central Valley and the Owens Valley. The range of this virus may correspond with the range of *Aedes melanimon* in California. Campbell et al. (1989) detected CE antibodies in only 1 of 337 deer sera tested from both high and low elevation areas of California.

Jamestown canyon virus (JC): JC virus is a human pathogen in other parts of the United States, and has been incriminated as the cause of CNS disease in about 40 cases. Recently, Campbell et al. (1989) reported that 23% of deer serum samples from high elevation areas in California were positive by neutralization test to JC, but only 9% of those from low elevation areas. Antibodies to this virus also have been detected in horses, and most recently in cattle from Modoc County. Based on these serological studies, Campbell et al. (1989) concluded that JC virus and JS virus were so closely related as to be synonymous.

Over the past 2 years, more than 31,000 mosquitoes were collected from high elevation areas and tested for virus. The bulk of these collections were *Aedes communis* DeGeer and were from Alpine County in the Carson Pass area. Six virus isolates were obtained from these mosquitoes, all indistinguishable from the prototype JC virus. Four of the isolates were from *Ae. communis*, one was from *Aedes cataphylla* Dyar collected as larvae and reared to the adult stage in the laboratory, then pooled for testing, and one was from a collection of *Aedes hexodontus* Dyar.

These isolations represent:

(1) Evidence that JC virus is transovarially transmitted in *Aedes* mosquitoes in California.

(2) The first isolates of an arbovirus from snow pool mosquitoes in California.

(3) The first confirmed isolates of JC from *Ae. hexodontus* or *Ae. cataphylla* anywhere.

Because of the probable involvement of *Ae. communis* in JC virus ecology in forested high mountain environments in California, we began a series of ecological studies of this mosquito. In the summer of 1989, we marked and released three series of females of this species in the Carson Pass area. Based on regression analysis of recaptures, we calculated daily survival rates of 0.91, 0.91, and 0.88 per day. These rates are very high and indicative of a relatively long adult life, and thus a high degree of vector capacity.

We have also begun vector competence studies with this species, and a comprehensive study of genetic variation in populations from the entire western United States.

Other California serogroup viruses: During 1988 and 1989, we collected over 16,000 mosquitoes from low elevation areas of California and Oregon and tested them for virus. Most of these collections were *Aedes squamiger* (Coquillett), *Aedes increpitus* Dyar, and *Cs. inornata*. Five strains of a yet-unidentified CAL serogroup virus were isolated from *Ae. squamiger* collected as larvae near Morro Bay, San Luis Obispo County. The virus appears to be more closely related to CE virus than to JC virus. Further testing may show it to be CE virus, or a previously unknown virus.

These isolation attempts represent the first significant tests of *Ae. squamiger* as an arbovirus vector, and the isolates represent the first arboviruses ever isolated from the species. The isolations were not entirely unexpected, since *Ae. squamiger*, although restricted in range to California coastal habitats, is closely related to the snow pool species *Aedes fitchii* (Felt and Young) and *Ae. increpitus*. All three species belong to the *Aedes stimulans* (Walker) group of species. Ironically, we have failed to isolate any viruses from *Ae. fitchii* or *Ae. increpitus*. Because of the potential public health significance of these isolates from coastal salt marsh environments, we plan further studies of *Ae. squamiger*. To better understand virus-vector relationships, we analyzed genetic variation and relatedness among California members of the *Ae. stimulans* group. We concluded that *Ae. squamiger* is most closely related to coastal populations of *Ae. increpitus*; more distantly to *Ae. fitchii*.

Recent studies: Bunyamwera serogroup viruses.

Recent information about Bunyamwera serogroup viruses in California has come from the serosurveys of vertebrate animals already mentioned and the re-testing of partially identified or unidentified virus isolates from mosquitoes.

Cache Valley virus (CV): This virus is the only Bunyamwera serogroup virus ever isolated from Oregon (from *Cs. inornata*). It has never been isolated from California. The serosurvey of deer in California (Campbell et al. 1989) failed to provide convincing evidence of CV activity in high or low elevation areas.

Lokern virus (LOK): Since the original isolation of LOK in Kern County from *Cx. tarsalis* in 1962, most subsequent isolations have been from *C. variipennis* (Karabatsos 1985). Our studies have shed no additional light on vector nor vertebrate host relationships of this virus in California.

Main Drain virus (MD): Numerous additional isolations of MD virus have been made from *C. variipennis* and from jackrabbits since the original isolation in 1964. Occasional isolations have been made from mosquitoes. MD was isolated from the brain of a horse with encephalitis (Emmons 1968). In our serological survey, low seroprevalence rates in deer sera were detected; rates were somewhat higher in low elevation areas than in high elevation areas.

Northway virus (NOR): Until recently, NOR virus was known only from Alaska and the adjacent provinces of Canada (Calisher et al. 1974). There it was associated with snow pool *Aedes* mosquitoes and antibodies had been detected in human serum samples. Surprisingly, the extensive serosurvey of deer in California provided evidence of extensive infection due to this virus. Seroprevalence rates were about the same from high elevation samples (26%) as from low elevation samples (23%). The retesting of partially identified or unidentified virus isolates from mosquitoes collected in California has already been mentioned. Five isolates from mosquitoes collected in Butte County in 1970-71 (4 *Anopheles freeborni* Aitken, 1 *Aedes sierrensis* (Ludlow)) were found to be very closely related to the prototype strain of NOR virus. The isolation from *Ae. sierrensis* is the first isolation of an arbovirus from this species.

Many new details of the ecology of CAL and BUN serogroups viruses in California have been discovered and will be extended in future studies. We still know little about the public health importance of these viruses in California.

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PASSERIFORM AND COLUMBIFORM ARBOVIRUS ACTIVITY

IN ORANGE COUNTY, CALIFORNIA, 1989

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ABSTRACT

The Orange County Vector Control District (OCVCD) has been conducting a study of the wild bird population in Orange County since 1987 in order to better understand their role in the overall encephalitis cycle and also to test the feasibility of using this type of activity as a surveillance/monitoring system for the future. In 1989, 24,155 birds were captured and 10,123 of those were sampled for antibodies to St. Louis encephalitis (SLE) and western equine encephalomyelitis (WEE). A total of 155 (1.54%) showed positive for SLE virus activity. Positive testing birds have been collected in the county for 30 consecutive months, thus showing that the virus is present year-round at low levels.

Introduction.

As stated in Gruwell et al. 1988, the project concentrated on three species of birds; House Finch (*Carpodacus mexicanus*), House Sparrow (*Passer domesticus*), and Rock Dove (*Columba livia*). White-crowned Sparrows (*Zonotrichia leucophrys*) were also tested during their migrations and winter residency in the county. These four species accounted for 98 percent of the samples (Tables 1, 2 and 3). The first three are the most numerous, most easily captured, and best available virus reservoirs found in the peridomestic avifauna of the county. The White-crowned Sparrows were negative for viral antibodies on first testing and developed positive reaction to testing only after having been in the area for some time and then being resampled.

Materials and methods.

Passerine birds were collected in modified crow traps and columbine (Rock Doves) were obtained by shooting. Detailed descriptions of collecting and bleeding techniques have been provided by Gruwell et al. (1988).

The blood sera samples were analyzed with Hemagglutination Inhibition (HAI) tests conducted by Ms. Carrie Fogarty at the Orange County Public Health Laboratory. The elapsed time from submission of samples to complete testing and results averaged about five days although when necessary, results were received within 48 hours.

Thirteen crow traps were placed around Orange County including five private residences, four park maintenance yards, one suburban fire station, one community college horticultural station, one rural duck club, and one rural site just north of the Orange County/San Diego County line on Camp Pendleton Marine Corps Base (Fig. 1). Rock Doves were collected by shotgun at Bonita Canyon (Coyote Canyon) County Landfill in Irvine (Site XIV, Fig. 1).

Results.

SLE-positive samples were collected all over the county (Fig. 1). The majority (78.7%) were taken from Rock Doves which constituted only 25.9 percent of the total samples (Table 4). Individual positives are listed in Table 5. This was the first time Rock Doves were collected weekly year-round and positives shown every month (Fig. 2). There was some viral activity almost continually with House Finches (Fig. 3) and only for about six months with House Sparrows (Fig. 4).

Discussion.

With the end of 1989, we now have three consecutive years of data (56,310 birds captured; 24,179 sampled) and can begin to see some patterns, problems and possible solutions.

First, it is obvious from the overall positive rate (Fig. 5), that SLE virus is active at varying low levels year-round in the small birds in the county. The gap in April and May of 1987 is probably due to small sample size and the fact that the project was just beginning and techniques were being honed. Indeed, the thirty consecutive months of

Table 1. Small Bird Species Sampled in Crow Traps by Month, 1989.

Common Name	Scientific Name	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	% Total Samples
House Finch	*Carpodacus mexicanus*	158	235	275	152	228	660	978	744	321	419	354	331	4,855	47.96
House Sparrow	*Passer domesticus*	113	68	29	61	211	314	384	346	139	108	86	82	1,941	19.20
Rock Dove	*Columba livia*	190	173	262	120	224	209	198	236	155	202	229	425	2,623	25.90
White-Crowned Sparrow	*Zonotrichia leucophrys*	96	143	87	60	22	10	2	-	-	12	39	36	506	5.00
Brown-Headed Cowbird	*Molothrus ater*	-	-	3	5	17	9	8	1	3	-	-	-	46	0.45
Say's Phoebe	*Sayornis saya*	-	1	-	8	5	7	9	7	6	-	-	-	43	0.42
Common Crow	*Corvus brachyrhynchos*	-	-	-	-	-	-	-	-	8	3	17	-	28	0.28
Song Sparrow	*Melospiza melodia*	5	5	5	1	1	1	-	-	-	-	-	-	18	0.18
Scrub Jay	*Aphelocoma coerulescens*	1	1	-	-	-	4	-	-	-	-	-	-	6	0.06
Mourning Dove	*Zenaidura macroura*	1	-	-	-	-	-	-	-	-	-	-	-	1	-
Ground Dove	*Columbigallina passerina*	5	-	20	12	-	-	-	-	-	-	-	-	37	0.37
Loggerhead Shrike	*Lanius ludovicianus*	2	-	-	-	-	-	-	-	-	-	-	-	2	0.02
Audubon's Warbler	*Dendroica auduboni*	2	-	-	-	-	-	-	-	-	-	-	-	2	0.02
Red-Winged Blackbird	*Agelaius phoeniceus*	2	-	9	-	-	-	-	-	-	-	-	-	11	0.11
Starling	*Sturnus vulgaris*	-	-	-	-	1	-	-	-	-	-	-	-	1	-
Mockingbird	*Mimus polyglottos*	-	-	-	-	1	1	-	1	-	-	-	-	3	0.03
	TOTAL ALL BIRDS	574	626	690	419	710	1215	1579	1335	632	744	725	874	10,123	100.00

Table 2. Recaptured Small Bird Species Taken in Crow Traps by Month, 1989.

Common Name	Scientific Name	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	% of all Recaptures
House Finch	*Carpodacus mexicanus*	521	403	571	324	477	826	921	1,025	1,034	966	1,022	831	8,921	63.58
White-Crowned Sparrow	*Zonotrichia leucophrys*	441	326	319	190	125	51	5	-	-	7	32	61	1,557	11.10
House Sparrow	*Passer domesticus*	135	97	73	65	239	363	461	443	415	359	279	236	3,155	22.49
Song Sparrow	*Melospiza melodia*	14	10	8	4	7	-	-	-	-	-	-	-	43	0.30
Ground Dove	*Columbigallina passerina*	25	20	55	49	40	22	-	-	-	-	-	-	211	1.50
Scrub Jay	*Aphelocoma coerulescens*	4	4	-	-	-	9	-	-	-	-	-	-	17	0.12
Red-Winged Blackbird	*Agelaius phoeniceus*	3	-	-	-	-	-	-	-	-	-	-	-	3	0.02
Say's Phoebe	*Sayornis saya*	-	-	-	6	4	21	24	32	18	4	-	-	109	0.78
Brown-Headed Cowbird	*Molothrus ater*	-	-	-	-	11	5	-	-	-	-	-	-	16	0.11
	TOTAL ALL BIRDS	1,143	860	1,026	638	903	1,297	1,401	1,500	1,467	1,336	1,333	1,128	14,032	100.00

Table 3. Resampled (blood) Small Bird Species by Month, 1989.

Common Name	Scientific Name	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	% of all Resamples
House Finch	*Carpodacus mexicanus*	65	109	125	79	76	155	190	264	232	218	244	201	1,958	64.26
White-Crowned Sparrow	*Zonotrichia leucophrys*	43	96	61	53	22	10	2	-	-	1	8	16	312	10.24
House Sparrow	*Passer domesticus*	84	44	18	13	49	68	82	82	75	65	59	81	720	23.63
Song Sparrow	*Melospiza melodia*	3	3	3	1	-	-	-	-	-	-	-	-	10	0.34
Ground Dove	*Columbigallina passerina*	3	-	9	10	-	-	-	-	-	-	-	-	22	0.72
Scrub Jay	*Aphelocoma coerulescens*	-	1	-	-	-	1	-	-	-	-	-	-	2	0.06
Say's Phoebe	*Sayornis saya*	-	-	-	3	2	2	3	4	5	-	-	-	19	0.62
Brown-Headed Cowbird	*Molothrus ater*	-	-	-	-	4	-	-	-	-	-	-	-	4	0.13
	TOTAL ALL BIRDS	198	253	216	159	153	236	277	350	312	284	311	298	3,047	100.00

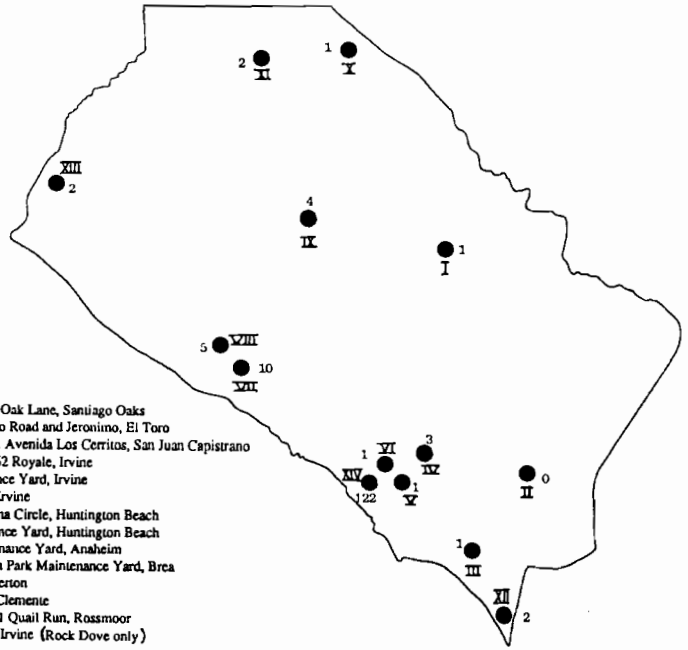

Figure 1. Existing Crow Trap sites (roman numerals) and SLE positives for each site in Orange County, California, 1989. A total of 155 SLE positives were obtained from the fourteen sites.

Table 4. Total Positive Birds by Species, Orange County, 1989.

Rock Dove *(Columba livia)*	- 122
House Finch *(Carpodacus mexicanus)*	- 19
House Sparrow *(Passer domesticus)*	- 14
Total	- 155

Total Samples three species = 9,419

Percent positive Rock Doves = 4.65
Percent positive House Finches = 0.4
Percent positive House Sparrows = 0.72

Overall percent positive for 1989 = 1.65

Table 5. Positive Bird Sera 1989.

Catalog No.	Species	Sex Age	Locality	Date Collected	Date Reported	Titer SLE	Titer WEE
JAG 89 - 33	House Finch	Ad	Central Pk, Hunt. Bch	3 Jan.	9 Jan.	1:20	
44	Rock Dove	Ad	Bonita Canyon	4 Jan.	11 Jan.	1:20	
57	Rock Dove	Ad	Bonita Canyon	4 Jan.	11 Jan.	1:40	
109	Rock Dove	Ad	Bonita Canyon	11 Jan.	19 Jan.	1:40	
124	Rock Dove	Ad	Bonita Canyon	11 Jan.	19 Jan.	1:20	
137	Rock Dove	Ad	Bonita Canyon	11 Jan.	19 Jan.	1:40	
307	Rock Dove	Ad	Bonita Canyon	18 Jan.	26 Jan.	1:20	
440	Rock Dove	Ad	Bonita Canyon	25 Jan.	30 Jan.	1:40	
443	Rock Dove	Ad	Bonita Canyon	25 Jan.	30 Jan.	1:80	1:40
	Total Samples January = 574, Total Positives = 9 SLE, 1 WEE Percent Positive = 1.74 Total Rock Dove Samples January = 190, Total Positive Rock Doves = 8 SLE, 1 WEE Percent Rock Dove Positive = 4.7						
JAG 89 - 719	Rock Dove	Ad	Bonita Canyon	8 Feb.	10 Feb.	1:40	
748	Rock Dove	Ad	Bonita Canyon	8 Feb.	10 Feb.	1:20	
756	Rock Dove	Ad	Bonita Canyon	8 Feb.	10 Feb.	1:20	
835	Rock Dove	Juv	Bonita Canyon	15 Feb.	28 Feb.	1:20	
837	Rock Dove	Ad	Bonita Canyon	15 Feb.	28 Feb.	1:20	1:20
849	Rock Dove	Juv	Bonita Canyon	15 Feb.	28 Feb.	1:20	1:20
992	Rock Dove	Juv	Bonita Canyon	22 Feb.	3 Mar.		1:20
1007	Rock Dove	Juv	Bonita Canyon	22 Feb.	3 Mar.	1:80	1:20
	Total Samples February = 626, Total Positives = 7 SLE, 4 WEE Percent Positive = 1.76 Total Rock Dove Samples February = 173, Total Positive Rock Doves = 7 SLE, 4 WEE Percent Rock Dove Positive = 6.37						
JAG 89-1207	Rock Dove	Ad	Bonita Canyon	1 Mar.	10 Mar.	1:20	
1239	Rock Dove	Ad	Bonita Canyon	1 Mar.	10 Mar.	1:20	
1334	Rock Dove	Ad	Bonita Canyon	8 Mar.	15 Mar.	1:20	
1358	Rock Dove	Ad	Bonita Canyon	8 Mar.	15 Mar.	1:20	
1374	Rock Dove	Ad	Bonita Canyon	8 Mar.	15 Mar.	1:20	
1501	Rock Dove	Ad	Bonita Canyon	15 Mar.	28 Mar.	1:20	
1639	Rock Dove	Ad	Bonita Canyon	22 Mar.	4 Mar.	1:40	
1642	Rock Dove	Ad	Bonita Canyon	22 Mar.	4 Mar.	1:20	
1759	Rock Dove	Juv	Bonita Canyon	29 Mar.	11 Mar.	1:20	
1766	Rock Dove	Juv	Bonita Canyon	29 Mar.	11 Mar.	1:40	
1788	Rock Dove	Juv	Bonita Canyon	29 Mar.	11 Mar.	1:20	
1838	House Finch	Ad	Mason Park, Irvine	30 Mar.	11 Mar.	1:20	
	Total Samples March = 690, Total Positives = 12 SLE Percent Positive = 1.74 Total Rock Dove Samples March = 262, Total Positive Rock Doves = 11 SLE Percent Rock Dove Positive = 4.2						
JAG 89-1922	Rock Dove	Juv	Bonita Canyon	5 Apr.	18 Apr.	1:20	
2061	House Finch	Ad	Challet, Irvine	13 Apr.	20 Apr.	1:20	
2239	Rock Dove	Ad	Bonita Canyon	26 Apr.	2 May	1:40	1:20
2291	House Finch	Ad	San Mateo Pt, S. Clem.	27 Apr.	2 May	1:20	
	Total Samples April = 419, Total Positives = 4 SLE, 1 WEE Percent Positive = 1.19 Total Rock Dove Samples April = 120, Total Rock Doves Positive = 2 SLE, 1 WEE Percent Rock Dove Positive = 2.5						

Table 5. Positive Bird Sera 1989 - continued.

Catalog No.	Species	Sex Age	Locality	Date Collected	Date Reported	Titer SLE	Titer WEE
JAG 89-2353	Rock Dove	Ad	Bonita Canyon	3 May	10 May	1:20	
2365	Rock Dove	Ad	Bonita Canyon	3 May	10 May	1:20	
2380	Rock Dove	Ad	Bonita Canyon	3 May	10 May	1:40	1:40
2587	Rock Dove	Juv	Bonita Canyon	17 May	25 May	1:20	
2594	Rock Dove	Ad	Bonita Canyon	17 May	30 May	1:40	
2612	Rock Dove	Juv	Bonita Canyon	17 May	30 May	1:20	
2615	Rock Dove	Juv	Bonita Canyon	17 May	30 May	1:80	
2621	Rock Dove	Juv	Bonita Canyon	17 May	25 May	1:20	
2807	Rock Dove	Juv	Bonita Canyon	24 May	1 June	1:20	
2808	Rock Dove	Juv	Bonita Canyon	24 May	1 June	1:20	
*2844	House Finch	Fledg	San Mateo Pt, S. Clem.	25 May	1 June	1:40	
2905	House Finch	Fledg	Cole, San Juan Cap.	29 May	8 June	1:20	
2945	House Finch	Fledg	Pett, Huntington Bch	30 May	8 June	1:20	
	Total Samples May = 710, Total Positives = 13 SLE, 1 WEE						
	Percent Positive = 1.97						
	Total Rock Dove Samples May = 224, Total Rock Doves Positive = 10 SLE, 1 WEE						
	Percent Rock Dove Positive = 4.91						
JAG 89-3157	Rock Dove	Juv	Bonita Canyon	7 June	13 June	1:20	
3172	Rock Dove	Juv	Bonita Canyon	7 June	13 June	1:40	
3175	Rock Dove	Ad	Bonita Canyon	7 June	13 June	1:20	
3178	Rock Dove	Juv	Bonita Canyon	7 June	13 June	1:20	
3535	Rock Dove	Ad	Bonita Canyon	14 June	22 June	1:80	
3539	Rock Dove	Ad	Bonita Canyon	14 June	22 June	1:160	
3732	Rock Dove	Juv	Bonita Canyon	21 June	28 June	1:80	
3738	Rock Dove	Juv	Bonita Canyon	21 June	28 June	1:20	
3757	Rock Dove	Ad	Bonita Canyon	21 June	30 June	1:20	
3762	Rock Dove	Ad	Bonita Canyon	21 June	30 June	1:80	
3770	Rock Dove	Juv	Bonita Canyon	21 June	30 June	1:80	
3776	Rock Dove	Ad	Bonita Canyon	21 June	30 June	1:80	
3778	Rock Dove	Juv	Bonita Canyon	21 June	30 June	1:40	
4008	Rock Dove	Ad	Bonita Canyon	28 June	6 July	1:80	
4010	Rock Dove	Juv	Bonita Canyon	28 June	6 July	1:20	
4011	Rock Dove	Juv	Bonita Canyon	28 June	6 July	1:20	
4012	Rock Dove	Ad	Bonita Canyon	28 June	6 July	1:40	
4031	Rock Dove	Ad	Bonita Canyon	28 June	6 July	1:40	
4033	Rock Dove	Ad	Bonita Canyon	28 June	6 July	1:80	
4051	Rock Dove	Juv	Bonita Canyon	28 June	6 July	1:20	
*4206	House Finch	Imm	Central Pk, Hunt. Bch	30 June	11 July	1:20	
*4221	House Sparrow	Imm	Central Pk, Hunt. Bch	30 June	11 July	1:20	
*4231	House Sparrow	Imm	Central Pk, Hunt. Bch	30 June	11 July	1:20	
	Total Samples June = 1,215, Total Positives = 23 SLE						
	Percent Positive = 1.89						
	Total Rock Dove Samples June = 209, Total Rock Doves Positive = 20 SLE						
	Percent Rock Dove Positive = 9.57						
JAG 89-4247	House Finch	Imm	Byers, Santiago Oaks	3 July	13 July	1:20	
4349	Rock Dove	Ad	Bonita Canyon	5 July	13 July	1:20	
4354	Rock Dove	Ad	Bonita Canyon	5 July	13 July	1:40	
4641	Rock Dove	Ad	Bonita Canyon	12 July	19 July	1:20	
4658	Rock Dove	Juv	Bonita Canyon	12 July	19 July	1:40	
4673	Rock Dove	Juv	Bonita Canyon	12 July	19 July	1:20	
4861	House Sparrow	Imm	Brown, Rossmoor	14 July	21 July	1:20	
5096	Rock Dove	Juv	Bonita Canyon	19 July	28 July	1:80	
5114	Rock Dove	Juv	Bonita Canyon	19 July	28 July	1:20	

Table 5. Positive Bird Sera 1989 - continued.

Catalog No.	Species	Sex Age	Locality	Date Collected	Date Reported	Titer SLE	Titer WEE
5124	Rock Dove	Juv	Bonita Canyon	19 July	28 July	1:40	
5270	House Sparrow	♂Ad	Brown, Rossmoor	21 July	31 July	1:20	
5360	House Sparrow	Imm	Fullerton College	25 July	2 Aug.	1:20	
5458	Rock Dove	Ad	Bonita Canyon	26 July	2 Aug.	1:20	
5487	Rock Dove	Ad	Bonita Canyon	26 July	2 Aug.	1:40	
	Total Samples July = 1,579, Total Positives = 14 SLE Percent Positive = 0.89 Total Rock Dove Samples July = 198, Total Rock Doves Positive = 10 SLE Percent Rock Dove Positive = 5.05						
JAG 89-5878	Rock Dove	Juv	Bonita Canyon	2 Aug.	15 Aug.	1:20	
5887	Rock Dove	Juv	Bonita Canyon	2 Aug.	15 Aug.	1:20	
6009	House Sparrow	Imm	Modjeska Park, Ana.	4 Aug.	15 Aug.	1:20	
6210	Rock Dove	Juv	Bonita Canyon	9 Aug.	17 Aug.	1:20	
6220	Rock Dove	Ad	Bonita Canyon	9 Aug.	17 Aug.	1:20	
6244	Rock Dove	Ad	Bonita Canyon	9 Aug.	17 Aug.	1:20	
6253	Rock Dove	Ad	Bonita Canyon	9 Aug.	21 Aug.	1:20	
6263	Rock Dove	Juv	Bonita Canyon	9 Aug.	21 Aug.	1:40	
*6377	House Sparrow	Imm	Modjeska Park, Ana.	11 Aug.	21 Aug.	1:20	
*6602	House Finch	♂Ad	Pett, Huntington Bch	18 Aug.	28 Aug.	1:20	
6772	Rock Dove	Juv	Bonita Canyon	23 Aug.	30 Aug.	1:20	
6780	Rock Dove	Juv	Bonita Canyon	23 Aug.	30 Aug.	1:40	
6824	Rock Dove	Ad	Bonita Canyon	23 Aug.	30 Aug.	1:20	
7105	Rock Dove	Ad	Bonita Canyon	30 Aug.	8 Sept.	1:20	
	Total Samples August = 1,335, Total Positives = 14 SLE Percent Positive = 1.05 Total Rock Dove Samples August = 236, Total Rock Doves Positive = 11 SLE Percent Rock Dove Positive = 4.66						
JAG 89-7202	House Finch	Imm	Pett, Huntington Bch	5 Sept.	12 Sept.	1:40	
7334	Rock Dove	Juv	Bonita Canyon	13 Sept.	26 Sept.	1:20	
7546	House Sparrow	Imm	Fullerton College	22 Sept.	4 Oct.	1:20	
7632	Rock Dove	Ad	Bonita Canyon	27 Sept.	4 Oct.	1:20	
7639	Rock Dove	Ad	Bonita Canyon	27 Sept.	10 Oct.	1:40	
7647	Rock Dove	Ad	Bonita Canyon	27 Sept.	10 Oct.	1:40	
7649	Rock Dove	Ad	Bonita Canyon	27 Sept.	4 Oct.	1:20	
7669	Rock Dove	Juv	Bonita Canyon	27 Sept.	4 Oct.	1:40	
7676	Rock Dove	Ad	Bonita Canyon	27 Sept.	10 Oct.	1:160	
7681	Rock Dove	Juv	Bonita Canyon	27 Sept.	10 Oct.	1:20	
7761	House Finch	Ad	Central Pk, Hunt. Bch	29 Sept.	4 Oct.	1:20	
	Total Samples September = 632, Total Positives = 11 SLE Percent Positive = 1.74 Total Rock Dove Samples September = 155, Total Rock Doves Positive = 8 SLE Percent Rock Dove Positive = 5.16						
JAG 89-7787	House Finch	Ad	Carbon Canyon	3 Oct.	10 Oct.	1:20	
7824	Rock Dove	Juv	Bonita Canyon	4 Oct.	10 Oct.	1:40	
7839	Rock Dove	Juv	Bonita Canyon	4 Oct.	10 Oct.	1:20	
7962	Rock Dove	Juv	Bonita Canyon	11 Oct.	18 Oct.	1:20	
8173	Rock Dove	Ad	Bonita Canyon	18 Oct.	26 Oct.	1:20	
8196	Rock Dove	Juv	Bonita Canyon	18 Oct.	26 Oct.	1:80	
8200	Rock Dove	Ad	Bonita Canyon	18 Oct.	26 Oct.	1:40	
8310	Rock Dove	Ad	Bonita Canyon	25 Oct.	31 Oct.	1:20	

Table 5. Positive Bird Sera 1989 - continued.

Catalog No.	Species	Sex Age	Locality	Date Collected	Date Reported	Titer SLE	Titer WEE
8318	Rock Dove	Ad	Bonita Canyon	25 Oct.	31 Oct.	1:20	
8443	House Sparrow	Imm	Pett, Huntington Bch	27 Oct.	3 Nov.	1:20	
8444	House Sparrow	Imm	Pett, Huntington Bch	27 Oct.	3 Nov.	1:40	
8460	House Finch	Ad	Challet, Irvine	30 Oct.	3 Nov.	1:20	
	Total Samples October = 744, Total Positives = 12 SLE						
	Percent Positive = 1.63						
	Total Rock Dove Samples October = 202, Total Rock Doves Positive = 8 SLE						
	Percent Rock Dove Positive = 3.96						
JAG 89-8638	Rock Dove	Ad	Bonita Canyon	8 Nov.	15 Nov.	1:40	
*8680	House Sparrow	Imm	Pett, Huntington Bch	10 Nov.	17 Nov.	1:20	
*8716	House Finch	Ad	Challet, Irvine	13 Nov.	17 Nov.	1:20	
8954	House Finch	Ad	Pett, Huntington Bch	21 Nov.	28 Nov.	1:40	
8969	Rock Dove	Ad	Bonita Canyon	22 Nov.	4 Dec.	1:20	
9148	Rock Dove	Juv	Bonita Canyon	29 Nov.	6 Dec.	1:20	
	Total Samples November = 725, Total Positives = 6 SLE						
	Percent Positive = 0.83						
	Total Rock Dove Samples November = 229, Total Rock Doves Positive = 3 SLE						
	Percent Rock Dove Positive = 1.31						
JAG 89-9384	Rock Dove	Ad	Bonita Canyon	8 Dec.	15 Dec.	1:20	
9400	Rock Dove	Ad	Bonita Canyon	8 Dec.	15 Dec.	1:20	
9409	House Sparrow	Imm	Pett, Huntington Bch	8 Dec.	15 Dec.	1:20	
9461	Rock Dove	Ad	Bonita Canyon	11 Dec.	15 Dec.	1:40	
9477	Rock Dove	Ad	Bonita Canyon	11 Dec.	15 Dec.	1:40	
9526	Rock Dove	Ad	Bonita Canyon	13 Dec.	20 Dec.	1:40	
9590	Rock Dove	Ad	Bonita Canyon	13 Dec.	20 Dec.	1:20	
9691	Rock Dove	Ad	Bonita Canyon	14 Dec.	22 Dec.	1:40	
9694	Rock Dove	Ad	Bonita Canyon	14 Dec.	22 Dec.	1:40	
#136 *9738	House Finch	Ad	Pett, Huntington Bch	15 Dec.	22 Dec.	1:20	
9770	Rock Dove	Ad	Bonita Canyon	18 Dec.	22 Dec.	1:40	
9821	Rock Dove	Ad	Bonita Canyon	20 Dec.	27 Dec.	1:20	
9841	Rock Dove	Ad	Bonita Canyon	20 Dec.	27 Dec.	1:40	
9858	Rock Dove	Ad	Bonita Canyon	20 Dec.	27 Dec.	1:20	
9860	Rock Dove	Ad	Bonita Canyon	20 Dec.	27 Dec.	1:40	
9864	Rock Dove	Juv	Bonita Canyon	20 Dec.	27 Dec.	1:40	
9866	Rock Dove	Juv	Bonita Canyon	20 Dec.	27 Dec.	1:40	
9903	Rock Dove	Ad	Bonita Canyon	21 Dec.	27 Dec.	1:20	
9942	Rock Dove	Ad	Bonita Canyon	21 Dec.	27 Dec.	1:20	
*10020	House Sparrow	Imm	Modjeska Park, Ana.	27 Dec.	4 Jan.	1:20	
*10024	House Sparrow	Imm	Modjeska Park, Ana.	27 Dec.	4 Jan.	1:20	
#283 *10042	House Finch	Ad	20 Ranch, Irvine	28 Dec.	4 Jan.	1:20	
#152 *10115	House Finch	Ad	Pett, Huntington Bch	29 Dec.	4 Jan.	1:20	
	Total Samples December = 874, Total Positives = 23 SLE						
	Percent Positive = 2.74						
	Total Rock Dove Samples December = 425, Total Rock Doves Positive = 17 SLE						
	Percent Rock Dove Positive = 4.0						

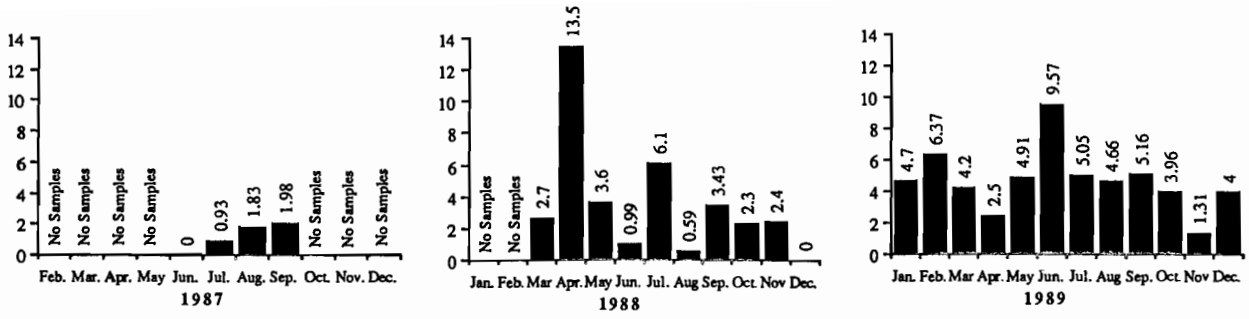

Figure 2. Percent positive Rock Doves (*Columba livia*) per month; 1987-1989.

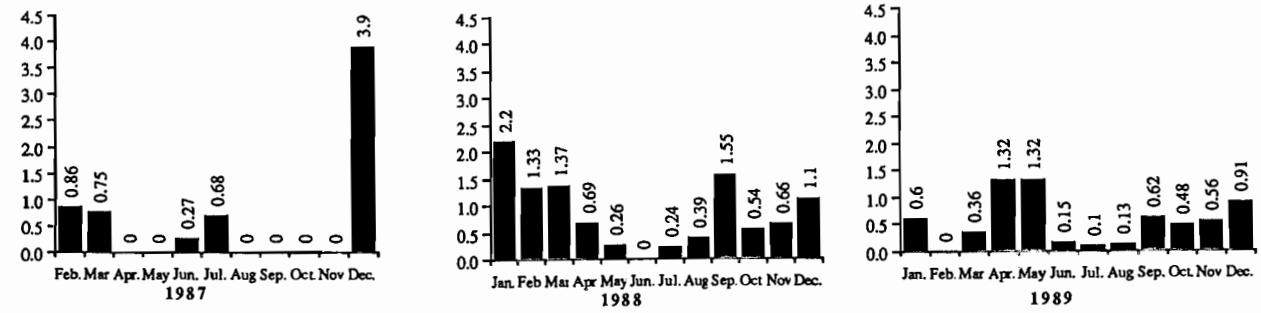

Figure 3. Percent positive House Finches (*Carpodacus mexicanus*) per month; 1987-1989.

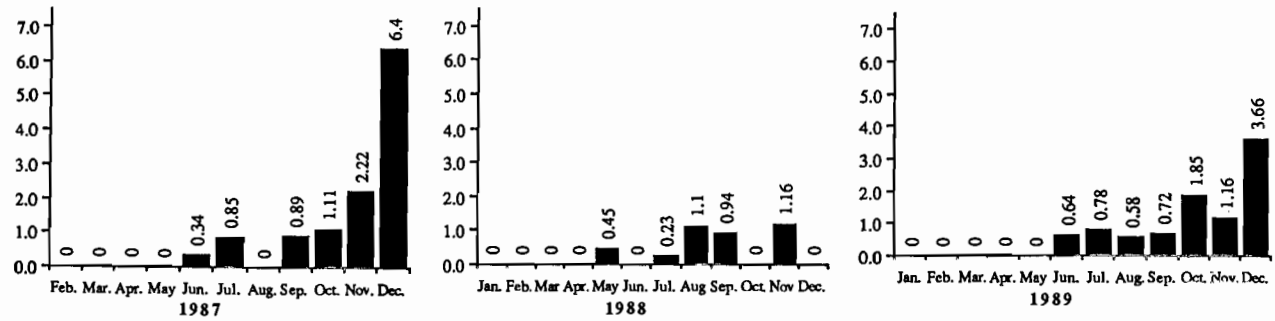

Figure 4. Percent positive House Sparrows (*Passer domesticus*) per month; 1987-1989.

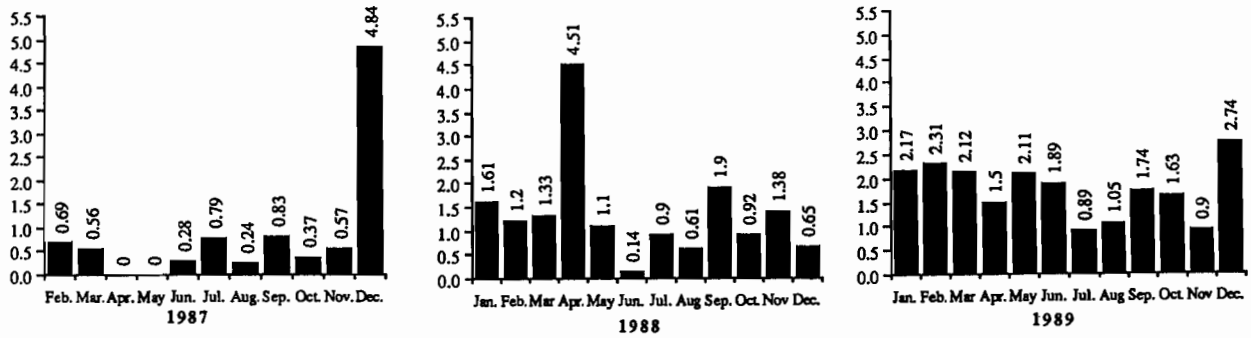

Figure 5. Percent positive for combined Rock Doves, House Finches, and House Sparrows per month; 1987-1989

positive birds has carried over into 1990. It should also be noted that the percent positive has increased from 0.6 in 1987 to 0.99 in 1988 to 1.54 in 1989; suggesting an upsurge in the avian virus cycle.

Second, looking at individual species does seem to show some wide patterns and observations:

Rock Dove (*Columba livia*) (Fig. 2). Based on 4,450 samples, it seems (with one major drawback) to be the best indicator of virus activity, averaging about 4 to 5 percent annually. All the birds were collected at the same site which is a landfill. They fly into the landfill to forage and fly out again in the afternoon. Thus, when a bird shows positive, we know only that there is virus in the county as they are obviously being infected at their roosting sites and not at the landfill. These are feral Rock Doves (i.e. unbanded and not part of someone's flock) so they probably roost in old buildings, overpasses, bridges, etc. Preliminary plans for 1990 have been made to live trap and radio track some of these birds in order to locate their home sites and possible virus foci.

House Finch (*Carpodacus mexicanus*) (Fig. 3). Based on 11,264 samples, Finches show general year-round viral activity at a low level with a definite winter carryover in first and second year adults. The lowest positive levels occur from May to August when most of the samples are fledglings and immatures. In August we began using individual numbered bands on Finches in our four most productive traps in order to gather more detailed information on recapture rates, relocation, and hopefully, duration of antibody in individual birds. With the help of Ms. Kathy Vanderpool (Fullerton State College) 400 birds have been banded and results will be available soon.

House Sparrow (*Passer domesticus*) (Fig. 4). Based on 5,694 samples, we found the peak activity in Sparrows during the last six months of the year with no apparent winter carryover. It seems that only immature and first year birds are involved although not many plumage-mature adults are captured.

In conclusion, virus activity in present year-round in about one percent of the passerines sampled and about 4-5 percent of the columbines. We are at the point now where we can fine tune the sampling and tracking processes in order to learn more about the virus cycle in birds and coordinate this information with other approaches, such as mosquito pools, feeding preferences, sentinel flocks, meteorological data, etc.

Acknowledgements.

The authors express their gratitude to the staff of the Orange County Vector Control District and the Orange County Public Health Laboratory for their support and assistance.

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EVALUATION OF MOSQUITO AND ARBOVIRUS ACTIVITY IN ORANGE COUNTY, CALIFORNIA, 1989

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In 1989, the Orange County Vector Control District (OCVCD) continued its mosquito and encephalitis virus surveillance. Mosquitoes were collected all year at twelve permanent sites throughout the county (Fig. 1). Twenty-one CDC/CO_2 traps were utilized as well as eleven gravid female traps (six were discontinued in May). A total of 14,042 mosquitoes was collected from which 442 pools were submitted for virus testing. The collection included 256 pools of *Culex quinquefasciatus* Say, 146 pools of *Culex tarsalis* Coquillett, 23 pools of *Culex stigmatosoma* Dyar, 10 pools of *Culiseta incidens* (Thompson), and 7 pools of *Culiseta inornata* Williston. None of the pools tested positive for St. Louis encephalitis (SLE) or western equine encephalomyelitis (WEE) viruses. In addition to the above mosquitoes, *Culex erythrothorax* Dyar and *Culiseta particeps* Adams were also taken from rural localities, but not pooled. Sentinel chicken flocks included one large flock of 25 at 20 Ranch Duck Club in Irvine and four mini-flocks (five chickens each) located in Fullerton, Buena Park, Cypress, and Los Alamitos. Seroconversions for SLE antibodies occurred in Buena Park on August 21, and again on November 13-15, at Buena Park and Fullerton.

Wild bird sera collected by Dr. John Gruwell and Ms. Becky Brown were tested for SLE and WEE antibodies at the Orange County Public Health Laboratory. Over 24,000 birds were trapped and 10,123 blood samples taken during 1989. The summary of results is given in Table 1. Rock Doves (*Columba livia*) (pigeons) from Irvine showed the highest number of SLE and WEE positives at 4.38 and 0.27 percent, respectively.

House Finches (*Carpodacus mexicanus*) and House Sparrows (*Passer domesticus*) comprised a small percentage of the total. Compared to last year (Gruwell et al. 1988), SLE activity doubled in Rock Doves (2.1% in 1988), increased 2.5 times in House Sparrows (0.3% in 1988), and decreased in House Finches (0.5% in 1988). Western equine encephalomyelitis infections decreased in all the above species (1.1%, 0.1%, 0.2%, respectively in 1988). In addition to the Rock Doves sampled fromBonita Canyon Landfill in Irvine, 95 samples were also obtained from John Wayne Airport. All of these tested negative for SLE and WEE. Trends in the wild bird populations as shown in Figures 2 and 3 are similar to 1987 and 1988 (Webb et al. 1988); positive birds were found almost every month of the year, including December and January.

Once again, wild birds appeared to be a very effective sentinel for virus activity in the Los Angeles Basin. There were consistent seroconversions months before the positive mosquito pools or chicken seroconversions (Fig. 2). The highest peak in wild bird SLE activity occurred in mid-June (a human case occurred in mid-August), while the first positive mosquitoes were at the end of July or first of August; the first chicken seroconversions were in August, just prior to and after the human case. Two more peaks occurred in September and October before the next seroconversions in November. These results were very similar to data from 1987 and 1988.

Figure 3 illustrates the separation of SLE-positive Rock Doves from Finches and Sparrows. Except for January and February, SLE activity had approximately the same temporal distribution in both groups only at a lower level in wild House Finches and Sparrows.

Mosquitoes were collected from seven suburban and five rural sites. The CDC/CO_2-light traps were used at all 12 sites and 11 of the 12 sites had gravid female mosquito traps and Australian Crow traps in place. Two of these sites (20 Ranch Duck Club in Irvine and Fullerton College) also had sentinel chicken flocks. The San Joaquin Freshwater Marsh had only CDC/CO_2 traps. *Culex quinquefasciatus* was the predominant mosquito species in suburban sites and was present through the fall and winter months, while *Culex tarsalis* was predominant in rural areas and, in most cases, was gone by October. This is illustrated by comparing a residential backyard site in Irvine (Fig. 4) with two rural sites in Irvine (Figs. 5 and 6).

Culex quinquefasciatus started to increase at the Irvine residential site by the end of March, whereas, at the nearby rural site (20 Ranch Duck Club) *Culex quinquefasciatus* started a month earlier. Both of the above sites had peaks of *Culex quinquefasciatus* activity occurring in mid-April,

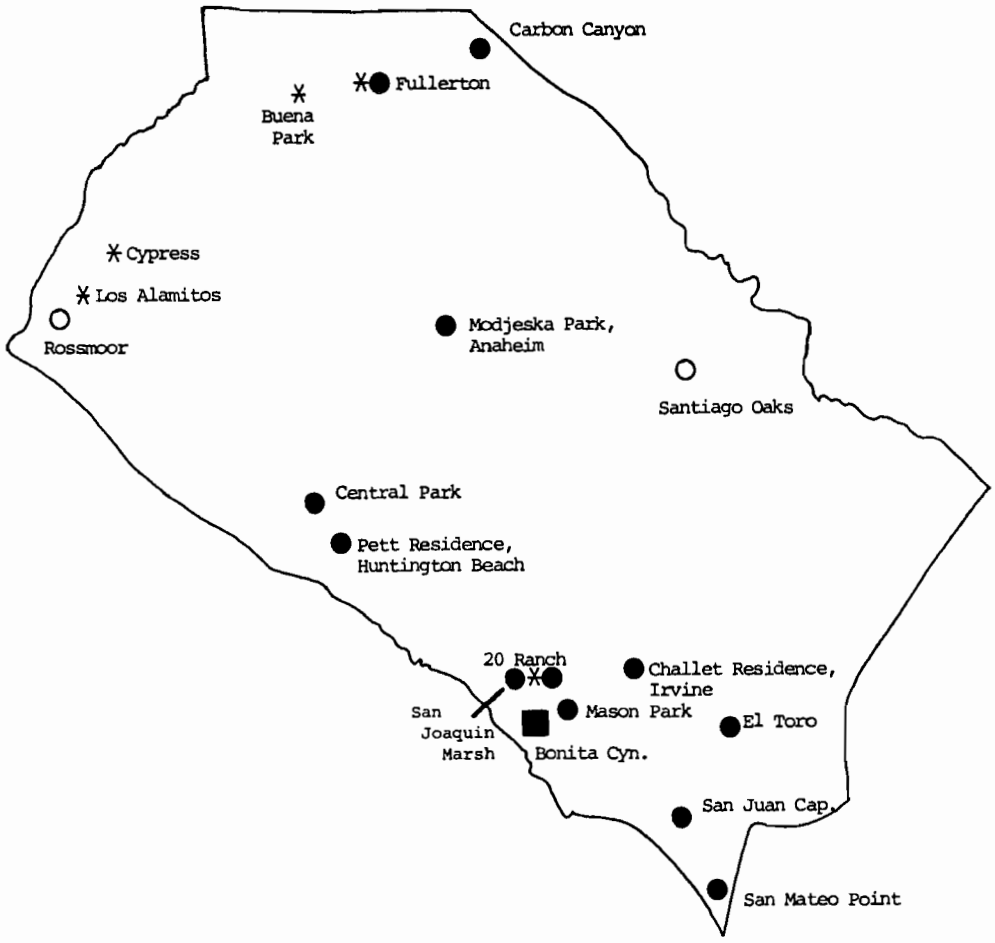

Figure 1. Locations of study sites containing crow traps, CDC traps and gravid female traps (•); crow traps only (o); sentinel chicken flocks (*); and pigeon collections (▪) in Orange County, California.

Table 1. SLE and WEE seroconversions in small bird sera samples from Orange County, California.

Species	SLE	WEE	No. Blood Samples	Percent SLE	Percent WEE
Rock Dove	115	7	2,623	4.38	0.27
House Sparrow	14	0	1,941	0.72	0.00
House Finch	19	0	4,855	0.39	0.00
White-crowned Sparrow	0	0	806	0.00	0.00
Others	0	0	198	0.00	0.00
Totals	148	7	10,123	1.46	0.07

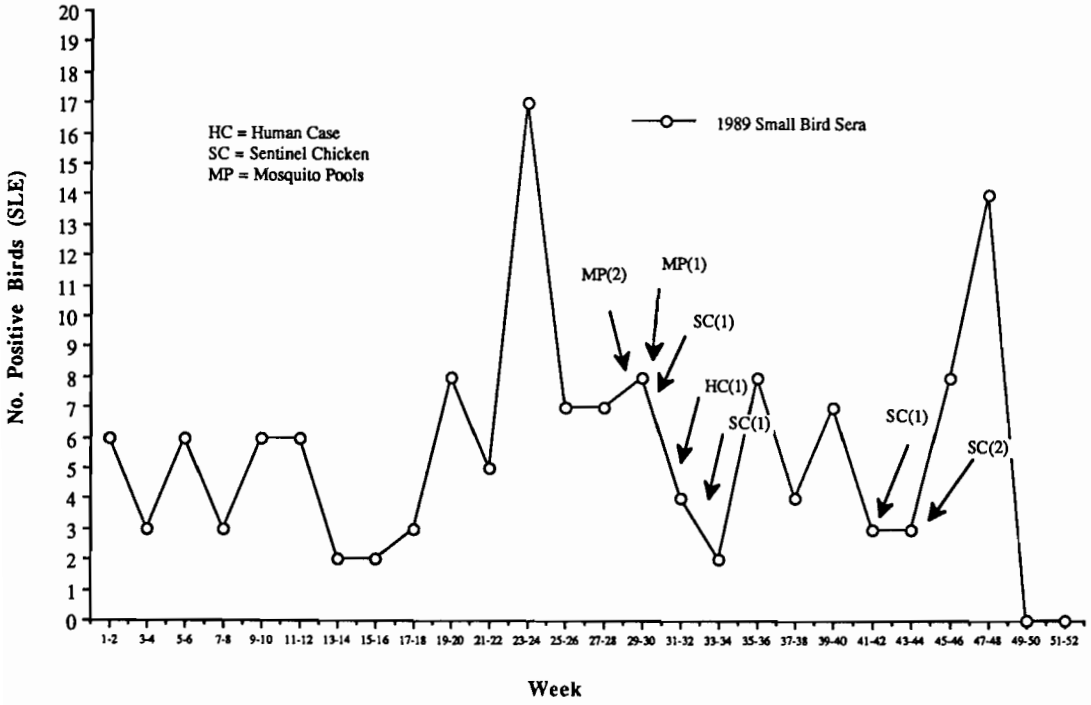

Figure 2. SLE virus activity in the Los Angeles Basin, 1989.

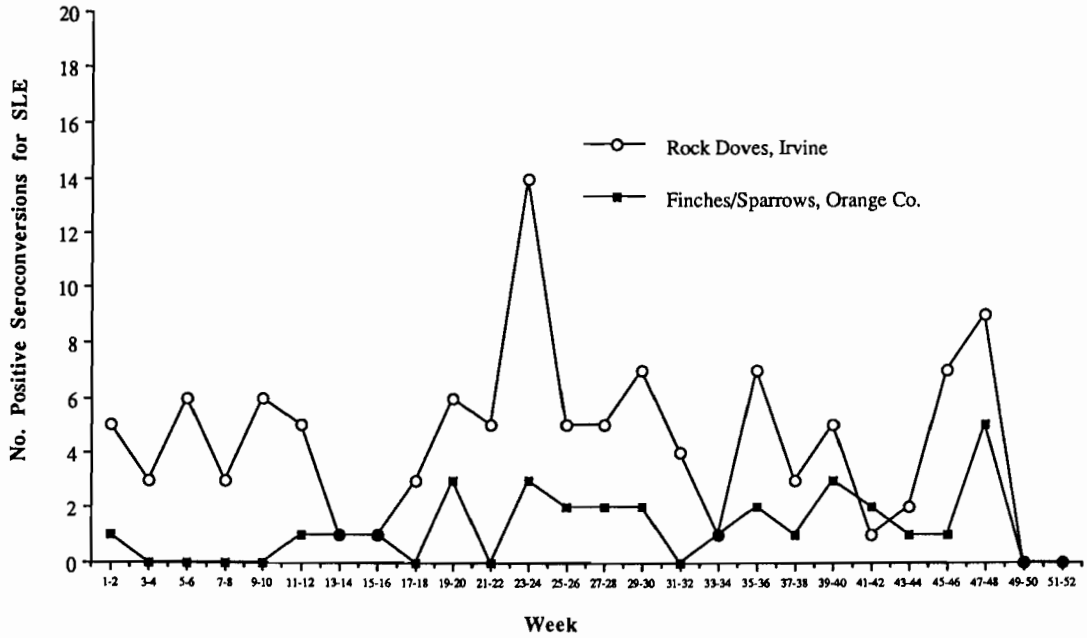

Figure 3. SLE virus activity in wild birds from Orange County, 1989.

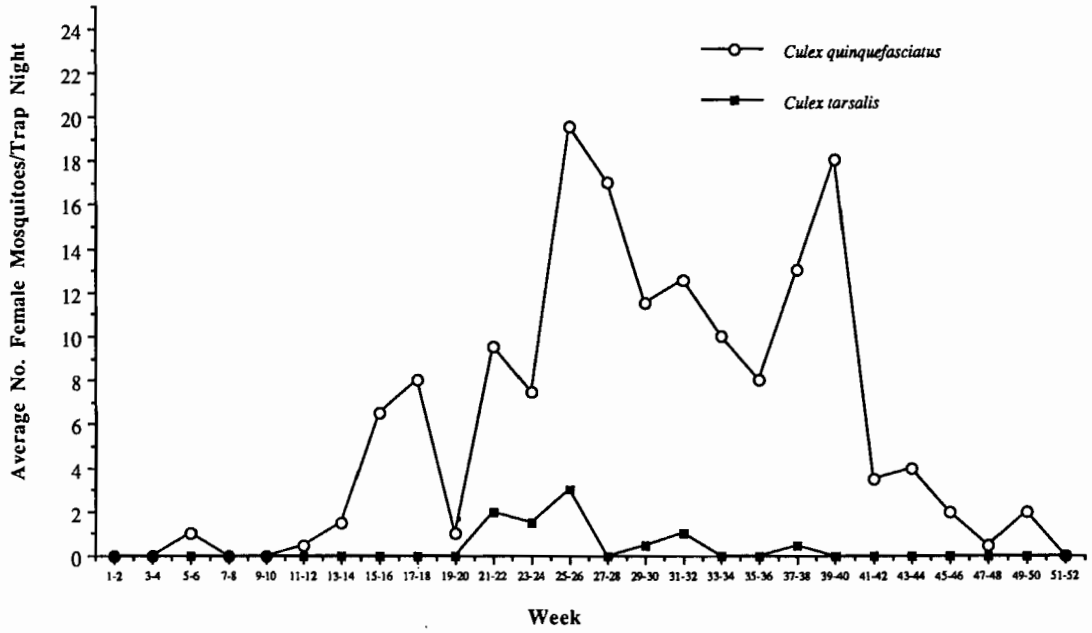

Figure 4. Host-seeking female mosquito activity at a suburban residence in Irvine, 1989 - CDC/CO_2 trap.

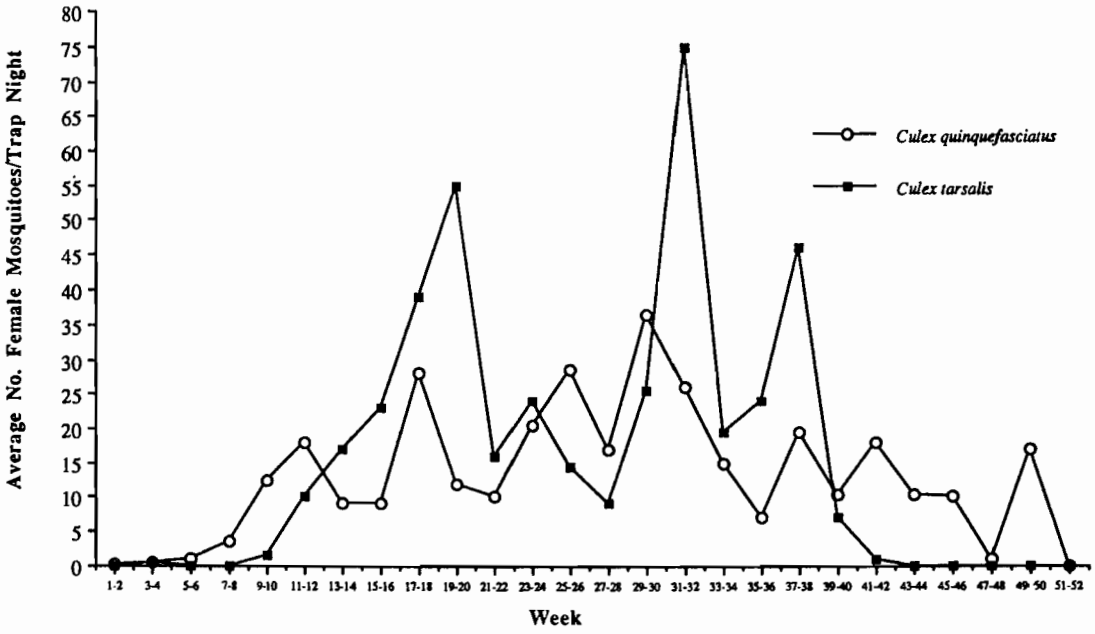

Figure 5. Host-seeking female mosquito activity at 20 Ranch Duck Club, Irvine, 1989 - CDC/CO_2 trap.

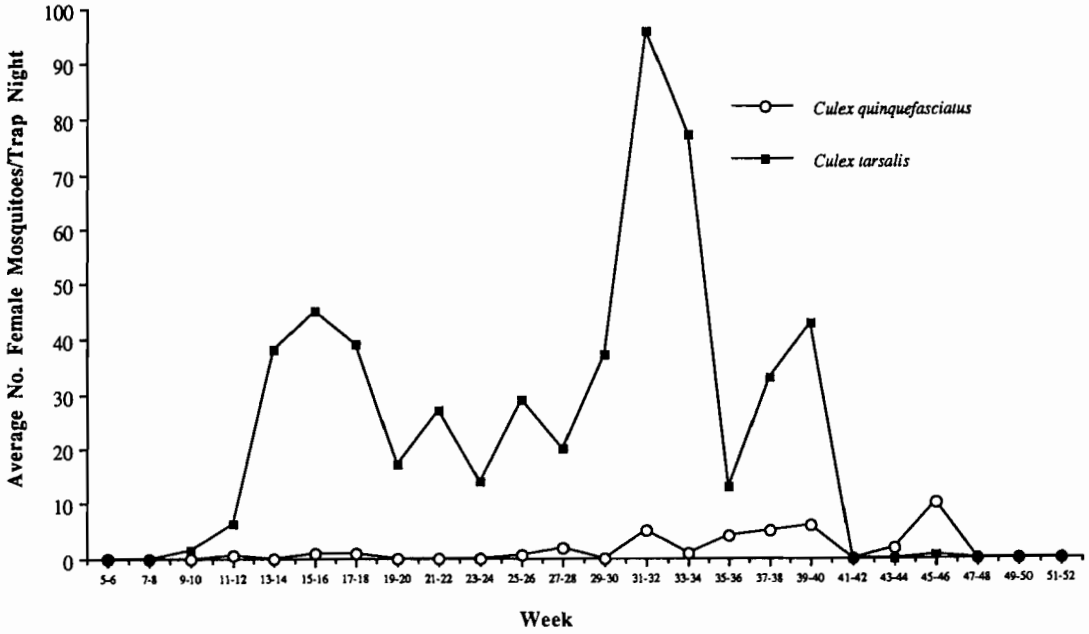

Figure 6. Host-seeking female mosquito activity at San Joaquin Marsh, Irvine, 1989 - CDC/CO_2 trap.

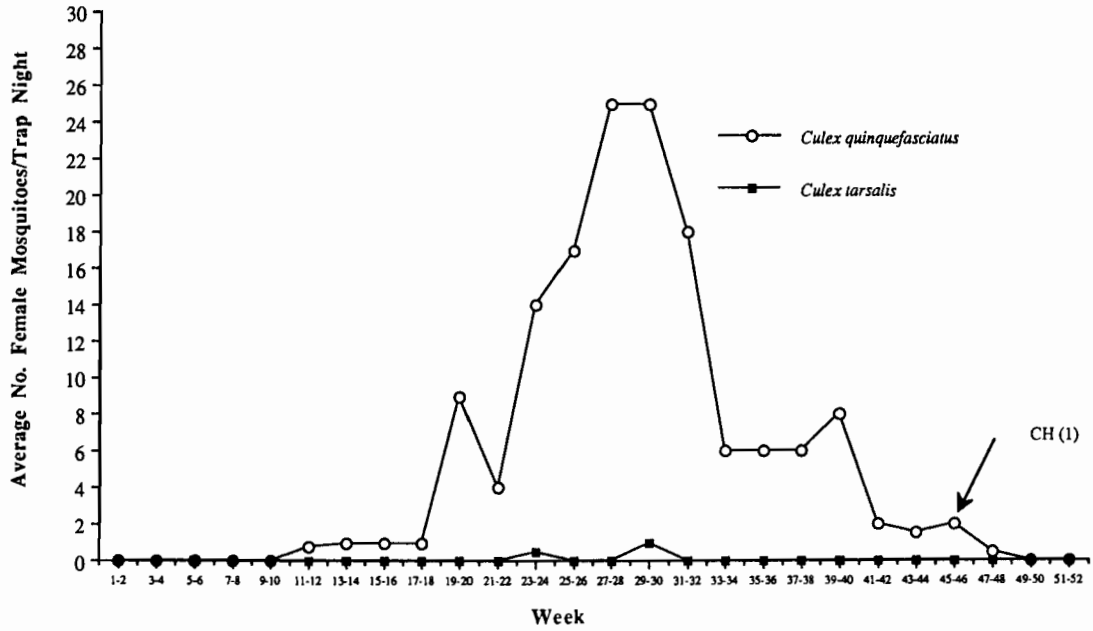

Figure 7. Host-seeking female activity in Fullerton, 1989 - CDC/CO_2 tap. CH = sentinel chicken SLE seroconversion.

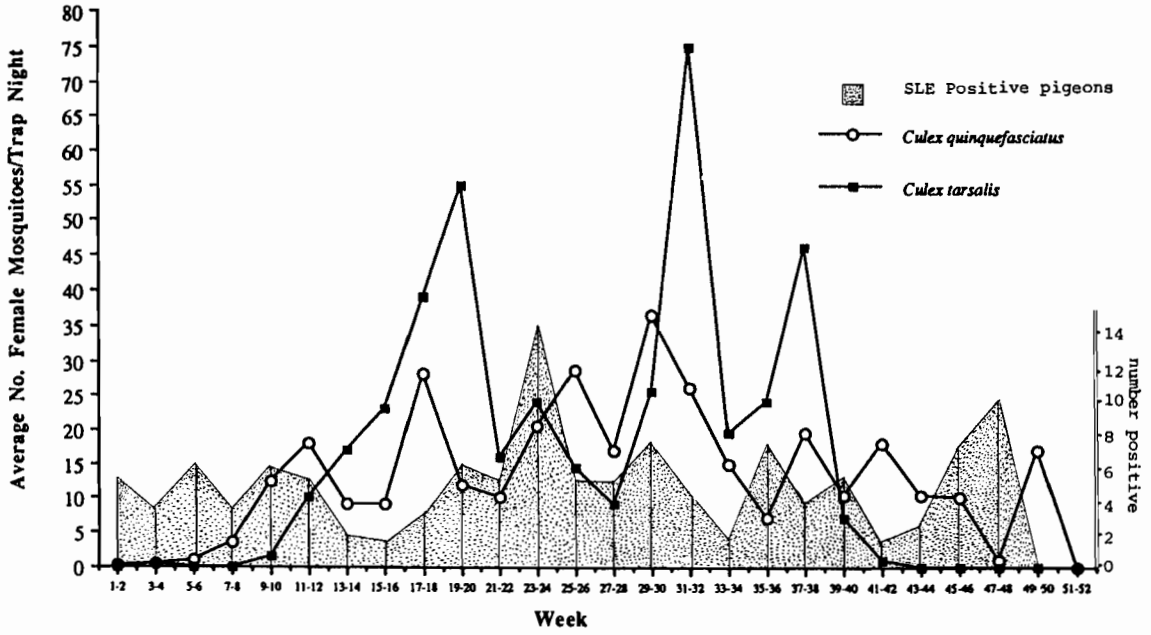

Figure 8. Host-seeking female mosquito activity (20 Ranch Duck Club) and SLE positive pigeons (Bonita Canyon) from Irvine, 1989 - CDC/CO_2 trap.

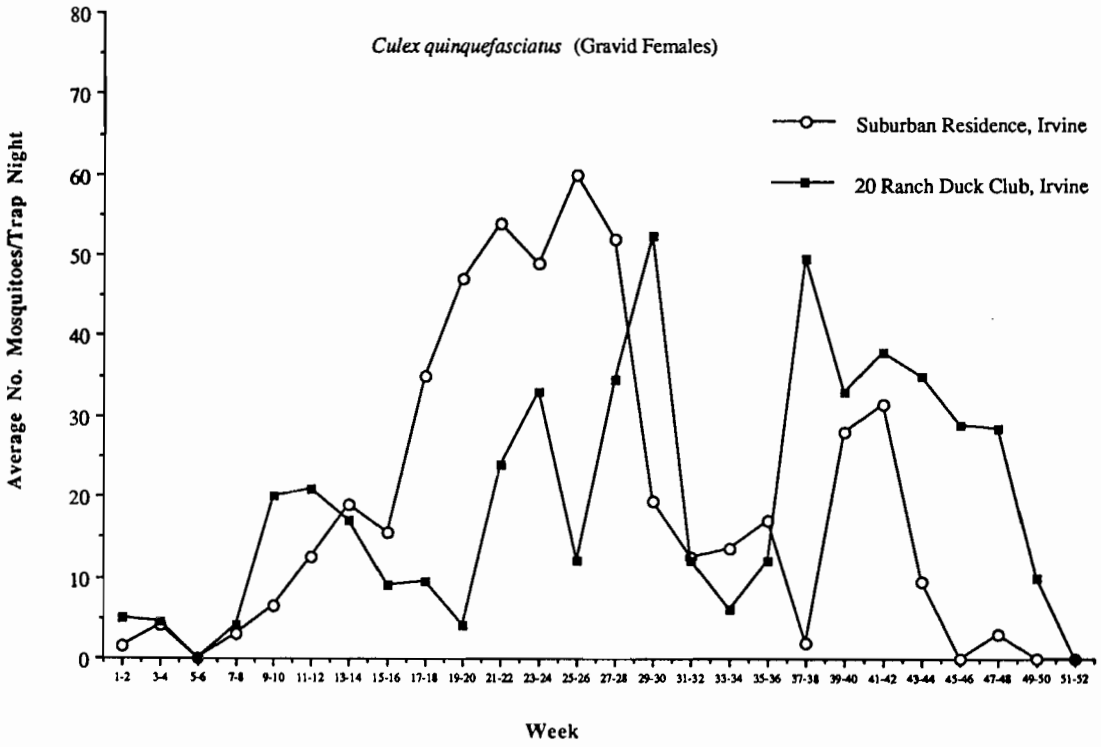

Figure 9. Gravid female mosquito activity in Irvine, 1989 - 7 Reiter trap.

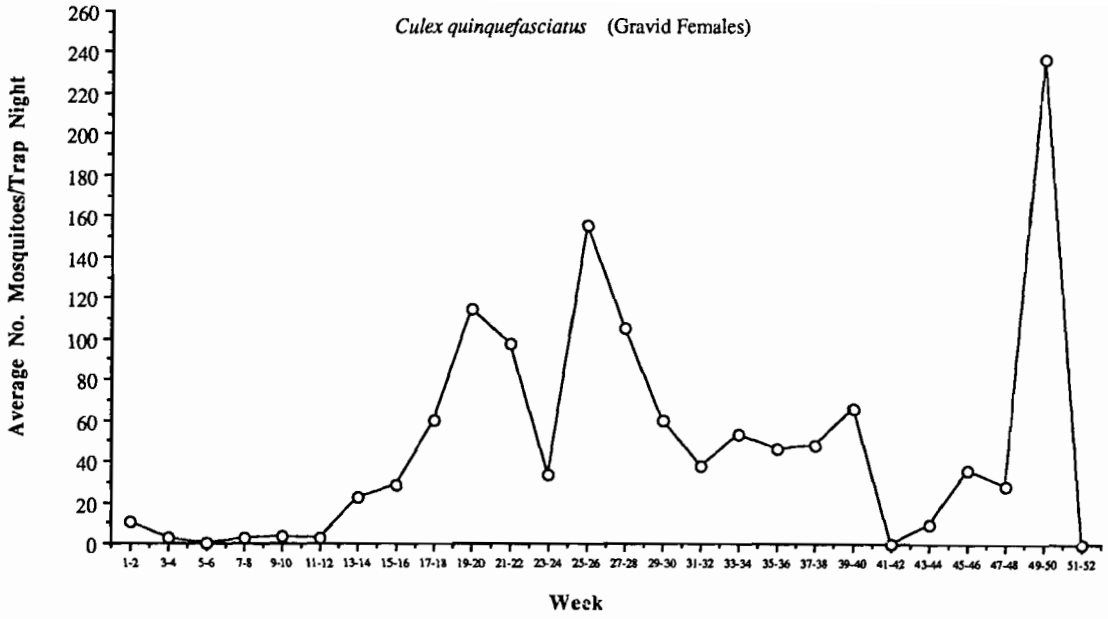

Figure 10. Gravid female activity at Central Park, Huntington Beach, 1989 - Reiter Trap.

mid-June, mid-August and mid- to late September. Numbers ranged from 1-20 mosquitoes per trap-night at the rural sites. *Culex quinquefasciatus* occurred in very low numbers in the San Joaquin Marsh during 1989 (0-10 per trap-night). *Culex tarsalis* in the marsh averaged 20-40 per trap-night from April through July and peaked in August (100 per trap-night). By mid-October, *Cx. tarsalis* was gone. In adjacent 20 Ranch Duck Club, *Cx. tarsalis* activity followed similar trends. Compared to 1988, *Cx. tarsalis* numbers were considerably lower in the San Joaquin Marsh. Although the April peak of activity did not occur in 1988, the mid-May peak reached 75 per trap-night (30 per trap-night for 1989), and the August peak reached approximately 400 per trap-night (100 per trap-night for 1989). *Culex quinquefasciatus* averaged 10-20 per trap-night starting in June, 1988, whereas, this species was almost completely absent in 1989. At the Irvine residential site, *Cx. tarsalis* only reached 2-3 per trap-night between May and July. Figure 7 illustrates the mosquito activity at a suburban site in Fullerton. *Culex quinquefasciatus* is most abundant from June through August (25 per trap-night) and remains present through November.

An interesting observation was made in mid-November when a sentinel chicken at this site seroconverted for SLE at a time when host-seeking *Cx. quinquefasciatus* were still present but *Cx. tarsalis* had been absent since mid-July. *Culex stigmatosoma* was present in low numbers until November (1 per trap-night). The number of *Cx. tarsalis* never exceeded two per trap-night during the entire year. A similar correlation can be seen between SLE positive pigeons and *Cx. quinquefasciatus* from Irvine (Fig. 8). After October, *Cx. tarsalis* had disappeared, while *Cx. quinquefasciatus* persisted through the winter along with pigeon seroconversions. *Culex stigmatosoma* and *Culiseta incidens* were also present until mid- November. However, from April through September peaks for *Cx. tarsalis, Cx. quinquefasciatus* and pigeon seroconversions corresponded relatively closely.

Gravid female *Cx. quinquefasciatus* were obtained from ovipositional traps at both suburban and rural sites. Figure 9 illustrates mosquito activity from the 20 Ranch Duck Club in Irvine and a residential site. Peak activity periods occurred at approximately the same time of year, although a few mosquitoes were taken after November at the residential site. Between April and July, greater numbers were collected from the backyard source (35-60 per trap-night), but after September more were taken from the rural site (30-50 per trap-night). Data from these two localities for 1988 differed considerably. Fewer *Cx. quinquefasciatus* were taken in June and July, 1988 (only 1-5 per trap-night) and in September (25-30 per trap-night) at 20 Ranch Duck Club. Greater numbers of mosquitoes were present from mid-July, 1988 (140 per trap-night), August (90 per trap-night) and September (80 per trap-night) at the residential site. The highest numbers of gravid female *Cx. quinquefasciatus* were obtained from a suburban park in Huntington Beach where 110 per trap-night were collected in May, 160 per trap-night in June,

and 240 per trap-night in December (Fig. 10). This December increase seems to correspond to a hot weather pattern that was present in mid-December, although none of the other localities show such a dramatic change.

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MOSQUITO ABUNDANCE AND ARBOVIRAL ACTIVITY IN SAN BERNARDINO COUNTY DURING 1989

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ABSTRACT

Of the 29,513 mosquitoes collected in New Jersey light traps and dry ice-baited traps in San Bernardino County during 1989, *Aedes vexans* (49.7%), *Culex tarsalis* (30.6%) and *Culiseta inornata* (19.5%) were the main species collected in the desert region. *Culex tarsalis* (35.0%), *Culex stigmatosoma* (29.2%), *Culex quinquefasciatus* (16.5%), and *Cs. inornata* (12.2%) were predominant in the valley region. In general, mosquito activity was the highest (88.1%) in the rural habitats of the desert region. This also directly contrasted the mosquito activity found in the valley region where more mosquitoes were collected in the urban and suburban sites than in the rural locations. Seasonally, different individual species of mosquitoes prevailed from April through November, depending on region and habitat.

During our studies on the arboviral activity, none of the mosquito samples or blood sera from sentinel chicken flocks were found positive for arbovirus activity.

Introduction.

As part of the California encephalitis virus surveillance (EVS) system, San Bernardino County, through its Vector Control Program, has been carrying out EVS and mosquito control activities in order to protect the well-being of its residents. Geographically, the county consists of three distinct regions: the desert, mountain and valley regions. Demographically, the valley region houses over 80% of the nearly 1.3 million county population with the remaining scattered over various parts of the desert and mountain regions. Historically, however, cases of both Saint Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) have been reported in the desert and valley regions from time to time.

After the encephalitis outbreak of 1984 (which resulted in 26 human cases of SLE in southern California), the next human case of encephalitis (SLE) in California was reported from the city of San Bernardino in 1987 (Emmons et al. 1988). Of the two human cases in 1988, one case was reported from the same site in San Bernardino (Anonymous 1988). During the same period, both SLE and WEE virus activities were reported in the desert region, especially Needles and adjourning areas along the Colorado River. Due to the periodic incidence of encephalitis in the two regions, mosquito control and EVS activities have been routinely carried out in the desert and valley regions of this county. The data generated in routine EVS activities are evaluated here in relation to the population dynamics of adult mosquitoes and arboviral activity in San Bernardino County during 1989.

Material and Methods.

The general procedure for EVS studies in San Bernardino County during 1989 consisted of the following three main components:

Adult Mosquito Population Dynamics: The abundance of various mosquito species was monitored on a weekly basis by operating a number of New Jersey Light Traps. In the valley region, the traps were stationed at six locations (Yucaipa Regional Park, Fifth Street in San Bernardino, Randall Basin in San Bernardino, Fontana Regional Park, Ontario and Upland). In the desert-Needles area, three traps were operated along the Colorado River.

Adult mosquitoes collected in these traps were counted and identified to species and sex. The Adult Mosquito Occurrence Report was sent to the California Department of Health Services (CDHS).

Arboviral Activity in Female Mosquitoes: To monitor arboviral activity in local mosquito populations throughout both the desert and valley regions,

dry ice-baited traps were used overnight to collect host-seeking adult female mosquitoes. Eight or more traps were operated on a biweekly (valley region) or monthly (desert region) basis.

Female mosquitoes collected in overnight traps were anesthetized using triethylamine. They were counted, identified to species, sexed and pooled by species with 10-50 adults per labelled vial. All pools (vials) were stored in dry ice in the field or at -60° F in the laboratory before being shipped in dry ice-packed containers to the State of California Department of Health Services' Viral and Rickettsial Disease Laboratory (VRDL) in Berkeley for viral determination.

Arboviral Activity in Sentinel Chicken Flocks: Both wild and domestic birds are known to play a significant role in the epidemiology of mosquito-borne encephalitides by acting as reservoir hosts for the encephalitis virus(es). Therefore, two sentinel flocks each consisting of 20 white leghorn chickens were maintained in the valley region during 1989. One flock was stationed near the Randall Basin in the city of San Bernardino. This site had a history of one human SLE case each in 1987 and 1988. The second flock was maintained at the San Bernardino County Vector Control Program's headquarters, also in the city of San Bernardino. Both flock sites had New Jersey light traps operated nearby. Blood serum samples from all sentinel chickens were taken on pre-determined dates during the mosquito season and sent to the VRDL for detection of arboviral activity. No chicken flocks were maintained by us in the desert region-Needles area. This region had a chicken flock located near a golf course in Needles which was maintained by the University of California Arbovirus Research Group.

Results and Discussion.

Of the total 29,513 mosquitoes collected in New Jersey light traps and dry ice-baited traps at various sites in the county during 1989, the most abundant culicine species in both the desert and valley regions was *Culex tarsalis* Coquillett (Table 1). In the desert region, *Aedes vexans* Meigen constituted 49.7% of the total collection, and was followed by *Culex tarsalis* (30.6%) and *Culiseta inornata* Williston (19.5%). Other species totaling <1.0% of the total number of mosquitoes included *Aedes increpitus* Dyar, *Anopheles franciscanus* McCracken, *Anopheles freeborni* Aitken, *Anopheles punctipennis* Say, *Culex erythrothorax* Dyar and *Culex quinquefasciatus* Say. Earlier studies in this area, however, have shown *Cx. tarsalis* as the most abundant species comprising as much as 72%, 62% and 86% of the total mosquitoes collected in 1986, 1987 (Reisen et al. 1988) and, more recently, 1988 (Mian, unpublished data), respectively.

Table 1. Faunal composition of mosquitoes caught in San Bernardino County during 1989.

Species	% composition[a] Desert	Valley
Aedes vexans	49.7	<0.1
Anopheles franciscanus	0.1	1.9
Culex erythrothorax	<0.1	0.7
Culex quinquefasciatus	<0.1	16.5
Culex stigmatosoma	0	29.2
Culex tarsalis	30.6	35.0
Culiseta incidens	0	4.1
Culiseta inornata	19.5	12.2
Culiseta particeps	0	0.3
Total collected	25,272	4,241

[a] Other species found in small numbers included *Ae. increpitus*, *An. freeborni* or *hermsi*, and *An. punctipennis*.

In the valley region of the county, mosquito composition by species was dominated by *Cx. tarsalis* (35%), *Culex stigmatosoma* Dyar (29.2%), *Cx. quinquefasciatus* (16.5%), *and Cs. inornata* (12.2%). These species were follwed, to a lesser extent, by *Culiseta incidens* (Thompson) (4.1%), *An. franciscanus* (1.9%), *Cx. erythrothorax* (0.7%), *Culiseta particeps* Adams (0.3%) and *Ae. vexans* (<0.1%). In the west end of the valley (Chino area) the three culicine species in order of their relative abundance have been reported to be *Cx. quinquefasciatus*, *Cx. stigmatosoma* and *Cx. tarsalis* (Pfuntner 1988). The Chino area is composed of various agricultural biotopes including, but not limited, to dairy farming; these biotopes provide ideal habitats for the breeding of mosquito species in the aforementioned order.

Based on the New Jersey light trap data, the distribution of mosquitoes by habitat in the desert region was predominantly rural (88.1%), whereas in the valley region, mosquitoes were found in higher numbers in both suburban (60.7%) and urban (33.1%) habitats (Table 2). This distribution pattern could be attributed to the proximity of trap sites to mosquito breeding habitats ranging from

Table 2. Distribution of mosquitoes caught in New Jersey light traps operated at various locations in San Bernardino County during 1989.

Trap Location	% mosquitoes/trap-night	
	Desert	Valley
Rural	88.1	6.2
Suburban	4.1	60.7
Urban	7.8	33.1
Total collected	21,383	2,217

residential swimming pools to flood control structures in the urban and suburban habitats, or to seepage water in ponds, ground depressions and irrigation ditches in agricultural areas along the Colorado River.

Data on the seasonal abundance of mosquitoes show a sharp rise in mosquito populations appearing in April through August in almost all three habitats (Fig. 1). The drop in mosquito population in June at the urban and suburban habitats was most probably due to larvicidal treatments with Teknar® (containing *Bacillus thuringiensis* var. *israelensis* serotype H-14) and adulticidal applications of Pyrenone® MAGC (a mixture of pyrethrins, and piperonyl butoxide) during the later part of May, 1989. The drop in mosquito abundance at the rural site could be largely attributed to the changes brought about in the duration and frequency of flood irrigation of pasture fields at the Havasu National Wildlife Refuge across the Colorado River from the city of Needles. Mosquito faunal composition by season in the desert area showed that *Ae. vexans* and *Cx. tarsalis* were the most prevalent species during the spring and summer months, whereas *Cs. inornata* prevailed during the fall and winter months (Table 3). Mosquito abundance as measured by CO_2-baited traps revealed an almost similar pattern with *Cx. tarsalis* as the most abundant species during April through September, *Ae. vexans* as the main species during April, and *Cs. inornata* dominating during November (Table 4).

In the valley region, both urban and suburban sites had higher mosquito activity than that of rural sites during the period of April through August; the latter had a higher number of mosquitoes than those of the former sites during the colder months, especially November and December (Fig. 2). Mosquito activity at these habitats during April

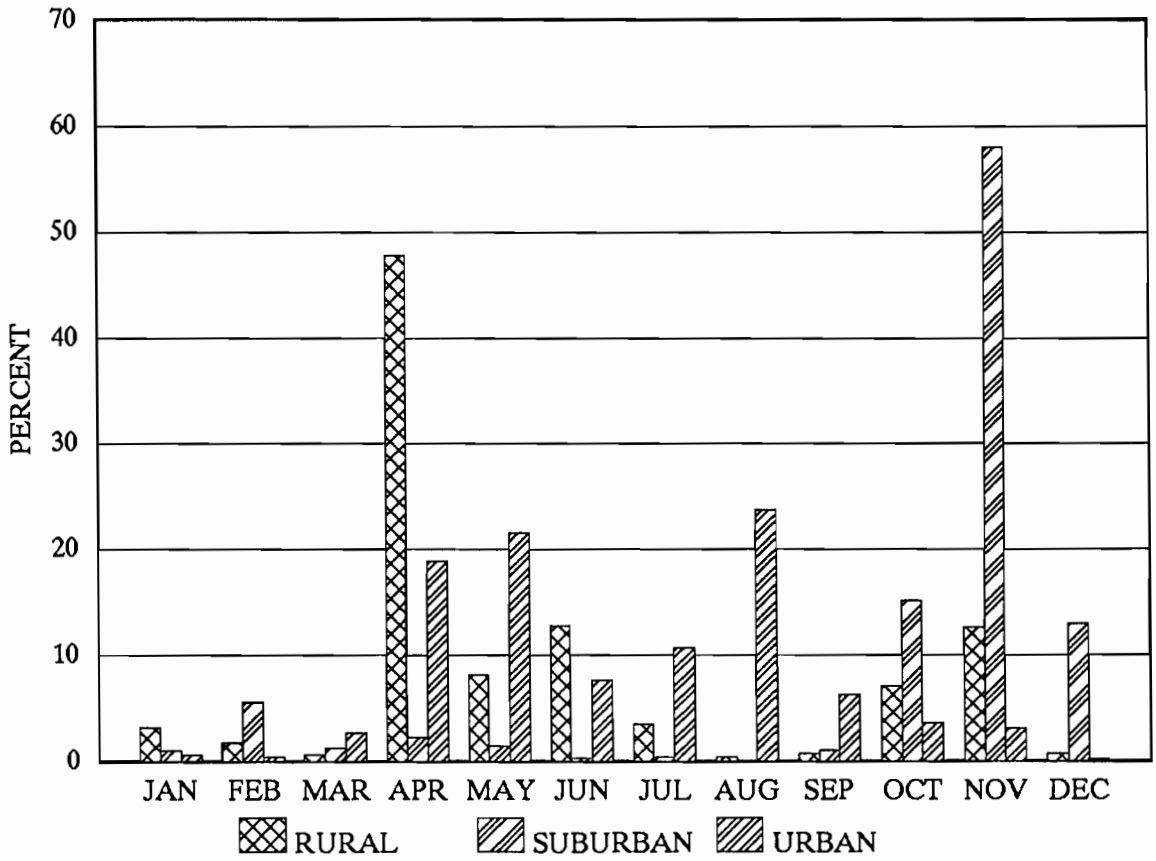

Figure 1. Seasonal abundance of mosquitoes by trap location in the desert region of San Bernardino County during 1989.

Table 3. Seasonal abundance of mosquitoes collected in New Jersey light traps from the desert region of San Bernardino County during 1989. Percentages are based on a total of 21,383 mosquitoes collected.

Month	Percent mosquitoes/trap-night by species					
	Ae. vexans	An. franscicanus	Cx. erythrothorax	Cx. tarsalis	Cs. inornata	Total
January	0	0	0	0.3	2.8	3.1
February	0	0	0	0.9	13.8	14.7
March	<0.1	0	0	0.5	0.3	0.8
April	26.3	0	0	4.7	0.1	31.1
May	2.4	<0.1	0	4.7	<0.1	7.2
June	14.5	<0.1	0	1.6	<0.1	16.2
July	2.6	0	0	0.9	0	3.5
August	0.2	0	0	1.9	0	2.1
September	0.1	<0.1	0	0.4	0.3	0.8
October	3.1	<0.1	<0.1	0.2	4.7	8.1
November	0.1	<0.1	<0.1	0.3	10.5	10.9
December	0	0	0	<0.1	1.5	1.5

Table 4. Seasonal abundance of mosquitoes collected in CO_2-baited traps from the desert region of San Bernardino County during 1989. Percentages are based on a total of 3,889 mosquitoes collected.

Month	Percent mosquitoes/trap-night by species					
	Ae. vexans	An. franscicanus	Cx. erythrothorax	Cx. tarsalis	Cs. inornata	Total
April	1.7	0	<0.1	29.4	0	31.1
July	0.1	0	0	22.0	0	22.1
August	0.2	0.1	0	42.6	0	42.9
September	<0.1	0	0	2.1	0	2.1
November	0.1	0	0	0.1	1.6	1.8

Table 5. Seasonal abundance of mosquitoes collected in New Jersey light traps from the valley region of San Bernardino County during 1989. Percentages are based on a total of 2,217 mosquitoes collected.

Month	Percent mosquitoes/trap-night by species							
	An. francis.	Cx. erythro.	Cx. quinque.	Cx. stigmato.	Cx. tarsalis	Cs. incidens	Cs. inornata	Total
April	0	0	0.4	3.5	0.3	0.4	0	4.6
May	0	0	0.7	13.6	1.7	1.5	0	17.5
June	0	0	0.9	10.3	3.1	1.1	0	15.4
July	<0.1	0.1	1.9	6.5	10.0	0.4	0	19.0
August	<0.1	0.1	2.4	2.0	4.1	0.3	<0.1	5.0
September	0.2	0.1	1.7	0.8	1.4	0.2	0.6	5.0
October	0.1	0.1	1.0	2.4	1.0	0.3	3.2	8.1
November	<0.1	0	0.3	0.3	0.1	0.2	19.5	20.5
December	0	0	0	0.1	0	0	0.7	0.8

Table 6. Seasonal abundance of mosquitoes collected in CO_2-baited traps from the valley region of San Bernardino County during 1989. Percentages are based on a total of 2,024 mosquitoes collected.

Month	Percent mosquitoes/trap-night by species									
	Ae. vexans	An. francis.	Cx. erythro.	Cx. quinque.	Cx. stigmato.	Cx. tarsalis	Cs. incidens	Cs. inornata	Cs. part.	Total
May	0	0	0	0.6	4.1	2.8	0.6	0	0	8.1
June	0.9	<0.1	0.6	1.0	2.7	5.0	1.3	0.2	0.6	12.3
July	2.7	0	0.4	3.6	2.8	25.9	1.6	0.1	0	37.1
August	0	0	0	5.6	3.9	6.2	0.1	0	0	15.8
September	<0.1	0	0	12.6	5.6	8.0	0.1	0	<0.1	26.3
October	0	0	0	0.2	<0.1	0.1	<0.1	0.1	0	0.4

through September was mainly due to *Cx. quinquefasciatus*, *Cx. stigmatosoma* and *Cx. tarsalis*, whereas during the later part of the year (i.e., November and December) *Cs. inornata* was the most prevalent species (Table 5). Similarly, data on mosquitoes caught in dry ice-baited traps present the same pattern of mosquito abundance with *Cx. quinquefasciatus*, *Cx. stigmatosoma*, and *Cx. tarsalis* as the predominant species during May through September (Table 6).

Regarding the arboviral activity in San Bernardino County, none of the mosquito pools (98) sent from the desert region were found positive for arboviral activity. However, serum samples from the sentinel chicken flock near Needles which was maintained by the U.C. Arbovirus Research Group, showed one WEE sereconversion during the month of July (Anonymous 1989). Similarly, mosquito pools as well as serum samples from both sentinel chicken flocks maintained in the valley area did not show any arbovirus activity during the 1989 season.

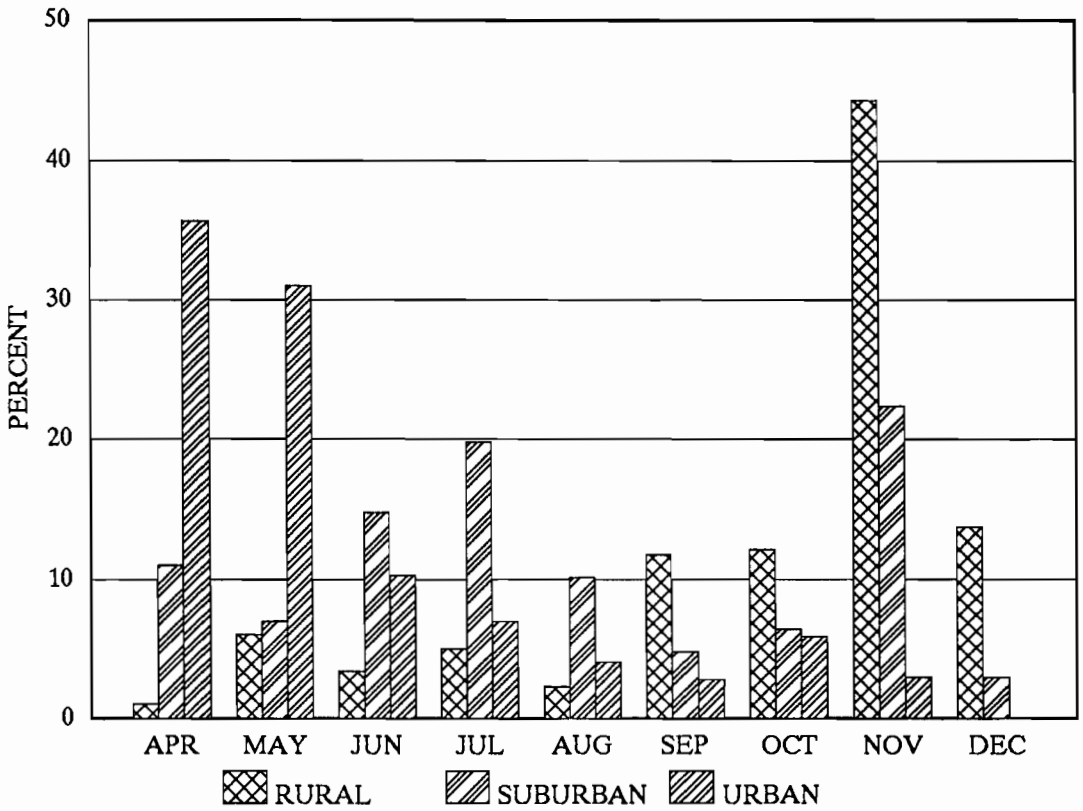

Figure 2. Seasonal abundance of mosquitoes by trap location in the valley region of San Bernardino County during 1989.

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The authors thank M.J. Bernstein, Program Manager, for logistics and other support during the course of these studies. The authors also appreciate the field assistance provided by D.E. Gomsi, L. Ainsworth, R.A. Berg, J. Krygier, and J.G. Lane. Cynthia DeChaine is thanked for her help in computer graphics. The typing and proofreading of this manuscript by S. Miller and P. Felts are also acknowledged.

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WEE PROGRAM IN WASHOE COUNTY, NEVADA:

ENCEPHALITIS SURVEY STUDY - 1989

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The following is a report on the 1989 encephalitis survey study conducted by the mosquito abatement program, Washoe County District Health Department.

Introduction.

Western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) are diseases caused by arboviruses (arthropod-borne viruses). These viruses are maintained in wild bird and rodent populations which serve as reservoirs of infection for mosquitoes. Mosquitoes then transmit the disease to other wild and domestic animals, horses and humans. Horses and humans are known as "dead-end" hosts. They develop a clinical disease but they do not develop high enough viral concentrations in their blood for the viruses to be passed on to uninfected mosquitoes. Birds and rodents, however, do not develop clinical disease, but they do develop a viremia which allows infection of mosquitoes and therefore, transmission of the virus to other animals and humans.

WEE is prevalent in the western United States and is chiefly vectored by the mosquito, *Culex tarsalis* Coquillett. Both horses and humans are susceptible to this disease. In endemic areas, human infection is common but usually only one in 1,000 infections actually results in clinical illness. However, in young infants (less than one year of age), one in 25 infections may cause severe illness and 60% of survivors usually have permanent neurological impairment (Sherris 1984). Mortality from WEE is estimated at 3-7% (Smith et al. 1985).

SLE is also a major cause of encephalitis in the United States. It has a geographic distribution similar to WEE and it is also known to be vectored by *Culex tarsalis*. SLE usually occurs in the late summer and fall and WEE usually occurs in early or mid-summer. This is due to a slower rate of growth of the SLE virus in the mosquito vector (Anonymous 1988). Unlike WEE, SLE infects but does not cause disease in horses and its highest attack rate in humans occurs not in infants but in adults over the age of 40 years of age. The overall case fatality rate for SLE is 9% (Smith et al. 1985).

Purpose.

The 1989 encephalitis study was undertaken to monitor the level of WEE and SLE viral activity in various locations in Washoe County. Monitoring in this area was deemed appropriate because the necessary factors for WEE and SLE outbreaks are present. These factors are as follows:

1. Washoe County is located within the known geographical distribution areas of WEE and SLE (the western United States).
2. There are large numbers *Culex tarsalis* mosquitoes in this area.
3. Birds and rodents are the known reservoirs for WEE and SLE and Washoe County currently sustains large populations of both of these.

Procedures.

In July, sentinel chicken flocks were established in four locations (Fig. 1 and Table 1). These flocks consisted of domestic chickens which were born on May 1, 1989 and raised by mosquito abatement personnel until they were 8-weeks old. The chickens were then tagged with leg bands and the flocks were placed in each of the four locations on either June 28 or June 29, 1989. The flocks were bled one week after placement and these blood samples were tested for antibodies to WEE and SLE to establish that all chickens used were free of previous exposure to WEE and SLE. Blood was then drawn at monthly intervals in August, September and October.

The chickens were kept outside in pens where they were protected from weather and predators but were freely exposed to mosquitoes. The leg bands were used for repeated identification and the chickens were checked and bled according to a schedule. A 27-gauge tuberculin syringe was used to obtain a one milliliter (1 ml) blood sample per chicken. The blood was withdrawn from the wing vein after the skin had been disinfected with alcohol. The blood samples were not diluted; they were allowed to clot and a serum sample was drawn off after centrifugation. The serum samples were then shipped to California State Health Department's Viral and Rickettsial Disease

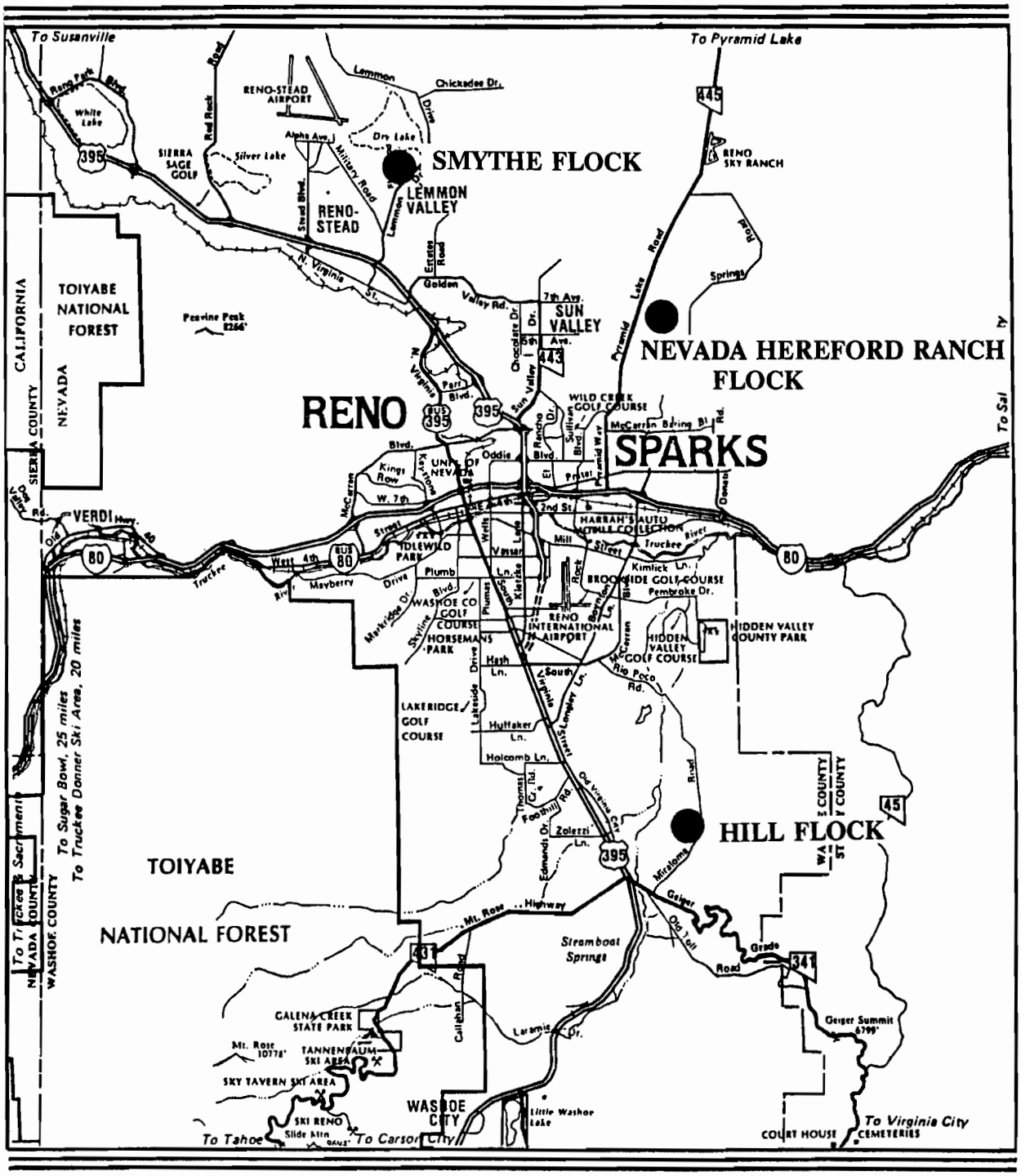

Figure 1. Sentinel chicken flock locations used in Washoe County during 1989 (Gerlach not shown).

Table 1. Sentinel chicken flock locations used in Washoe County during 1989.

Flock location	Tag numbers	Chickens
Nevada Hereford Ranch 7000 Pyramid Highway Sparks, Nevada 89431	1-20	18
Mr. and Mrs. Don Hill Old Mira Loma Road Reno, Nevada 89506	21-40	20
William and Dorothy Smythe 9415 Fleetwood Drive Reno, Nevada 89506	41-60	19
Pfaff's Welding and Fabrication 215 West Sunset Boulevard Gerlach, Nevada 89412	61-77	17

Laboratory in Berkeley, California where they were tested for WEE and SLE antibodies using the enzyme-linked immunoassay (ELIZA) test.

Additionally, New Jersey Light Traps were maintained near the sentinel flocks to monitor mosquito activity.

Results.

A total of 292 serum samples were tested for antibodies to WEE and SLE viruses. All of the samples tested were negative.

However, there was a positive SLE antibody sero-conversion in a chicken from the Churchill County Mosquito Abatement District's sentinel flock (documented: Jennifer Penner, Churchill County M.A.D.).

There is also an unconfirmed report of a human case of SLE in a farm worker in Yerington, Nevada that occurred a few weeks ago. Dr. Deborah Brus, Nevada State Health Department has been contacted and is checking into the case. She has agreed to notify us of the results of her report.

Future recommendations.

It is recommended that the encephalitis testing program be continued. Although no positive samples were found in Washoe County, there is evidence of SLE activity in Nevada this year (Churchill County and possibly Yerington). Also, the necessary factors for an outbreak; the vector, the reservoir and the host are present.

Through the use of sentinel chicken flocks and blood testing, the activity of WEE and SLE can be estimated. The finding of antibody in sentinel flock blood samples will provide warning of the possibility of transmission to humans. It will also allow for monitoring of the disease with respect to time and location. As a result, mosquito control efforts could be intensified in target areas to decrease or prevent any human cases.

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MOSQUITO-TRANSMITTED MALARIA IN CALIFORNIA: 1988 - 1989

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There were six episodes of mosquito-transmitted malaria in the State in 1988 (3) and 1989 (3), all due to *Plasmodium vivax*. These six episodes involved 40 cases residing in four counties. All other cases of malaria reported to the Department of Health Services in these two years (1988 = 331; 1989 = 378) were acquired outside the U.S.

Case histories.

Episode 1, El Dorado County: A 16-year old student from the Placerville area had onset of illness on 6/23/88 but was not diagnosed and confirmed as *P. vivax* malaria until 7/22. This was the first case of malaria reported in the county in three years. She had no history of foreign travel, blood transfusions or intravenous (IV) drug use. She lived with her family in a well screened house surrounded by open space used for cattle grazing. One and one-half months after her illness onset a few *Anopheles freeborni* Aitken were trapped near a large cattle pond about one mile from her house. No migrant workers (MW's) or immigrants from malaria endemic countries could be identified in the county before or after she became ill. Intensive local publicity and active surveillance of health care providers failed to reveal other cases. Her only other known possible exposure to malaria had occurred in the spring and fall of 1987 when she visited Yuba City in nearby Sutter County. Seven cases of *P. vivax* malaria were reported from this area in 1987, all in travelers or residents from northern India where temperate zone long-incubation strains of *P. vivax* (up to 12 months) have been documented.

Episode 2, San Diego County: On 8/2/88 a MW from Mexico was diagnosed and treated for *P. vivax* malaria at a clinic in northwestern San Diego County. Later that day a San Diego Health Department public health nurse accompanied the worker to where he was living in an isolated canyon encampment east of Rancho Santa Fe where she found 11 more MW's with malaria-like symptoms. All had *P. vivax* confirmed the following day. Subsequent investigations by the health department in two adjacent makeshift encampments identified 30 outbreak-related symptomatic *P. vivax* cases with onsets between 7/24 and 9/18. Of the 30, two cases were in a local family living in a new housing development next to the involved MW encampments and 28 were in MW's living in two encampments located off the Del Dios Highway between Rancho Santa Fe and Lake Hodges. The majority of the MW's were employed in local agriculture. All patients denied recent blood transfusions, IV drug use and previous malaria. Light traps placed near worker encampments (shelters made of cardboard and plastic tarps that were situated near water sources diverted from a canal and an irrigation pond) yielded high counts of *An. hermsi* Barr and Guptavanij, a competent malaria vector. Control measures included detection and treatment of cases, chloroquine prophylaxis of MW's and vector controls measures that included larviciding in areas with standing water and aerial fogging with insecticide.

Episode 3, Butte County: A 48-year old office worker in the County's Environmental Health Unit had onset of malaria symptoms on 9/11/88 which was confirmed as *P. vivax* infection a week later. She had no history of foreign or significant domestic travel, blood transfusion or IV drug use. Her case was the only one reported in the county in 1988. She lives on an almond and walnut ranch, 3 miles west of Chico and 3 miles east of the Sacramento River. The area around her home is a known habitat for *An. punctipennis* (Say). *Anopheles* mosquitoes were abundant in Butte County during the summer. In early August she recalled being bitten in the evenings by mosquitoes which apparently entered via a defective window screen. Migrant workers from Mexico had worked near her home during the almond harvest in August, however retrospective and prospective active surveillance by the Butte County Health Department did not reveal any missed or suspect cases of malaria in MW's or local residents.

Episode 4, San Diego County: On 7/28/89 a 52-year old banker who resides in a new development in Rancho Santa Fe had onset of malaria symptoms confirmed as *P. vivax* infection one week later. He had no history of foreign travel, blood transfusions

or IV drug use. He lives less than 2 miles southwest of the two local residents who had *P. vivax* infections in the 1988 outbreak (see above). On 8/10 a MW living in an isolated canyon near the junction of the San Diego River and Lusardi Creek, about 1 mile from the home of the local resident, was diagnosed with *P. vivax* infection. On 8/11 the San Diego County Health Department confirmed *P. vivax* in three of 40 MW's screened who lived in the same encampment. The four MW cases had onsets between 7/27 and 8/7. All denied transfusions, IV drug use or previous malaria. On the night of 8/10, five light traps placed at the encampment yielded 27 *An. hermsi*. Control measures included larviciding, adulticiding of mosquitoes and chloroquine prophylaxis for MW's living in the area.

Episode 5, San Diego County: On 8/22/89 a 32-year old engineer who resides in Rancho Penasquitos (6 miles southeast of Episode 4, above) had onset of chills and fever; *P. vivax* infection was confirmed one week later. He had no history of foreign travel, blood transfusions or IV drug use. On 8/31, 36 *An. hermsi* were collected in eight light traps within a one mile radius of his house. Earlier, on 7/7 and 7/30, two MW's from Mexico had onsets of malaria-like illness and were confirmed as *P. vivax* infection when they came to medical attention on 7/24 and 8/1 respectively. These MW's had been living in a small unprotected encampment in Penasquitos prior to their illness with a group that dispersed in July. At the time of his onset, the MW who became ill on 7/30 had been living in a lean-to within one mile of the local resident case. Control measures included larviciding and adulticiding *An. hermsi* habitats.

Episode 6, Kings County: On 9/9/89 a 37-year old teacher from Hanford had onset of fever, chills and headache but was not confirmed as *P. vivax* infection until 9/14. Her only foreign travel in the previous four years had been a one day visit to Tijuana, Mexico. She denied blood transfusions or IV drug use. Entomological investigations around her residence, school and her parents home in nearby Reedley showed no evidence of *Anopheles* habitats or activity.

She was most likely exposed at a family birthday outing held at a county park within one mile of the Kings River on the afternoon of 8/19. She recalls being bitten on her legs by mosquitoes before leaving the park. She also remembered seeing large numbers of Hispanic MW's encamped near the park which is flanked by peach and plum orchards, and grape vineyards. None of the other 15 persons who attended the party became ill. Light traps set out in the Burris Park-Kings River area on 9/28 yielded 30 *An. punctipennis* (Say) and one *An. freeborni*. Retrospective and active surveillance for other possible cases of malaria in Kings County during 1989 did not detect any other cases.

Discussion.

Since 1950 California has experienced 16 episodes of introduced autochthonous malaria (malaria acquired by mosquito transmission in an area where malaria does not occur regularly) accounting for 120 cases, all due to *P. vivax* (Fig. 1). Ten counties have been the sites of exposure with 7 in the Sacramento Valley and adjacent Sierra Foothills (Butte, El Dorado, Glenn, Nevada, Sacramento, Sutter, and Yolo), 2 in the San Joaquin Valley (Fresno and Kings) and San Diego County along the State's southernmost coast. Only two counties have experienced more than one episode, Sutter (4) and San Diego (4). The confirmed or presumptive sources of introduction were an army veteran just returned from Korea and agricultural workers from India (4 episodes) or Mexico (8 episodes). In three introductions, the source cases were uncertain but most likely from India or Mexico.

Transmission of malaria occurred from May to September, with three anopheline species being the likely vectors (*An. freeborni* and *An. punctipennis* in the Central Valley and *An. hermsi* in San Diego County). The largest of these outbreaks was in 1952 when 35 cases occurred in a group of Campfire Girls exposed in Nevada County. The second and third largest episodes were in 1986 and 1988 involving 27 and 30 cases respectively in San Diego County. The remaining 13 introductions resulted in 1 to 5 cases each.

Since 1986 there have been several important changes in the epidemiology of introduced malaria in California. The incidence of introductions has risen sharply; 9 (56%) of the 16 introduced episodes since 1950 have occurred in the last four years. Before 1986 all episodes (7) had occurred from Sacramento County northward and in 5 (71%), the source(s) of introduction were associated with immigrants recently arrived from northern India. Since 1986, activity has shifted with 6 of the 9 (67%) introductions occurring south of Sacramento County and 8 of 9 (89%) being associated with MW's from Mexico. Until 1986 all outbreaks of mosquito-transmitted malaria had involved only permanent California residents. Since 1986, the great majority of cases (59/71) have occurred in migrant workers, though local residents have also been involved in all outbreaks.

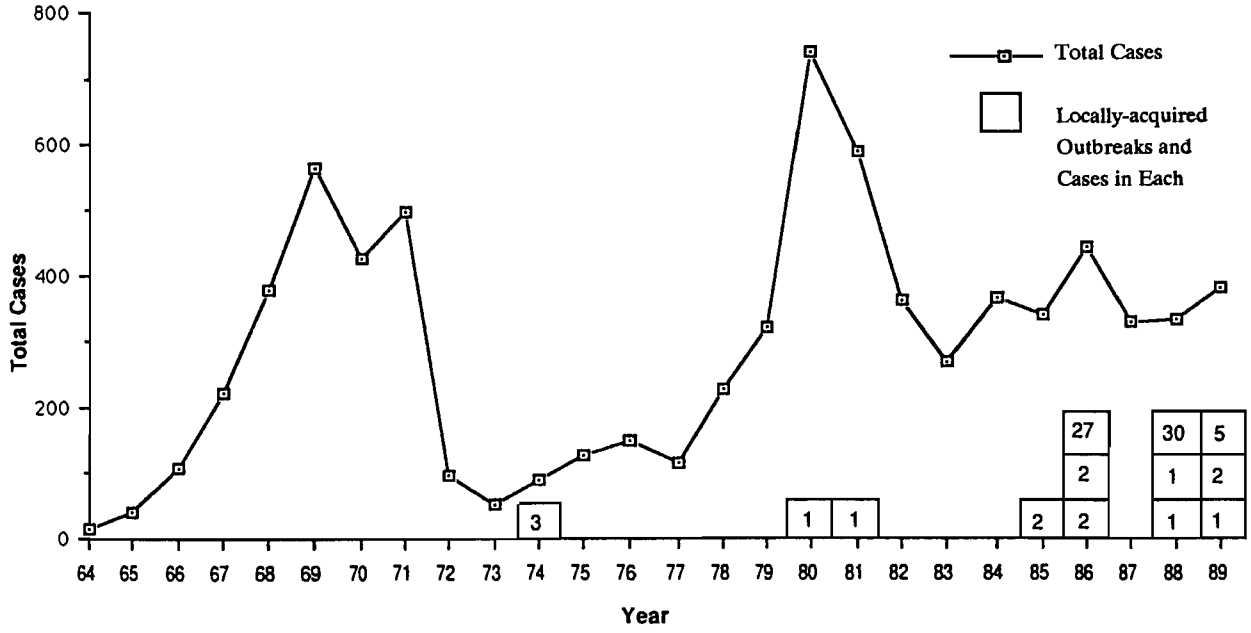

Figure 1. Annual reported malaria cases (total and locally-acquired) in California, 1964-1989.

Paralleling these trends in the epidemiology of introduced malaria in California has been a sharp rise in the incidence of malaria in Mexico and the number of imported malaria cases in persons entering the State from that country. Malaria cases reported in Mexico have risen steadily from 25,774 in 1980 to 166,271 in 1988 (>6 fold increase) while the number of California malaria cases reported in travelers and immigrants from Mexico has risen steadily from 12 in 1980 to 83 and 81 respectively in 1988 and 1989 (>6 fold increase). The episodes of local mosquito-transmitted *P. vivax* malaria since 1986 (particularly in San Diego County) have features in common which include 1) remotely located encampments, 2) inadequate shelters for MWs residing in areas with *Anopheles* mosquito vectors capable of transmitting malaria and 3) the reluctance of MW's to seek medical care because of limited access and concerns about being identified as undocumented aliens. Once a parasitemic individual introduces malaria in such settings, these factors may allow substantial transmission of malaria to evolve before outbreak foci can be identified and control measures instituted.

Prevention of mosquito-borne malaria transmission in these settings would be extremely difficult because of virtually insurmountable sociological, ecological, environmental and logistical factors. However, effective control to limit transmission can be achieved through 1) education of employers and immigrant workers at risk, 2) alert of health care providers to consider the diagnosis of malaria in febrile patients who live in malaria receptive areas, 3) prompt treatment and reporting of cases to public health authorities and 4) coordinated actions between local health departments and mosquito abatement agencies in the investigation and control of suspected foci of malaria transmission.

Acknowledgments.

M. Ginsberg (San Diego County Health Department), S. Minkin (Kings County Health Department), C.L. Ward (Butte County Health Department) and C.E. Weidmer (El Dorado County Health Department).

MALARIA IN MEXICO

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Malaria is one of the principal public health problems in Latin America where over one million cases occur every year. Ten percent of these cases are reported in Mexico. Developmental projects in malarious areas, human migrations in search of work or productive lands, improvised settlements with precarious household conditions, the overuse of insecticides that led to vector resistance, several financial and administrative inadequacies and insufficient knowledge of the epidemiology and dynamics of the disease transmission are only some of the components that make malaria control in the continent difficult.

The epidemiological situation of malaria in Mexico has shown several changes since 1942. Until the middle of the century, malaria constituted the fifth leading cause of death in the country, then a significant decrease in malaria incidence occurred and its place was taken by chronic diseases. A counter-transition was marked by an abrupt increase in malaria cases since 1960. It is possible to distinguish three phases along this period; one prior to the malaria eradication campaign; another during the eradication activities and finally, one post-eradication which is characterized by multiple epidemics.

In the first phase, even before the institution of the Malaria Eradication Campaign in 1955, mortality and morbidity due to malaria were decreasing. This probably resulted from a combination of factors like socio-economic development of the country and the availability of new methods against parasites and vectors. During the years of the eradication campaign (1956-1960), which consisted mainly of insecticide spraying all houses in the malarious area, the lowest number of cases was registered. In 1959 the eradication program achieved its maximum effect. The reduction in the number of cases was the result of good control in localities where the conditions for parasite transmission were precarious and in other localities with favorable but not optimal environment for transmission. However, only a moderate control was obtained in those areas where the ecologic conditions were favorable for the vectors. These locations became finally permanent residual malaria foci.

Since 1960, the number of malaria cases has begun to increase. In 1970 more than 57,000 cases were recorded and in the following 15 years, malaria morbidity reached epidemic proportions. During this period, the number of cases increased 5 to 6-fold, with a maximum of 133,698 cases in 1985. This figure represents the highest number of cases recorded since the initiation of antimalarial activities. In spite of the increase in the number of cases, the last death caused by malaria (an infection with *Plasmodium falciparum*) was seen in 1981.

It has been argued that the decline in spraying operations was the cause of malaria resurgence. The spraying index (IRC or number of sprays per 1,000 people) was reduced from 91.49 in 1972 to 3.54 in 1985. The slope of this reduction through time is very steep; however, the number of cases began to increase considerably in 1981, when the IRC reached 16.03. It is probable that in many areas, control measures had been successful in interrupting malaria transmission without completely eliminating the vector and the re-introduction of people with malaria in these areas was sufficient to initiate the epidemic.

Going back to 1970, when the rise of cases was evident, following the recommendations of W.H.O., the malaria problem was approached on a regional basis according to epidemiological, operative and administrative criteria. As a result, control measures were intensified in the Gulf of Mexico and the Yucatan Peninsula, but the activities in the Northwestern and Southeastern Pacific Ocean regions were not modified.

The world-wide malaria situation was at that time also clearly deteriorating. The spectacular result of eradicating malaria in 37 countries was achieved with the application of tremendous amounts of insecticides and the massive distribution of antimalarial drugs. This had some prices: the appearance of resistance of the vectors to the former and of the parasites to the latter. Technical problems appeared conspicuously and the number of cases (without considering the Africans) rose from slightly more than 3 million in 1972 to more than 10.7 million in 1977. Latin America, with its increasingly demanding economic problems, was not the exception.

Since 1985, in spite of the country's economic crisis, the operational budget was increased from

2,480.6 million pesos provided in 1985, to 26,674.6 million pesos in 1988; representing more than a ten-fold increase. The number of houses sprayed increased almost two-fold during the same period. In addition, coverage levels that were around 40% during 1984 and 1985 have recovered to levels of over 85%.

The number of recorded cases indicates that the epidemic was controlled since 1986. As a result of the control measures, malaria transmission has been focused again in areas with different ecological conditions. To date, almost 70% of all recorded malaria cases are limited to a territorial extension equivalent to less than 20% of the Country involving 13 states. In these states, malaria is transmitted in nearly 150 municipalities (less than 10% of all municipalities in the country). There are around 40,000 localities in these municipalities and a population at risk of around 15 million people. But it should be noted that from the total number of localities at risk, only one fourth (less than 10,000) had malaria cases in 1987. The average parasite incidence in this group of municipalities is approximately five times higher than the incidence in the rest of the country. On the other hand, the number of localities informing of cases (an indicator of malaria dispersion) is increasing, indicating the need to extend the control measures even more.

Malaria vector control with indoor application of residual insecticides is the principal measure. DDT spraying, the insecticide most widely used, has increased over the last four years, reaching a coverage of more than 1 million houses. The consumption of other insecticides has also increased, although in smaller proportions than DDT; among these insecticides, bendiocarb, malathion and fenitrothion are the most important. Resistance of anopheline vectors to DDT, organophosphates and carbamates has been documented in a few places in the Pacific Ocean coast.

Some state control programs had started to diversify control measures incorporating larvacide applications, mass and selective drug administration, some forms of spatial spray and environmental management with community participation to reduce vector breeding sites. Larvacides currently in use are organophosphate compounds (temephos and fenthion). Larvicidal treatments are particularly used in large population settlements with economic of tourist importance where breeding places are clearly identified but not amenable to elimination. Mass drug administration (as part of other control measures) is provided to localities or groups of localities whose annual incidence is higher than 5%. In spite of the multiple control alternatives, to date, all intents of integrated control have been isolated efforts and are so far not significant.

The effectiveness of control measures can be assessed by the reduction in the observed number of cases with respect to the number expected if the trends were maintained. Thus, using a mathematical model, it can be estimated that the application of extraordinary measures since 1985 produced an overall 45% reduction in the number of expected cases for 1988. Regionally, the levels of reduction were variable: 80% in the Gulf of Mexico and Yucatan Peninsula and 35% in the Pacific Coast. In the Central and Northern areas the number of observed cases was practically that which was expected. The comparison of the incidence between 1987 and 1988 in individual states, indicates that there was a reduction in all the states along the Gulf of Mexico, in Morelos and Hidalgo in the Central area, and in Chiapas and Sinaloa on the Pacific Coast. In all these areas, excepting Sinaloa and Morelos, *Anopheles albimanus* Wiedemann is the main vector.

Six of the states involved in the epidemic (Chiapas, Oaxaca, Sinaloa, Michoacan, Guerrero and Nayarit) have recorded most of the cases since 1972. There was also an evident increase in another four states (Campeche, Veracruz, Tabasco, Quintana Roo) in 1982, which eventually reached almost 40,000 cases in 1985 (30% of the total). The epidemic began in Chiapas two years before it reached the rest of the Country. It can be suggested that population movements to the coastal area of Chiapas from other malarious areas in Central America could have been responsible for the introduction of significant number of cases.

Twenty-five anopheline species have been identified in the malarious areas of Mexico. Among these, *Anopheles pseudopunctipennis* Theobald and *An. albimanus* have been involved as the main malaria vectors. *An. pseudopunctipennis* is the most widely distributed species and has been found from sea level up to 2,000 meters in altitude. It is found in three quarters of the whole malarious area and with the exception of the coastal area of Chiapas, this species is the most important vector along the coast of the Pacific Ocean. *An. albimanus* is found from sea level up to 600 meters in altitude. It is widely distributed in the coastal plains of the Gulf of Mexico and the Yucatan Peninsula and the rain forest and coastal plains of Chiapas. Although these two species have traditionally been considered uniform populations, their extensive geographical distribution and the diverse ecological systems where they are found, have conditioned the

appearance of regional variations with regard to their behavior, survival and vectorial capacity. Recently, *Anopheles vestitipennis* Dyar and Knab has been incriminated as the main vector of *Plasmodium vivax* during the rainy season in the Lacandon Rain Forest of Chiapas.

Three different human plasmodia species are found in Mexico: *P. vivax*, *P. falciparum* and *P. malariae*. *P. vivax* has been, by far, the principal cause of malaria in the country, with more than 95% of the recorded cases. The great majority of the cases due to *P. malariae* (on the average less than 20 cases per year) have been diagnosed in the hospitals among blood donors or in people with a history of blood transfusion.

The transmission of *P. falciparum* is limited to the southeastern states, in order of importance: Chiapas, Tabasco, Campeche, Veracruz and Oaxaca. Infections due to this parasite have always represented less than 5% of the total number of cases. Since 1978, nearly 90% of these cases have been registered in Chiapas. Malaria incidence due to this species has followed similar epidemic trends as *P. vivax*, but with more drastic changes. Increases in the number of cases was registered in 1970, 1978, 1983, followed by immediate reductions when control measures were intensified. Between 1976 and 1977, only one case of *P. falciparum* malaria was diagnosed in the Country; however, in the middle of 1978 this parasite was re-introduced in the coast of Chiapas, with 200 recorded cases. The problem in the coast was reduced gradually and the last case in the area was seen in 1987. Between 1982 and 1983, a new activation (reaching 1,554 cases) was the result of a massive arrival of refugees from Central America to the Lacandon Rain Forest. Refugee camps were re-settled in other areas but transmission, although reduced, persists in this zone. To date, the clinical response of *P. falciparum* infected cases to chloriquine treatment has not shown evidence of resistance to this drug.

Some serological studies in different areas with persistent transmission indicate that, in spite of their proximity, only some localities have evidence of transmission. Additionally, population antibody patterns suggest that transmission follows epidemic cycles, which produce age-independent or age-dependent patterns according to the duration of epidemics and the intervals between them. During these epidemics all age-groups are affected, suggesting that all groups are exposed to the same risk in a given time and place.

It is important to mention that the presence of vectors which seldom feed on man (like *An. pseudopunctipennis, An. vestitipennis,* and *An. albimanus*), the existence of relatively high anopheline densities, anophelism without malaria, drastic seasonal changes, malaria incidence fluctuations and a variable immunity as result of changes in endemic conditions, are some factors which provide an unstable pattern to malaria transmission in Mexico.

The geographical distribution of malaria includes practically all ecological regions: coastal plains, rain forests, river valleys and mountain ranges. Several epidemiological patterns can be identified in these regions. For instance, one epidemiological pattern where malaria is transmitted by *An. albimanus*, is represented by the region of the Pacific coastal plains in the state of Chiapas (Pacific Coast). A second one where *An. pseudopunctipennis* is the vector, is represented by the foothill areas of the Western Sierra Madre Mountain range in the states of Sinaloa and Chiapas (Foothills). And finally, the rain forest areas, represented by the northeastern part of the state of Chiapas (Lacandon Forest) where *An. vestitipennis* is the main vector. These epidemiological regions are considered the most important in terms of malaria incidence.

The Pacific Coast in the state of Chiapas is a largely modified area devoted to agriculture and cattle raising. It has a hot sub-humid climate, with an average rainfall of 2,152 mm. The agricultural land is used for the production of bananas, soy-beans, sesame and fruits. Potential sources for anopheline breeding are varied and include large marshes, fresh water lagoons and an extensive canal system used to irrigate banana plantations. The excess irrigation water, along with fresh water lagoons provide ideal sites for mosquito breeding during the dry season and although the rainy season only extends from mid-May to October, significant mosquito breeding occurs year-round. *An. pseudopunctipennis, An. vestitipennis, An. punctimacula* Dyar and Knab and *An. albimanus* have been found in the area, but the latter is by far the most abundant. *P. vivax* is actively transmitted in this area year-round, however, its incidence peaks during the rainy season.

The Foothills of Chiapas are also a modified area devoted mainly to agriculture. They are located at altitudes between 50 and 350 meters. They have a hot and humid climate with an average rainfall of 3,800 mm. Coffee plantations conform the characteristic landscape. the relative numbers of mosquitoes increase during the dry season (November to May). *P. vivax* is the only parasite transmitted in this area, and *An. pseudopunctipennis* appears as the most important vector. Although the

Pacific Coast and the Soconusco are contiguous regions, they have distinct malaria histories, with long-term persistence in the former and only a recent outbreak in the latter. In addition, the incidence of malaria in the Pacific Coast tends to increase with rainfall, while cases are concentrated in the dry season in Soconusco.

The Lacandon Forest is located in northeastern Chiapas at a mean altitude of 500 m, in an area of rolling hills on the eastern slopes of the Sierra Lacandon mountain range. It is a very humid rain forest, with a mean annual temperature and relative humidity of 26° C and 85%, respectively, and a mean rainfall of 3,000 mm. the rainy season occurs from mid-May to early November, when anopheline density levels are also highest. A vast area in this region is experiencing considerable environmental changes due to agricultural expansion, extensive cattle raising and forestry. The majority of the inhabitants are settlers from different endemic and non-endemic areas of Mexico that began inhabiting the area during the last 15 years in small and scattered villages (particularly along river courses). Malaria epidemics have been reported to coincide with the arrival of both new settlers and refugees from Central America. Both *P. vivax* and *P. falciparum* are actively transmitted in this area, in spite of the use of traditional control measures (indoor spraying with DDT and mass drug administration). Preliminary reports from entomological studies have confirmed the presence of *An. albimanus, An. darlingi* Root, *An. pseudopunctipennis, An. punctimacula*, and *An. vestitipennis*. As mentioned before, *An. vestitipennis* has been incriminated as the main vector of *P. vivax* during the rainy season, which is also the period of higher malaria incidence. This information has just recently been incorporated into mosquito control measures in order to further the efforts of the Malaria Control Program in Mexico.

IXODES PACIFICUS AND LYME DISEASE IN ORANGE COUNTY, CALIFORNIA, 1989

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The Orange County Vector Control District (OCVCD) began studies to determine the presence and distribution of ectoparasites including *Ixodes pacificus* Cooley and Kohls in Orange County in the winter months of 1983-1984 (Geck et al. 1984); only *Ixodes spinipalpis* Hadwen and Nuttall was recovered. In a later study three nymphal and one larval *I. pacificus* removed from an alligator lizard (*Gerrhonotus multicarinatus* (Blainville)) taken along Laguna Canyon Road (Laguna Beach) were examined for spirochetes under darkfield microscopy and were found to be negative (Webb and Bennett 1984, unpubl. data).

In 1986, Sergent conducted a survey of ticks in Orange County and tested 201 of them for *Borrelia burgdorferi* Johnson, Schmidt, Hyde, Steigerwalt and Brenner using darkfield microscopy methods. All were negative for spirochetes; *Dermacentor occidentalis* Marx (101 males, 83 females), *Dermacentor variabilis* (Say)(4 males, 1 female), *Ixodes pacificus* (4 males, 2 females) and *Argas cooleyi* Kohls and Hoogstraal (6 nymphs).

In 1987, Medina and Webb (unpubl. data) processed 161 *I. pacificus* (42 males, 119 females) collected in January, February and March from three Orange County localities. All specimens were negative for spirochetes. Clover (1987, unpubl. data) also obtained negative spirochete data from 79 *I. pacificus* specimens from three separate sites.

In 1988, Ryan (1989) tested 100 ixodid ticks for *B. burgdorferi* using darkfield microscopy, inoculation of tick midguts into BSK medium, and indirect fluorescent antibody (IFA) test. None, including 11 *I. pacificus*, were positive for spirochetes.

Table 1 summarizes the tick species tested for *B. burgdorferi* spirochetes by the OCVCD laboratory from 1984 through 1989. Table 2 outlines the results of IFA analysis of sera obtained from mule deer taken in Orange and San Diego Counties. Seven of nine deer sera indicated significant positive *B. burgdorferi* seroconverisons. Although these results may represent a cross reaction with related *Borrelia* species, further investigative field studies are needed to determine the presence or absence of *B. burgdorferi* in Southern California.

During the course of the tick and small mammal collection, it became clear that the most efficient system for surveillance in Orange County is to trap small mammals and test their sera for *B. burgdorferi* antibodies. Trapping, bleeding, and sera analysis takes significantly less time than flagging and lab analyzing the ticks. Table 3 outlines the protocol for a surveillance system for *B. burgdorferi* in Orange County.

Acknowledgements.

Our gratitude is extended to a number of people who were instrumental in collecting ixodid ticks and/or their hosts. They are Rudy Geck and Gary Reynolds (Orange County Vector Control District), Vern Reichard and James Clover (California Department of Health Services), John Kirchberg (California State University, Long Beach), and Slader Buck (Natural Resources Department, Camp Pendleton, USMC Base, San Diego County, California).

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Table 1. Orange County tick species collected and tested for *Borellia burgdorferi* (1984-1989). DF= Darkfield microscopy. DFA= Direct fluorescent antibody test.

Species	Larvae	Nymphs	Adults		Total	*B. burgdorferi* results	
			Males	Females		DF	DFA
Ixodes pacificus	5	7	47	49	108	0	0
Dermacentor occidentalis	28	0	100	130	258	0	0
Dermacentor variabilis	0	0	4	3	7	0	0
Dermacentor albipictus	0	0	(19)*		19	0	0
D. albipictis nigrolineatus	0	0	3	0	3	0	0
Argas cooleyi	0	6	0	0	6	0	0
Totals	33	13	154	(19)* 182	401	0	0

*Adults; sexes not determined.

Table 2. Results of IFA seroanalysis of mule deer (*Odocoileus hemionus*) in Orange and San Diego Counties, California, 1989.

Locality - Date Collection Number	IFA titer
Orange County	
Crow Canyon - 10/7/1989	
#1	1:128
#2	1:128
Fox Canyon - 9/10/1989	
#1	1:128
#2	1:256
San Diego County	
Camp Pendleton - 9/10/1989	
#116	1:128
#117	1:256
#118	1:64
#123	1:512
Camp Pendleton - 9/17/1989	
#141	1:512

Table 3. Detection system for *Borrelia burgdorferi* in Orange County, California.

I. Preliminary tick (*Ixodes pacificus*) collection.

II. If area positive for *I. pacificus*, then small mammals (*Peromyscus* spp.) are trapped.

III. Small mammal sera IFA-tested.

IV. If mammals positively seroconvert, then ticks are extensively collected.
   A. Removed from mammal hosts.
   B. Flagged from vegetation.
   C. CO_2 trapped.

V. Test ticks for spirochetes.
   A. Direct fluorescent antibody test.
   B. Darkfield microscopy.
   C. BSK medium inoculation.

DISTRIBUTION OF HEARTWORM IN CALIFORNIA DOGS AS RELATED TO MANAGEMENT, ELEVATION, GEOGRAPHY AND VECTOR DISTRIBUTION

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Introduction.

Since its introduction into the Gulf Coast region of North America, the filarial nematode, *Dirofilaria immitis* (Leidy), has spread across the continent until it now can be found throughout all fifty states and Canada. This nematode first migrated up along the Atlantic seaboard, slowly expanding its range westward into the Central, Plains, and Rocky Mountain States and on into Canada. The parasite has more recently appeared in California and Hawaii and has become endemic throughout the West Coast.

Within the last 20 years the coastal, foothill, and valley regions of northern California have become enzootic for *D. immitis* and its accompanying disease, canine cardiovascular dirofilariasis (dog heartworm). Filariasis was first isolated in native California dogs in 1946 (Roberts and Roberts 1946), but few actual cases were seen until the 1970's. Weinmann and Garcia (1980) were able to show that the native coyote population in northern California was harboring the parasite and was probably acting as a reservoir of infection for domestic dogs. Soon thereafter, Walters and Lavoipierre (1982) isolated advanced stages of the filarial larvae from vector mosquitoes in northern California, and thus established that the disease had become enzootic.

Dog filariasis in California is due to two worms, *Dipetalonema reconditum* and *Dirofilaria immitis*. The former species is a flea-transmitted pathogen which is generally not considered very pathogenic. However, it is quite similar to *D. immitis* in appearance and careful microscopic identification with the proper diagnostic techniques is necessary to differentiate between the two.

The second species, *Dirofilaria immitis*, is a mosquito-transmitted pathogen which occurs in dogs and other canids, occasionally in cats and rarely in humans. Adult worms, measuring 12-20 cm in the male and 25-31 cm in the female, are found in tangled, string-like masses in the right ventricle of the heart and in the pulmonary artery. They restrict the circulation, leading to loss of exercise tolerance, chronic cardiac insufficiency and heart failure. Infected dogs often become more sluggish as the disease progresses, and many dogs die of asphyxia, embolism, or dilation of the heart. The adult worms release tiny worms called microfilariae into the circulating blood of the host dog. These unsheathed microfilariae show a nocturnal periodicity in the circulating blood with the major number of larvae in circulation between 10:00 p.m. and 2:00 a.m.

When the microfilaria are ingested by mosquitoes they escape from the midgut into the haemocoele and develop in the Malpighian tubules. This is in contrast to the human filarial worms, *Wuchereria bancrofti* and *Brugia malayi,* which develop in the mosquito's thoracic muscles. Development is completed in a temperature-dependent 8-16 days at which time the infective larvae migrate into the head and enter the labium from which they escape when the mosquito is feeding. Passage of the infective larvae is through contaminative transmission whereby the infective larvae are deposited on the surface of the vertebrate host's skin in a pool of hemolymph and later enter through rubbing or scratching actions of the host or through their own migratory abilities. Mature worms reach the heart in three to four months and microfilariae are produced in six to eight months.

While earlier studies (Acevedo and Theis 1980; Walters et al. 1981; Walters and Lavoipierre 1984; Wright and Boyce 1989) have focused on the incidence, prevalence and distribution of canine

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heartworm in limited geographic areas of California, the present analysis represents three years (1984, 1985, 1986) of an ongoing study designed to examine the geographic, spatial, and temporal distribution of canine heartworm throughout California.

Methods.

Collection of data concerning the prevalence of dog heartworm in California was routinely performed by the Pet-Prevent-a-Care Mobile Vaccination and Diagnostic Clinic, headquartered in Santa Rosa, California. The survey considered all dogs treated at the mobile clinic and was composed of two parts. The first part consisted of a standard acid phosphatase diagnostic test which was coupled with a nuclepore filter. This test was used because it gave concentration sensitivity and specificity. The staining reaction for *D. immitis* and *D. reconditum* was very distinct and easily allowed differentiation between the two.

The second part of the survey consisted of a standard questionnaire for the dog owners to complete. In addition to questions on the geographic location of the owner's residence the questionnaire sought additional information on the age, sex, and breed of the dog; whether it had ever been administered anti-filarial preventative medicine or not; whether the dog had any recent travel history out of the county, state or nation; and whether the dog typically spent all its time indoors, outdoors or was allowed outside during the day or night only.

Results and Discussion.

As mentioned before, this study is continuous and ongoing, the data analyzed here represents a three year segment (1984-1986) of the study period. During this period, a total of 18,759 dogs were examined by the mobile clinic for filariasis and are thus the basis for this analysis.

Diagnostic separation of *D. immitis* and *D. reconditum* infections shows a relative difference in infection rate based on age of the dog (Fig. 1). Filarial infections with *Dipetalonema reconditum* are more likely to occur earlier in the dog's life than infections with *Dirofilaria immitis* but after one year of age the prevalence of *D. reconditum* infections are lower than that of *D. immitis* throughout the dog's lifetime. In some cases this may be due to the infection of the newborn puppies from the mother's infected fleas while still relatively confined in the safety of the nesting area. It is not until the puppies are old enough to roam about, particularly

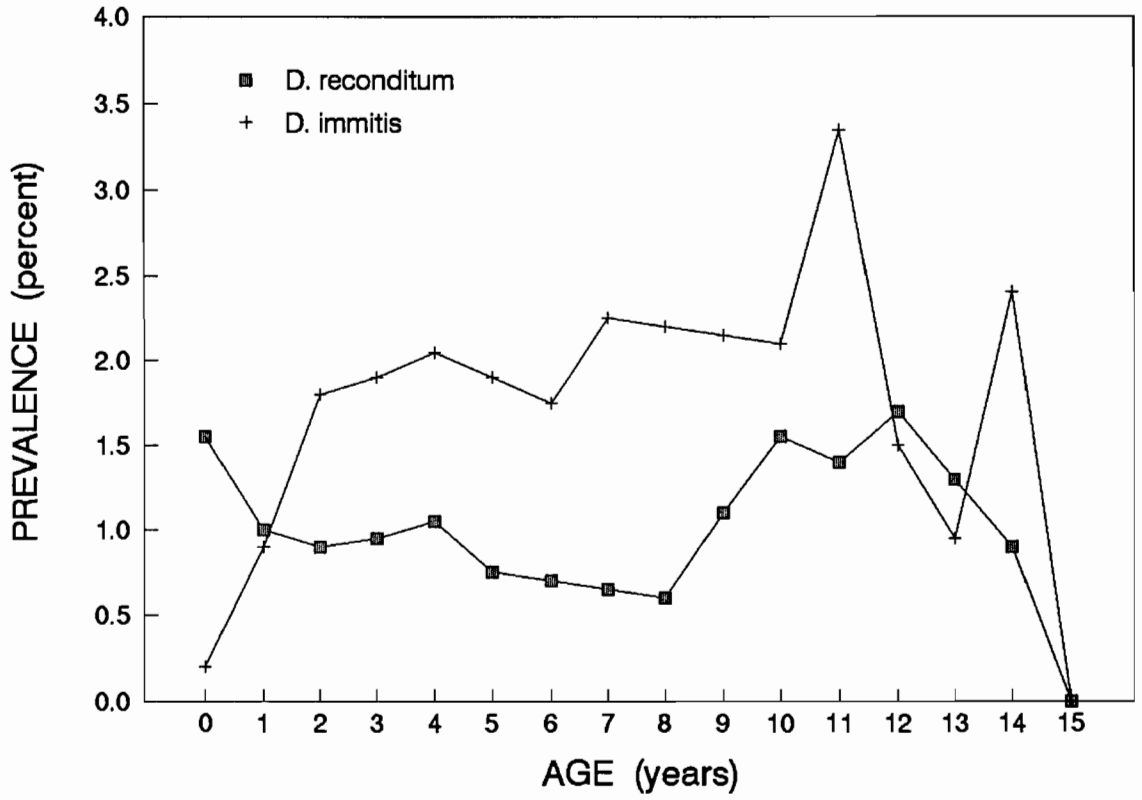

Figure 1. Comparison of *D. immitis* and *D. reconditum* infections in California dogs, 1984-1986.

at night, that they stand a greater chance of encounters with vector mosquitoes and thus are at risk of infection with *D. immitis*. The prevalence of *D. reconditum* infections may drop off during the first year because of the dispersal of these newborn puppies to different environments either without the necessary infected fleas or with greater sanitation measures (i.e., flea control). Since infections with *D. reconditum* are generally not considered very pathogenic, we focus the remainder of our analysis to filarial infections with *D. immitis*.

Of the total of 18,759 dogs examined for filariasis over the three year period, there was an overall prevalence rate of 1.8% (Fig. 2). This overall rate dropped from a high in 1984 of 2.1% to a low in 1986 of 1.4%. While not statistically different from each other, the overall prevalence rates in 1984 and 1985 were statistically greater than that observed in 1986 ($P>0.01$). This was probably not so much an indication that filariasis was on the decline as much as it was an indication that the drought experienced in California was having an effect on the production and availability of vector mosquitoes. Indeed, many mosquito abatement districts across the state reported a reduction in mosquito activity starting in 1986. The underlying cause was presumed to be the drought situation.

These data do not support the opinion at the time expressed in the news media that dog heartworm was on the increase in California. It may be that with the diminished vector populations, more publicity and hence recognition was devoted to the "secondary" public health concerns.

In examination of the effects of various factors in determining the infection risk to any particular dog, there was no significant difference between male and female dogs so they were combined in the statistical analyses. This is somewhat different than the findings of Wright and Boyce (1989) who found a slightly greater infection rate in male dogs and attributed it to increased nocturnal roaming in defense of territorial areas.

During the three year period, only 12.6% of all dogs were given preventative medicine by their owners. The prevalence rate in dogs stated by their owners to be on preventative medicine was about 0.8% during the three years. The prevalence rate in dogs not on preventative medicine was about 2.0% during the same period.

The fact that a small percentage of dogs whose owners had stated were on preventative medicine still became infected is probably not as much an indication of the failure of the prophylactic drug therapy as much as it's a reflection of the owners

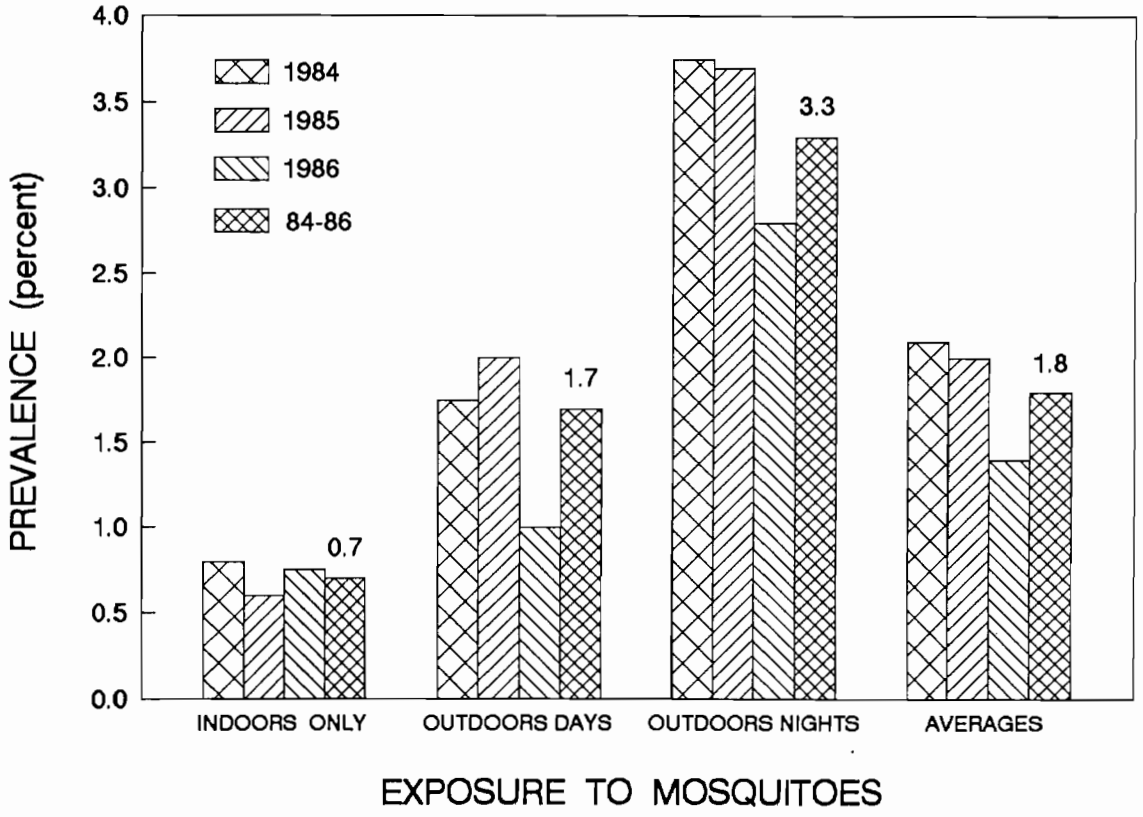

Figure 2. Prevalence of *D. immitis* in California dogs based on exposure to mosquitoes, 1984-1986.

negligence in strict administration of the drugs. At the time of the study D.E.C. was the only anti-filarial drug available. Ivermectin (Heart Gard) was not on the market until May, 1987. D.E.C., while effective, requires a daily dosage and as few as two or three days of missed therapy could result in infection.

Of all the factors examined, the one with the greatest influence on infection prevalence appears to have been whether the dog was allowed outdoors for extended periods of time, particularly at night (Fig. 2). Of those dogs kept indoors all the time the infection rate was only 0.7% (n = 5,708), while those dogs kept outdoors both day and night experienced the highest infection rate of 3.3% (n = 4,670). The dogs kept outdoors during the day but allowed indoors at night experienced an intermediate infection rate of 1.7% (n = 3,671). This overall trend of greater at-risk status for those dogs kept outdoors both day and night was also seen by Wright and Boyce (1989).

There also appears to be a differential risk factor to dogs based upon the elevation of their residence with the highest prevalence rates at elevations that encompass the foothills of the Coastal and Sierra Nevada Mountain Ranges (400-899 meters). Valley floor and coastal elevations show much lower prevalence rates. High elevations, above 1,000 meters, also show much lower prevalence rates.

There was a markedly distinct difference in the geographic distribution of infections throughout the state (Fig. 3). The foothill communities in northern California had a much higher prevalence rate than the valley, urban, coastal and southern communities. These prevalence rates ranged from 8.4% in Butte County to 0% in Los Angeles County. One hypothesis is that the greater infection prevalence rates seen in the foothill communities can be attributed to the greater overall abundance of vector mosquitoes (Table 1). With the single exception of Marin-Sonoma Counties, this trend held for all the areas examined. The lack of adequate trapping methods for *Aedes sierrensis* (Ludlow), the principle filarial vector in much of California, may explain why Marin-Sonoma Counties do not fit the expectations. In fact, mosquito collections in California, in general, are not aimed at *D. immitis* vector populations.

Of the more than 200 mosquito species known to support *D. immitis* development, 12 are found in California. Of those, only six or seven species are likely to comprise major vectors of *D. immitis*. These species are: *Aedes sierrensis*, *Aedes vexans*

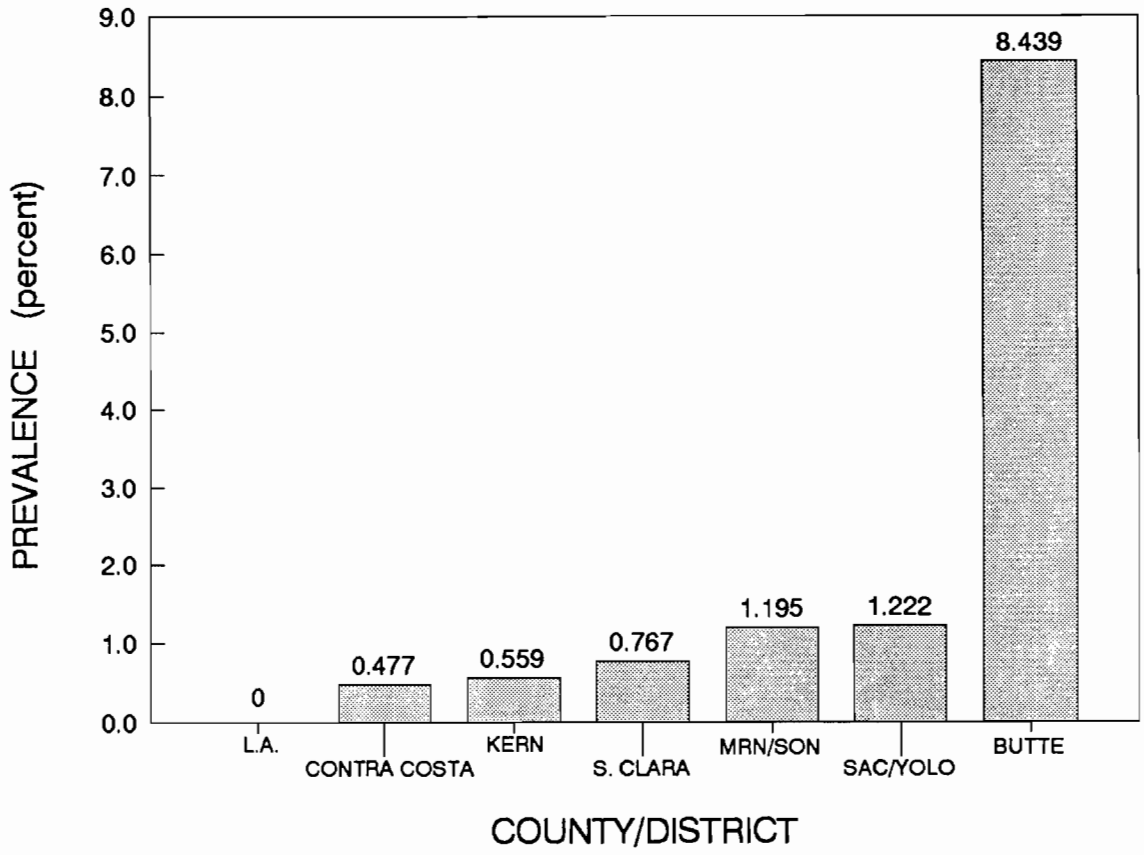

Figure 3. Prevalence of *D. immitis* in California dogs based on geographic region, 1984-1986.

Table 1. Relationship between dog heartworm (*D. immitis*) prevalence and vector mosquito abundance for seven geographical areas of California.

County(s)	Mosquitoes/ trap-night	Prevalence (%)
Butte	43.4	8.4
Sacramento/Yolo	4.7	1.2
Santa Clara	4.5	0.8
Kern	2.5	0.6
Contra Costa	2.1	0.5
Los Angeles	0.7	0
Marin/Sonoma	0.3	1.2

Meigen, *Anopheles freeborni* Aitken, *Anopheles punctipennis* Say, *Culex pipiens pipiens* L., *Culex pipiens quinquefasciatus* Say, and *Culex tarsalis* Coquillett.

The relationship between *D. immitis* and its mosquito vectors is complex. The microfilariae have not only a nocturnal periodicity which coincides with the feeding habit of many of its vectors, but in northern temperate regions it also has a seasonal cycle with a five- to ten-fold increase in microfilariae in the circulating blood in August and September, when some mosquito species are most abundant.

It is hoped that through a complete statewide survey of filariasis such as this, the complex relationship between *Dirofilaria immitis*, host dogs and the vector mosquitoes will become a little clearer.

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THE *AEDES ALBOPICTUS* PROBLEM IN THE UNITED STATES: MYTH OR REALITY

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ABSTRACT

Aedes albopictus was introduced into the United States sometime in the early 1980's. By the time the infestation was discovered in 1985, the species had already become wide spread in the country. The Centers for Disease Control responded by implementing programs to prevent further importation of mosquitoes in used tires, to reduce the spread of *Ae. albopictus* via interstate commerce, to improve surveillance, to increase research on the public health impact of *Ae. albopictus* in the United States, and to work with the Pan American Health Organization (PAHO) to prevent the export of *Ae. albopictus* to other countries in the region. A field laboratory was established in New Orleans to carry out research on behavior, biology and control of *Ae. albopictus*.

It is clear now that *Ae. albopictus* is well established and will not be eradicated from the country in the near future. However, the public health risk of *Ae. albopictus* may not be as great as predicted by some. Dengue is the most frequently mentioned disease threat. Nearly all major epidemics of this disease in the world, however, have been transmitted by *Aedes aegypti*, which has been present in the United States for over 200 years. In areas where introduced *Ae. albopictus* has replaced *Ae. aegypti* such as Hawaii and Guam, there have been no recent dengue epidemics. In terms of epidemic dengue transmission, therefore, the collective data suggest that we are at lower risk with *Ae. albopictus* than with *Ae. aegypti*.

On the other hand, the presence of this species in La Crosse virus endemic areas could increase the risk of human infection with that virus.

There is no doubt that the presence of *Ae. albopictus* in the United States poses a potential public health problem. The Centers for Disease Control recognize that potential and are working to decrease the risk. However, shrinking resources must be balanced with other health priorities such as Lyme disease.

MOSQUITOES OF NEVADA - PAST AND PRESENT

Harold C. Chapman[1] and Richard C. Hicks[2]

PAST

I was attached to the University of Nevada in Reno as a USDA/ARS medical entomologist from 1958-61. During those three years I had a position that many young entomologists dream about. The boss (Gaines Eddy) was far away in Oregon, I could do any kind of mosquito research I wanted to and I had to only please one technician. I wriggled into many abandoned mines, often climbed the Sierra Nevada Mountains in the west and the Ruby Mountains in the east, and visited the Amargosa Desert in the south. The mean elevation in the principal valley of Nevada averages 4,000-5,000 feet with other elevations ranging from 1,000 feet in the south to about 13,000 feet in the mountains. Except in the south, most precipitation occurs as snow in the winter.

During my three years in Reno, I covered many aspects of mosquito research including their distribution, ecology, biology and taxonomy which ultimately resulted in 13 publications and one bulletin.

Twenty-three mosquitoes were recorded from Nevada prior to my arrival. Another eight species were added during my tenure which were *Aedes communis, Ae. hexodontus, Ae. nevadensis, Ae. pullatus, Ae. schizopinax, Culex apicalis, Cx. territans* and *Psorophora confinnis.* Thus, 31 species were listed in my bulletin entitled "The Mosquitoes of Nevada" that was published in 1966. Three other mosquitoes have since been reported from Nevada and include *Culex quinquefasciatus, Culiseta alaskaensis* and *Psorophora signipennis*.

Probably the most interesting observations to me during these three years were the overwintering of large larval populations of *Culex erythrothorax* and the presence of autogeny in populations of 10 species of mosquitoes (*Aedes* -7, *Culiseta* -1, and *Culex* -2).

PRESENT

The distribution of mosquito species within the state is still poorly known. The counties possessing the most species (Clark, Douglas, Lyon, Ormsby and Washoe) are those nearest to my former headquarters in Reno and to Richard Hicks' headquarters. More distant counties, such as Esmeralda and White Pine, have only four and five species reported, respectively. Only *Culex tarsalis* and *Culiseta inornata* have been collected from all 17 counties.

The habitats that produced the most species of mosquitoes in the late 1950's in descending order were snow-melt in the mountains, irrigation, permanent ponds and streams and freshwater springs. Often the number of species is relatively unimportant and Nevada is no exception, since many of the species may be rare or non-biters of man and animals. The most pestiferous biting mosquitoes in Nevada, then and probably now, are *Aedes dorsalis, Ae. nigromaculis, Ae. melanimon* and *Ae. vexans* (all multivoltine) which result from irrigation in the valleys. Acreage under irrigation has only increased by 44,000 acres, from 735,000 in 1960 to the present total of 779,000 acres.

Hikers and campers might dispute the importance of these four multivoltine *Aedes* species since seven univoltine *Aedes* species (*cataphylla, communis, fitchii, hexodontus, increpitus, nevadensis* and *pullatus*) are often severe biters in the Sierra Nevada or Ruby Mountains.

A habitat not abundant in the 1950's, but which has become much more common in the past 20 years (according to Richard Hicks) is polluted waters around urban areas. This apparently has caused a large increase in the numbers of *Cx. quinquefasciatus* and *Cx. stigmatosoma* and a corresponding decrease in populations of *Cx. tarsalis*. This change in species abundance has resulted in much fewer human complaints, presumably due to their lesser biting habits, especially by *Cx. stigmatosoma.*

Richard Hicks has also noted that *Ps. toltecum,* which I first collected in Clark County in 1959 (as *Ps. confinnis*), is still a rare species and has not become a serious biting pest. Perhaps Nevada needs to plant and irrigate date groves like California to enhance populations of this species.

It is obvious that a number of other mosquitoes should occur in Nevada. *Culex pipiens* is reported from all northern and central states

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except Nevada and surely must exist in urban areas in the north and central part of the state. Both *Aedes sticticus* and *Coquillettidia perturbans* are known from all of the continental states except Arizona and Nevada. *Culex restuans* is reported from all five states that border Nevada. *Orthopodomyia signifera* and *Aedes flavescens* occur in all of the bordering states except Idaho and Arizona, respectively. *Aedes ventrovittis* is common in the Sierra Nevada Mountains of California and should occur in those same mountain ranges in Nevada.

Numerous name changes of Nevada mosquitoes have occurred since the 1960's and 1970's, thanks to busy taxonomists and are as follows: *Aedes nevadensis* for *Ae. communis nevadensis*; *Anopheles franciscanus* for *An. pseudopunctipennis franciscanus*; *Culex quinquefasciatus* for *Cx. pipiens quinquefasciatus*; *Cx. peus* for *Cx. thriambus*; *Cx. stigmatosoma* for what was called *Cx. peus*; and probably *Ps. toltecum* for *Ps. confinnis*.

There are probably six counties (Clark, Churchill, Douglas, Lyon, Humboldt and Washoe) with at least small mosquito control districts whereas only one existed in the early 1960's.

My single biggest disappointment (after the fact) during my three years in Nevada was that I was not aware of and lacked the ability to detect larvae patently infected with pathogens and parasites. In 1961, I was assigned by the USDA to the California State Department of Health Laboratory in Fresno which was directed by Ralph Barr and later by Bill Kellen. In the early 1960's, the laboratory staff of Bill Kellen, Truman Clark and Bill Wills pioneered studies on pathogens and parasites, particularly microsporidia (Protozoa) that infected mosquitoes. After I learned what infected larvae looked like, I made several quick trips back into Nevada and collected six species infected with microsporidia, then called *Thelohania*, but now referred to as *Amblyospora* and one species infected with an iridovirus (MIV). I certainly missed out on a great opportunity to determine the abundance and distribution of such diseases in the mosquito fauna of Nevada.

RECENT ADDITIONS TO THE MOSQUITO FAUNA OF SOUTHEASTERN CALIFORNIA

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Mosquito and arbovirus surveillance along the lower Colorado River in recent years has resulted in the discovery of isolated populations of *Aedes (Kompia) purpureipes* Aitken and *Aedes (Ochlerotatus) thelcter* Dyar that are apparently unique to that region of southern California. Captures of adult females collected by various CO_2- baited traps have been reported by Jakob et al. (1985) and Meyer et al. (1987) for *Ae. purpureipes* and by Meyer et al. (1988) for *Ae. thelcter*. The discovery of these two species has increased the number of currently recognized species indigenous to the state of California to fifty-one. This paper summarizes the distribution of these species in North America, identification, habitat associations, seasonality, larval breeding requirements, and associated mosquito species.

The occurrence of *Ae. purpureipes* and *Ae. thelcter* along the lower Colorado River has resulted in a westward range extension of *Ae. purpureipes* by ca. 300 km (Meyer et al. 1987) and *Ae. thelcter* by ca. 750 km (Meyer et al. 1988). Collection records for *Ae. purpureipes* in California are from the Havasu National Wildlife Refuge opposite Needles, San Bernardino County. Additional specimens have been collected from scattered localities in LaPaz (Parker) and Yuma Counties (Yuma Proving Grounds), Arizona (Jakob et al. 1985). Records for *Ae. thelcter* in California include the Bard Valley (Haughtelin Lake and Winterhaven), Imperial County, and Blythe (S.L. Durso pers. comm.), Riverside County. Just recently, Maloney and Reid (1990) have reported the capture of three female *Ae. thelcter* in CDC traps baited with dry ice in the cantonment area of the Yuma Proving Grounds (20 km NNE Bard), La Paz Co., Arizona. Previous westernmost records for *Ae. purpureipes* have been from the Baboquivari Mountains in central Pima County, Arizona. *Aedes thelcter* has been taken as far west as the Trans-Pecos region of west Texas and extreme southeastern New Mexico (Darsie and Ward 1981).

Sporadic searches across the Chihuahuan Desert in New Mexico and Arizona (*Ae. thelcter*) and along the Gila River in Arizona (*Ae. purpureipes*) indicate that existing environmental conditions may not support colonization by either species (Miller et al. 1964, McDonald et al. 1973). Therefore, the distribution between populations along the lower Colorado River and eastern populations must be considered disjunct until populations are discovered that link demes from both geographic areas.

The number of adult females collected to date indicates that populations of both species are relatively small. This factor may have contributed to previous oversight or in the misidentification of *Ae. thelcter* as *Aedes vexans* (Meigen). At a glance, the female of *Ae. thelcter* can be overlooked among a number of female *Ae. vexans*. However, upon closer examination, gross differences become apparent. The legs of *Ae. thelcter* are counter-shaded with dark scales dorsally and light scales beneath the femur, and the tarsi lack the pale narrow apical bands that are characteristic of *Ae. vexans*. Dorsal abdominal markings also are quite different. The abdominal bands of Ae. vexans are emarginate (indented) while those of *Ae. thelcter* are broadly triangular and abdominal terga VI and VII are marked dorsally with an "hour glass" patch of pale scales. The female of *Ae. thelcter* is keyed in Darsie and Ward (1981) at couplet #64 (p. 64) using the above abdominal characteristics. Compared to *Ae. thelcter*, females of *Ae. purpureipes* are striking and cannot be confused with any other *Aedes* indigenous to California. The integument of the thorax is bright orange, pleural scale patches are silver and the legs are jet black with iridescent reflections (Carpenter and LaCasse 1955). The female of *Ae. purpureipes* is keyed at couplet #38 (p. 49) (Darsie and Ward 1981) on the basis of the absence of postspiracular setae.

The occurrence of either of these species in California is somewhat unexpected considering their ecological association with more mesic environments found in southeastern Arizona (*Ae. purpureipes*) and southern Texas (*Ae. thelcter*). Throughout its range in southeastern Arizona, *Ae. purpureipes* breeds in water that collects in tree holes and rot cavities that form in a variety of different tree species (i.e., hackberry, *Celtis*; sycamore, *Platanus*; oak, *Quercus*). Although larvae of *Ae. purpureipes* have yet to be collected from tree hole sources near adult capture sites along the Colorado River, Meyer et al. (1987) speculated that

larvae would most likely be found in tree holes associated with willows (*Salix*) growing near the river margin (Fig 1A). Willows are phreatophytic (i.e., capable of maintaining a relatively stable water level within rot holes and cavities) and plants growing near the river may receive ample water from the ground table to sustain the water level within either tree or rot holes. Other possible sources would be tree hole or rot cavities that form less frequently in mesquite (*Prosopus*), salt cedar (*Tamarix*), and palo verde (*Cercidium*).

Along the Pecos River in southeastern New Mexico, *Ae. thelcter* breeds in alkaline overflow pools in salt cedar marshes. Most captures of *Ae. thelcter* from the Bard Valley were at sites located next to the flood plane of the Colorado River (Fig 1B). Soils of the flood plane are highly alkaline and support a salt tolerant flora that includes, in addition to salt cedar, dense thickets of arrowweed (*Pulchea*) and saltbush (*Atriplex*). Like *Ae. purpureipes*, larvae of *Ae. thelcter* have not been collected from suspected breeding sources. Breeding undoubtedly occurs in the alkaline ground pools within the flood plane, however, the vegetation forms impenetrable thickets that are too dense to accommodate a thorough search for larvae.

All captures of female *Ae. purpureipes* and *Ae. thelcter* have been during the months of August and September coincidental with the period of the summer monsoon (Fig. 2). The region along the lower Colorado River receives the greatest incremental increase in seasonal rainfall during this period from isolated heavy thundershowers (Mallery 1936). Thus, the observed seasonality of *Ae. purpureipes* and *Ae. thelcter* is restricted to late summer as a consequence of the rainfall pattern and fortuitous flooding of alkaline ground pools and tree holes. Elevated late summer temperatures also are essential to assure that larval development is completed before floodwater sources dry between thunderstorms. Tree hole breeding requirements of *Ae. purpureipes* perhaps lessen the need for more frequent rainfall in comparison to the vulnerability experience by *Ae. thelcter* if floodwater sources dry before being recharged by runoff.

Females of *Ae. purpureipes* and *Ae. thelcter* have been collected in association with *Anopheles franciscanus* McCracken, *Aedes dorsalis* (Meigen), *Aedes taeniorhynchus* (Weidemann), *Aedes vexans* (Meigen), *Culex erythrothorax* Dyar, *Culex quinquefasciatus* Say, *Culex tarsalis* Coquillett, *Psorophora signipennis* (Coquillett), and *Psorophora toltecum* (Dyar and Knab)(= *confinnis*) (Lynch- Arribalzaga) (Jacob et al. 1985, Meyer et al. 1987, 1988, Meyer unpublished data). Based upon adult associations, it can be presumed that larvae of *Ae. thelcter* coexist with other floodwater species following heavy thunderstorms in August and September. Alkaline ground pools that are flooded at that time are known to be colonized by all three *Aedes* species and by both species of *Psorophora* (Bohart and Washino 1978). The larvae of *Ae. thelcter* can be separated from the other *Aedes* by the following characters: head hairs 5C and 6C single in *Ae. thelcter* with multiple branches in *Ae. vexans* and distal pectins (1-3) on siphon detached in *Ae. thelcter* and attached (uninterrupted row) in *Ae. dorsalis* and *Ae. taeniorhynchus* (Darsie and Ward 1981).

Diversification of tree hole breeding mosquitoes in the continental United States reaches a maximum of nine species in the mountains of southeastern Arizona (Cochise and Santa Cruz Counties). Zavortink (1972) has reported that larvae of *Ae. purpureipes* in southeastern Arizona are most commonly associated with *Aedes burgeri* Zavortink, *Aedes monticola* Belkin and McDonald, and *Aedes muelleri* Dyar. Associations with other tree hole breeding species along the lower Colorado River are unknown since larvae of *Ae. purpureipes* have yet to be collected. During January of 1987, R.P. Meyer and W.K. Reisen attempted to locate possible sources of *Ae. purpureipes* by sampling tree holes at the base of willows growing within 200 m of the Colorado River at Needles (Havasu National Wildlife Refuge). Previously, larvae have been collected during the winter months in southeastern Arizona (Zavortink 1972). Our searches failed to detect the presence of *Ae. purpureipes*, however, larvae of *Orthopodomyia signifera* (Coquillett) were collected from several tree holes in willows growing near the river. If *Ae. purpureipes* and *Or. signifera* are eventually collected from the same tree hole source, the larvae can be separated easily by overall shape and by examining the cuticle. The larval cuticle (vestiture) of *Ae. purpureipes* is unique in that the entire surface is clothed with microtrichia (aculeate) that give the larva a velveteen appearance (Darsie and Ward 1981).

Continued mosquito surveillance along the lower Colorado River should result in further additions to the mosquito fauna of the region and state. Increased human activity and habitat modification could result in providing conditions favorable to colonization by some other *Aedes*

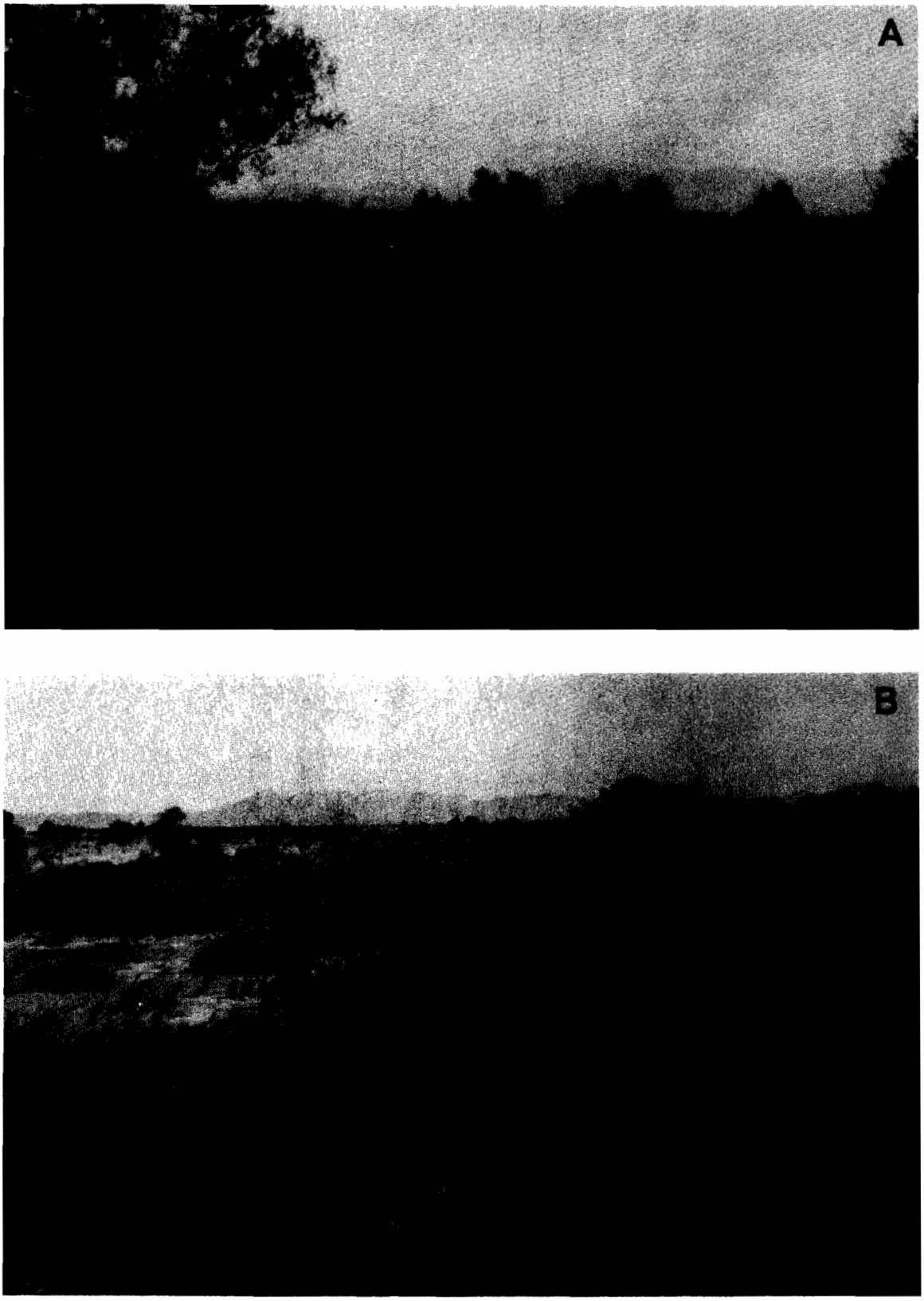

Figure 1. A: Riparian habitat along the Colorado River at Needles, California, where *Aedes purpureipes* females were collected by CO_2 traps during September 1986. B: Flood plain of the Colorado River near Haughtelin Lake *where Aedes thelcter* females were collected by CO_2 traps during September 1987.

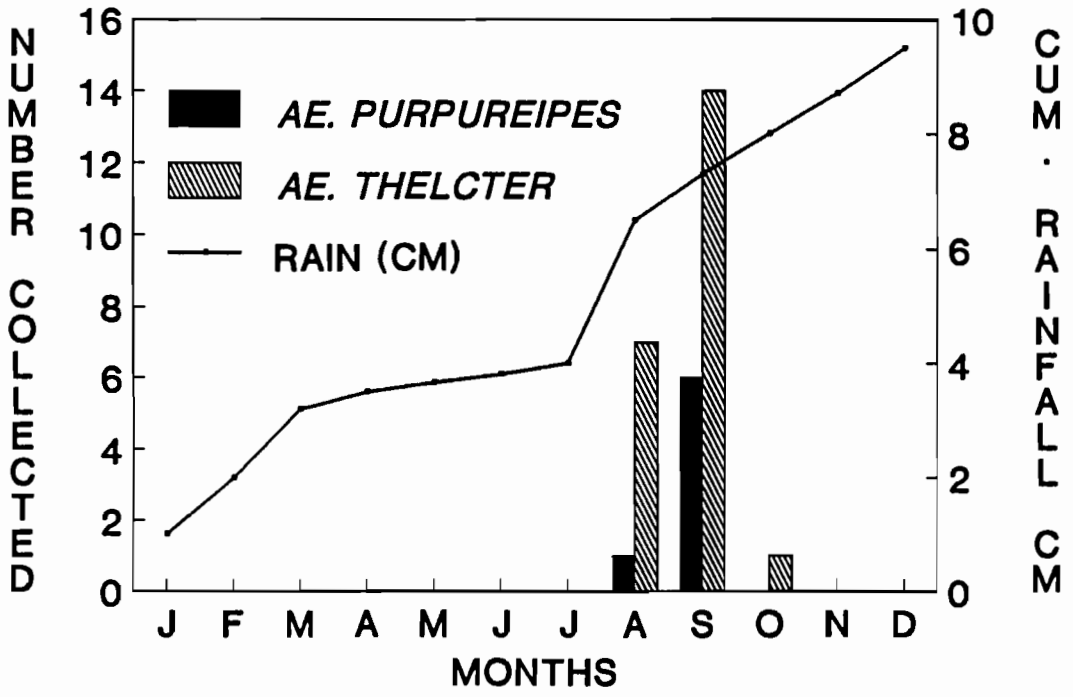

Figure 2. Relationship between cumulative rainfall and the seasonal occurrence of *Aedes purpureipes* and *Aedes thelcter* along the lower Colorado River in California. Cumulative rainfall represented by the 30-year mean for Needles and Blythe, California and Yuma, Arizona.

species (i.e., *Aedes sollicitans* (Walker) and *Aedes trivittatus* (Coquillett)) found further east in central and southeastern Arizona.

Acknowledgements.

The author thanks Dr. W.K. Reisen, V.M. Martinez, and B.R. Hill of the Arbovirus Field Station, University of California, Berkeley, for their contribution in the capture of *Aedes purpureipes* and *Aedes thelcter* at study sites in San Bernardino and Imperial Counties, and S.L. Durso, Coachella Valley Mosquito Abatement District, who collected a small series of female *Ae. thelcter* at Blythe, Riverside County, in September 1989.

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STUDENTS OF THE 1990's: BLENDING PRACTICALITY WITH IDEALISM

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By way of introduction, I hold the position of Affirmative Action Officer for the College of Agricultural and Environmental Sciences at U.C. Davis, where my dealings are primarily with faculty and staff. While I do work intensely with students in the summer when our special summer programs are in session, I don't work with them on a daily basis throughout the year. It would be inappropriate for me to come here today and give you the impression that I know everything about our students.

I have been working at U.C.D. for 25 years, so I do have some ideas and perceptions. And to make sure they weren't based solely on my own experiences, I spoke with my colleagues, the staff members and faculty who have constant contact with students. I'd like to share what I found out with you, which I hope will make clear why I chose this particular theme for my talk.

I've seen quite a progression in my 25 years of working for the University. The 1960's and 1970's brought a whole new breed of young people to college campuses, ours included. Where it may have been true that the greatest concern for the college student of the 1950's was getting a date for the prom, in the 1960's students worried whether the draft lottery would take them or their friends to the war in Vietnam. We had our demonstrations, our sit-ins, our rallies and near-riots. Students (including science students) wanted to change the world, make it a better place. This was a whole new breed of young people; unafraid to challenge the status quo, sometimes for better and sometimes for worse.

It was in the 1960's and early 1970's that young people joined the Peace Corps in droves. In college they majored in social work, philosophy, art and sociology. But 1970 would mark the beginning of the "environmental decade" and enrollment in the sciences in our College and elsewhere would fall into serious decline. Where few had even heard the word "ecology" before, concern for the environment was a natural conclusion for a generation dedicated to becoming one with its world. Science was equated with "the Establishment" and technology, with war and pollution. It just didn't jibe with their concerns.

Then along came the late 1970's and early 1980's. A lot of the happy-go-lucky youth of the 1950's were now the financially-struggling parents of college students. Don't think our kids didn't see this. In the 1980's, students stopped worrying about saving the world and turned to domestic concerns, to their own financial futures. Major national polls showed that at the beginning of the Reagan years, when people were asked what their biggest concerns were, they didn't say "war." They said "inflation".

Students backed away from art, drama, philosophy and psychology. They went into computer science, business, economics and pre-law or pre-medicine programs. And when asked what was their most important goal in life, they weren't the least bit embarrassed to answer, "Making money." Make no mistake about this. Even if they said, "Getting a good job," their definition of a good job did not include personal satisfaction or opportunities to help mankind or personal growth. To them, a good job was defined by the size of the accompanying paycheck. And since science is viewed, per se, as a high-money occupation, they returned to the sciences, with interest in the biological sciences but only insofar as it was a good lead into medical school and a lucrative career.

Traditionally hosting at least some liberal faction, student bodies became more and more conservative, politically and socially. Membership in Young Republican clubs increased as did college-aged support of political figures such as Ronald Reagan; something which would have been unheard of in the 1960's. We teased the young radicals of the 1960's and 1970's about giving up their idealism to have nice cars, homes, clothes and belongings. And we lost the "Peace and Love Generation" to the "Me Decade".

Which brings us to the science students of today; the students of the 1990's. It seems that the pendulum is swinging once again. The media report a general dissatisfaction with lives based solely on money and material possessions. People are giving up fast-track urban careers in exchange for lesser-paying, slower-paced country living. It seems that the self-centeredness of the "Me Decade" may be giving way to a return to the idealism we saw 20 years ago.

I think our college students today are an interesting reflection of these changes. They are a blending of styles; a blending of practicality with idealism. Like the students of 20 years ago, they want to save the world. They want to help people. They want to make a difference. But they are also realistic and pragmatic. They are planners; thinking things out, planning ahead and working through to the future.

In one of our College brochures, we say to students, "You can make a good living. And more. You can make a difference." We thought this would communicate to the students that financial security does not necessarily conflict with living one's ideals. They are realistic enough to know that they must financially support themselves. But financial security can also mean financial independence- the means to go out and achieve other personal goals; goals that may make a difference in their world (our world).

Take an individual who, 20 years ago, may have joined the Peace Corps. This person may today sign up for our program in International Agricultural Development and become a highly-trained expert in engineering, social work, advances in land use and agri-business. This individual will arrive in a Third World nation equipped to supervise and share up-to-date agricultural techniques with the local population, with an eye for maximizing that community's agricultural potential, with the skills to appreciate and respect those people and their culture. Our students recognize that a desire to help is not enough. They must know how to help. And they know that college (an education in the sciences) is one way to do just that.

The kind of student you will see graduating and entering the job market for you is, and must be different.

We have one survey which shows why students didn't want to come to an agriculture school in the 1980's. For example, the image of agriculture had too much to do with the foreclosure of so many family farms. We still struggle with our enrollment in this area. We are, however, seeing gains in student interest in the environmental sciences, as interest in the environment comes once again into world focus with issues like the impacts of acid rain, the depletion of the ozone layer and the destruction of the rain forest. But take our Land, Air and Water Resources Department. It sounds like an environmental department, but is also known as one of our most powerful agriculture departments. Some of our most respected scientists are there and they are dealing with land, air and water as it affects agriculture. This is important. This is what "sustainable agriculture" is all about; farming practices compatible with the environment. Students gain insight into the place of this interaction of agricultural science and environmental science in life in the 1990's. So we may see an increase of students in departments like this one who think they're only interested in conservation and ecology, who come to learn they are concerned with the agricultural sciences as well. We have students coming into the College who never dreamed they would be in an "ag school." In fact, in some years as much as 40 percent of our recruitment turns out to be students who switch from Letters and Science to Agricultural and Environmental Sciences.

The old battle cry to students was, "Specialize!" Specialization is no longer the watch word. In today's world technology and scientific information changes too rapidly. Specialtists with too narrow a focus (too narrow a background) will find themselves obsolete, unless their background in the basics is both solid and broad. Recently, about a hundred of our campus researchers gathered to discuss the future of biotechnology on our campus. Outside speakers representing federal government, state government and industry attended this workshop, and one of them had this to say: "In the rapidly changing field of biotechnology, education and training must be in the fundamentals; physics, chemistry and biology."

In a recent campus interview about trends in career opportunities for our students, the coordinator of our Career and Internship Office indicated:

> *"We are beginning to see a trend toward more emphasis being placed on the breadth of capabilities in a student. Employers look at the whole individual rather than at specific technical skills. Is this individual quick, bright and adaptable? Will this student be able to learn the specifics I decide to give him or her?"*

He went on to say:

> *"This trend toward hiring the generalist means employers play a more active role in the specific training of their new employees. [Stasulat] says that there will continue to be technical entry doors for certain specific disciplines but there will be more generalists under the larger umbrella of scientist."*

Finally, he said this:

> *"Fifteen years ago, I saw more separation between soil scientist, plant scientist and food scientist. Today an employer comes to Campus looking for a microbiologist and finds that individual in any number of departments. In the past, during interview days, you would see*

more students standing in line by major. Today they're all mixed in together."

It's clear that in the opinion of industry, government and other forward thinkers, an excellent education is a broad-based education. We will stress that in our teaching programs in the hope of delivering a truly well-prepared individual to the world.

For two years now the College of Agricultural and Environmental Sciences at U.C.D. has been working under the auspices of a long-range strategic plan known as Project 2000. A mission of this plan is as follows: *The vision for undergraduate teaching in the College is to graduate individuals possessing the highest level of competence in the scientific disciplines as well as the qualities of character necessary to pursue scientific inquiry, instilling in these students both the ability and the desire to be valuable contributors to the public good.*

As a veteran of several decades in the evolution of society and the evolution of the "Ag School", I am moved to have witnessed this change. The spectrum of affect of blending practicality with idealism is broad, and continues to propel the faculty, staff and students in this direction.

Thank you.

AN EVOLVING COMPUTER SIMULATION (ECOSIM) OF MOSQUITOES TO SUPPORT A LARVAL CONTROL PROGRAM IN ALAMEDA COUNTY, CALIFORNIA

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Introduction.

The Alameda County Mosquito Abatement District (ACMAD) conducts a comprehensive mosquito control program of physical, biological and chemical control. A computer simulation program has been developed to support decisions made in the larval treatment component of that program. The reason this area of the program was selected was because the greatest number of decisions, and perhaps the most complex decisions, are being made in the larval treatment program. A simulation to support larval treatment decisions, therefore, appears to offer the greatest potential for increasing efficiency and cost-effectiveness.

The problem of establishing an efficient larval treatment program is complicated by a number of factors. The District has approximately 1400 major mosquito sources in 815 square miles. Seven technicians are assigned the responsibility of inspecting and treating an average of about 200 major sources in zones of about 100 square miles each. During the spring and summer months, as many as six species of mosquitoes may be designated for larval treatment. The size of the District, number of mosquito sources and number of pest and vector mosquito species combine to create an enormous scheduling problem.

In recent years, environmental, safety and cost considerations have further complicated the task for technicians by increasing requirements for information. A decision whether to treat a mosquito source now entails the following information:

1. Location of the larval source.
2. Presence or absence of mosquito larvae.
3. Species of larvae present.
4. Number/dip of each developmental stage of larvae to compare to an established treatment-threshold.
5. Presence of beneficial predators.
6. Presence of wildlife (e.g. endangered species).

Ironically, at the same time that pressures were increasing to process more information about mosquito sources, financial constraints imposed by a tax reduction measure in the late 1970's caused the District to cut back the number of mosquito control technicians.

ACMAD data systems.

The District designed, developed and implemented an automated data processing system (ACMAD Data Systems) as one of a number of measures aimed at increasing efficiency to compensate for the loss of staff (Roberts 1984). The systems analysis and construction of computer programs was accomplished by a team of District employees (Rusmisel et al. 1983). All District programs have been written in BASIC programming language of Tandy computers. Our current host computer is a Tandy 4000 operating on SCO Xenix software and currently supporting six terminals at various workstations in the District offices. The center piece of the District's computer system is a data base describing the physical and biological characteristics of all major mosquito sources in the District.

A number of computer programs operating in ACMAD Data Systems directly support the larval treatment program (Fig. 1). An environmental simulation program (ESP) determines which species of mosquitoes in the District would be active in the larval stage at any given time. Each day ESP compares input information (date, tide, rainfall, maximum and minimum temperatures) to conditions coded in the program which predict the beginning or end of larval activity of each important mosquito species in the District. When a species of mosquito becomes active in the larval stage, the data base of all mosquito sources is searched by ESP to create a "hot" file of all sources where larvae of the species have been found. The "hot" files are updated each day with inspection and treatment data recorded by the technicians on the previous day. A scheduling program (ZING) searches all "hot" files on a weekly basis or upon request to create zone inspection and treatment information about the location of the mosquito sources, and the results of the most recent inspections or treatments.

The District's computer system, at the above stage of development, provided valuable assistance to mosquito control technicians. ESP provided the answer to "what" species of mosquitoes were active and "when". ZING listed the specific larval sources "where" the larvae could be expected to be found. It was felt, however, that still another increment of efficiency could be added by assisting technicians in deciding more precisely "when" a particular source in a "hot" file should be inspected. Ideally, the best time to inspect a source would be when larvae were present at the established treatment-threshold level. If high quality information were provided to the technicians predicting when threshold would be reached in each source, inspections could be more efficiently scheduled; and savings could be accrued by avoiding unnecessary inspections.

Evolving Computer Simulation of mosquitoes.

The District began construction of a computer program in 1986 with the express purpose of providing a date when threshold is reached at each active larval source. Since the major factor determining the time of threshold is the rate of larval growth, the computer program took the basic form of a simulation of larval growth. Other components have been added to the simulation as necessary to refine the output. The program is best described as an evolving computer simulation of mosquitoes designated by the acronym ECOSIM. It has been constructed by a team of District employees. The employees gained much of the technical expertise necessary to construct the simulation by assisting in the development of the computer simulation of Coyote Hills Freshwater Marsh (Schooley et al. 1982).

Description of the simulation.

ECOSIM is called up daily by a control program in ACMAD Data Systems to simulate larval growth in all "hot" sources (Fig. 1). The simulation occurs following input of the previous day's operational and weather data to insure that the most recent input data is available to the simulation. For mosquito sources that have already been inspected and found to have early instar larvae present, ECOSIM simulates the development of the instars (and pupae) and predicts a date when treatment threshold is reached. When sources are treated, found without larvae, or have not yet been inspected, oviposition is simulated. The simulation proceeds through hatching and larval growth until simulated threshold is reached. Three companion papers will describe the temperature subroutine, the oviposition subroutine and the use of the simulation in the District (Conner and Roberts 1990; Mead et al. 1990; Rusmisel and King 1990).

Behavior of the simulation.

The initial input to a larval growth component of the simulation may be provided by a field inspection of the source or by simulated oviposition. Standard inspection procedures during field inspections establish the number of larvae (and pupae) per pint dipper in each instar. All of the individuals in each instar are considered a distinct cohort to begin the simulation. Simulated oviposition may add eggs to the source each day resulting in additional cohorts as the simulation progresses.

The computer begins the simulation on day one which is the date of the last inspection, the date of the last treatment of the source, or if neither of the preceding, the date when the "hot" file was created. An increment of growth is added to each cohort for that day based upon the temperature of the source. The growth rates and the relative duration of each larval stage used in the simulation have been determined locally for each species being simulated (Mead and Conner, 1987). The growth is calculated by use of a rate summation technique and added to each cohort as a fraction of total growth from eggs to pupae (Wagner et al. 1985; Fig. 2). The grown cohorts are then transformed back to number per dip to determine if threshold has been reached. If threshold has not been reached, another day is incremented and the cohorts are processed through the growth loop again (Fig. 3).

If the simulation reaches threshold, the number of days of growth are added to the initial date of inspection and a predicted date of threshold is created. This date is then made available to each technician by placing it next to the appropriate source in the zone inspection guidelines.

Figure 3 describes the essentials of the simulation.

Method of construction.

ECOSIM has been developed by a bottom-up approach. This approach was given a substantial boost by Alan Berryman during discussions at a computer modeling workshop at the 1987 conference of the American Mosquito Control Association held in Seattle, Washington. He said that simulations are accomplished to learn about the dynamics of the system being simulated or to obtain some very practical information. He suggested that if the simulation were to be aimed at a practical application, the model should be as simple as possible, adding complexity only as necessary. Other

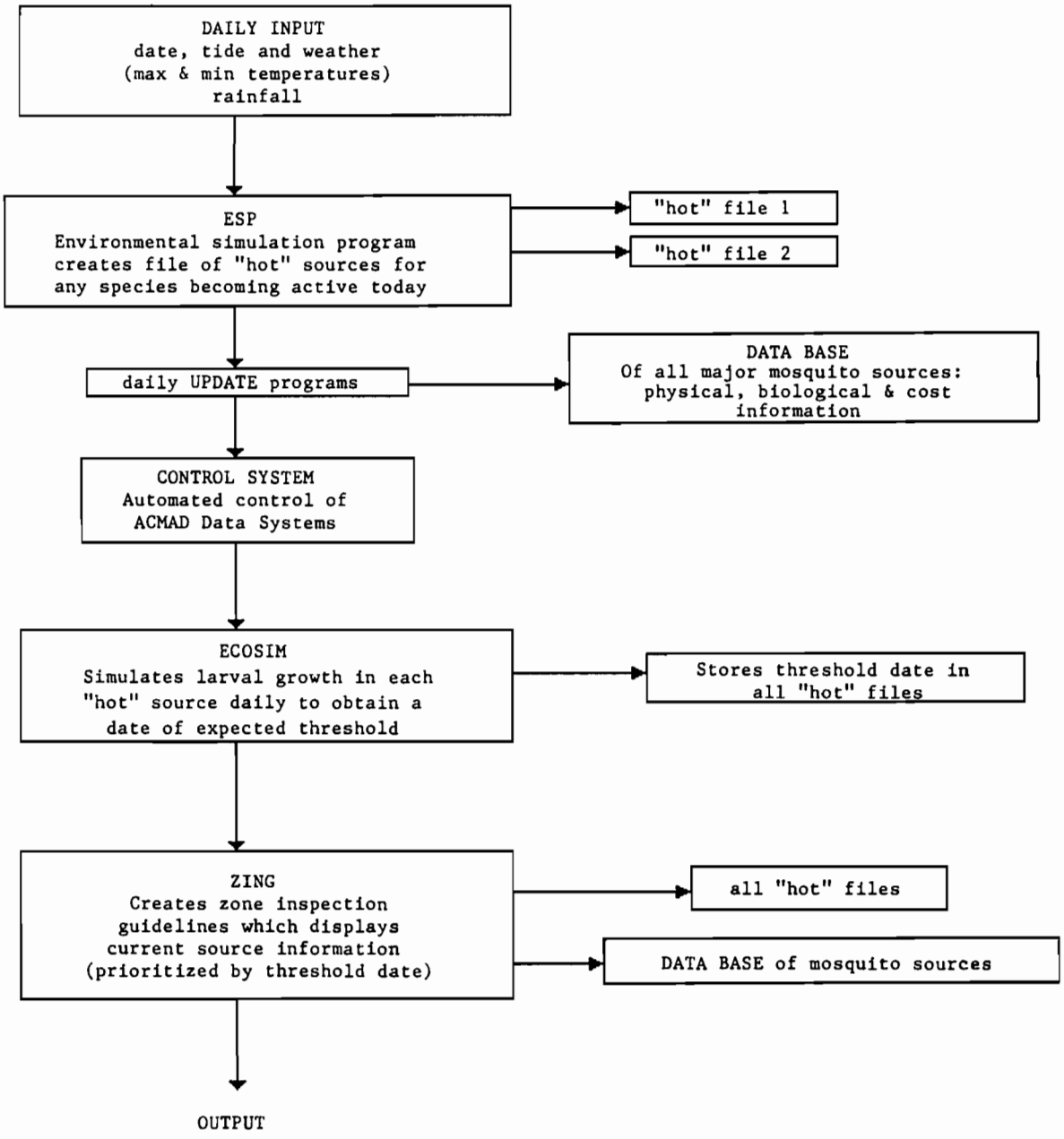
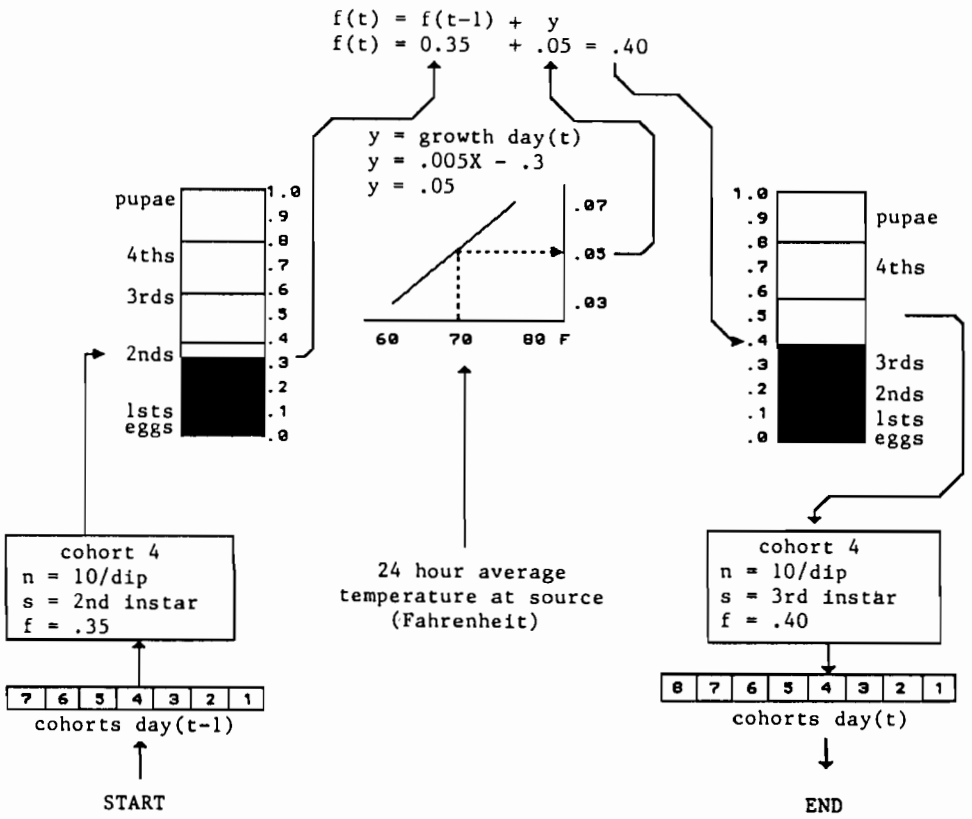


Figure 1. Flow chart of the operations of ACMAD DATA SYSTEMS emphasizing support provided to the larval control program.



Cohort = Individual mosquitoes are segregated into cohorts according to their age. Mosquitoes collected at the source and identified as distinct instars (and pupae) are segregated into separate cohorts. All subsequent oviposition occurring during the simulation creates an additional cohort (cohort #8).

n = number of individuals in a cohort

s = stage of larval development of the individuals in a cohort (eggs, larvae or pupae)

f = fraction of accumulated growth completed by a cohort between egg and emergence as adult mosquitoes

y = .005X - .3 is an equation used to determine the fraction of growth of a cohort in one day at specified temperature X. The values are for illustrative purposes only. The slope of the line below the equation graphically represents the growth rate (see Mead and Conner, 1987) and is used to estimate a solution (y = .005(70) - .3 = .05 = fraction of growth for one day).

t = current time of the simulation (in days). t-1 = yesterday.

f(t) = f(t-1) + y is the rate summation equation used to increment growth of each cohort each day.

Figure 2. An illustration of the rate summation method used to simulate one day's growth of immature mosquitoes in ECOSIM (one cohort).

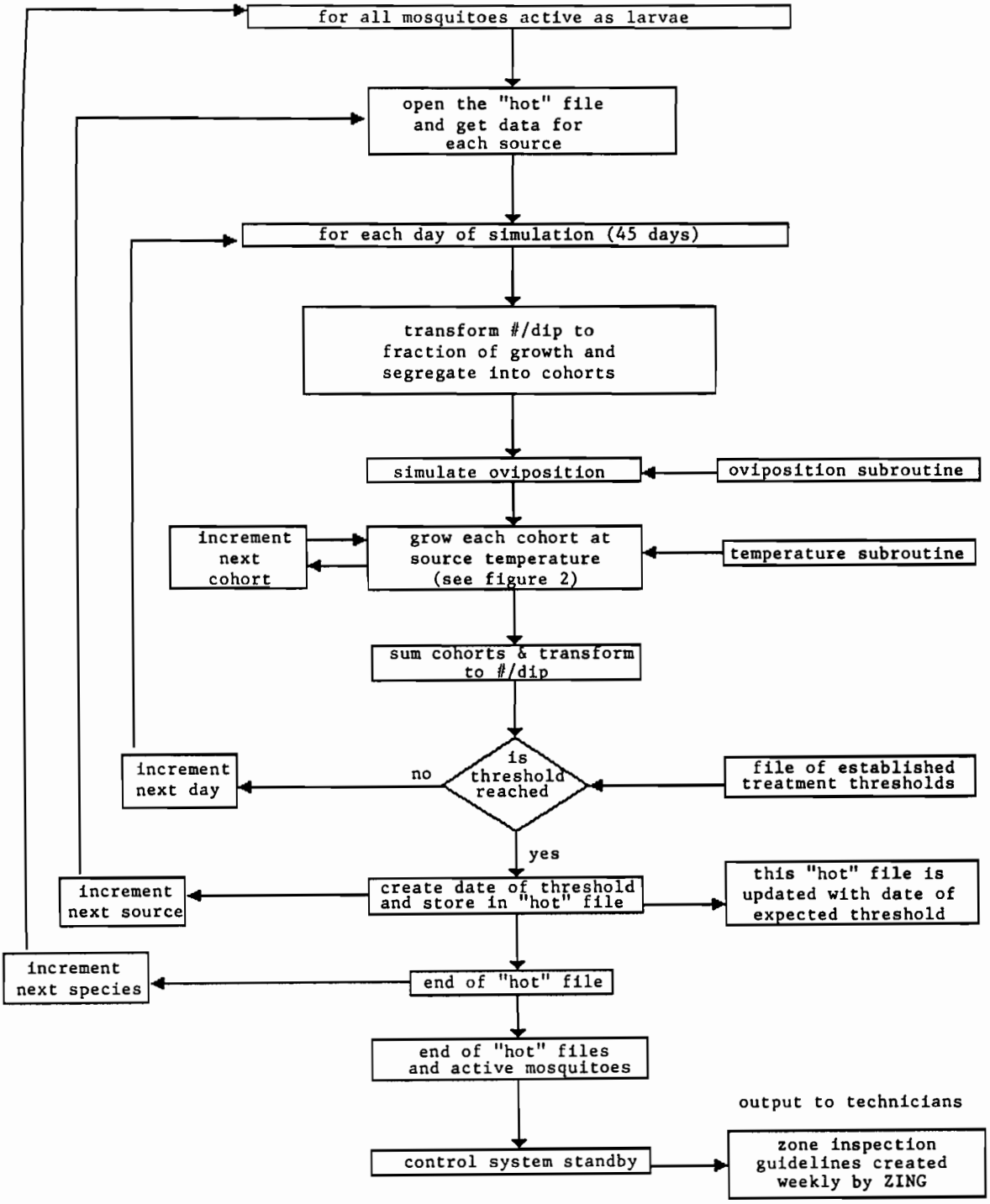


Figure 3. Flow chart of the operation of ECOSIM in ACMAD DATA SYSTEMS.

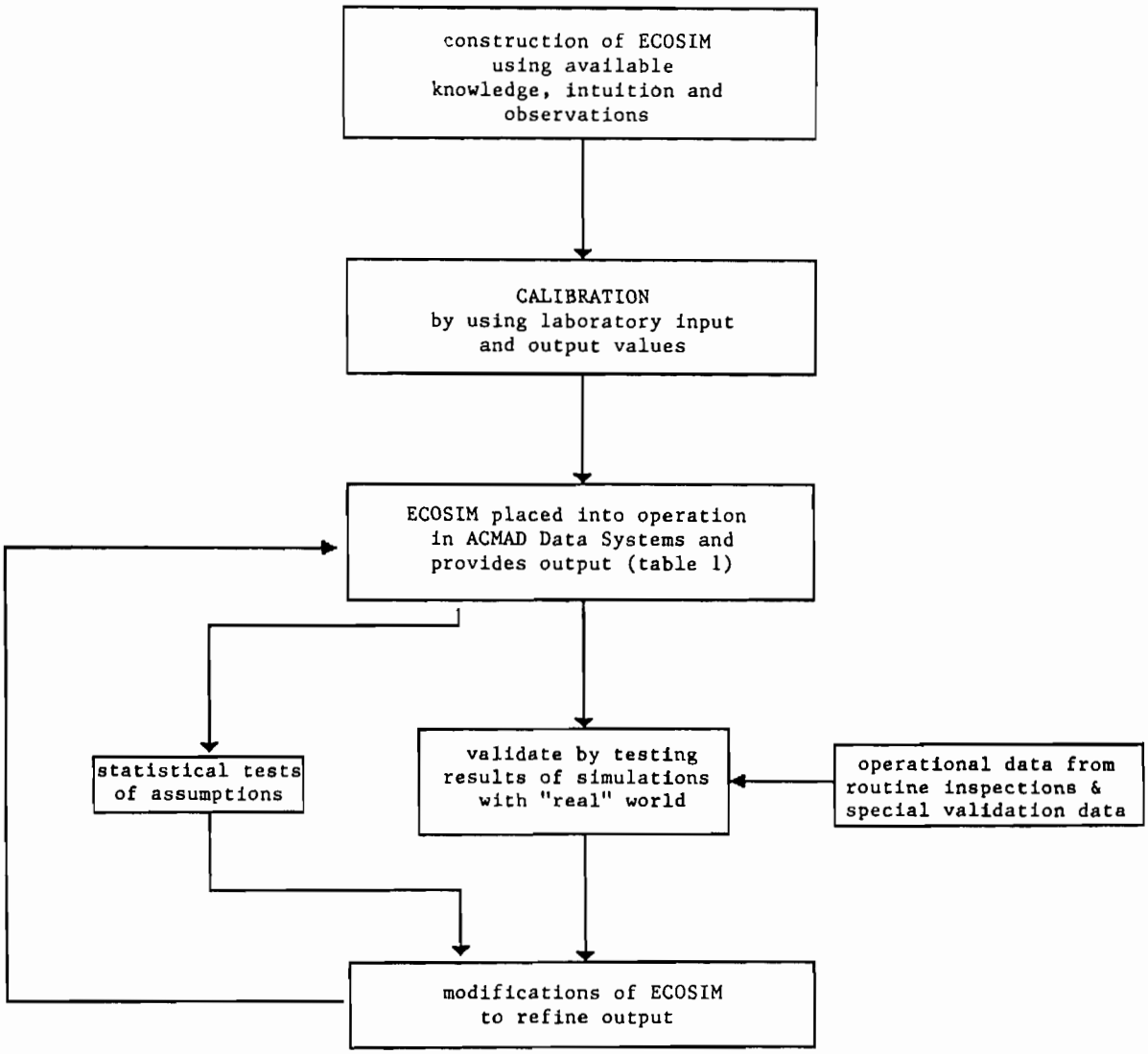


Figure 4. A flowchart of the validation procedures for ECOSIM.

wise, he felt we might waste resources dealing with an unnecessary level of complexity.

A significant advantage of developing the simulation from the bottom was that it became useful almost immediately. The basic component of the model was the simulation of larval growth. Once it had been built and was calibrated, it was providing useful information. The simulation, therefore, was placed into operation in late 1986 in a relatively primitive state of development. The simulation has continued to evolve into a more complex program as complexity was added to further refine the output (Table 1).

Validation of ECOSIM.

According the Shannon (1975) confidence in any simulation is established by repeated cycles of construction followed by verification. He suggests that verification should include statistical testing of assumptions of the model as well as comparing input-output transformations of the model to those of the real world. Our approach to validation has included an initial calibration, testing of the output with the real world and statistical analysis of assumptions. A companion paper by Mead et al. (1990) will conduct statistical analysis of the temperature subroutine.

The model was initially tested by comparing simulated growth of the larvae with growth of larvae in controlled laboratory conditions. The laboratory data used in the test was the same data used to calculate the growth rates used in the simulation. Using this data assured us that the input and expected output to the simulations were correct. The

results of the simulation were then compared to the laboratory results and the simulation was modified as necessary (calibrated) to reduce error. By this method, we were able to establish a level of intrinsic error of 2.4% that was built into the simulation. It appeared that the error was primarily associated with the length of the time interval chosen in the simulation. Since a one day time interval was as small an interval as we could practically support in the simulation, we were forced to accept that level of error and would expect it to increase in warm temperatures and decreased with cooler temperatures.

An on-going system has been established to accomplish evaluation of how well the output of ECOSIM represents the real world. The "reality check" of the simulation is accomplished, for the most part, by utilizing data collected routinely in the larval treatment program (Fig. 4). Some data were also collected by frequently repeated inspections of sources made for the specific purpose of validating the simulation. These inspections were time consuming and expensive, however, causing us to rely primarily on data collected through routine inspection.

Criteria has been established to validate the simulation. The results of the simulations run in 1987, 1988 and 1989 were compared with data collected from the field by the routine larval inspections. The following validation system was established:

1. Operational data were searched to find inspections of sources where two or more inspections had indicated growth of larvae through at least two instars.

Table 1.-Evolving complexity of ECOSIM.

Program Components	Date Installed	Date Replaced	Useful Output to Technicians
Larval Development	1/87	In use	Minimum development time of larvae to threshold in inspected sources found with larvae (maximum growth rates).
Ambient Temperature Subroutine	2/87	6/87	None. No measurable improvement.
Source Temperature Subroutine	6/87	In Use	Probable actual development time of larvae to threshold in each positive source.
Temperature Related Mortality	8/87	12/87	None. Output not validated. To be installed in the future.
Oviposition Subroutine	12/88	In Use	Probable development time egg to threshold in sources not inspected or without larvae.
Predation Subroutine	future ?		Refinement of predicted #/dip to trigger threshold.
Stochastic Component	future ?		Determine probabilities for predicted thresholds.
Adult Population	future ?		Determine numbers of adult mosquitoes based on simulated larval development in all sources. (Predict complaints).

Table 2.-Results of validation tests on ECOSIM.

Parameter Measured	Year 1987	1988	1989
Successful Simulations *	48.0% n=33	79.3% n=29	80.8% n=43
Average Number of Days Error	5.9 days n=17	5.0 days n=6	4.3 days n=9

* Simulation predicts development within one day (see text).

2. If simulation had successfully predicted within one day the number of days required for the observed growth, the simulation was considered successful.
3. The total number of days error was established for unsuccessful simulations.

The percentage of successful simulations and the average number of days error were used as parameters to evaluate the effectiveness of the simulations (Table 2).

Results of validation tests indicate the output of the simulation is improving. A significant improvement made in the simulation between 1987 and 1988 can primarily be attributed to the implementation of a program to monitor larval source temperatures. The program is discussed in detail in a companion paper (Mead et al. 1990). It is expected that the statistical analysis accomplished on the temperature subroutines will provide the basis to gain another significant level of improvement in the simulations in 1990.

The future of ECOSIM.

Our goal in the development of ECOSIM is to increase the accuracy of the output to a level where it will be a highly reliable tool. We would like to continue the validation process to attempt to reach a 90% success level and a reduction in the average error (Table 2). The simulation, operating at the level of reliability, should be a powerful scheduling tool for the technicians. It would be expected to provide yet another increment of efficiency to the larval control program.

The desire to improve the accuracy of ECOSIM seems to require the addition of more components. This evolution toward more complexity brings ECOSIM that much closer to providing output beyond that of just predicting treatment-threshold. Table 1 lists the subroutines now being considered to be added and describes the desired output. It appears feasible in the future for ECOSIM to predict activity and numbers of adult mosquitoes based upon simulated levels of adult emergence. The structure of ECOSIM provides an opportunity to accumulate the number of emerging adults from each source during the simulation. A component to sum and store the number of emerging adults could easily be placed in the simulation loop (Fig. 3). An adult mosquito simulation could then be built to use the emergence data, as well as other available data, to provide needed information concerning adult mosquitoes.

The development of a promising new simulation may preclude the need to develop ECOSIM to the level of adult mosquito populations. A simulation of mosquito populations has been developed for Orange County, California. The simulation is accomplished by RAM, an artificial life system developed at UCLA for modeling populations (Taylor et al. 1987). Our District is in the process of putting it into operation to support the *Culex pipiens* L. control program. It is particularly appealing because it simulates mosquito populations and would support decision at the District-wide level. ECOSIM, on the other hand currently only supports individual decisions at each source. RAM and ECOSIM operating together would create valuable decision support to both the supervisor of the control programs as well as the individual mosquito control technicians in each zone. If RAM were to be easily modified to also support

decisions at the individual source level, it may well replace ECOSIM altogether. It should be remembered that computer programs compete in an environment of differential selection (Beniger 1986). They either continue to evolve and remain competitive or they become extinct.

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USE OF AN EVOLVING COMPUTER SIMULATION (ECOSIM)

TO SUPPORT LARVAL CONTROL - AN ENTOMOLOGICAL PERSPECTIVE

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The overall goal of our District's computer ECOSIM is to increase the efficiency of the larval control program (Mead et al. 1990; Roberts et al. 1990; Rusmisel and King 1990). The payoff is to inspect only sources that are positive for larvae.

From the point of view of the entomologist in charge of control programs, one of the questions in my mind must be, is the model providing a help to the technicians? Does the model help them accomplish their work in a more efficient manner?

Alameda County Mosquito Abatement District encompasses 825 square miles of territory with a population of approximately 1,200,000 people. This provides a heavy workload for seven technicians.

The technicians are assigned zones within which they are responsible for mosquito control. These zones obviously have variances in size, number of breeding sources, temperature, population, ecological diversity, diversity of mosquito species, and differing rates of mosquito larval production and growth rates.

One of the programs developed by our District is the Zone Inspection Guidelines (ZING) (Roberts et al. 1990). Its objective is to generate a list of sources in each zone on a weekly basis, with information available to describe their most current condition and then to display them in a logical inspection and treatment schedule for review by each technician.

I believe that the weekly zone inspection guidelines do help the technicians with their daily workload. Obviously, a good technician knows the mosquito production taking place in his or her zone throughout the year, but the ZING printout is a good reminder or backup for memory.

The ZING printout prioritizes the breeding sources, highest priority at the top of the page and lowest priority at the bottom. The priority is established by the simulation providing a projected date of threshold. This allows the technician to make a judgment on which sources must be attended to immediately, which sources he may be able to ignore and for how many days he may ignore them without problems.

The ZING program predicts when the mosquito larval threshold has been reached and when the source must be treated. The simulation actually has a conservative view, in-so-far as when a source has been inspected and found to be negative for larvae, the model assumes oviposition will occur the next day.

A number of years ago (1974 - 1975) management was concerned with resistance and with the number of insecticidal treatments that were occurring during the normal mosquito-breeding seasons. We made attempts to reduce insecticide pressure, lower costs and reduce the technicians' workload, yet still have an efficient and effective mosquito control program. We moved toward this goal by establishing a larval treatment threshold. Larvae would need to be in sufficient numbers and develop to a certain instar before treatment was to occur. A threshold was established for each mosquito species and the threshold could be altered to accommodate the program in case of emergency or other unusual circumstances. ECOSIM is a tool designed to utilize this approach and move us closer to those original goals.

Time spent in the field is the best way for technicians to accumulate knowledge about mosquito control. Our technicians have accumulated a great amount of field experience which they use to good advantage. The computer simulation model is an added benefit and is designed to be a helpful tool. Technicians use the ZING program, but they must also use their field knowledge. By prudent use of the ZING program, the technicians could make better allocation of their time; for example, more time for thorough sampling of breeding sources. Also, they could become more involved in computer programming; thereby insuring they understand how ZING works and its strengths and limitations.

Secondary benefits of the simulation model are that the entomologist and technicians, by helping develop the model, have learned much more about mosquitoes and the need for good reliable data. The effectiveness of the simulation is contingent upon it being structured in a realistic way analogous to the "real mosquito world". It is dependent upon data from the environment. These demands, met by

the technicians, have provided a valuable learning experience. The required monitoring system has also increased the knowledge about new and more efficient monitoring techniques. The model demands these things and we therefore must provide it. As you have heard from the speakers before me, we must depend upon specific individuals to do certain specialized jobs as well as meeting their zone responsibilities. Therefore, the overall job becomes more interesting and learning is accelerated. The bottom-up method of computer modeling has created an opportunity for everyone to learn from the beginning.

The use of biorational pesticides that do not persist in the environment means more intrusions on a habitat, more inspections and more frequent treatments. We feel using the computer prioritization of the sources can help us better time the applications and reduce the frequency of inspections and treatments.

The computer makes more specific information available to the entomologist. In the past, the data was of a more general nature. Now all the data is being utilized for a specific purpose in the decision-making process. For example, light trap data is used in the simulation to determine threshold on a certain day. This information is available and the entomologist has that data for decision making. Light trap data is now, therefore, utilized routinely in decision making.

The computer simulation model appears to be a valuable tool that helps increase our efficiency and effectiveness in mosquito control.

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PREDICTION OF LARVAL SOURCE TEMPERATURES BY ECOSIM

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Alameda County Mosquito Abatement District currently uses a computer simulation to assist technicians in determining when treatment of breeding sources should occur (Roberts et al. 1990). ECOSIM (Evolving Computer Simulation of Mosquitoes) was designed to predict the number of days required for larvae to reach an established treatment-threshold. Initial developement of ECOSIM focused on determining temperature-related growth rates of *Culex pipiens* L., *Culex tarsalis* Coquillett, *Culiseta inornata* (Williston) and *Culiseta incidens* (Thomson) (Mead and Conner 1987). Following this, a temperature model, which operates as a subroutine in ECOSIM, was developed to determine the temperature of mosquito breeding sources. This study describes the development of the temperature model, compares the results with actual values, and makes recommendations to improve the accuracy of the temperature predictions.

Thermal characteristics of sources.

Studies of the Coyote Hills Freshwater Marsh provided a starting point in understanding the thermal characteristics of aquatic habitats (Collins and Meyer 1985). Temperature fluctuations of the marsh occured on a daily cycle, with the high reached by mid-afternoon and the low by early morning. The correlation between ambient and source temperatures established in the study was initially used by ECOSIM as the means to predict all larval source temperatures in the county. This approach failed, as many of the sources in the county did not have the same thermal characteristics as the Coyote Hills Marsh.

To overcome this problem, source types were studied and grouped according to their similar response to ambient temperature. An Onsite Weather Logger (OWL) with a Tandy 102 portable computer facilitated in the analysis of the source types by recording ambient, surface, and 6" depth temperatures at hourly intervals for 24 hours. The data collected produced temperature profiles by which three groups were defined, based on the response of surface temperature to ambient temperature (Fig. 1):

1) Sources in which temperatures rose or fell readily with a rise or fall in ambient temperature.
2) Sources that tended to respond less dramatically with changes in ambient temperature.
3) Sources in which little or no rise in temperature occured with the daily increase or decrease in ambient temperature.

Sources in group 1 will be referred to as "shallow", though this group also included some deep sources which had profiles similar to shallow sources. Sources within this group were generally less than one foot in depth, had a static flow and were 50% or more sunlit. Sources greater than one foot in depth tended to contain dense submerged vegetation throughout.

Sources in group 2 will be referred to as "deep", though this group also included shallow sources. Sources within this group were generally greater than one foot in depth with clear water. Any sources in this group less than one foot in depth were flowing and were 50% or more shaded.

Group 3, the "subterranean" group, included all manmade underground sources such as catch basins, storm drains and utility vaults.

The temperature model.

The temperature model was based upon certain assumptions. First, the growth rates of the larvae are influenced mainly by the temperature at the 1/2" depth (Stewart 1974). Second, mosquito growth rates are driven by the 24-hour mean temperature with no significant influence caused by temperature fluctuation (Milby and Meyers 1985). Third, the average temperature derived from the daily high and low is not significantly different from that derived by the 24 hourly temperatures. And last, warming of the water surface is assumed to be correlated with ambient temperature, and can be represented by a constant.

The model requires, as input, high ambient and low source temperatures. To collect the low source temperatures, a county-wide monitoring program was established, dividing the county into four regions with three monitor sources in each. Low source tempertures were collected three times weekly between 6 and 9 AM. These values were

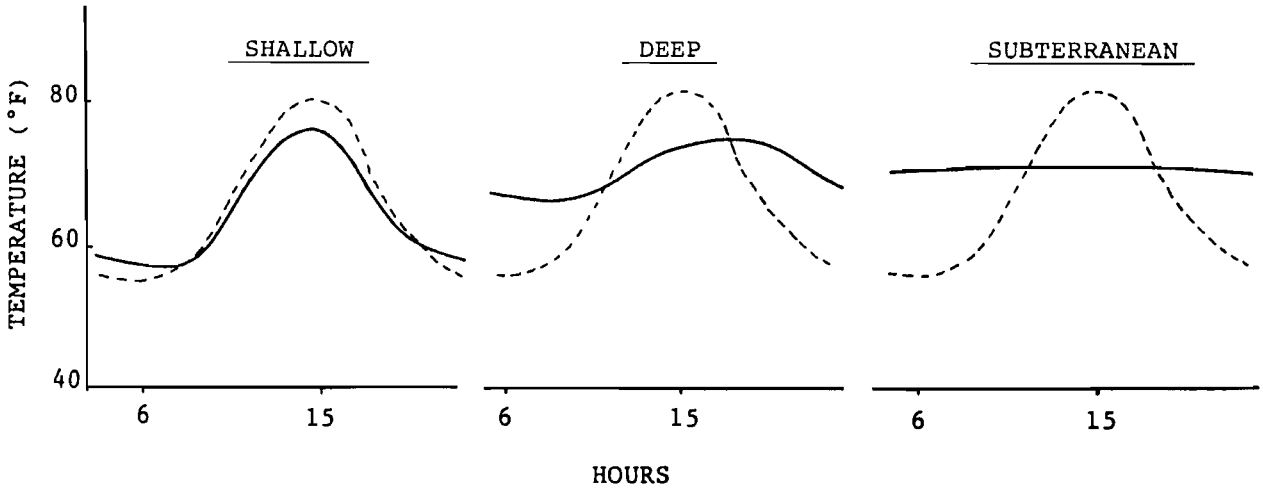


Figure 1. Typical daily temperature profiles (----- water surface, - - - air) for the three groups of sources.

used by the temperature model as the low source temperatures for similar sources in each region. The high ambient temperature reported by the National Weather Service was collected daily for eight cities.

The model derives the high source temperature by using the high ambient and the low source temperatures. The high ambient temperature is considered the potential high a source may reach; the actual increase, however, is proportionate to that potential, and is determined by a warming coefficient. The high source temperature is then averaged with the low to provide the 24 hour mean (Fig. 2).

Three warming coefficients were established, from sample OWL profiles representing the three source groups. The coefficient is derived by dividing the actual increase in temperature (high source minus low source) by the potential increase (high ambient minus low source).

Comparison of results.

The temperatures predicted by the model were compared to the actual temperatures recorded by the OWL, which was placed in 25 locations for a minimum of two days each, over the course of one year. Afterwards, a simulation was run for each location. The values compared in the analysis were the 24-hour mean, low source, high source, and high ambient temperatures. Low source and high source temperatures were at the surface (1/2" depth). The 6" depth temperatures were not used in this study.

Twenty-four hour mean temperature. In Figures 3 through 5, the plots are shown with an ideal correlation line and limit lines of plus or minus five degrees Fahrenheit. The limit lines were based on the temperature-related growth rates of the species simulated (Mead and Conner 1987), resulting in a one to two day treatment-threshold error.

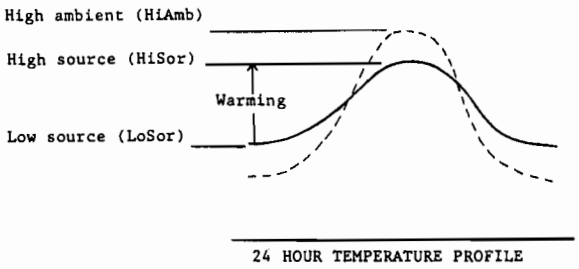
Shallow sources showed a strong correlation between the actual and simulated temperatures (Fig. 3). The majority of data fell within the limit lines. The slope, however, indicated that at the lower temperatures (cooler months) the simulated temperatures were warmer than the actual values, while at the higher temperatures (hotter months) the simulated temperatures were cooler than the actual values.

The deep sources also showed a strong correlation between the actual and simulated temperatures (Fig. 4). The majority of data fell within the limit lines.

No correlation existed for the subterranean group (Fig. 5). About half of the data points fell outside of the limit lines. It was found that these points represented data from storm drains and utility vaults, while the data inside the limit lines represented catchbasins.

Low source temperatures. Monitored low source temperatures used in each of the simulations were compared to the actual temperatures recorded by the OWL (Table 1). For each source group, the differences were significant using the paired comparisons test at a 95% confidence level.

High ambient temperatures. The high ambient temperatures, as reported by the National Weather



Warming coefficient = WC

Warming = (HiAmb - LoSor)x WC

HiSor = LoSor + warming

24 hr mean = (HiSor + LoSor)/2

Figure 2. Derivation of the 24-hour mean temperature of a larval source.

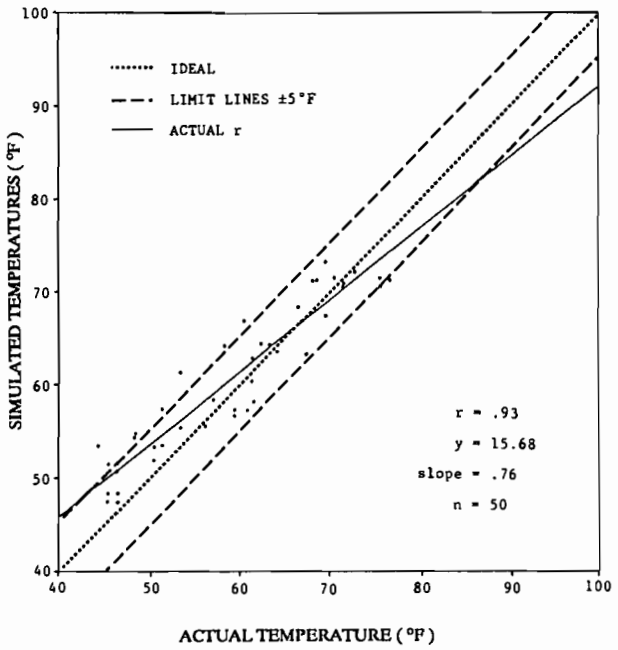


Figure 3. Comparison between simulated and actual 24-hour mean temperatures for shallow sources.

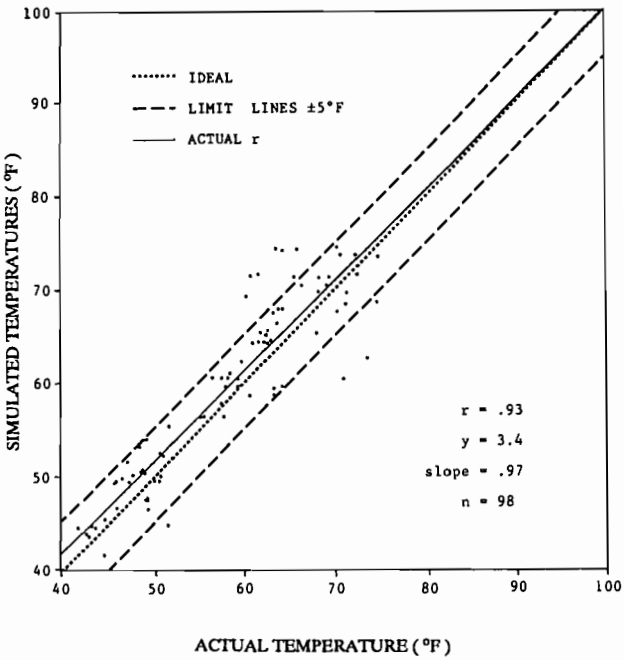


Figure 4. Comparison between simulated and actual 24-hour mean temperatures for deep sources.

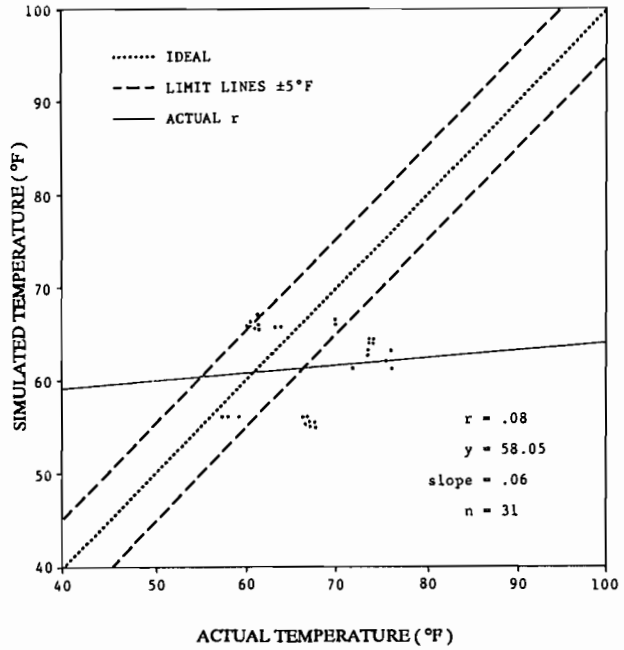


Figure 5. Comparison between simulated and actual 24-hour mean temperatures for subterranean sources.

Table 1. Differences between monitor source temperatures used in the simulation and actual low source temperatures (° F). M_D= Mean of the differences (simulation - actual). SD= Standard deviation.

Group	Pairs	M_D	SD
Shallow	50	5.1	3.6
Deep	98	1.8	4.6
Subterranean	31	-4.4	7.7

Table 2. Differences between simulated high source temperatures and actual temperatures (° F). M_D= Mean of the differences (simulation - actual). SD= Standard deviation. WC= Warming coefficient.

Group	Pairs	M_D	SD	WC
Shallow	50	-5.1	8.1	.545
Deep	98	-2.7	3.5	.255
Subterranean	31	-1.6	1.8	.053

paired comparisons test. OWL data was subtracted from weather service data, and resulted in a mean difference of 0.35 and standard deviation of 5.8. The differences were not significant at the 95% confidence level.

High source derivation. The warming coefficients were tested for accuracy by first eliminating known sources of error. The simulations were rerun using OWL data for the low source and high ambient temperatures, and then the derived high source temperatures were compared to the OWL high source temperatures using the paired comparisons test (Table 2). The test indicated a significant difference between the derived and actual temperatures at the 95% confidence level for each warming coefficient.

Discussion.

Shallow sources. Errors in simulated temperatures for shallow sources need correction in both the warming coefficient and monitor source values. Error in the calculation of the 24-hour mean was sometimes reduced by the process of averaging the low source and the high source temperatures. The negatively skewed error of the warming coefficient was offset by the positively skewed error of the monitor source temperatures (Tables 1 and 2). A negative high source difference indicates that the value for the warming coefficient should be larger. Analysis of monitor source data indicated that error was introduced by using the shallow edge of a lake to monitor temperatures rather than using sources with an average shallow depth. This resulted in significant discrepancy at these locations; that is, the low source temperatures were high, since deep sources cool at a slower rate. Shallow monitor sources should be carefully selected to insure the temperatures collected represent those of other shallow sources in the region. The difficulty of keeping a shallow monitor source year around may possibly be solved by the use of an artficial source which is currently being investigated. Data from deep vegetated sources were not sufficient to be included in this study.

Deep sources. The temperature model currently provides acceptable levels of accuracy for deep sources. However, with the additional OWL data gained since the warming coefficients were established, revision of the coefficients could provide even greater accuracy. Data from turbid sources were not included since they seemed to have characteristics that were common to both the shallow and deep groups. Further study is needed before turbid sources can be simulated.

Subterranean sources. Although individual sources in the subterranean group varied only slightly in temperature in the daily cycle, they varied significantly in temperature from source to source. The error in temperature simulation was due primarily to the monitor source temperatures. Subterranean sources should be studied to determine if it is necessary to create additional classes within this group based upon their low source temperatures. Minor adjustment to the warming coefficient should also improve the results.

Conclusions.

The following is recommended: revision of the warming coefficients, reselection of some monitor sources, and further investigation of subterranean, turbid, and vegetated sources.

The purpose of the statistical analysis was not to measure the magnitude of error as much as it was to point out where error could be reduced. The required level of accuracy for the temperature model in relation to ECOSIM has yet not been established. The predictions of the ECOSIM model are currently at acceptable levels 80% of the time,

with an ultimate goal of 90% (Roberts et al. 1990). Improvement of the temperature model should increase the accuracy of the predictions and bring the ECOSIM model closer to its stated goals.

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PRELIMINARY ASSESSMENT OF THE OVIPOSITION COMPONENT OF AN EVOLVING COMPUTER SIMULATION (ECOSIM) OF MOSQUITOES

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Introduction.

Since 1987, Alameda County Mosquito Abatement District (ACMAD) has been using a computer simulation of mosquitoes called ECOSIM (Evolving Computer Simulation) to assist mosquito control technicians in timing their inspection and treatment of sources (Mead et al. 1990; Roberts et al. 1990; Conner and Roberts 1990). Initially, the simulation operated effectively on sources that were found to be positive for mosquitoes in their early stages of development by simply simulating the growth of larvae until they reached threshold. The date of threshold would then become the date of proposed reinspection and/or treatment. For those sources where no mosquito larvae were detected, however, an oviposition subroutine was necessary in order to generate an inspection date. Four species of multivoltine mosquitoes were chosen for the initial phase of this project: *Culex pipiens* L., *Culex tarsalis* Coquillett, *Culiseta incidens* (Thompson), and *Culiseta inornata* Williston.

Monitoring methods.

Monitoring oviposition traps, New Jersey light traps, and fixed station larval monitoring were used to determine if oviposition was taking place. Oviposition traps (also known as gravid female traps) were used for *Culex pipiens*. The traps used were modified from a design by Robert Cummings, Biologist/Engineer at the Los Angeles County West Mosquito Abatement District. His design is an improved version of Reiter's trap (Reiter 1983). The main improvement being the placement of the fan on the exit tube to avoid the mutilation of the specimens. A complete description of this trap design is soon to be published by Mr. Cummings. A fermented alfalfa infusion is used as the attractant for *Culex pipiens*. When the infusion has aged, lost its odor and turned red, it attracted *Culiseta incidens* females almost exclusively. Formulations that attract other mosquito species are being sought. Between three and six traps were put out overnight once a week. The traps were distributed throughout the county; three being in fixed locations where *Culex pipiens* is the dominant mosquito species.

The second means of detecting oviposition was through the use of adult light traps. The District operates eight New Jersey light traps in fixed locations throughout the county year round (since 1988; previously April-October). These traps run seven nights a week from 6 p.m. to 6 a.m. Adult mosquitoes are collected from the traps and identified weekly.

Fixed station larval monitoring is also being used to detect oviposition. Larvae are monitored at twelve locations throughout the county weekly. The monitoring is done by a single individual to standardize the sampling. The sources are treated when they reach threshold, and a new cycle of monitoring is begun.

Operation of the subroutine.

Operation of the subroutine currently uses a deterministic approach to predict oviposition. Initial values of the monitoring inputs have been established to trigger oviposition in the simulation. When input from any of the three methods of monitoring is greater than the established values, oviposition is assumed to be occurring and is triggered in the simulation. The initial values selected to trigger oviposition are five individuals in the adult light traps or ten percent of the fixed larval stations positive for larvae. When oviposition is triggered in the simulation, it occurs at a rate of 200 eggs per dip each day.

Results and discussion.

The oviposition subroutine, when operating properly, should provide us with information about the likelihood that oviposition is occurring by a particular species at a particular source. It should also indicate if the level of eggs being deposited will ultimately reach numbers of late instar larvae sufficient to trigger threshold. Our current level of knowledge, however, does not allow us to predict threshold by oviposition. For this reason, we have selected relatively low values for the inputs to trigger oviposition and high number of eggs to be oviposited. By using this approach, we expect to be able to provide fairly accurate, but conservative, information to the technicians. The threshold date would represent the minimum development time

necessary for oviposition to trigger a threshold. We have adopted this conservative approach to use until we feel we can establish a correlation between the number of eggs being oviposited (in number per dip) and the various values of the monitoring inputs.

We currently feel that light trap data is not an accurate indicator of the oviposition, but that it may indicate when a species first becomes active at the start of a new breeding cycle. Light trap data indicating *Culiseta inornata* activity in the fall can be very useful when combined with rainfall information. If a technician inspects a source that has already been activated by the computer and finds no larvae at that source, the computer will start oviposition at that source on the following day unless the source was recorded as dry. If on a subsequent inspection the technician had found larvae, the computer would start the growth simulation from that point, since field numbers have priority. This is a self-correcting function of the program assuring that the most recent field data is used. Another conservative feature of the simulation occurs because there is currently no mortality or predation component in the simulation. The numbers of larvae in the simulation are likely, therefore, to be greater than what actually occurs in the field. The simulation is being validated by comparing larval numbers and threshold dates that are predicted with actual numbers from the field (as recorded on the technicians' daily reports). The values of the oviposition indices are changed as necessary during an on-going process of fine tuning.

Conclusions.

With continued study, we hope to more accurately predict the time intervals between successive generations of larvae as they progress through their breeding season. The generations become shorter in time as day length and temperature increase. We would like to move to a more stochastic approach to oviposition in the future. There are two goals we are aiming for: (1) To quantify oviposition in order to determine the number of eggs per dip. (2) To predict the probability that each source may be positive for larvae. The main reason for trying to improve the scheduling program is to save the technician time by only going to a source when it is necessary. This savings in time for the technicians also translates into savings for the District in fuel, insecticide and manpower costs.

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A COST ANALYSIS FOR VOLUNTARY COMPLIANCE WITH THE ENDANGERED SPECIES ACT FOR *AEDES SQUAMIGER* CONTROL IN TWO CALIFORNIA COUNTIES

Ronald D. Keith[1] and Glenn E. Conner[2]

Introduction.

The Endangered Species Act (ESA) has impacted both Marin/Sonoma Mosquito Abatement District (MSMAD) and Alameda County Mosquito Abatement District (ACMAD) as well as other districts throughout California and the United States. The impact on the two San Francisco bay area districts has manifested itself in increased pesticide costs and the need for additional manpower to control mosquito populations. Hidden costs are also incurred by the districts as well as to the environment with the implementation of the act. It is the intent of this paper to outline some of the costs for voluntary compliance with the ESA.

History.

In many ways protecting endangered species and controlling pest and disease carrying mosquitoes is a zero-sum effort. Increased expenditures for third generation pesticides consumes savings generated through long term source reduction projects. There are costs and benefits to be realized with a variety of approaches for control. It is our goal to balance the reduction of mosquito populations with the various costs while minimizing the impact on endangered species.

The ESA requires that the Environmental Protection Agency (EPA) ensure that registered pesticide use will not jeopardize endangered species or adversely modify critical habitats. At the outset of the program, 1980-1984, EPA consulting with the U.S. Fish and Wildlife Service (USFWS) evaluated specific pesticides on a case-by-case basis for potential biological harm. This approach was taken to task in a 1986 report titled "The Environmental Protection Agency's Implementation of the ESA with Respect to Pesticide Registration" (Anon., 1986). The report stated that the previous process was too slow and recommended a "cluster" approach be implemented. This grouped similar use materials together for biological evaluation.

On May 8, 1988 an amended plan was reported in the Federal Register outlining the new program. After each pesticide in a cluster was evaluated EPA would ask the USFWS to review the clusters and provide biological opinions on whether jeopardy existed. As a result of public input that highlighted various inequities, the cluster approach was discarded in favor of a species based model for biological consultations. This program is outlined in the Federal Register, June 21, 1989.

Under this program endangered species will be ranked on factors detrimental to their existence. Those species needing the greatest protection will be given priority consideration. There are three major differences between the cluster approach and the new species based approach. Instead of issuing a "may affect" determination for a particular use of a pesticide based on the highest registered application rate, EPA will use this rate only as a screening mechanism for further evaluation. If the highest rate application indicates that the pesticide "may affect" an endangered species, EPA will then determine the lowest registered application rate that "may affect" the species. Also under the cluster approach, once a "may affect" determination was made EPA would request from the USFWS a biological opinion only for that use. If a suspected jeopardy existed, all products of that pesticide for that use were subject to use limitations. In contrast, under the species based program, once the lowest registered application threshold is determined this rate will be used in consultation with USFWS and will be limited only to the specific application rates that "may affect" a listed species.

Under the new program, the EPA will also take into consideration application methods, timing, and use patterns and integrate this information to provide the USFWS with a more realistic package with which to make biological determinations.

Local Response.

Previous efforts by both districts to control *Aedes squamiger* (Coquillett) have relied on two major approaches, source reduction and

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organophosphate pesticide applications with an emphasis on source reduction. Over the year source reduction projects to minimize *Aedes squamiger* populations have been undertaken by both districts with a significant decline in the overall number of this species. Alameda County MAD placed an emphasis on disking cracked-ground seasonal wetlands, previously known as dry-diked areas while Marin/Sonoma MAD concentrated on constructing tidal recirculation ditches to allow for increased tidal exchange in stagnant pothole areas within the Petaluma Marsh and other tidal marsh areas. Both techniques disrupted the ovipositional substrate making it unacceptable for breeding habitat.

It is this same disruption of habitat, especially with regard to disking that is being called into question by advocates of more stringent regulations to protect endangered species. These recent environmental restrictions and changes in philosophy concerning source reduction as viewed by regulatory agencies have limited the amount of new source reduction projects and in some cases eliminated them. Source reduction has been an accepted alternative to chemical application for mosquito reduction by both the districts and regulatory agencies for many years.

Cost effective mosquito control could be achieved by utilizing the proper tools out of the source reduction toolbox for sound water management practices. This is especially evident from the success of the Petaluma Marsh project in Sonoma and Marin counties. Source reduction has also been shown to be the most economically sound approach to mosquito abatement versus a chemical control approach (Sarhan et al. 1980). In the Petaluma Marsh from 1968-1972 tidal recirculation ditching was responsible for reducing pesticide use by over eighty percent (Telford and Rucker 1973). Fenthion usage in 1972 was reported, at that time as only nine gallons for all mosquito suppression. Over five thousand acres of tidal salt marsh was enhanced by better tidal flow. With increased scrutiny by regulatory agencies and the elimination of some source reduction tools both districts fell back to a pesticide approach for *Aedes squamiger* control in the late 1970's and early 1980's.

With the concern expressed by the May 1988 ESA proposal, organophosphate based pesticides were discontinued by both districts during the 1988-1989 season to voluntarily comply with the provisions of the Act as modified in 1989. The districts' response to these restrictions has been to control the remaining *Aedes squamiger* populations using the "third generation" pesticides or biological control methods. *Bacillus thuringiensis* var. *israelensis* (serotype H-14) and Methoprene are becoming the mainstay of both districts' pesticide treatment regime.

In the past inexpensive organophosphate insecticides proved to be the most immediately effective method of reducing larval populations of this mosquito. The shift to biological control agents has increased pesticide budgets almost eight to ten fold in some cases. There are also a number of indirect environmental costs associated with the biological control approach, especially for reducing saltmarsh mosquito populations, specifically *Aedes squamiger*, in that multiple applications are necessary to achieve total control.

The reason for this will become evident with a short discussion of *Aedes squamiger* biology. *Aedes squamiger* is a univoltine species with larval activity beginning after fall rains. Eggs aestivate during the summer and hatch on high tides and the first rains of the winter season. Successive cohorts of eggs develop on each storm event as water levels rise to flood previously dry areas. Eggs are deposited by the previous winter's adult population along contour lines defined by receding flood water. The interval pattern of rain/high tide and length of dry periods affect where and at what height eggs will be deposited in the marsh. Six distinct hatches of *Aedes squamiger* were observed following high tides and heavy rains from October to February, 1953 to 1954 (Telford 1958), but previous observations found only three hatches.

Organophosphate control efforts over the years were planned to treat fourth instar larvae late in the breeding season, taking into consideration the synchronization of pupation which generally begins in early February. Quick effective control could be realized using low cost organophosphate pesticides with a one treatment approach just prior to pupation.

Bacillus thuringiensis var. *israelensis* (*Bti*) was chosen by both districts as the major operational control agent in voluntarily compliance with the ESA. Methoprene was used on a restricted basis at test plot locations since it was a member of the original larvicide cluster and had generated questions regarding potential detrimental affects on food chain organisms. *Bti*, a consumable mosquitocide needs to be applied at specific times to maximize its effectiveness. Variable feeding rates based upon water or ambient temperature and larval physiology directly affect mortality rates. Through previous studies it was found that there is an inverse relationship between mortality rate and increased physiological age, i.e younger instar larvae are more susceptible to *Bti*. Higher application rates of *Bti* are necessary due to the slow physiological development and treatment at ambient temperatures of 8-11° C. As a result, multiple treat-

ments at high dosage rates were required during the 1988-1989 season to achieve acceptable *Aedes squamiger* mortality rates in contrast with a one time application (at low dosage rates) of a contact organophosphate insecticide.

Increased disturbance of breeding habitat is inherent using the "acceptable" or "biorational" control approach. Direct increased costs to the districts were sustained in both increased surveillance time and higher pesticide cost as well as the indirect environmental costs of increased perturbation of breeding habitat which is coexistent with endangered species habitat.

For the purpose of comparison we have chosen two locations, one in Alameda County (Ora Loma) and Hales Property in Marin County. Data were compiled from the three previous control seasons (October - April); 1986-1987, 1987-1988, and 1988- 1989. Prior to the 1988-1989 season, organophosphates and Golden Bear oils were used in the marshes by both districts while only biologicals and oils were used during the 1988-1989 season. Due to the importance of the independent variable, rainfall, on hatching success of *Aedes squamiger*, direct comparisons cannot be inferred from such a small data set. Additional data from subsequent years will be necessary to make statistically significant conclusions.

Nevertheless, certain trends and comparisons in costs for treating these areas can be examined with this in mind. The usage of fenthion, GB-1111, and *Bti* for the three previous *Aedes squamiger* control seasons in Marin County demonstrate the continuing trends. Fenthion usage dropped from one gallon in 1986-1987 to zero in 1988-1989, while International Toxic Units (ITU's) of *Bti* substantially increased from less than one billion during the 1986-1987 and 1987-1988 control seasons to over 900 billion during 1988-1989. Golden Bear oil use remains relatively stable between 500 and 1,750 gallons since this is the product of choice for *Culiseta inornata* (Williston) treatment. Some use of oil was reserved for treating *Aedes squamiger* pupae but this was minimal due to the increased effort to treat early instars.

Alameda County MAD experienced a similar trend in pesticide use for *Aedes squamiger* control over the same period (Fig. 1). Once again, there is a general decline in organophosphate and larviciding oil usage and a corresponding increase in biorational insecticide use.

Intrusion frequency is also increased with the use of biological control agents. Examination of ACMAD's work experience over the past two seasons at the Ora Loma property shows 31 inspections and three treatments during the 1987- 1988 season and 29 inspections and eight treatments

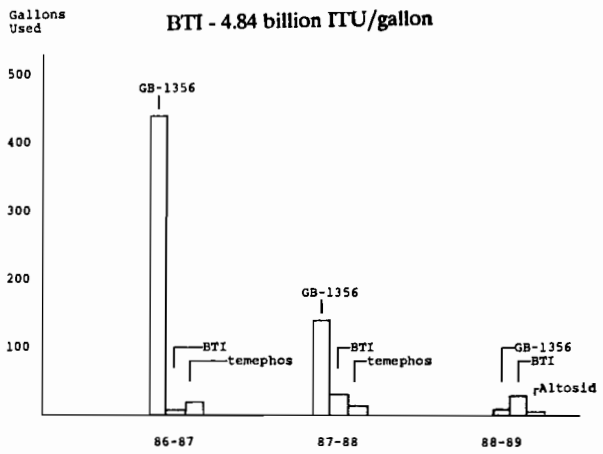

Figure 1. ACMAD's insecticide usage over the last three seasons for the control of *Ae. squamiger*.

during the 1988-1989 season. A similar experience was noted by MSMAD at the Hales property in Marin County with two inspections and one treatment during the 1987-1988 season and five inspections and four treatments during the 1988- 1989 season.

Surveillance time is increased significantly using the biorational approach to larviciding. Subsequent trips (post treatment) are necessary to evaluate the effectiveness of the biological application. Greater presence in the marsh with all- terrain vehicles (ATV's) increases the indirect costs of marsh disturbance, short-term damage to resident vegetation, an increased potential for oil or hydraulic fluid leaks or spills, disruption of endangered species habitat, greater potential for incidental take of endangered species, and greater risk for employee injury, (increased man-hours in a higher risk category resulting in increased insurance costs).

Since the biological control philosophy is not restricted to just *Aedes squamiger* control, the increased costs for biologicals is reflected in the districts' overall pesticide budgets. Figure 2 shows the dollar amount spent by Marin/Sonoma MAD on pesticides for the past ten years. The dramatic increase in funds allocated to pesticide acquisition is principally due to the high cost of these biologicals and the need for more frequent applications.

A cost per acre comparison for fenthion and other non-organophosphate alternatives is shown in Table 1. It is obvious that biorational insecticides are several times greater in cost than the organophosphate, fenthion.

If we look at a price comparison between fenthion with other non-organophosphates in Table 2, we see a low of three times to a high of sixty-two

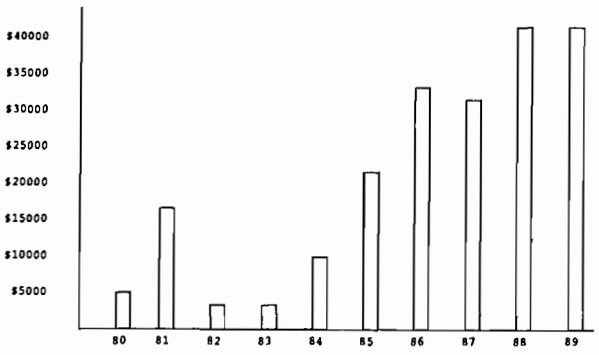

Figure 2. MSMAD's pesticide expenditures over the last ten years.

Table 1. Cost/acre values for various insecticide treatments used in the control of *Aedes squamiger*.

Treatment	Application Rate Low	High
Fenthion Liquid	$0.30	$1.18
Altosid 150-day Briquets	$3.48	$4.64
Vectobac-G Granules	$2.33	$18.60
Vectobac-12AS Liquid	$1.77	$7.06
GB-111 Oil	$5.04	$8.40
Bti-Sand Granules	$4.15	$16.60
Altosid-Sand Granules	$3.31	$4.10
Vectobac-12AS + Altosid Briquets	$5.25	$11.70

Table 2. Comparative costs of fenthion against biorational and oil insecticides at two different application rates for the control of *Aedes squamiger*.

Treatment	Fenthion (0.025 lbs AI/A) Low	High	Fenthion (0.050 lbs AI/A) Low	High
Altosid 150-day Briquets	12X	15X	6X	8X
Vectobac-G Granules	8X	62X	4X	31X
Vectobac-12AS Liquid	6X	24X	3X	12X
GB-111 Oil	17X	28X	8X	14X
Bti-Sand Granules	14X	55X	7X	28X
Vectobac-12AS + Altosid Briquets	18X	39X	9X	20X

times the cost for materials alone using the biological control approach.

Caveat.

We have all been reassured that there will be no new federal taxes, but the fact remains that our constituents are bearing the burden of increased pesticide budgets and manpower needs to comply with the Federal mandate of the endangered species act. The public will probably have to pay for protecting endangered species indirectly since a recent survey of southern California residents conducted by the Los Angeles Times (December 10, 1989) found that the majority would "tolerate significant life style and economic inconveniences to protect the environment but would not pay higher taxes to help save endangered wildlife". Similarly voluntary contributions from California tax returns are not encouraging. Approximately one million dollars was contributed in tax year 1987 and approximately $909,000 in tax year 1988. These funds are for the protection of all endangered species, plant and animal, within California.

The Saltmarsh Harvest Mouse (*Reithrondontomys raviventris*) and California Clapper Rail (*Rallus longirostris obsoletus*) were originally placed on the endangered species list primarily due to loss of habitat and not as a result of detrimental affect by pesticides. Recent articles in the San Francisco Chronicle (January 22, 1990) report continued illegal destruction of wetland habitat by developers and private land owners around the bay area.

It is the goal of the mosquito abatement districts to voluntarily comply with the ESA before it becomes mandatory to exhibit good faith and willingness to work with the USFWS, California Department of Fish & Game and EPA to protect endangered species while suppressing saltmarsh mosquito populations. It is, however, our constituents who are indirectly footing a portion of the bill to protect these species.

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A NEW VECTOR CONTROL PROGRAM

FOR THE CITY OF MORENO VALLEY, CALIFORNIA

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In 1989 the Moreno Valley City Council decided to provide it's residents with a Vector Control Program (VCP) to fill a function that the Riverside County Environmental Health Services Division had formerly provided, prior to the city's incorporation in 1984. The VCP is administered through a combination of the Public Works Department (Maintenance Operations) and the Building and Safety Department (Code Enforcement). The 1989-90 budget provided $137,000 for one Vector Control Technician and one Code Enforcement Officer and the accompanied costs of spray equipment and vehicles. The Vector Control Technician would provide the administration and delivery of mosquito, chironomid, rodent and fly control services. Right-of-way weed control was added to the list later on.

In October 1989, I was hired to set up and operate the vector and weed control programs. The city had generated a basic job outline of the services desired. From that document I drafted a detailed program outline that described the mission, specific tasks, goals and objectives and proposed control methodology. This program outline is updated every six months to insure progress. The updates address problem areas and the status of the program. These reports are delivered to the Maintenance Operations Manager and Director of Public Works. It is essential to keep the organization apprised of the status of the program.

Mosquito control.

The City of Moreno Valley, with a population of 120,000, is one of the fastest growing cities in the United States with an estimated growth of 20,000 people per year. The city contains a number of developments surrounded by undeveloped land. This type of patchwork development yields numerous street drainage areas throughout the city. As development occurs, these drains will give way to catch basins and underground drainage. Currently, I am treating 900 catch basins with Altosid XR (methoprene) which has yielded excellent larval control with minimal expenditure of time. Where applicable, mosquitofish (*Gambusia affinis*) are planted in street drains and 100% control is easily accomplished. I am currently using *Bti* in the form of Vectobac 12AS in all other sources. Those sources breeding high numbers of pupae are treated with Golden Bear 1356.

We are bordered on our northern perimeter by a range of foothills that produce drainage to the south. In dry years the drains are of minor significance. The same applies to six flood retention basins within the city. We also have a series of duck clubs outside of our control area that produce substantial numbers of *Aedes dorsalis* Meigen, that filter into residential areas.

In 1990 we had one suspected St. Louis encephalitis case in Moreno Valley. I immediately instituted an Encephalitis Virus Study (E.V.S.) for pooling mosquitoes for submission to the Viral and Rickettsial Disease Lab (VRDL) in Berkeley, California. The resident had immigrated from India some two years prior to moving to Moreno Valley and had no recent travels to other areas. It was finally decided, by Dr. Richard W. Emmons of VRDL, that the virus was probably contracted at some earlier time and location.

Rodent control.

Our Rodent Control Program is performed on a service request basis. The Vector Control Technician receives the call and then inspects the property for infestation. I provide the residents with a written checklist of factors that may influence rodent harborage on their property. The checklist identifies items that need attention or repair. I stress exclusionary methods and environmental manipulation. Presently, we have very isolated infestations of the Roof Rat (*Rattus rattus*). Our control is limited to backyards and out-buildings and we extend printed information about rodent control to our resident callers. House Mouse (*Musculus*) infestations seem to be related to the urbanization of farm ground. These mice primarily enter through garages and move into walls and attic spaces via poorly constructed walls or pipe openings.

Fly control.

The need for fly control has been primarily due to three remaining poultry ranches near the eastern and undeveloped perimeter of the city. I developed a fly source inspection form by which I can checklist problem areas and recommend steps for control. The real backbone of this process is the development of a cooperative effort with poultrymen that will allow us to gain control of flies. Every effort is made to avoid legal abatement procedures and work quickly to solve problems.

Weed control.

The Vector Control Program is also responsible for the weed control efforts in the city, comprised of over five-hundred and twenty-eight miles of roadway. This is accomplished by the application of residual herbicides in the winter and translocated herbicides in the warm months. This effort has dramatically reduced the mechanical and hand removal of pest weeds. The herbicide-treated areas include roadsides, parkways and drainage ditches. This portion of the program is in cooperation with the Street Maintenance Division of Public Works, and currently utilizes 20% of our labor hours.

In the past year I have received service requests about pests that really don't fit into the vector category. These pests range from birds, snakes (including rattlers), opossums, ground squirrels, assorted spiders and scorpions and numerous insects. This part of the Vector Control Program is more of a public relations effort. I will identify the pest, research the biology and control and then extend that information to the resident. I keep some forty different "Pest Control Bulletins" about specific pests on hand for distribution to residents. With basic pest information, the homeowner can make better decisions about controlling pests themselves.

In Moreno Valley we have a growing problem with rockdoves (*Columba livia*). Resident calls seem to follow a pattern. The caller has a tile roof with an overhang that provides an excellent nesting niche. A pair of rockdoves arrived one day, nest and soon the roof population is eight, and then twenty. "Enough!", the human hosts complain. I will assist by inspecting the roof eave areas and recommending remedies for any problem areas found.

We have yet to provide actual control of rockdoves, although I am studying trapping methods in regards to E.V.S. blood-sera samples. I am using a wire rockdove trap to collect these birds. The trap measures two feet by six feet with a height of eight inches. Due to our trapping on pitched roofs, a plywood floor with a fourteen inch landing platform was added. Affixed to the plywood are two braces that hold the trap level on a pitched roof. The rockdoves land on the plywood that is baited with grain and then are drawn into the trap by additional feed or a decoy. On the far end of the trap there is a frame that holds four one-gallon containers. Two are filled with water and two with pigeon feed. Once in the trap, the rockdoves have enough food and water for approximately five days. Rockdoves are leery about entering these traps unless a decoy is placed inside or until they get use to the trap placement, which takes two to three days. Catch numbers are frequently up to ten birds a day in a heavily infested area.

Because of the unique status Moreno Valley holds as being one of the few cities that provide vector control, I have submitted a proposed ordinance to the City Attorney that is based on the State Public Health and Safety Codes. The latter work well for vector control districts, but need to be edited for municipal programs. I removed all references to district boards and entered the appropriate authority that would perform those functions. The different authorities would include the city council, department heads, code enforcement officers and middle managers. This organization is needed in case of any legal actions on the part of the Vector Control Program. The ordinance also provides for assessing a service charge on a per parcel basis. Since our budget for vector control is generally funded, we are investigating the use of such a service charge.

In order to survive in the nineties, we are going to have to be flexible in the service that we provide. No longer will a mosquito abatement district be able to provide just mosquito control. The complaints that you handle at this time will be your support in the planning of full vector control services for the future.

Acknowledgements.

I would like to thank Don J. Womeldorf, Charles M. Meyers and Minoo B. Madon of the Environmental Management Branch for their assistance and notes concerning the drafting of an ordinance for the City of Moreno Valley. I would also like to thank Barry McClellan, Director of Public Works and Charles A. Hatfield, Maintenance Operations Manager for the City of Moreno Valley, for their continued interest and support of the Vector Control Program.

THE ROLE OF SCCVCD IN LYME DISEASE SURVEILLANCE

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Our District has been very involved in Lyme Borreliosis (LB) surveillance since we started receiving many phone calls about LB last spring when Newsweek and other publications began publicizing the importance of the disease.

Our District program includes the following six elements:

1) We receive telephone inquiries concerning basic questions about ticks, disease risk, and a few medical questions about the tests for Lyme disease. Physician-related questions are referred to the Deputy County Health Officer, our epidemiologist.

2) We are notified of new cases by our Health Officer, and when appropriate, we will be doing tick surveys of the area where the patients believe they were exposed to ticks. In 1989, we had 18 cases reported in Santa Clara County. We hope that our input will help the Health Officer screen the reports to determine if they are Lyme disease cases.

3) We recently created the position of Community and Education Resource Coordinator (a senior technician level assignment). Her projects on LB include the following:

   a) Develop a LB pin-up for physicians which will include color photographs of the "typical rash" along with symptoms. This will be for our 2,000 physicians since it is exetremely difficult to diagnose LB and early treatment is critical.

   b) Prepare a presentation for use by District staff to medical groups during May and June.

   c) Prepare a new LB brochure for the public to replace the CDHS brochure. This effort will be coordinated with Jim Clover at the CDHS.

   d) Participate in our county fair to educate the public on LB and other aspects of vector control. We had over 7,700 attend our booth in 1989. We have received valuable assistance from San Mateo County Mosquito Abatement District in preparing our booth.

   e) Continue to answer telephone inquiries as well as give presentations to schools and other civic groups requesting services.

4) We identify ticks for anyone bringing ticks to us. We receive these requests about once a week. If we receive a request from a physician, we go to their office and pick up the tick.

5) We are doing tick surveys of our parks (County, City, and State) to familiarize ourselves with the terrain, and if people call about a particular area, we can tell them if we have picked up ticks in that area. After a recent conversation with Jim Clover, we will develop a vegetation map of the County and sometime in the future designate deer populated areas to further designate high risk areas.

6) We are in the process of providing education to the park rangers and County Health Department's Communicable Disease Committee for obvious reasons and the Public Health Nurses because they interview and screen all LB cases reported to the Health Department.

Two other points I need to address are the following:

1) CDHS sent the new CDC Lyme disease survey report forms along with the pending case definition of LB to each County Health Officer last December. Additional copies are available after this presentation.
2) Please reach out and touch your County Health Officer to determine what role your District can assume in LB. Your entomological expertise is vital in the diagnosis of this disease which requires epidemiological information for diagnosis. This is not a disease that can be accurately diagnosed just by a laboratory test.

We must work together with the medical community to protect the people of California from this very important vector-borne disease.

TWELVE YEAR UPDATE ON THE ONTARIO ROOF RAT ENVIRONMENTALLY IMPROVED BLOCK MAINTENANCE PROGRAM

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ABSTRACT

The City of Ontario, California, realized they had a rat problem in the late 1950's. The San Bernardino County Department of Environmental Health Services implemented a federally funded roof rat control program during 1978-1980. This program utilized environmental manipulation as well as standard rodent control measures in a 193 block target area in the city. From 1981 to today this program continues via a contract with the city. Roof rat numbers were lowered and kept at low numbers by reducing their required harborage and food.

Introduction.

The City of Ontario, California, with a population of over 124,000, is located in the San Bernardino Valley approximately thirty miles east of Los Angeles and twenty-five miles west of San Bernardino. Rat complaints started to increase in the late 1950's and in 1958 the City of Ontario instituted a sanitary sewer rat control program which consisted of rodent baiting with poison. In 1964, the City of Ontario asked the State Bureau of Vector Control and the San Bernardino County Health Department to evaluate the rat population infesting the city sewers and to recommend a program for their control. During the initial study, all rats observed were the roof rat, *Rattus rattus* L. (Rohe 1966a and 1966b). Eighty-four (37%) of the 229 sewer manholes that were checked were shown to be positive for rat infestation.

Rat complaints continued to increase steadily even after the city implemented control measures. The San Bernardino County Department of Environmental Health Services conducted a rodent survey of the sewer system in 1971 and found the roof rat spreading to neighboring communities. All the rats found above ground and in the sewers were a single subspecies of roof rats, *Rattus rattus alexandrinus* Geoffroy St. Hilaire (Cox 1973). Approximately 15% of the homes in the entire city of Ontario were found to be infested with rats and 4.3% of the sewer manholes had evidence for rats (Zdunowski 1975). A target area of 191 blocks was outlined with an associated 35% infestation rate for rats. This target area was chosen on the basis of an unemployment rate greater than 10% and the average income being 15% below the mean for the State of California.

Roof rats can be a severe problem for the city. Not only do they cause concern by physically destroying objects but they are vectors of many diseases. Sylvatic plague is endemic to San Bernardino County and the roof rat with its associated fleas is a vector of the plague bacterium. Salmonellosis food poisoning and amoebic dysentery can be spread through rat excreta (Scott 1959). Murine typhus and leptospirosis are two other diseases that are transmitted in rat excreta. Rat-bite fever is transmitted to man by the bite of a rat and without treatment, can cause up to 10% mortality (Richter 1945). The tropical rat mite, *Ornithonyssus bacoti* (Hirst) is a common ectoparasite of roof rats in southern California and can cause a rat mite dermatitis (Brooks 1966).

It was the abundance and rising population of these rats in the city of Ontario with the potential for disease spread, that led the Department of Environmental Health Services to propose a demonstration roof rat control program. The federal government already had in existence funding for Norway rat control programs; but roof rats presented a new problem. The County of San Bernardino applied for, and received in 1977, a grant to implement a new program to control these rodents. This is a report on the efficacy of the program after twelve years of operation.

Materials and Methods.

Once it was established that roof rats were a problem for the city of Ontario a target area was

formed for the program area. Federally mandated guidelines were followed to delineate two target areas comprising 193 blocks total (in preliminary studies only 191 blocks were studied). Selection criteria were:

1. Active roof rat signs of 25% or higher (35% actually found).
2. The mean family income of families in the target area had to be $10,000 (later changed to $12,000).
3. Incidence of greater than 15% exposed garbage and 30% unapproved refuse at residences.

These 193 blocks consisted of 4,968 dwelling units housing 11,200 people. Block composition consisted of 85.5% residential, 12.8% business, 1.4% vacant lots, and 0.6% mixed business and residential (Fig. 1).

Allocated personnel included a program manager, one community health educator, two first line supervisors, seven field aides, and nine part-time summer field aides. Block survey inspections commenced early in the first quarter of 1978. Various publications were available at the time of the implementation of this program advising on rat control (Howard and Marsh 1976; Littig et al. 1969) but the procedures in this program were those of Davis et al. (1974).

The Ontario Roof Rat Control Program utilizes environmental manipulation which is based on the premise that not until significant householder behavioral modification takes place will a neighborhood control the rats and prevent their successful re-entry (Long et al. 1980). The three major components of environmental manipulation are sanitation, exclusion, and suppression. Sanitation is the removal of exposed garbage, replacement of unapproved refuse containers, and the removal of outside accessible animal food sources. Exclusion is designed to keep rats away from man and to keep rats away from commodities which they require. Suppression is chemical, physical, biological, or any other form of direct killing. Two other factors were necessary for continued rodent control: (1) human behavioral modification utilizing a health education approach to help the residents modify their environment to such an extent that the rat carrying capacity was lowered, and (2) continued long-term maintenance of that modified habitat. Without continued attention a neglected premise could become a rat population focus.

Surveys of the blocks were conducted by one or two person teams methodically inspecting each home. Along with the inspections, rodent baiting and trapping were also carried out. Each team recorded individual home information on premise correction notices giving the home owner a copy (Fig. 2). These forms were informative to the homeowners as well as served as official notices should further legal action be needed if appropriate cleanup measures were not followed. This information plus other pertinent data was recorded on Exterior Sanitation and Rat Survey Block Record Forms (Fig. 3).

Staffing of the program never reached full

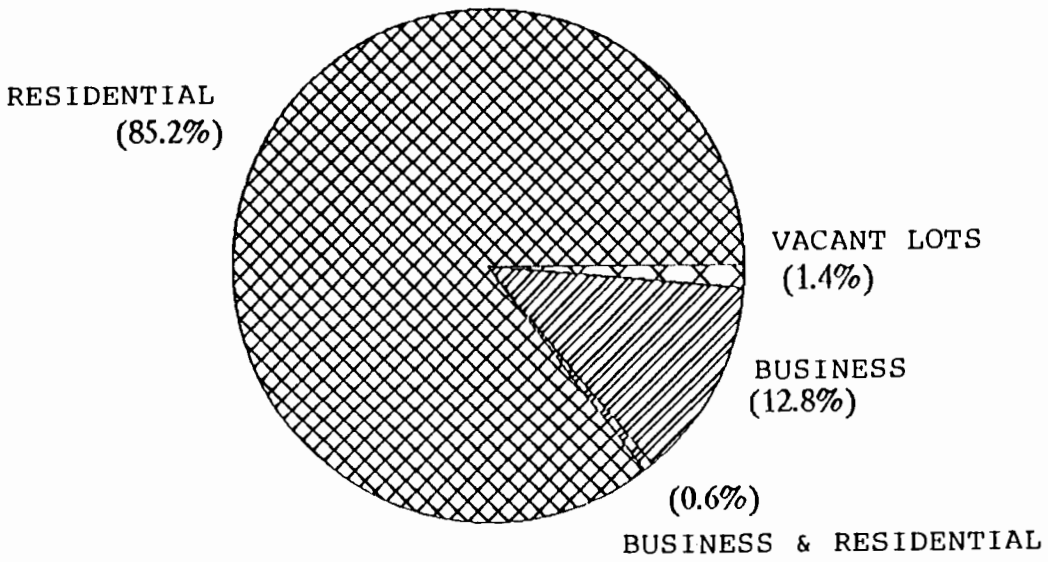

Figure 1. Ontario Roof Rat E.I.B. Maintenance Program block compostion.

1853

County of San Bernardino — Environmental Public Works Agency
DEPARTMENT OF ENVIRONMENTAL HEALTH SERVICES
☐ 2355 East Fifth Street • San Bernardino, Ca 92415 • (714) 383-3200
☐ 320 East "D" Street, Ontario, CA 91764, (714) 988-1208
☐ 15505 Civic Drive, Victorville, CA 92392, (619) 243-8141

RODENT CONTROL
PREMISES CORRECTION NOTICE

Date_____

Name_____ Regarding Premises Located At:_____

Address_____

An inspection of the above premises discloses conditions which must be corrected in order to assure that the house, the yard, and the neighborhood will be kept free of rodents. These measures are necessary to control and eliminate these animals and the ectoparasites which are a nuisance and can carry diseases.

THE ITEMS CHECKED [X] MUST BE CORRECTED:

I. Building(s) in need of ratproofing to prevent the entrance of rats through openings in foundations, siding, vents, etc.

a. ☐ Crawl holes, foundation vents, attic vents not effectively screened. (Use ¼" mesh wire, such as hardware cloth.)

b. ☐ Rats can enter at roof intersections, or under eaves.

c. ☐ Rats can enter through holes in foundation, loose or broken siding, or around holes for pipe or conduits.

d. ☐ Overhanging vegetation needs to be pruned away from building(s) and/or wires.

II. Rat harborages, where rats may hide and/or nest in protected places, which this Department found to exist on the premises.

a. ☐ Wood piles, loose lumber, metal, and miscellaneous items, must be stored 18 inches off the ground, with a clear area below, and 12 inches away from walls, buildings, or fences, or be removed from premises.

b. ☐ Rubbish piles, weeds, or brush to be removed from premises.

c. ☐ Heavy ground cover, ivy, elephant grasses, heavy vertical vines, etc., need to be pruned, thinned, or removed.

d. ☐ Palms or other trees to be pruned and/or thinned.

III. Potential food sources found to exist on premises.

a. ☐ Garbage improperly stored or not stored in fly and rodent-tight garbage containers.

b. ☐ Fruits and nuts not harvested and lying about on the ground.

c. ☐ Pet and bird foods left outside.

d. ☐ Improperly stored foodstuffs such as fruits, vegetables, nuts, pet foods, grains, etc.

IV. Other _____

Your cooperation in correcting the checked items, within _____ days and by _____, 19____, will be greatly appreciated. Failure to comply with this notice within the specified time may subject you to penalties as provided by State and County Ordinances. If additional information is needed, please call the office checked above (Vector Control Section).

RICHARD L. ROBERTS, R.S., MPH, Director
Department of Environmental Health Services

By_____
Vector Control Consultant

DISTRIBUTION: Original — Resident or Owner
Yellow — Field File

Figure 2. Ontario Roof Rat E.I.B. Maintenance Program Rodent Control Premise Correction Notice.

EXTERIOR SANITATION AND RAT SURVEY
BLOCK RECORD OR SUMMARY FORM
(composite sheet)

City_____ County_____ Date_____ Inspector_____ Page____ Of_____

ADDRESS BLOCK #s	# of Premises Inspected	Number of Premises	PREMISES INFORMATION							RAT FOOD					RAT HARBORAGE															ACTIVE RAT SIGNS			POTENTIAL RAT ENTRY		
													Plant Related							PLANT RELATED														
			Residential	Business and Residential	Business	Vacant Lot	Food Business	Vacant	Dwelling Units	Unapproved Refuse Storage	Exposed Garbage	Animal Food	Fruit/Nut Trees	Other	Abandoned Automobiles	Abandoned Appliances	Lumber on Ground	Other Large Rubbish	Outbuildings	Board Fences and Walls	Ivy-horizontal	Ivy-vertical	Vegetative Overhangs	Honeysuckle	Pyracantha	Palm Trees	Grass and Weeds	Other	Exterior (Use Code)	Interior (Use Code)	Sewers on Premises	Structural	Sewers on Premises	TOTAL VIOLATIONS PER PREMISES
SHEET TOTAL																																	
COMPOSITE TOTAL																																	
% PREMISE INSP.																																	
% TOTAL PREMISES																																	

Figure 3. Exterior Sanitation and Rat Survey Block Record Form.

capacity for various reasons (promotions, school, leaving, etc.). The proposal for the program listed twenty full- or part-time positions to be incorporated in the project. During 1978 eleven employees were actually hired. An average of eight employees were working on the project during 1979 and 1980. The federally funded program ended December 31, 1980. In subsequent years (1981 - 1989) staffing for the program was maintained at one to three people.

After the federally funded program ended, the San Bernardino County Department of Environmental Health Services proposed a plan to maintain the status of the blocks based on certain criteria. Maintenance-level criteria was that a block had two percent or less of the premises with active rat signs and either: (1) fifteen percent or less of the premises with exposed garbage, or (2) thirty percent or less of the premises with unapproved refuse storage. The plan would incorporate minimum use of human resources, materials, and services while interfacing with other municipal agencies and departments. Environmentally Improved Blocks (E.I.B.'s) were defined as "contiguous blocks (or an entire subtarget area) where maintenance has been achieved and sustained for a minimum of twelve (12) months" (Long 1980). The Environmental Improvement Agency would be the lead agency, and Environmental Health Services Department, the lead department, for controlling and maintaining all E.I.B.'s. The City of Ontario would be the lead municipal agency with operations disseminating from the City Building Department, Solid Waste Department, Fire Department, Street Maintenance Department, and Community Relations Department. Activities were divided for each group to minimize the pressures incurred by a single organization being solely responsible for control and maintenance of environmental and public health conditions.

Results.

Before the Ontario Roof Rat E.I.B. program began over 35% of the homes in the target area were infested with rats. At the end of the first comprehensive survey of Target Area A (blocks #121-193) in 1978, 18% of the homes were found to be infested with roof rats. Table 1 lists the projected and realized premise prevalence rate deficiency reductions for the program.

Target Area B (blocks # 1-120) was surveyed and rat control measures were enacted during the fourth quarter of 1978. After this survey, an infestation rate of 6% for rats was found for Target Area B. Procedures which lowered the rat numbers included code enforcement, baiting and trapping, palm tree baiting, placement of refuse bins, and community health education.

Objectives for Target Areas A and B were to have been as listed in Table 2 by December 31, 1978 and December 31, 1979, respectively.

Table 3 is a compilation of two year, five year, and twelve year data. By March 1979, 93% of the blocks in Target Area A had met maintenance criteria and 100% of the blocks were in maintenance on September 30, 1980. By December

Table 1. Premise prevalence rate of deficiencies as found over the past twelve years.

Deficiency	Premise prevalence rate (% of total inspected) 1978	1980	1989
Unapproved Refuse	57	5	1
Exposed Garbage	50	3	1
Ivy - Vertical	20	4	8
Ivy - Horizontal	13	2	4
Vegetative Overhang	48	7	33
Lumber on Ground	36	5	8
Other Large Rubbish	37	5	11
Outbuildings	38	5	4
Structural Defiencies	53	10	49

Table 2. Program objectives for Target Areas A and B. Figures are percent of objective.

Objective	Area A	Area B
Maintenance Level	95	95
Active Rat Signs	<2	<2
Exposed Garbage Reduction	30	15
Unapproved Refuse	15	10
Ivy - Vertical	<10	3
Ivy - Horizontal	<8	5
Lumber on Ground	<20	5
Outbuildings	20	10
Vegetative Overhang	20	15
Structural Deficiencies	20	15

1979, 86% of the blocks in Target Area B were in maintenance. As of December 31, 1980, all goals were reached and active rat signs were reduced to a prevalence rate of less than 1%. A total of 34,443 premises has been inspected during this program. Figure 4 shows percentages of exterior signs along with some factors affecting their population. Figure 5 depicts the decline in the number of rats over the years.

Six randomly chosen blocks outside the target areas were surveyed during early 1990. Rat prevalence was below 1% but occurrence of some rat harborage items were high; such as lumber on ground (18%), other large rubbish (23%), and vegetative overhang (50%). Selected listing of exterior sanitation deficiencies for the blocks outside the target areas are presented in Table 4.

Discussion.

The Ontario Roof Rat Environmentally Improved Block Maintenance Program had a specific goal, namely to lower the roof rat prevalence rate to 2% or less of the homes in the target area. This goal was achieved and maintained (excluding a rise to 3% in 1988). Deficiencies noted relating to rat harborage and rat food were lessened and remain so. Efficiency of the program is high with the current field technician surveying approximately 25-30 premises per day. Survey techniques remained constant by use of guidelines detailing methodology.

Rat numbers were low in both the targetted area as well as in the blocks surveyed outside the

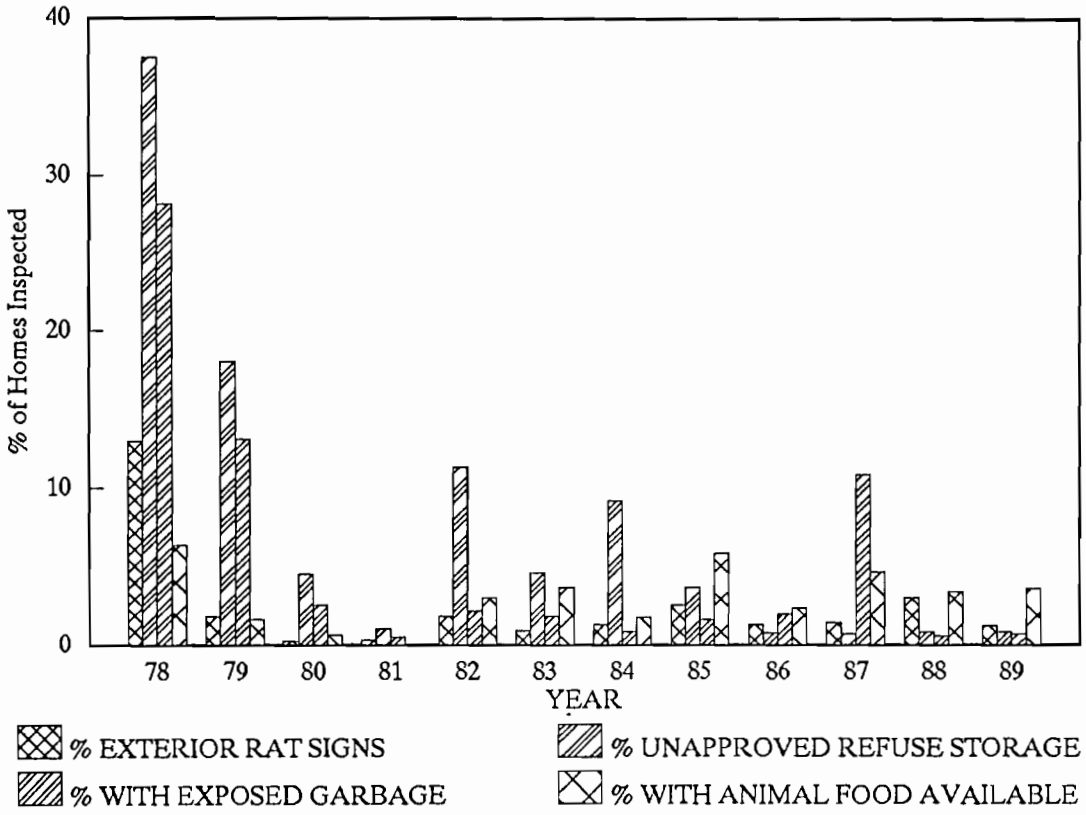

Figure 4. Selected factors affecting Roof Rat populations in the target areas.

Table 3. Two, five, and twelve year inspection data (and associated percentages of total premises inspected).

Inspection Criteria	twelve years (%)	first 2 years (%)	first 5 years (%)	last 5 years (%)	last 2 years (%)
No. of premises	2,870	9,566	5,362	1,345	1,499
Unapproved Refuse	592 (21%)	3,376 (35%)	1,400 (26%)	15 (1%)	14 (1%)
Exposed Garbage	486 (17%)	2,700 28%)	1,103 (21%)	62 (5%)	12 (1%)
Animal Food	102 (4%)	445 (5%)	186 (3%)	55 (4%)	55 (4%)
Fruit and Nut Trees	986 (34%)	4,194 (44%)	2,063 (38%)	288 (21%)	264 (18%)
Other Rat Food	209 (7%)	1,127 (12%)	475 (9%)	26 (2%)	7 (0%)
Lumber on Ground	462 (16%)	2,217 (23%)	936 (17%)	166 (12%)	136 (9%)
Other Large Rubbish	445 (16%)	2,219 (23%)	937 (17%)	127 (9%)	118 (8%)
Outbuildings	472 (16%)	2,329 (24%)	982 (18%)	135 (10%)	69 (5%)
Ivy - Horizontal	198 (7%)	909 (10%)	398 (7%)	72 (5%)	92 (6%)
Ivy - Vertical	253 (9%)	1,197 (13%)	526 (10%)	75 (6%)	103 (7%)
Vegetative Overhang	688 (24%)	3,150 (33%)	1,366 (25%)	271 (20%)	393 (26%)
Exterior Rat Signs	120 (4%)	636 (7%)	259 (5%)	27 (2%)	40 (3%)
Interior Rat Signs	6 (0%)	24 (0%)	10 (0%)	5 (0%)	8 (1%)

Table 4. Exterior sanitation deficiencies for blocks surveyed outside the target area during 1990.

Deficiency	% of total[a]
Abandoned Appliances	6
Lumber on Ground	18
Other Large Rubbish	23
Vegetative Overhang	50
Palm Trees	10
Grass and Weeds	9
Other Rat Harborage	7

[a] 162 premises were surveyed on seven blocks.

target area. But this is not a verified control area. The Ontario Roof Rat Program was publicized in newspapers and city publications which covered the whole city. Rat control measures were outlined and people were advised to call the San Bernardino County Vector Control Program if they suspected rats were present on their property. The vector control technicians conducted premise inspections, sewer inspections (with subsequent rodent control measures implemented) and block surveys outside the target area in areas where people complained of rats.

Rat complaints were not an accurate indicator of rat abundance. The more the public would know about the rat control program (press releases, television segments about rats, clean-up campaigns, etc.), the more phone calls would come in. What did happen is that rat numbers and associated food and harborage have all been lessened throughout the whole San Bernardino Valley.

Today the Ontario Roof Rat Environmentally Improved Block Program continues to educate residents in the target area. Blocks are routinely surveyed, trash clean-up programs conducted, community talks given, and press articles published when necessary. The free distribution of rodent bait ceased in 1986, but people are advised where to purchase appropriate rodent bait. Staffing currently consists of one vector control technician, one vector ecologist and one supervisor; all employees of the San Bernardino County Vector Control Program with other duties along with the Ontario Roof Rat Program. The Program is currently funded through a contract with the City of Ontario.

What is the next step? A new survey form will be used (Fig. 6) which will eliminate non-significant

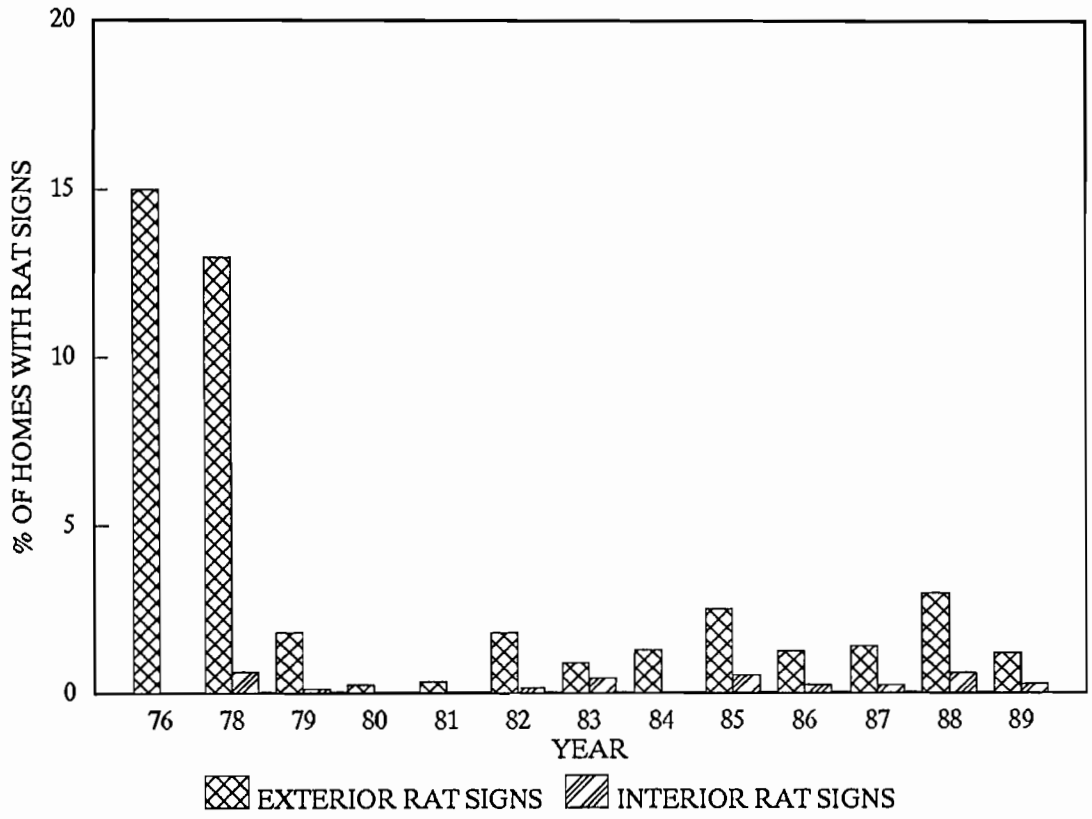

Figure 5. Percentages of homes with exterior or interior Roof Rat signs.

EXTERIOR SANITATION AND RAT SURVEY
BLOCK RECORD OR SUMMARY FORM
(composite sheet)

City_____ County_____ Date_____ Inspector_____ Page____ Of_____

ADDRESS BLOCK #s	# of Premises Inspected	PREMISES INFORMATION						RAT FOOD					RAT HARBORAGE								ACTIVE RAT SIGNS		POTENTIAL RAT ENTRY	
		Number of Premises	Business and Residential	Business	Food Business	Vacant	Dwelling Units	Unapproved Refuse Storage	Exposed Garbage	Animal Food	Plant Related		Abandoned Appliances	Lumber on Ground	Other Large Rubbish	Outbuildings	PLANT RELATED				Exterior (Use Code)	Interior (Use Code)	Structural	TOTAL VIOLATIONS PER PREMISES
											Fruit/Nut Trees	Other					Ivy	Vegetative Overhangs	Palm Trees	Other				
SHEET TOTAL																								
COMPOSITE TOTAL																								
% PREMISE INSP.																								
% TOTAL PREMISES																								

Figure 6. New Exterior Sanitation and Rat Survey Block Record Form.

information areas. Instead of a smaller target area, the whole city plus sufficient buffer area surrounding the city, needs to be included. The surveys do not need to be completed as often on each block; many people remembered the last visit and still practiced good rat prevention techniques. A complete survey every three to four years would appear to be adequate after initial rat control measures have been started. This program will remain in effect in the City of Ontario with the revisions that have been noted here.

Acknowledgements.

The author acknowledges Jeffrey Lane for his work on computerizing the data and all the staff of the San Bernardino County Vector Control Program, Department of Environmental Health Services, that have worked on this program over the years.

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CAN MARSHES BE MANAGED TO CONCURRENTLY REDUCE MOSQUITO DENSITIES AND ENHANCE WATERFOWL HABITAT?

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ABSTRACT

Seasonally flooded marshlands in California are major waterfowl habitats, but can also produce large numbers of mosquitoes. Our on-going experiments to examine whether seasonally flooded marshlands can be managed to limit mosquito densities and concurrently enhance densities of invertebrates consumed by waterfowl have been conducted in twelve experimental ponds (each 11 X 22 meters) located at Grizzly Island Wildlife Area in Solano County. These ponds were designed to mimic seasonally flooded marshes dominated by pickleweed, *Salicornia virginica*.

The plant cover in one-half of each of the twelve ponds was reduced 50% by mechanical mowing. Following flooding in September, densities of *Culex tarsalis* mosquito larvae were significantly lower in the 50% plant-cover treatment. In contrast, densities of hydrophilid beetle larvae were higher in the 50% plant-cover treatment areas. During October and November, these beetle larvae apparently suppressed densities of a third dominant species, chironomid midge larvae (*Cricotopus sylvestris*) resulting in significantly higher midge densities in the 100% plant-cover treatment areas.

By January, however, high *C. sylvestris* densities primarily occurred in the 50% plant-cover treatment areas; the reverse of the earlier pattern. This distribution of midges was correlated with algal production in the experimental ponds. Because waterfowl in California only consume invertebrates from December to March, higher densities of *C. sylvestris* midge larvae and hydrophilid beetle larvae were available for waterfowl consumption in the 50% plant-cover treatment areas; the 50% plant-cover treatment also resulted in fewer *Cx. tarsalis* mosquito larvae (i.e. October densities).

A VERSATILE CAGE CULTURE SYSTEM FOR THE MOSQUITOFISH, *GAMBUSIA AFFINIS* (BAIRD AND GIRARD)

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P.O. Box 726
Yuba City, California 95992

Introduction.

Many bodies of water throughout California could serve as useful rearing sites for mosquitofish, but are not being utilized at present for a variety of reasons. Common examples are the State's numerous wastewater treatment impoundments, which are often productive, but sometimes present serious obstacles to effective fish management and harvest activities. For instance, immense pond area and depth, excessive aquatic vegetation or manmade barriers to seining, such as floating or submerged aeration units often create problems for mosquito abatement biocontrol technicians. Other potential fish rearing habitats contain predacious fish populations typically represented by bass and other sunfish which commonly interfere with the survival or population growth of introduced mosquitofish. In some impoundments, these larger fish have completely eradicated mosquitofish earlier stocked for ongoing vector problems.

One practical aspect of fish rearing related to operational vector control is that of maintaining readily available stocks of fish in or near a technician's specific zone of operation. Small ponds, streams or drains stocked with fish for mosquito control purposes in the spring may often produce significantly more fish than would ordinarily be required for efficacious mosquito control throughout the summer months. These surplus fish could be advantageously employed in other nearby mosquito sources if only they could be easily retrieved from these productive waters. All too often it's simply impractical or too labor intensive to attempt recovery of these stocks of fish from former mosquito sources. Yet, one could consider it a waste of these typically eutrophic habitats to not be able to fully exploit at least some of them as alternative production sites for mosquitofish.

This project was designed to take advantage of this incidental fish production and develop various methods to best propagate mosquitofish in an essentially unmanaged aquatic habitat. One of the easiest ways to accomplish this is to create relatively small areas of confinement for stocks of mosquitofish at some convenient site within these productive waters. To do this, one could screen off a small area as a production site for captive mosquitofish. Polyvinyl chloride (PVC)/fiberglass window screen will usually suffice as the isolating barrier; although unusually small fry maybe able to penetrate new or clean fly screen, especially during the fall when noticeably smaller fry often appear in offspring populations. This is generally not of critical importance as mosquito-related fish stockings taper off at this particular time of the year. However, the mesh size selected should be large enough to permit the unrestricted entrance of microorganisms from the outside habitat to serve as forage items for the newborn. In extremely nutrient-rich habitats, these organisms may also be of sufficient abundance to nourish brood fish caged within the fry containment area; although many unfortunate young will usually be cannibalized shortly after birth.

Gravid female brood stock are communally maintained within this enclosure in large-mesh baskets, but kept isolated from the offspring in the outer enclosure to help reduce parent - offspring cannibalism and to facilitate the manipulation of the brood fish themselves. Periodic replacement of female stock within the brood baskets may be desired to assure maximum yields from this type of aquaculture system. In addition, scheduled removal of all offspring from the outer containment area will likely be required every few weeks to minimize cannibalism there as well.

Bird predation of captive mosquitofish is a distinct possibility; thus exclusionary cover netting will be a worthwhile option in most installations. The adult backswimmer (Notonectidae) is another important predator that often adversely affects mosquitofish production, especially in wastewater stabilization impoundments. Usually, weekly surface applications of Golden Bear Larviciding Oil (GB-1356)[a] or other suffocating oils will temporarily reduce backswimmer populations within the screened enclosures to manageable levels where they have formerly been a serious problem.

[a]GB-1356 - Witco, Golden Bear Div., Oildale, CA 93388-5446.

This brief experiment was designed to provide practical information on the use of a cage aquaculture system installed at a local secondary municipal wastewater facility where previous in situ bioassays had been conducted and had demonstrated that there was suitable quality water for long-term mosquitofish survival and reproduction.

Materials and Methods.

For this trial a very large, screen-covered rectangular framework was constructed of 200 psi, PVC pipe and schedule 40 PVC fittings. Assembled, the framework was 224" (569 cm) long, 78" (198 cm) wide and 40" (102 cm) deep. For more convenient transportation, the framework was constructed in two identical, sections which were joined together at the study site with the use of PVC unions. A fine-mesh screen box to fit the interior dimensions of the pipe framing was sewn together using two large sections of Aquascreen netting;[b] a PVC/fiberglass material with a mesh size slightly smaller than the ordinary window screen it closely resembles. Grommets were fastened to the screen enclosure along all its exterior edges and nylon cordage was used to attach these grommeted areas to the pipe framing. To suspend this large cage in the water, six, 0.5 ft^3 (14,160 cc) styrofoam floats were tied to the framework with cord and adjusted to provide approximately 16" (41 cm) of freeboard. Finally, a little water would be poured into the pipe framework to provide some necessary ballast and the completed outer cage would be ready for use.

A second, much smaller cage covered with larger-mesh plastic screen was constructed to serve as the breeding trap for gravid female stock. The selection of a suitable mesh for this spawning cage facilitated the passage of newborn fry into the confines of the outer screened enclosure previously described, while retaining all brood females. Although a cubic structure could have been employed, a wedge-shaped pen was constructed of 0.5" (1.3 cm) PVC pipe and covered with 0.125" (0.3 cm) high-density, polyethylene screening (Internet #XV-1670).[c] The overall measurements for the breeding trap were: 96" (244 cm) length, 20" (51 cm) width and 15" (38 cm) depth at waterline. The 8 ft^3 (0.23 m^3) screened breeding trap received an interior lining of three-dimensional, plastic matting (Enkamat #7020).[d] This 0.7" (18 mm) thick material served as temporary refuge for the offspring to further protect them from cannibalistic females before they had developed enough vigor to swim through the breeding trap's mesh out into the larger enclosure. Buoyancy for the breeding trap was afforded by a rectangular, sealed 4" PVC drain pipe support collar attached to the enclosed trap by means of nylon cable ties. Lastly, a black shade cloth cover was employed to both shield the trap from excessive sunlight and prevent adults from jumping into the outer enclosure.

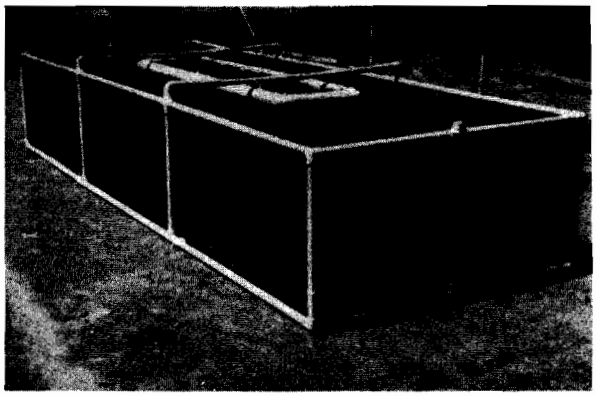

Figure 1.-Complete cage system ready for testing.

On August 22, 1989 the complete cage system (Figure 1) and brood stock were installed in Pond #5 at the Municipal Wastewater Treatment Facility of Live Oak, California. Initial brood females were collected at the Sutter-Yuba Mosquito Abatement District fish farm and 500 presumably gravid females that could not pass through a 0.20" (5 mm) bar grader were selected for stocking in the breeding trap. A few fry appeared in the outer enclosure almost immediately and an estimated 1000 more were observed several days later. However, on September 5th, a call was received from a sewage treatment plant operator stating that vandals had set the cage system adrift in the impoundment. Upon inspection that day, no overt damage was detected and 462 thriving females were removed from the spawning trap. They were replaced with 500 unspawned females on September 6th.

On September 19th, 485 surviving brood females from the September 6th stocking were removed and replaced with new spawners from similarly-graded stocks harvested from Pond #7 of this facility. All offspring were collected from the outer enclosure at this time and preserved in 10% formalin. While removing fry, a small hole in the Aquascreen was spotted near the bottom edge of the outer enclosure and patched. After examining

[b]Aquacreen® - Menardi-Criswell Corp., Augusta, GA 30903.
[c]InterNet #XV-1670 - InterNet Inc., Minneapolis, MN 55427.
[d]Enkamat #7020 - American Enka Co., Enka, NC 28728.

this 0.25" by 0.75" (10 mm by 30 mm) hole, it was concluded that a muskrat's chewing had likely caused the damage. The hole's shape, size and location was judged to be inconsequential with regard to the possible loss of offspring from the outer enclosure.

Periodic surface applications of larvicidal oils were required throughout the study, as notonectids were extremely abundant in this series of ponds and were able to fly into the outer enclosure where they pursued newborn fish. These treatments inside and outside the cage system never appeared harmful to any of the fish, as no female or fry mortality was observed during the term of this experiment. As the fall season arrived, significantly lower water temperatures were recorded and by November 1st, the pond water temperature had dropped to 57° F (13.9° C) and the experiment was terminated at this time. All fry were harvested, preserved as before and 308 surviving female brood fish were removed from the spawning basket and stocked into the waters of the experimental study pond - the disposal site for all other earlier spawned females. The entire cage system was then removed from the water, disassembled and returned to the District where it was cleaned and stored. On this date a tiny new, but very similarly shaped hole in a bottom crease was noticed. As before, it was presumed to be muskrat damage and again in a location not likely to result in any significant loss of fish.

Representative samples of offspring were later examined, sexed (when possible), measured and enumerated to provide information to help evaluate the success of this informal trial.

Results and Discussion.

Experimental data derived from this brief trial are depicted in Table 1. In terms of results, two separate stockings of 500 female brood fish contributed 2,678 offspring that were harvested from the outer enclosure; subsequently, a single stocking of 500 females produced the remaining 1,760 young that were collected the last day of the study (Nov. 1st). In all, 4,438 fish were produced in this system employing untried materials configured in a previously untested manner.

The effect of approaching fall and winter seasons played an obvious and significant role in the reduced growth rates of young produced from the last (Sept. 19th) brood fish stocking. Unfortunately, there was no way to estimate the extent of loss of newborn caused by 32 brood female escapees that were recovered from the outer enclosure at the termination of the study. Nor was it possible to quantify the effect a varying, but often abundant population of adult notonectids inhabiting the outer enclosure exerted on the young fish. The mean number of young produced per stocked female for this trial was only 2.96; which

Table 1.-Brood Female Management and Offspring Harvest Data.

Date	Number Brood Females Stocked	Number Spawned Females Removed	Number Offspring Harvested From Cage	Mean Offspring Per Brood Female	Mean Offspring Length, mm Male/Female	Gender Offspring Male/Female (%)
08/22	500					
09/05		462				
09/06	500					
09/19	500	485	2,678	2.68	18.5/18.9	36/64
11/01		*340	1,760	3.52	12.3**	n/a***
Totals/ Means:	1,500	1,287	4,438	2.96	15.8**	n/a

* Included in this value were 32 brood females that escaped from the spawning basket into offspring enclosure.

** All offspring combined, regardless of gender.

*** Sex ratio not reported; most offspring were too immature to accurately identify gender.

was extremely low. For the first two stockings, no obvious attempt was made to individually select only those females obviously gravid; instead, this particular bar grader was chosen because it quickly provided the 500 largest and presumably gravid mosquitofish available at the time from our source of adult fish.

As the fall season neared it became increasingly difficult to obtain any large female fish; thus some slight bias may have been inadvertently introduced in the selection of the last batch of brood females over the two previous selections. This may possibly help explain why more offspring were produced by the last group of females than the two previous stockings (3.52 vs 2.68 fry per female, respectively). Another possibility is that there may also have been a more pronounced behavioral tendency for the last group of females to complete spawning, as demonstrated by the comparatively smaller offspring observed in the enclosure after September 19th. Perhaps the onset of the fall season serves to provide some natural cue to near- term gravid females to develop and release smaller, poorer nourished fry just before the end of their normal reproductive season.

Recommendations.

Overall, this brief trial demonstrated that this cage system has merit; although some refinements are definitely needed to help improve its management. The cage system constructed this year was really too large and cumbersome for easy manipulation during regularly scheduled harvests. At least two workers were needed to move the outer enclosure into shallower water where enough of its bottom could be exposed to guarantee that all fry were actually being removed. Thus, the primary cage design to be tested next summer will be much smaller so that a single technician can easily and rapidly harvest all offspring.

Some other design modifications to this system would undoubtedly result in a configuration that could be more closely be adapted to the varying physical requirements of different aquatic sites. For example, instead of constructing a screened PVC box for the outer enclosure as done for this study, placement of vertical screening along a single edge and corner of a pond could simplify and speed up installation, especially if the bottom edge of the fence-like outer barrier was extended a short distance below the water surface and then across the pond horizontally onto the shoreline, thus creating a trough-like enclosure. To harvest, technicians would merely pull most of the submerged netting shoreward leaving as a result only a tiny submerged area from which all fish could be harvested. After this maneuver, the screening would again be drawn out into the pond to its original position.

Additionally, several female spawning traps could be placed within a single offspring enclosure to accelerate fry production. Finally, in some suitably turbid waters, various types of natural or synthetic harborage materials could be inserted in the outer enclosure and would be exploited by the fry, thereby aiding in reducing large offspring - smaller offspring cannibalism, especially when frequent offspring harvests are deemed impractical.

PRELIMINARY RESULTS OF AN ELISA TEST FOR DETECTION OF ORGANOPHOSPHATE-RESISTANCE IN *CULEX* POPULATIONS DUE TO INCREASED DETOXIFICATION BY ESTERASE

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ABSTRACT

An ELISA procedure that enables the detection of increased activity of esterase B1 in organophosphate-resistant *Culex* mosquitoes is described. Preliminary results indicate that the procedure adequately identifies resistant individuals in laboratory as well as field strains. The quantity of esterases can be estimated through serial dilution of homogenate.

Introduction.

Two types of detoxifying esterases, A and B are responsible for resistance to a variety of organophosphate insecticides in *Culex* mosquitoes. Both types have been purified and shown to be structurally different: esterases type B (i.e., B1, B2 and B3) are monomeric molecules of 67 kDa, whereas esterases type A (i.e., A1, A2, and A3) are dimeric molecules of 120 kDa containing a polypeptide of 60 kDa (Fournier et al. 1987, Mouchès et al. 1987). Using antisera raised against the 60 kDa polypeptide of esterase A1 and the 67 kDa polypeptide of esterase B1, Mouchès et al. (1987) demonstrated that increased activity of both esterase types is due to an increased production of the protein.

In the present investigation, we report preliminary results of an ELISA procedure for detecting and quantifying increased production of esterases B in single mosquitoes, and we discuss the potential use of this procedure in the detection and monitoring of organophosphate resistance in natural populations.

Materials and methods.

Mosquito strains: The mosquitoes used were from laboratory strains as well as field collections of *Culex quinquefasciatus* Say and *Culex pipiens* L. (Table 1). These mosquitoes are susceptible or resistant to organophosphorus insecticides due to various esterases. The type of the esterase present and the proportion of insects carrying that esterase were determined by starch gel electrophoresis of single insect homogenates following the procedure of Pasteur et al. (1981).

Sample preparation: Homogenates were prepared by grinding insects in 0.05 M sodium carbonate/bicarbonate buffer pH 9.6 (1 ml/mosquito) in 1.5 ml polypropylene microcentrifuge tubes with a teflon pestle. They were centrifuged at 16,000 g for 30 sec. The supernatant was either used directly or after further dilution in the same buffer.

Enzyme-linked Immunosorbent Assay (ELISA): Anti-esterase B1 antiserum prepared by Mouchès et al. (1987) was purified and part of it was conjugated to alkaline phosphatase (Sigma) as described by Clark and Adams (1977).

Initially, three ELISA procedures (Crook and Payne 1980) were tested. The direct and double antibody sandwich methods used the conjugate prepared as described above. The indirect method utilized commercially prepared goat anti-rabbit antibodies conjugated to alkaline phosphatase (Boehringer Mannheim Biochemicals, Indianapolis, IN) or horseradish peroxidase (Bio-Rad Laboratories, Richmond, CA). Results of initial tests comparing the three procedures, and the limited availability of antiserum B1 prompted us to choose the indirect method.

The ELISA procedure used was as follows: 200 µl volumes of adult mosquito homogenate were

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Table 1.-Laboratory strains and field collections of *Culex* investigation.

Strain	Species	Origin	Active esterase (and frequency)	Reference
S-Lab	*Cx. quinquefasciatus*	Lab. (California)	none	Georghiou et al. (1966)
S54	*Cx. pipiens*	Lab. (France)	A1 (100%)	Pasteur and Sinègre (1978)
SeLax	*Cx. quinquefasciatus*	Lab. (California)	A2/B2 (100%)	Wirth et al. (1989)
Simpson	*Cx. quinquefasciatus*	Field (California)	B1 (100%)	Georghiou et al. (unpublished data)
Berkeley	*Cx. quinquefasciatus*	Field (Illinois)	B1 (50%)	Georghiou et al. (unpublished data)
Tem-R	*Cx. quinquefasciatus*	Lab. (California)	B1 (100%)	Ranasinghe and Georghiou (1979)

introduced in wells of polystyrene ELISA plates (Immulon I-Dynatech, Dynatech Laboratories, Inc., Alexandria, VA), and incubated overnight at 4° C. Plates were washed three times for 3 min with PBST (0.02 M sodium phosphate, 0.15 M NaCl, 0.02% sodium azide, 0.05% Tween-20 (v:v), pH 7.4), followed by introduction in each well of 200 µl purified antiserum B1 diluted (1 µg/ml) in PBST-PVP (i.e., PBST containing 2% of Polyvinylpyrrolidone, MW 44,000). After incubation for 3 hours at 37° C, the plates were washed as described above, 200 µl of alkaline phosphatase-labelled goat anti-rabbit IgG diluted in PBST-PVP (1:2500) were added to each well, and incubated for 3 hours at 37° C. The plates were washed again in PBST, and 200 µl *p*-nitrophenyl phosphate solution (0.6 µg/ml of 9.8% diethanolamine (v:v) buffer adjusted to pH 9.8 with HCl) were delivered in each well. Plates were incubated at room temperature (~25° C) and the OD405 was measured with a BioRad EIA reader (model 2550) after 5, 15, 30, 45, 60 and 90 min incubation periods. Results were expressed as optical densities (OD's) after a given time of incubation or as the variation of OD per minute (OD/min). It was found that conversion of OD/min in units of alkaline phosphatase activity reduced considerably the day to day variation. This conversion was done in establishing, for each microtiter plate, a standard curve for alkaline phosphatase activity using 100 µl of 6 dilutions of goat anti-rabbit IgG conjugated with alkaline phosphatase (i.e., dilutions containing 0.00087 - 0.00690 units of enzyme) in PBST-PVP, and 100 µl of *p*-nitrophenol phosphate solution.

Results.

Detection of *Culex* esterases with Antiserum B1: Mass homogenates of mosquitoes from strains S-Lab, S54, SeLax, and Tem-R were tested by ELISA using the anti-esterase B1 antibody. As was expected, little difference was observed between S-Lab and S54 mosquitoes (Table 2) since the former strain has no esterase of high activity, and the latter possesses an esterase (A1) which does not cross-react with antiserum B1 (Mouchès et al. 1987).

The SeLax strain (with esterases A2 and B2) appeared to contain a slightly higher quantity of esterase cross-reacting with antiserum B1 than either the S-Lab or S54 strains (1.12 to 1.75-fold in 2 experiments). Due to experimental variations, it is difficult to determine whether this difference is significant, but it may be concluded that either cross-reactivity between esterase B2 and antiserum B1 is low, and/or that the increase in quantity of esterase B2 in the SeLax strain was barely detectable under the experimental conditions used.

On the contrary, Tem-R mosquitoes (with esterase B1) contained large quantities of esterase reacting with antiserum B1. Depending on the experiment, the difference between S-Lab and

Table 2.-Quality of esterase (expressed in units of alkaline phosphatase) cross reacting with the esterase contained in different *Culex* strains.

Strain	Quantity of esterase (AKP units)	Ratio over S-Lab
S-Lab	0.41	1.00
S54	0.58	1.41
SeLax	0.76	1.86
Tem-R	9.04	22.05

Tem-R varied from 8 to 44-fold. These results indicate that the indirect ELISA procedure was adequate to determine whether mosquitoes possessed or lacked highly active esterase B1.

Detection of esterase B1 in single insects: Single mosquitoes of two strains (S-Lab and Tem-R) and of two field collections containing different proportions of esterase B1 (Simpson, California, 100% and Berkeley, Illinois, 50%) were studied under the experimental conditions described above. The observed OD's/min were converted to alkaline phosphatase units and a histogram constructed (Fig. 1). As expected, susceptible S-Lab insects were clearly distinguishable from resistant Tem-R. Each of the two field collections, Simpson and Berkeley, contained a proportion of mosquitoes with low esterase quantities similar to those observed in S-Lab adults (one in 18 for Simpson and 11 in 18 for Berkeley). These proportions were not significantly different from those expected based on the frequency of the highly active esterase B1 as determined by electrophoresis. Some of the insects in these field collections displayed esterase quantities within the range of those observed in Tem-R insects. However, the majority had an esterase quantity intermediate between those of S-Lab and Tem-R, consistent with their resistance levels as expressed by LC95 values toward temephos (e.g. 0.22 ppm for Berkeley, 0.25 ppm for Simpson and 7.0 ppm for Tem-R). These results are in agreement with those obtained by dot-blot immunoassay in this laboratory (Beyssat-Arnaouty et al. 1989).

Quantification of esterase B1 in insects: Although the ELISA test procedure used in these experiments clearly permits the detection of insects with increased activity of esterase B1, it does not allow a precise determination of its quantity. This can be achieved by comparing variations in OD/min in relation to esterase B1 concentration using multiple Tem-R homogenates. As can be seen in Figure 2, OD/min reaches a plateau at values equal to ~45. With Tem-R insects, this occurs with homogenates containing $1/500^{th}$ of a mosquito. It is only below this OD/min that there is linearity with homogenate concentration, and where the quantity of esterase B1 can be estimated. In the experiments comparing single insects (see above) esterase quantity of Tem-R adults was therefore grossly underestimated in relation to S-Lab (about 20x). Use of dilutions, as described here shows that Tem-R insects contain some 1,000-fold more esterase B_1 than do S-Lab insects, a value very similar to the resistance ratio observed between the two strains (about 800x).

Discussion.

Immunological detection of esterase B in laboratory or field collections of *Culex* mosquitoes can be achieved by ELISA using an anti-esterase B1 antibody, at least when the mosquitoes considered contain an esterase B1. Detection, based on the use of constant dilution of homogenate (i.e., 0.2 mosquito) cannot be used for adequate quantification of esterase and, thus, for estimation of resistance levels. However, our study demonstrates that this can be achieved using serial dilutions.

Similar conclusions were reached by Beyssat-Arnaouty et al. (1989) using a dot-blot immunoassay. Comparision of the ELISA and dot-blot immunoassay suggest that, without additional

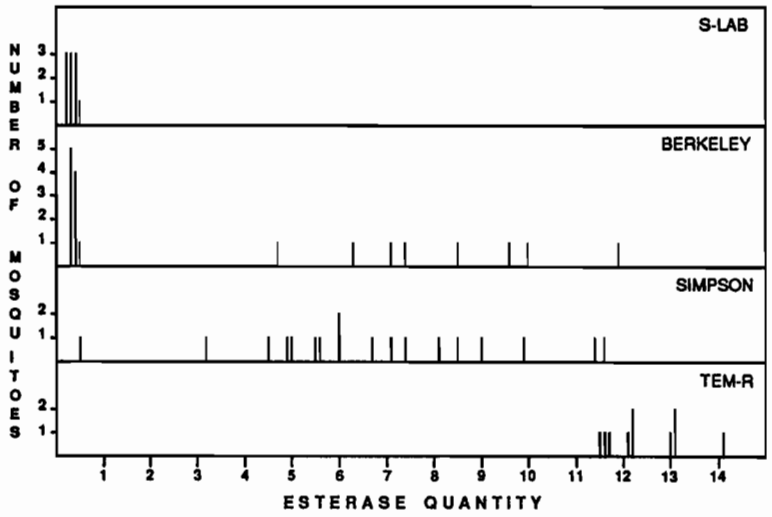

Figure 1.-Esterase quantity (expressed in units of alkaline phosphatase) observed in single mosquitoes of strains S-Lab (susceptible), Berkeley and Simpson (containing a mixture of mosquitoes with and without esterase B1), and Tem-R (resistant due to high quantities of esterase B1).

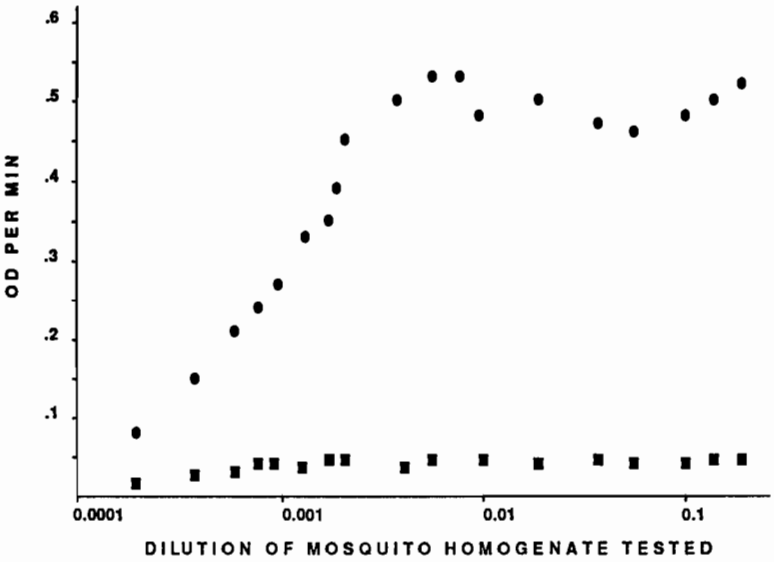

Figure 2.-Variation of OD/min in mosquito homogenates of strains Tem-R (●) and S-Lab (■) at various dilutions. Dilutions are expressed as fractions of mosquito.

improvement, ELISA is less suitable for field surveys due to the much more elaborate equipment it requires.

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SENSORY PHYSIOLOGICAL CORRELATES

OF HOST-SEEKING AND OVIPOSITION BEHAVIOR

IN *AEDES AEGYPTI*

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Air-borne chemical signals are vital in guiding hematophagous arthropods to sources of blood, plant nectar, and oviposition sites. The organism usually must orient to the source of these signals over relatively long distances and, in the case of a blood meal host, the odor-source is both mobile and capable of taking defensive action against the attacking organism. The goal of the research in our laboratory is directed toward a better understanding of the physiological mechanisms that underlie the control and modulation of some important odor-mediated behaviors in disease transmitting mosquitoes. The physiological mechanisms of specific interest include the role of peripheral olfactory organs in the control of specific behaviors, the physiological regulation of these peripheral sensory organs, and the mode of action of the insect attractants and repellents.

Correlation of sensory input with behavioral output.

In order to achieve this goal, we must be able to make precise correlations between a specific behavior, the responsiveness of its receptors, and the physiological state of the mosquito. Historical attempts by many biologists to correlate sensory physiology with overt behavior of the organism, even in the laboratory, were not particularly successful. The conclusion usually reached was that there was no such correlation; i.e., a behavior could not be explained on the basis of the activity observed in the sensory receptors. We believe this inability to demonstrate significant correlations can be attributed to attempts to compare data from different sources (experiments or labs) with insufficient regard to physiological or chronological age, time of day or season, and/or populations of insects (strains or colonies) as well as to assumptions that all insects within sample populations, even within cohorts, represented homogenous physiological and genetic conditions. Furthermore, averaged responses from a population of mosquitoes often will not permit precise correlations between physiology and behavior.

In order to avoid these pitfalls, we choose to make our correlations based on the analysis of data obtained from individual mosquitoes within each experimental and control group using the following routine procedures. First, we determine the specific behavioral responsiveness of an individual mosquito, then we determine the electrophysiological responsiveness of her chemosensory neurons to odors relevant to that behavior, and then we confirm the physiological state of the mosquito by subsequent microdissection and examination. Using this process, we have been able to make 1:1 correlations between physiological responsiveness of one receptor (specifically the one for the host-attractant, lactic acid) and the presence or absence of the behavior that it mediates (i.e., host-seeking behavior)(Davis 1984a, b).

Taking laboratory data to the field.

Even with this 1:1 correlation between a sensory receptor and its corresponding behavior, if we went to the field and tried to catch host-seeking mosquitoes with lactic acid, we would fail. Why? Even though lactic acid (in the presence of small amounts of CO_2) appears to be a critical stimulus for host attraction, lactic acid alone is not sufficient. There are other host odors, whose identities are not yet known, that are necessary to achieve the same degree of attraction displayed by the odor of a human hand. Some of these other host odors are in turn critical for the elicitation of the next piece in the total sequence of behaviors leading from host detection and orientation to probing and engorgement (Davis, unpublished observations). Without these other chemical signals, we cannot elicit the full behavioral sequence and would trap few, it any, host-seeking mosquitoes. However, there are several examples among hematophagous insects where it has been possible to take laboratory data directly to the field. One such example is the

host-odors octenol and acetone from African zebu cattle breath that have been successfully used in the field to attract avid tsetse flies in a trap (Vale et al. 1988). Another example involves oviposition site seeking behavior of mosquitoes. After a blood-meal, mated, gravid female *Culex quinquefasciatus* Say females are attracted to an oviposition site containing erythro-6-acetoxy-5-hexadecanolide, a pheromone found in the egg apical droplets of this species (Laurence and Pickett 1985; Otieno et al. 1988). With either of these systems, a precisely correlated laboratory study of physiology and behavior can aid in their improvement and further exploitation in pest control strategies.

Model of sensory-behavioral mechanisms.

In our attempt to understand the integration and regulation of endogenous and environmental signals necessary to co-ordinate and mediate the expressions of the appropriate behavior at the appropriate time, we developed a heuristic conceptual model of the changes that occur as the female mosquito goes from one behavior to another through her life cycle (Davis 1989). The model is based on our observations of the sensory responses of antennal olfactory receptors of individual female mosquitoes at various stages in their life cycle and correlating these responses with their established behavioral responsiveness and physiological status and on selected studies of behavioral and physiological correlations of others. It incorporates several internal physiological states known to influence certain behaviors; e.g., nutritional state and nectar feeding (Klowden 1981), abdominal distention inhibition of nectar and blood-feeding (Klowden and Lea 1979a, b), and the circadian influences of host-seeking and oviposition behaviors (Chadee and Corbet 1987).

An important component of the model involves the receptors for the host attractant, lactic acid, and the endogenous regulation of host-seeking behavior following a blood meal. In females that are actively seeking a host, the sensitivity of the lactic acid-excited neuron is high. Within 24 hours post-blood meal, the sensitivity of this neuron is depressed below the level of lactic acid emanating from a human hand and the female will no longer host-seek (Davis 1984a). The sensitivity of the lactic acid inhibited neuron does not change with blood-feeding. If one assumes, arguendo, that the input from these two receptors for lactic acid are summed in the female's central nervous system, the resulting sum in active host-seeking females will be positive, or excitatory, while in those females in which host-seeking is not observed, the sum is negative, or inhibitory. We feel that this may be the mechanism underlying the switching on and off of host-seeking behavior at the appropriate times.

Determinates of behavior: peripheral vs. central nervous system.

The notion that the peripheral sensory nervous system may mediate a specific behavior is contrary to the traditional notion that the central nervous system (CNS) is the sole site for determining which behavior will be expressed. Traditional theory holds that the peripheral sensory system provides the CNS with information about the organism's environment which the CNS can then use in selecting and carrying out a particular behavior pattern (Kandel et al. 1979). This theory assumes that the response characteristics of the peripheral sensory neurons are constant in all physiological states of the organism and that they provide the CNS with an uninterrupted and unaltered flow of sensory information relevant to all behaviors.

In contrast, our data demonstrate that the response characteristics of at least one receptor system change from one physiological state to another (specifically from non-blood fed to gravid) in *Aedes aegypti* (L.) females. The sensitivity of the lactic acid excited neuron is high when host-seeking behavior is present and low when it is inhibited. Thus our data strongly supports a hypothesis that a peripheral receptor, not just the CNS, may be directly involved in the selection of which behavior may be expressed as well as in the modulation of the behavior once selected.

Summary.

The fact that our model is based solely on the life cycle of the non-diapausing, anautogenous mosquito, *Ae. aegypti* raises several questions:

In the context of the basic model, what modifications would be necessary in order to understand how are the different forms of adult diapause mediated and regulated? Similarly, how are host-seeking and oviposition behaviors regulated in obligatory or facultative autogenous, mosquitoes? With respect to oviposition behavior, are there differences in the role of the sensory receptors among different species of mosquitoes, for example, in relation to oviposition site preference?

On another level, what is the mode of action of insect repellents? Do we know how they work? No, but with the above approach, we are making some inroads on how the repellent, deet, might work (Davis et al. 1987).

As is usually the case, we continue to raise more questions than we have answered. However,

we have an approach to making valid correlations between sensory physiology and behavior and to obtaining the fundamental information necessary to make some significant advances in our understanding of sensory-behavioral mechanisms in mosquitoes and how these mechanisms might be used to our advantage in pest control systems.

Acknowledgement.

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PERIPHERAL SENSORY RESPONSIVENESS AND ODOR-MEDIATED BEHAVIOR IN ADULT FEMALE MOSQUITOES

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Introduction.

Mosquitoes undergo changes in olfactory sensitivity to biologically-relevant volatiles that are correlated with physiological and behavioral state. Such changes are believed to be of adaptive significance in the control of odor-mediated behaviors such as host-seeking (Davis et al. 1987). Modulation of peripheral sensory responsiveness is an often-overlooked aspect of insect physiology that has potential implications for pest control strategies that manipulate insect behavior through the use of volatile attractants and repellents.

Here we present a few specific examples of how changes in peripheral receptor sensitivity are correlated with changes in olfactory-mediated behavior in diapausing and gravid females as well as in avid mosquitoes that are exposed to the repellent DEET.

Results and Discussion.

Antennal receptors that are sensitive to host attractants such as lactic acid display decreased sensitivity in mosquitoes that are not host-responsive. For example, diapausing *Culex pipiens* L. females do not host-seek and therefore do not blood-feed and develop eggs (Mitchell 1983; Bowen et al. 1988). The absence of host-seeking behavior in diapausing females is correlated with low sensitivity to host attractant lactic acid in both laboratory-reared (Bowen et al. 1988) and field collected, diapausing females. Furthermore, diapause termination is accompanied by the appearance of host-seeking and an increase in lactic acid receptor sensitivity.

Olfactory receptors that are sensitive to oviposition site-related volatiles display enhanced responsiveness in gravid *Culex pipiens* females. Receptors that are stimulated by ethyl propionate, for example, display an increase in the maximum response to this stimulus as compared to non-gravid females. In addition, the proportion of cells specifically responsive to ethyl propionate increases in gravid females; non-gravid and diapausing females possess many more non-responsive and non-specific neurons. These receptors have been examined only during the first gonotrophic cycle in non-diapausing *Culex pipiens*, so it is not known if the changes described here recur in a cyclical pattern during successive gonotrophic cycles.

The mechanism of repellent action presents an example of how mosquito behavior can be manipulated by effecting changes in peripheral input. DEET (n,n-diethyl-m-toluamide) is an effective mosquito repellent that suppresses the response to lactic acid in *Aedes aegypti* L. when both lactic acid and DEET are presented simultaneously (Davis 1985). Essentially, this results in a decrease in the net afferent output from that cell in a pattern that is very similar to changes in cell sensitivity observed in diapausing and gravid females. Not all repellents operate in exactly the same way, but it is striking that one of the most effective repellent formulations operates by interfering with the mosquitoes' ability to detect a host attractant.

Conclusion.

Sensory responsiveness in mosquitoes is a function of physiological state. The qualitative and quantitative perception of behaviorally relevant volatiles changes in concert with seasonal reproductive events such as diapause and gravidity. Sensory responsiveness is highly correlated, in large part, by altering sensory input, either endogenously by physiological processes related to events in the female life cycle, or exogenously through the presentation of repellents.

Electrophysiological and behavioral studies of other species, other attractants, and other receptors would greatly enhance our nascent understanding of odor-mediated behavior in the mosquito. Such findings are of obvious relevance to new repellent formulations as well as mosquito management strategies that employ volatile chemical attractants.

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BIOLOGY AND ECOLOGY OF LARVAL SNOW POOL *AEDES* IN THE SIERRA NEVADA MOUNTAIN RANGE

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ABSTRACT

In 1989 and 1990, a project on the population dynamics of *Aedes* mosquitoes found between elevations of 1,700 and 2,600 meters in California was initiated in an attempt to identify factors responsible for variability in mosquito control efforts. Studies conducted toward this end include: 1) observation of the seasonal succession of mosquito species, 2) identification of species composition, and 3) documentation of initial egg hatch and subsequent larval appearance at different elevations and in a variety of habitats.

In 1989, 18 different locations on both the eastern and western slopes of the Sierra Nevada mountains were monitored from initial thaw until pools became dry. Weekly dipping was done at each site and information on larval presence, developmental stage, number and species was recorded. We found that larval appearance was not only dependent on elevation, but that a temporal pattern of egg hatch from a single pool also exists. *Aedes cataphylla* Dyar, *Aedes hexodontus* Dyar, *Aedes ventrovittis* Dyar, and *Aedes communis* (DeGeer) tend to be early-season species. *Aedes schizopinax* Dyar, *Aedes fitchii* (Felt and Young), and *Aedes increpitus* Dyar appear mid-season, while *Aedes hemiteleus* Dyar is found later in the season. Within each of these species, a pattern of distinct multiple hatches was observed. Studies in 1990 confirmed this pattern; however, no correlation between a particular snow pool species and multiple hatching was found. Species inhabiting similar sites at one elevation with the same vegetation could exhibit both the multiple and single cohort pattern.

In addition to installment hatching during the single season "lifetime" (i.e. wet to dry period) of a snow pool, additional hatching was observed if re-flooding occurred within the same season. One week after severe thunderstorms, first instar *Ae. communis* larvae were recovered from a site that had been dry for two weeks. Late-season hatches without drying were also observed in *Ae. cataphylla* and *Ae. hexodontus* sites in which no mosquitoes had been collected for a month following treatment. These patterns could give the impression of treatment failures.

Attempts to correlate multiple hatching with weekly water depth and temperature measurements were inconclusive; however, 24-hour temperature monitoring yielded a possible cue for the initial egg hatch of the season. Data collected after snow melt, but while ice was still forming on pool surfaces, revealed a vertical temperature gradient. All larvae were clustered in the warmest bottom layer and remained there until the ice melted the following morning. When pools without larvae were monitored over a 24-hour period, it was discovered that most of the time the water was in a frozen "slushy" state from the surface to the bottom. Assuming equal conditioning of eggs, successful hatching might depend on seasonal temperatures increasing to the point where ice forms only on the snow pool surface overnight and a vertical temperature gradient forms allowing larval survival.

These findings are particularly significant to vector control districts which may need to adjust control protocols by increasing inspection visits to sites and resorting to multiple applications.

Acknowledgements.

We wish to thank the personnel of El Dorado County Service Area III for their assistance and cooperation. This study was funded by University of California Mosquito Research Funds.

STUDIES ON THE POPULATION BIOLOGY OF THE *AEDES DORSALIS* COMPLEX IN CALIFORNIA

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ABSTRACT

Aedes melanimon and *Aedes dorsalis* are closely related floodwater species which are important biting pests and have been associated with arbovirus activity. Studies conducted in the Sacramento Valley of California have suggested that *Ae. melanimon* has a high biological capacity to vector arboviruses based on high female survivorship, high seasonal abundance, low rates of expression of autogeny, a five-day interval between bloodmeals and the continued presence of parous females across the transmission season.

Though *Ae. dorsalis* and *Ae. melanimon* are considered to be closely related yet distinct species, the systematic relationship between different geographic populations of these species has not been determined. *Aedes dorsalis* exists in two allopatric populations, in intertidal brackish marshes along the Pacific coast and in extensive areas of the Great Basin. *Aedes melanimon* is found throughout lowland river valleys such as the Central Valley of California and in limited locations in the Great Basin. Preliminary studies of different geographic populations of these species were initiated in 1989.

Adult and larval collections of *Ae. dorsalis* and *Ae. melanimon* were made from 13 different geographic locations in the Western United States in 1989. The majority of specimens were frozen for subsequent genetic analysis using isozyme electrophoresis while genitalia mounts were prepared from material from nine of the collections. Superficial examination of these mounts showed no discernable differences in the genitalia of *Ae. melanimon* from the Central Valley, the Owens Valley in eastern California and the Klamath Basin in northeastern California. No differences were observed in the male genitalia of *Ae. dorsalis* from northern California coastal salt marshes and Salt Lake City, Utah, in the Great Basin.

Distinct morphological differences were found between the genitalia of Central Valley *Ae. melanimon* and coastal California *Ae. dorsalis*. These findings are consistent with a preliminary isozyme electrophoretic analysis of these populations which suggest that these populations belong to closely related yet genetically distinct species.

Population parity rates of *Ae. melanimon* females collected from the Owens Valley of California during August 1989 were found to be extremely high with 77 of 79 (97%) dissected females found to be parous and 41 of 79 (51%) having completed two or more gonotrophic cycles. Extensive flooding had occurred along the Owens River approximately one month previously suggesting that these females were members of aging cohorts produced in response to the flooding and that the age of the cohort was in large part responsible for the high parity rate. High biting activity combined with the high population parity rate does, however, suggest that the Owens Valley population of *Ae. melanimon* may have the capacity to be involved in arbovirus transmission.

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MARK-RELEASE-RECAPTURE STUDIES ON *CULEX* MOSQUITOES IN SOUTHERN CALIFORNIA, 1989[1]

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Introduction and Objectives.

Mark-release-recapture studies were initiated on *Culex* mosquitoes in southern California during 1988 to study the population ecology and dispersal of adults into and within residential habitats (Reisen et al. 1989). The specific objectives were to: 1) compare the dispersal and dispersion of adults emerging from peridomestic and peripheral breeding sources; 2) estimate the duration of the gonotrophic cycle and survivorship; 3) calculate population size, density and immigration and emigration rates; and 4) evaluate the effectiveness of CO_2 and gravid traps in different residential settings.

Releases of *Culex stigmatosoma* Dyar at Chino and Rossmoor during 1988 were inconclusive. Low recapture rates hampered our study at a dairy in Chino, where only 11 of 30,000 released female *Cx. stigmatosoma* were recaptured, and of these 11 recaptures, five were taken at traps positioned more than 1 km from the point of release. Similar low recapture rates (0.3%) were obtained for *Cx. stigmatosoma* released at Rossmoor in Orange County. In contrast, 11.4% of *Culex quinquefasciatus* Say were recaptured at Rossmoor which allowed the estimation of dispersal, survivorship and population density. The primary objective of our 1989 experiment at Chino was to determine if *Cx. stigmatosoma* has a teneral dispersive phase. The recapture rates of differentially marked teneral females collected as immatures were compared with conspecific host-seeking females collected by CO_2 traps and with *Cx. quinquefasciatus* females released in residential and dairy environments. Our secondary objective was to compare survivorship, size and rates of addition (immigration) and deletion (emigration) among the different *Culex* species.

Studies in Los Angeles County were designed to determine if the results obtained at Rossmoor during 1988 were representative of mosquito populations in residential environments throughout the Los Angeles metropolitan area.

Methods and Results.

Releases at Chino. Immature *Culex* were collected at three dairies, held to emergence, counted and marked with fluorescent dust. A total of 43,492 adults were released at the same dairy studied during 1988 and at St. Andrew's church about 1 km to the north, of which 492 (1.2%) were recaptured by 36 CO_2 and 12 gravid traps operated daily for 10 consecutive days. Overall, 70% of marked and 93% of the 102,765 unmarked females were collected by CO_2 traps. Recapture rates of *Cx. stigmatosoma* were highest for females released at host-seeking age at both the church (3.3%) and the dairy (8.3%). These females did not disperse and were recaptured at traps within 30 meters of the release point. Similar to our 1988 study, only 9 (0.1%) teneral females were recaptured, and three of these were recaptured at CO_2 traps located >1 km from the point of release (maximum distance =1.3 km).

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A complete presentation of 1988 and 1989 data will be submitted for publication in the Journal of Medical Entomology.

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In contrast, *Cx. quinquefasciatus* did not appear to have a teneral dispersive phase, since recapture rates for teneral and host-seeking age females were similar at both the church (4.7 and 4.7%, respectively) and dairy (0.3 and 0.7%, respectively). Most *Cx. quinquefasciatus* females released at the church did not disperse and were recaptured within 0.5 km of the release point. The uncorrected mean distance dispersed was 0.24 km for host-seeking females and 0.14 km for teneral females. In contrast, females released at the dairy readily dispersed into the residential area during host-seeking flights, and the mean distance dispersed was 1.03 km.

The numbers of marked nulliparous host-seeking, gravid and parous host-seeking *Cx. quinquefasciatus* females released as tenerals that were recaptured each day were plotted as a function of time in days after release to estimate the duration of the gonotrophic cycle. The interval between peak recapture of nulliparous females on Day 3 and parous females on Day 9 was six days. Population estimates were made for teneral females released at the church and recaptured at traps within an immediate 0.14 km^2 area. Based on a gonotrophic cycle of six days and a parity rate of 0.22, the vertical survivorship of unmarked females was calculated to be 0.78. Horizontal survivorship estimated by regressing the log of the recapture rate as a function of time was 0.67. Population size calculated by a modification of the Lincoln Index averaged 94,781 females per day with a daily addition rate of 0.47. The population was increasing at the rate of 0.14 females/female/day, and, in agreement, the number of unmarked females collected increased from 466 to 4,690 per day.

Releases at Lynwood. At Lynwood, Los Angeles County, only 5,280 teneral adult mosquitoes were released in the center of intersecting N-S, E-W transects of CO_2 traps, with recapture effort concentrated about the release point. Only 0.6% were recaptured, too few for further analysis. Most marked adults were recaptured by sweeping vegetation at houses near the release site, and only one host-seeking and one gravid *Cx. quinquefasciatus* were recaptured at traps. In contrast to Chino where most females were collected at CO_2 traps, 67% of the 7,273 unmarked females collected were captured in gravid traps.

Releases at Van Nuys and the Sepulveda Basin. A total of 14,866 teneral *Culex* adults were released at a residence in Van Nuys and at a pond in the Sepulveda Basin Wildlife Area about 1 km to the west, of which 186 (1.5%) were recaptured at traps arranged along transects radiating from the Basin release point and clustered near the release point in Van Nuys. The recapture rate of *Cx. quinquefasciatus* and *Cx. stigmatosoma* was higher for females released in Van Nuys (15.9 and 0.9%, respectively) than in the Sepulveda Basin (3.3 and 0.1%, respectively), while conversely the recapture rate of *Culex tarsalis* Coquillet was higher for females released in the Basin (3.6%) than in Van Nuys (0.5%).

Similar to Chino, female *Cx. quinquefasciatus* released in residential habitat remained clustered at traps adjacent to the release site (mean dispersal distance = 0.13 km). However, even though females remained clustered at the site of release, few host-seeking nulliparous or parous individuals were recaptured at CO_2 traps. Most marked females (72%) were recaptured in gravid traps 6-7 days after release. The duration of the gonotrophic cycle was considered to be one day shorter than the six days observed at Chino where maximal numbers were recaptured at gravid traps eight days after release.

Based on a parity rate of 0.41 for unmarked *Cx. quinquefasciatus* females collected by CO_2 traps in Van Nuys and a gonotrophic cycle length of five days, vertical survivorship was estimated to be 0.84. Horizontal survivorship was estimated to be 0.82 by regression using the decrease in marked females recaptured at CO_2 traps on Days 3 to 9 post-release. Mean population size for Days 6-8 for traps within a 0.15 km^2 area was calculated to be 3,912 females.

Concluding Discussion.

Culex stigmatosoma appears to have a teneral dispersive stage which results in the emigration of females from breeding sites. In agreement, the numbers of unmarked females collected at CO_2 traps in housing tracts was significantly higher than at dairy sites during both 1988 and 1989 studies. This dispersive stage appears to be unique for *Cx. stigmatosoma* and was not observed for *Cx. quinquefasciatus* during the present study or for releases of *Cx. tarsalis* in Kern County.

Culex quinquefasciatus released at peripheral dairy or wildlife habitats infiltrated adjacent housing tracts, whereas those released in residential environments remained near the point of release. Reasons for this movement remain unexplained, but may be related to host-seeking behavior.

Carbon dioxide-baited and gravid trap effectiveness varied markedly among experiments. CO_2 traps were effective when *Cx. quinquefasciatus* population density was high at Rossmoor (121,531 females/km^2) and Chino (671,634 females/km^2), but were ineffective at residential communities in Los Angeles where densities were low (36,612

females/km^2 in Van Nuys). Perhaps, large numbers of host-seeking females stimulated available avian hosts to elicit anti-mosquito behavior which made them refractory to blood feeding and diverted unfed females to CO_2 traps with CO_2 release rates which approached that of mammals.

The effectiveness of gravid traps appeared to be related to the availability of competing breeding sources. At Rossmoor and Chino where peripheral breeding sources were readily available and population densities were high, CO_2 traps collected many more *Cx. quinquefasciatus* than did gravid traps. In contrast, at Lynwood and Van Nuys where peripheral breeding sites were not found and population densities were low, gravid traps collected far more females than concurrently operated CO_2 traps.

Few *Cx. tarsalis* were released and recapture rates generally were lower than observed in our previous release-recapture studies in Kern County. Additional research is needed on the population ecology of this species in residential areas before the ecology of the arbovirus transmission in the Los Angeles Basin can be fully understood.

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MOSQUITOFISH REPRODUCTION:

INFLUENCE OF PHOTOPERIOD AND NUTRITION

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ABSTRACT

Mosquitofish (*Gambusia affinis*) constitute an important biological controller of mosquitoes in many parts of California, especially in agroecosystems such as rice fields. Mosquitofish use could be even more effective if more fish were available (e.g. from accelerated reproduction) for spring stocking. Two, 24-week experiments were conducted using 32 tanks in light-tight boxes to better understand the effects of photoperiod and diet on mosquitofish reproduction.

In the first experiment, fish at 15L:9D, and 13L:11D showed accelerated reproductive development of larvae compared with 11L:13D and control (naturally increasing) photoperiods. However, all fish were fed TetraMin flake diet alone, and most photostimulated females died, presumably from an insufficient diet, about one week before parturition.

In the second experiment, two photoperiods, 15L:9D and control, each incorporating two dietary regimes, flakes and tubificid worm-supplemented flakes, were included. Fish fed worm-supplemented flake diets showed greater survival and reproduction than fish fed only flake diets. Also, worm-supplemented flake diet fish at 15L:9D reproduced 11-12 weeks earlier than the worm-supplemented flake diet fish at control photoperiods.

THE INFLUENCE OF FLOODING DATE ON DENSITY AND AGE STRUCTURE OF THE THREE-SPINED STICKLEBACK, *GASTEROSTEUS ACULEATUS*, IN SUISUN MARSH, CALIFORNIA

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Introduction.

Suisun Marsh, located in Solano County, contains 12% of California's wetlands (Rollings 1981). This brackish water marsh is a system of permanent channels and seasonally flooded ponds that are typically flooded in autumn and then drained in early spring. Although this flooding creates waterfowl habitat, it can also create mosquito problems (Batzer and Resh 1988).

A potentially important fish predator of mosquito larvae in Suisun Marsh is the three-spined stickleback, *Gasterosteus aculeatus* L. Sticklebacks can significantly reduce invertebrate populations, including mosquito larvae (Worgan and FitzGerald 1981, Ward and FitzGerald 1983, Wootton 1984). Preliminary observations in Suisun Marsh indicated that fish introduced into ponds during flooding in early September reproduce during September and October and, if the ponds remain flooded long enough, have a second reproductive cycle in early spring (Batzer, unpublished data). Thus, the three- spined stickleback, which usually has a spring- summer breeding cycle (Moyle 1976, Wootton 1984), appears to also breed in the autumn months in Suisun Marsh.

If there are two breeding periods in Suisun Marsh, densities of sticklebacks in the ponds may be influenced by flooding date. In Suisun Marsh, flooding can be initiated as early as September or as late as December. Ponds flooded after the September-October breeding cycle will contain only the sticklebacks initially introduced during the flooding (the intake culverts are closed after the ponds are filled). Alternatively, stickleback populations in ponds that are flooded in early September may be augmented by September and October reproduction, and only these ponds may have sufficient numbers of fish to impact mosquito populations. The purpose of this study was to document whether the timing of initial flooding can influence the population size and age structure of sticklebacks in seasonal ponds of Suisun Marsh.

Materials and methods.

Three-spined stickleback populations were studied in six different seasonally flooded ponds in Grizzly Island Wildlife Area of Suisun Marsh. Three of these ponds were flooded in early September; the remaining three were flooded in late October. Pilot studies indicated that sticklebacks reproduced in September or early October; therefore, this flooding pattern allowed us to determine if ponds flooded after this reproductive event had significantly different stickleback populations than ponds flooded earlier.

Stickleback populations in each of the six ponds, ranging in size from 12 to 100 hectares, were monitored using minnow traps. Specific sites for trapping were selected in areas of each pond that contained pickleweed, *Salicornia virginica* L., and had water depth between 30 and 50 cm (in one pond, the trap location was moved in late February because of decreased water levels).

Two minnow traps lined with 1 mm fiberglass mesh were placed in each pond. The traps were checked weekly over an 18-week period, starting in late October and ending in early March. At each collection date, fish were counted and grouped into one of five classes: <10 mm, 10.1 to 20 mm, 20.1 to 30 mm, 30.1 to 40 mm, and >40 mm. The reproductive status of the sticklebacks was determined based on male coloration and gravid condition of females. All fish were then released. Sweep-net sampling was used to collect mosquito larvae.

Differences in densities and sizes of sticklebacks in two flooding treatments were analyzed using t-tests or Chi-square tests. Densities of sticklebacks and mosquito larvae were compared using Spearman's rank-correlation test.

Results and discussion.

In terms of the seasonal total of fish collected in each pond, two of the ponds flooded early in September had much higher densities of sticklebacks than the remaining four ponds. However, the third pond flooded in early September had very low fish density (Table 1).

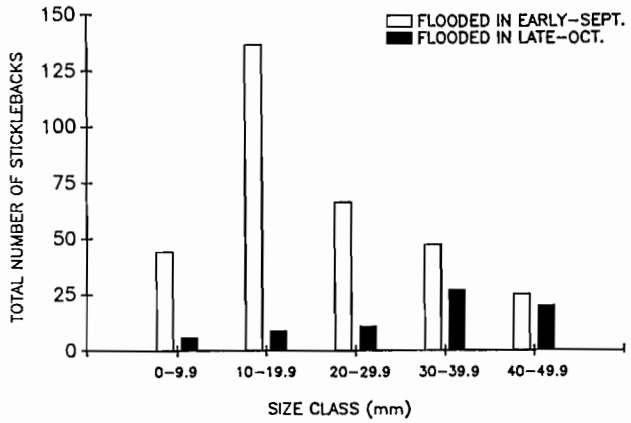

Figure 1. Total number of three-spined sticklebacks, *Gasterosteus aculeatus*, trapped in each of five size classes from six ponds in Suisun Marsh.

Significant differences did exist in the size class distribution of all fish collected when ponds flooded in early September were compared to those flooded in late October (Fig. 1, Chi-square, $p < 0.05$). Ponds flooded in early September had a significantly higher percent of the smaller fish (<20 mm) than ponds flooded in late October (arcsine transformed data, t-test, $p < 0.05$). Fish smaller than 20 mm would most likely have resulted from reproduction occurring within the ponds because all reproductively mature fish collected exceeded 30 mm. Reproductively mature males and females were found in trap collections in both treatments by the end of November but fry were not captured in the traps in the late-October flooded ponds; they were, however, observed along the edges of these ponds in March.

Table 1. Total number of three-spined sticklebacks, *Gasterosteus aculeatus*, and mosquito larvae, *Culiseta inornata*, collected in six ponds in Suisun Marsh over an 18-week period (October-March).

Flooding Date	No. of sticklebacks	No. of mosquitoes
early-September	350	6
	231	0
	18	353
late-October	53	16
	26	248
	35	29

Densities of sticklebacks and densities of the most commonly occurring mosquito larvae in the ponds, *Culiseta inornata* (Williston), were negatively correlated (Table 1, Spearman's rank-correlation, $R_s = -0.94$, $p < 0.05$). Whether this was caused by direct interaction between the two populations or by other factors is unknown. This study, though, does indicate that timing of flooding may influence population characteristics of sticklebacks. How these characteristics relate to the role of sticklebacks in control of mosquitoes in seasonal marshlands merits further study.

Acknowledgements.

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PHEONOLOGICAL SURVEY OF ABUNDANCE AND DIVERSITY OF AQUATIC FAUNA IN SACRAMENTO COUNTY VERNAL POOLS

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ABSTRACT

In 1988 and 1989, water samples were collected on a weekly basis from a group of nine vernal pools in Sacramento County to determine the population abundance and species distribution of microscopic zooplankton and macroscopic invertebrates inhabiting benthos and nekton microhabitats. Each microhabitat type contained a specific group of species, with some species distributed throughout all microhabitats within the pool. Abundance of most species increased after rainfall added new water into the pools. Most pools within the sample area contained the same faunal types. As the pools evaporated, temperature gradually increased while pH and dissolved oxygen (DO) decreased. In the final stages prior to complete evaporation, many characteristic faunal types disappeared and other characteristic types appeared; notably Culicidae and other low DO-tolerant dipterans. Protozoa and Rotifera abundance remained constant throughout the sample period. Recognizable algal blooms occurred at least twice and appeared to affect the DO concentration in the pools.

Introduction.

In recent years it has become increasingly important to be aware of endangered animal and plant species and their threatened communities when carrying out vector control activities. Federal and state laws presently exist to govern and protect such species and communities. These laws can often take precedence when it comes to the needs of city and county government or private development projects.

Ephemeral, or vernal, pools are probably the closest to extinction of all recognized endangered communities. As such, they fall under the watchful eye of several agencies. Specifically, they are classified an endangered community by the federal government and are therefore protected by the Endangered Species Act of 1973 (ESA) and the Federal Water Pollution Control Act and Amendments of 1972, specifically the Clean Water Act (CWA). In California, endangered species and endangered communities fall under the protective jurisdiction of the Resources Agency, Department of Fish and Game. However, vernal pools are also considered a potential mosquito breeding source and therefore can be a possible public health hazard and thus would become the responsibility of the Health and Safety Code, Mosquito Abatement Act and amendments of 1915. Many of the mosquito and vector control mandates can be in direct conflict with natural wetland habitat preservation laws, and thus create uncertainties for all agencies concerned. Because of the potential problems created by ambiguities in laws concerning public agencies, it has become important to know what animals are present, when they occur, and how many there are in the vernal pool habitat. In relation to this information, it is especially important to know what mosquito species occur and when they utilize the pools.

Vernal pools are almost exclusively a California phenomena, although they occur in some limited areas of Africa. They exist extensively with the proper geological conditions on the eastern border of the northern Sacramento and central San Joaquin Valleys and to a lesser extent in southern California. In Sacramento County, the pools occur on the eastern terrace soils above dense hardpan clays. These clays are so thick and dense that water from winter rains cannot percolate through into the lower soil column and therefore forms a shallow water table near the surface. The surface is covered with numerous small hills (mimia mounds) and depressions whose method of formation is of some debate. Most investigators suggest their formation is a result of the hardpan geology, either clay fracture patterns with groundwater pressure and/or expansion and contraction of lattice clay minerals, resulting in the characteristic mounds and

depressions (Holland and Jain 1977). The deeper depressions dip into the shallow water table forming the vernal pools. The pools are filled from rain water only; there are no inlets or outlets. The water level is regulated by the water table and is lowered as the pools evaporate. The landscape is typically a rolling foothill grassland or sparse woodland of oak generally below 3,000 feet in elevation.

Vernal pools generally fill during the first rains of November and December and are completely dry by May or June. During this time, water chemistry, temperature, and physical morphometry will change quite drastically. Due to the associated dynamic changes which occur in the microhabitats of the pools, and the subsequent faunal and floral adaption, and from the island-like isolation of individual pools from other water sources, many faunal and floral inhabitants have evolved into new and endemic forms specifically adapted to vernal pools in general, and often specific to a archipelago in particular.

There has been a great deal of work done on vernal pool flora, where greater endemicity exists, but little or no work on the fauna or on the frequency and types of mosquito species utilizing the pools as breeding sites. This survey attempts to list all the faunal types located in a cluster or archipelago of adjacent pools as they pass through the physical differences associated with their seasonal evolution. Species distribution within various microhabitats and individual abundance were also noted. Special emphasis was placed on the use of vernal pools by specific mosquito species and the associated physical conditions in which they occur.

Materials and Methods

In the springs of 1988 and 1989, ten weekly samples were taken to census an archipelago of small vernal pools in the eastern portion of Sacramento County. The surveyed pools are all located within the Buffalo Creek Quadrangle (T8N, R7E, S11). The archipelago consists of nine pools which occur within an approximate area of one-half acre.

Due to the constantly changing conditions of faunal microhabitats which alter sampling efficiency, several sampling methods were employed, each with many replicates. Because faunal activity varied with time of day, all samples were taken at roughly the same time (between 8:00 and 9:00 a.m.). As the water level decreased, and as plant and algal densities increased, collection efficiency was altered and some sampling methods could not be used. In the final weeks only small pipet samples could be taken, and no abundance rating could be determined as pools were reduced to puddles.

Water column samples were taken with a 12 cm gape nylon plankton net which was pulled across the pool along its longest axis. Five dips per site were taken with a standard 250 ml dipper. Smaller samples were taken randomly throughout the pool with a 10.0 ml column tube and a 1.0 ml Hensen-Stemple pipet. Soil samples were taken with a modified 10.0 pipet which was pushed approximately 1.0 ml into the substrate.

Nekton samples were taken with a floating surface sampler. Abundance values were determined using a 5.0 ml counting wheel, a 1.0 ml Sedgewick-Rafter counting chamber and a 0.1 ml Palmer counting cell. Chemical properties were measured with LaMotte Chemical Freshwater Test Kits. Dissolved oxygen (DO) and temperature were measured with a YSI Model 57 oxygen meter and temperature probe, and pH was measured using a Standard Digitsense pH meter. Water depth was measured at the same location each week at approximately the deepest point in the pool. Water's edge and other sampling locations were measured from a stationary datum point. Water turbidity was estimated using a white enamel dipper.

Identification of invertebrates was accomplished using information and keys from the following references: Merritt and Cummins 1984, Pennak 1978, Usinger 1956, and Ward and Whipple 1959. Information on the ecology of vernal pools was found in the following papers: Holland and Griggs 1976, Purer 1939, and Stebbins 1976.

Results and Discussion.

Pool Morphometry: Nine oblong pools formed the archipelago; all with a longer North/South axis and a narrow East/West axis. The greatest length of each pool was measured and found to be 26.9, 23.2, 16.1, 12.2, 10.4, 9.4, 8.5, 8.4, and 7.3 meters. Depth and temperature measurements were made on a weekly schedule at the pool center marker which was at roughly the deepest location. Depth was measured by subtracting the amount of change from a mark on a permanently set survey stake. Water temperature was taken at the surface and within the substrate.

On January 6, 1989, the largest pool had a North/South distance of 26.9 meters and a East/West distance of 16.4 meters and covered an area slightly greater than 0.05 acre. The depth of the pool at the center data point was 29 cm. For the first two weeks the depth of the pool remained constant. As ambient air temperatures increased,

the pool began showing signs of evaporation. By February 1, the depth had dropped to 25 cm and began dropping two and five centimeters per week thereafter. During 1989, two rain storms, one in February and one in March, restored the pool water depth to nearly the original level, adding over 11 cm total. After the last storm the water level remained constant for another three weeks until warm weather began in April. Within the next five weeks the water depth dropped from 21.6 cm to less than 1.0 cm in May.

Temperature ranged from 11°C in January to 16°C just prior to drying in May, but fluctuated significantly throughout the sample period. The pool shape was roughly oval with a slight invagination on the northeast side. At the beginning of the season the pool held an estimated 132,300 liters. Water visibility remained clear except after rain storms and during the last two weeks before drying. The eight other smaller pools, all located within a few meters of the large pool, were also monitored to note any differences in temperature, water quality and species abundance and diversity.

Chemical Conditions: Several conditions (total hardness, alkalinity, phosphorous concentration, ammonia-nitrogen, nitrate and nitrite levels) were monitored initially, and then only taken at the close of the sample period to note overall change. Dissolved oxygen and pH were taken each week. Water samples were drawn and tested from the same location and at the middle of the water column each week.

Among those conditions sampled for overall change, only the ammonia-nitrogen concentration changed significantly. Initial measurements were less than 0.2 ppm and increased to over 0.4 ppm. This large change is likely due to the presence of cattle in the area during the last weeks before the pool had evaporated. The dissolved oxygen level was found to decrease each week with the exception of directly following the two aforementioned rain storms at which time it would increase. The pH decreased each week throughout the sample period despite other conditions. In January, readings ranged between 9.7 and 9.5. During the last month of sampling the pH had dropped to 7.1 (Fig. 1).

Biological Conditions: The plankton net sample was allowed to settle and concentrate in a one liter flask. Using a 10 ml pipet, 1 ml aliquots were pipetted into a Sedgewick-Rafter counting chamber. A single strip from top to bottom of the chamber was counted and identified. The aliquots were taken from the middle region of the concentrate. Organisms were fixed in 70% isopropyl alcohol to

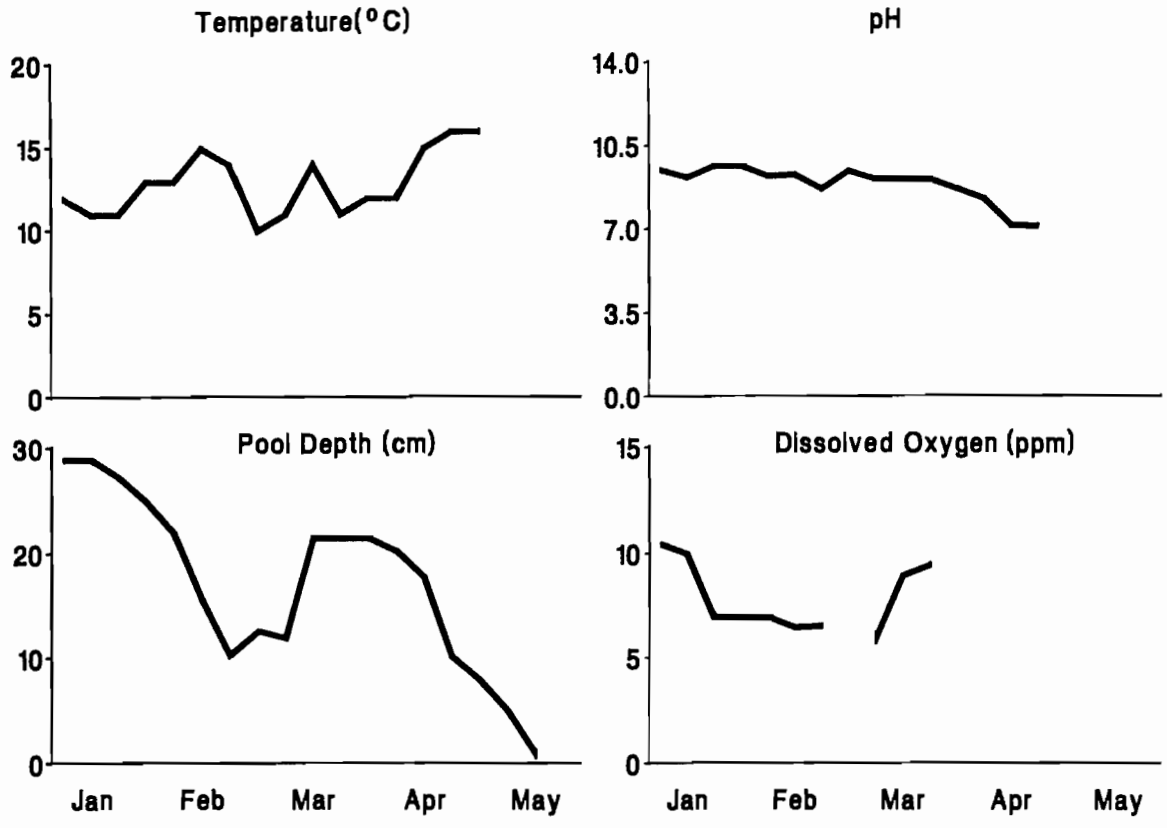

Figure 1. Changes in the physical characteristics of the vernal pool during the study period.

facilitate counting. Different organisms were counted and separated using a modified heme counter.

The plankton net sample could not be used effectively for determining species density because each aliquot contained many thousands of each species. When counting the concentrated sample, no significantly relative difference in numbers of planktonic classes could be found. The plankton net samples did prove to be valuable in determining species diversity, as other sampling techniques had built-in sampling selectivity (i.e., some techniques did not collect organisms that were in low densities).

Each week a single 1 ml Hensen-Stemple pipet sample, a surface sampler collection and five standard enamel dipper samples were taken from the pool. These samples were taken at random from outer margins and from within the central region of the pools. The dipper samples and surface sampler collections were split into 1 ml aliquots and pipetted into a Sedgewick-Rafter counting chamber. From these samples, organisms could be easily counted and therefore abundance could be determined.

An artificial demarcating system was used to make four abundance ratings. In dipping, pipet or surface samples containing no individuals of a specific organism, a rating of ABSENT was given for that organism. If the sample contained from one to ten of a specific organism, it was rated as LOW density; in samples containing 11 to 100 of a specific organism, the rating was a MODERATE density; and samples containing more than 100 organisms were rated as HIGH density. Organisms for density determination were only identified to their major class or subclass.

In any population sampling system it must be noted that sampling bias or selectivity and/or resolution of the collecting device may produce an artificial decline or increase in abundance curves. In this report it is hoped that some of these artifacts were eliminated through the practice of using several sampling devices.

Taxon Abundance, Diversity and Succession: The vernal pools were found to contain a great array of organisms, matching a representative in each aquatic class, but the diversity within each class was found to be lower than that which occurs in the more permanent wetland habitats (Table 1). Many species were the same as those found in the other pond and lake habitats, but their relative numbers were greater in the vernal pools. Some species found in abundance were those generally considered rare in most aquatic habitats. Others, which are abundant in pond and lakes were also very abundant in the vernal pools.

The most abundant microcrustaceans were the small cladocera and copepod species living on the water surface and within the water column. Their numbers were many hundred times greater than any of the other planktonic or benthic microzoans. Protozoans and rotifers were quite obviously the most abundant animals present, easily having numbers that far exceeded all other animals in the samples. Their numbers were frequently in the thousands with even the smallest of samples. Protozoans also showed the greatest diversity, having many times the amount of different species.

Among the algae, several groups existed in extremely large numbers. The most abundant were the unicellular algae such as euglenophytes, cryptophytes, chryophytes (especially diatoms),

Table 1. A list of aquatic taxa collected from nine vernal pools in Sacramento County.

- PROTOZOA
  - CILIATA
    - Peritricha
      - Vorticellidae
        - -numerous others
  - MASTIGOPHORA
    - Phytomonadina
      - Volvocidae
        - -numerous others
  - SARCODINA
    - Amoebina
      - Amoebidae
- ROTATORIA
  - MONOGONONTA
    - Ploima
      - Trichoceridae
      - Brachionidae
      - Notommatinae
      - Dicranophorinae
- GASTROTRICHA
  - Chaetonotoidea
    - Chaetonotidae
      - *Chaetonotus* spp.
- PLATYHELMINTHES
  - TURBELLARIA
    - Neorhabdocoela
      - Rhynchomesostominae
        - *Rhynchomesostoma* sp.
      - Dalyellipae
        - *Gieysztoria* sp.
      - Mesostomidae
        - *Mesostoma* spp.
- NEMATODA
  - APHASMIDIA
    - Enoplida
      - Dorylaimidae
- ANNELIDA
  - OLIGOCHAETA
    - Opisthopora
      - Haplotaxidae
        - *Haplotaxis* sp.
- MOLLUSCA
  - GASTROPODA
    - Pulmonata
      - Physidae
        - *Aplexa hyponum*
      - Lymnacidae
        - *Lymnaea* sp.
- TARDIGRADA
  - EUTARDIGRADA
    - Macrobiotoidea
      - Macrobiotidae
        - *Macrobiotus* spp.
- ARTHROPODA
  - CRUSTACEA
    - Cladocera
      - Macrothricidae
        - *Streblocerus sericaudatus*
      - Sididae
        - *Sida crystallina*
      - Daphnidae
        - *Daphnia pulex*
        - *Daphnia similis*
    - Eucopepoda
      - Diaptomidae
        - *Diaptomus franciscanus*
      - Cyclopidae
        - *Acanthocyclops vernalis*
      - Harpacticidae
        - *Chappuisius* sp.
    - Ostracoda
      - Cyprinae
        - *Ilyodromus pectinatus*
      - Limnocytherinae
        - *Limnocythere* sp.
    - Conchostraca
      - Lynceidae
        - *Lynceus* sp.
    - Notostraca
      - Lepiduridae
        - *Lepidurus packardi*
  - ARACHNIDA
    - Hydracarina
      - Eylaidae
        - *Eylais* sp.
  - INSECTA
    - Collembola
      - Smynthuridae
    - Diptera
      - Chironomidae
        - *Prodiamesa* spp.
        - *Chironomus attenuatus*
        - *Chironomus* spp.
      - Culicidae
        - *Culiseta inornata*
        - *Culex tarsalis*
    - Coleoptera
      - Dytiscidae
        - *Agabus* sp.
        - *Hydroporus* sp.

chlorophytes (especially desmids) and finally, the dinoflagellates.

Filamentous blue-green and yellow-green algae were also very abundant, especially during the final weeks before complete evaporation of the pool. During the first months, in January and February, different diatom blooms occurred each week. Later, in the spring, the chlorophytes had their blooms. During and after each algal bloom, a corresponding microcrustacean bloom occurred.

At the time of the initial survey on January 6, there had been sufficient rains for the pools to have stable water levels. The pools had been intermittently present and absent from the early-winter rains in November and December. During this time ground soils had not yet been saturated enough to create a sufficient water table to fill the pools. Cold weather had frozen what water existed and occasional sunny days evaporated the pools until they were nearly dry. Certain organisms were present from the very beginning, even in these early days of instability. Turbellarians were observed during the first week in January, along with nematodes and rotatorians. Protozoans were very abundant and remained so throughout the life of the pool. The first insects also appeared during this week. Two species of dytiscid beetles, *Agabus* sp. and *Hydroporus* sp., were observed entering and exiting the pools. Larvae of the chironomid, *Prodiamesa* spp., were also seen in abundance.

The first blooms appears to have occurred sometime in early January (Fig. 2). Samples collected contained both cladocera and copepod numbers exceeding 100 individuals per 1 ml aliquot. The most abundant species identified in these first samples were the copepods *Diaptomus franciscanus, Acanthocyclops vernalis* and the cladocera *Daphnia pulex.* The ostracod *Ilyodromus pectinatus* and the conchostracan *Lynceus* sp. were also at high densities. During the same week several algal groups, the diatoms (especially *Acanthes* sp. and *Navicula* sp.) and the desmids (especially *Closterium* sp. and *Cosmarium* sp.) were collected at the high density rate.

By the second collection week, after the heavy December rains, life in the pools seems to have exploded with abundance and diversity. Standing at the shore of the largest pool, the surface glistened red with the swarming bodies of the copepod *D. franciscanus*. Other copepods of less spectacular color (*Chappuisius* sp.) existed in nearly equal numbers. The bodies of thousands of ostracods (*I. pectinatus*) and the conchostracan *Lynceus* sp. appeared to bounce, suspended in the water column. New species of cladocera appeared for the first time in this sample. A rarely seen macrothricid cladocera species, *Strebloceras serricaudatus*, was collected along with a sidid species, *Sida crystallina*, while the earlier inhabiter, *Daphnia pulex*, was still present in large numbers. Suddenly, the previously barren bottom was clustered with the first newly emerging plant sprouts.

Filamentous chlorophytes (*Zygnemopsis* sp.) was also first noticed during this week along with numerous associated microzoans. The numerous varieties of rotifers were collected from around the submerged vegetation and algae. Protozoans and single-celled algae were extremely abundant, as were filaments of the blue-green algae *Anabaena* sp. The physid gastropod *Aplexa hyponum* appeared as soon as submerged vegetation did (Fig. 3). Two distinctive genera of turbellaria existed; a light brown type (*Rhynchomesostoma* sp.) found on the surface and a green type (*Gieysztoria* sp.) found associated with submerged vegetation.

Samples taken the third and fourth week showed a distinct decline in numbers of microcrustaceans. Probably sampling resolution and selectivity eliminated much of the diversity found in the previous bloom samples. However, concentrations of protozoans, microzoans and turbellarians remained constant. A substrate sample yielded a one-time collection of an oligochaete (*Haplotaxis* sp.), and unidentified species of nematode and another, smaller gastropod (*Lymnaea* sp.). Concentrations of algae continued to increase and vegetation began to emerge through the surface of the water. On February 18 and again on April 6, individual red water mites of the genus *Eylais* were collected.

A rain storm that occurred on February 25 added just over 2.0 cm of water to the pools. This influx of fresh water increased the concentration of dissolved oxygen and increased the pH of the pool. Collections made during the next week showed a corresponding increase in microcrustacean numbers; representing the second recorded population bloom. In most cases, populations did not reach numbers recorded in the first bloom, and its duration was very short. Conditions reversed by the second week after the storm and microcrustacean populations declined to an abundance level similar to that which existed prior to the storm.

A larger rain storm occurred on March 8, adding almost 10 cm of water to the vernal pools. As with the other storm, a microcrustacean bloom followed, but only cladocera and copepod populations increased. Other microcrustaceans, such as ostracods and conchostracans continued to decline after the storm. Other groups either

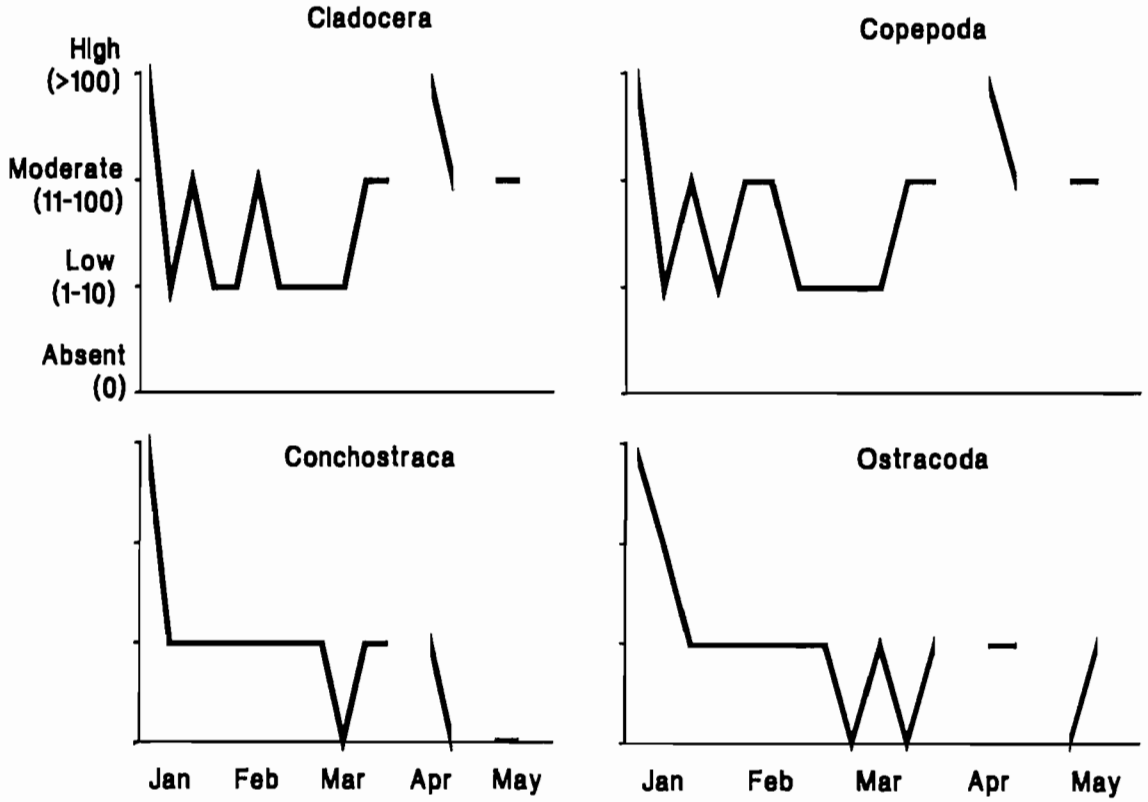

Figure 2. Microcrustacean population changes in the vernal pools during the study period.

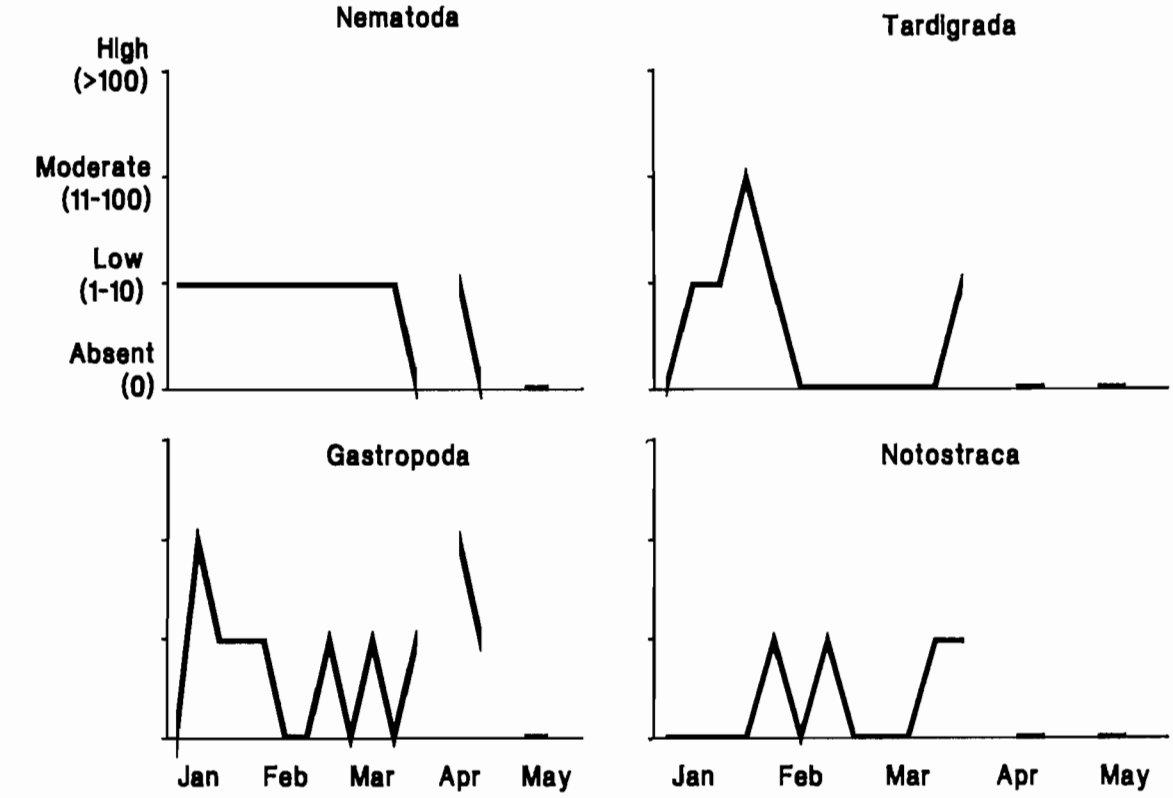

Figure 3. Selected invertebrate population changes in the vernal pools during the study period.

declined or did not show significant change or response to the storm.

On March 13 and 23, extensive activity of the notostracan *Lepidurus packardi* was noted. Young individuals had been collected earlier (February 8 and 25) and occasionally molted exuviae were seen floating, but no significant numbers had otherwise been observed or collected. The tadpole shrimp's activity of uprooting young plants had made the otherwise clear water very murky. Individuals were easily seen around the margins of the pools. Uprooted plants covered the surface of the pools and many exuviae collected in wind-driven piles at the southern margin of the pools. Individuals were easy to collect with standard random dipping, and many could be collected if specific effort were made to do so. After this two week period, few were observed and none were collected. It appeared that these animals, as with other vernal pool crustaceans, are closely synchronized to develop and mature together. These weeks of activity must have represented the mating and oviposition period, though mating was not observed.

In April, climatic conditions shifted to warmer temperatures and greater direct sunlight. Pool temperatures changed from an average of 11-12°C to an average of 15-16°C. Evaporation occurred at an accelerated rate and most faunal populations began to decline. The exception were the insects. During the final weeks of the pool's existence populations of predaceous and scavenger beetles increased (Fig. 4). Springtails blanketed the surface of the water during the last five weeks and mosquitoes appeared on April 13.

The first mosquitoes collected were *Culiseta inornata* Williston. They had not been seen in any of the surveyed pools throughout the winter months. From April 17 through May 2 *Culex tarsalis* Coquillett were collected in concentrations between 1 and 10 larvae per dip. Also, during the last four weeks, the red bloodworm, *Chironomus attenuatus*, were observed and collected, but were not observed prior to the April 13 collections.

The final two weeks before drying, only small pockets of water remained. Within these pockets existed thousands of copepods and other dying microcrustaceans. The other smaller pools were completely dry by April 17, and the largest pool was dry soon after May 2. A few days after the pools had dried, small reddish circles of dried zooplankton could be seen in the deepest depressions.

Conclusions.

None of the collected and identified animal

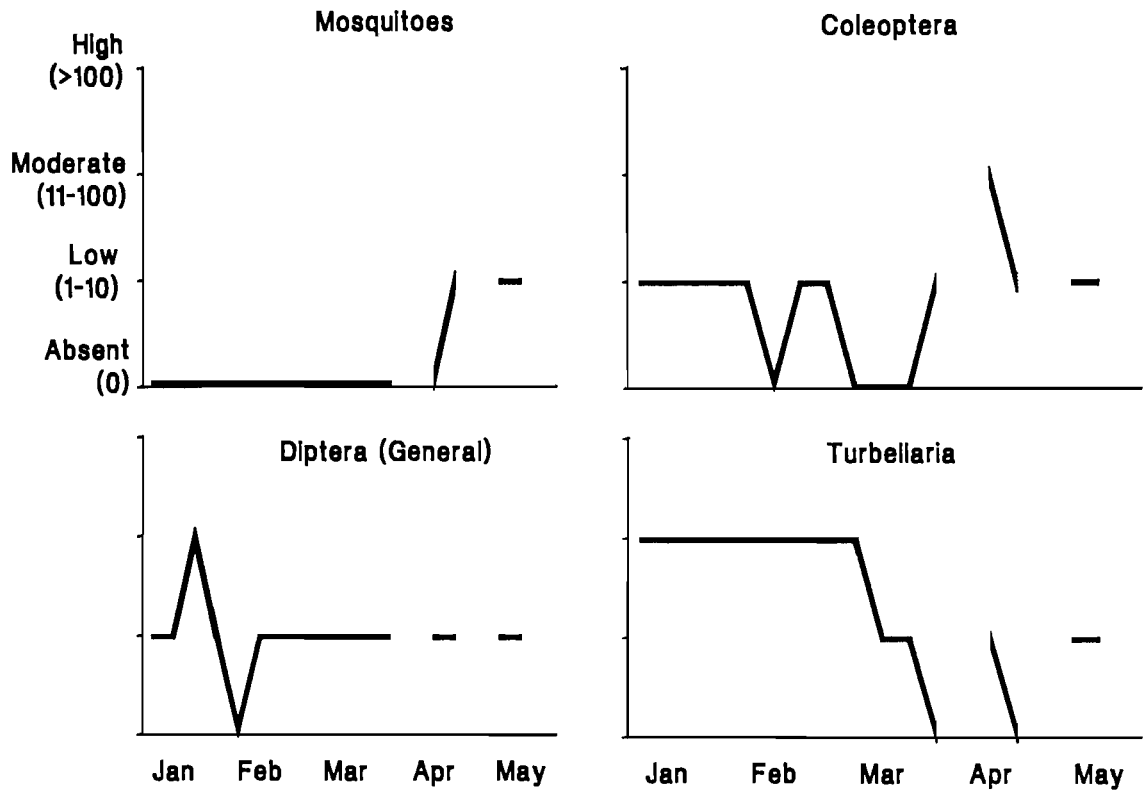

Figure 4. Insect and flatworm population changes in the vernal pools during the study period.

species were considered endemic to vernal pools, though several rarely encountered microcrustaceans were collected. Surprisingly, no anostracans were collected in the survey pools, yet they are known to be common in vernal pools elsewhere in the county. Unfortunately, cattle were allowed to graze in the area of the pools during the last weeks of the 1989 survey, which may have prematurely destroyed some delicate species by lowering the water quality.

From the observations made at this specific cluster of relatively undamaged vernal pools, mosquitoes and low dissolved oxygen-favoring midges were found only during the final weeks before drying. Pool conditions had been deteriorating during the last several weeks and normal populations were declining. Gravid female crustaceans were rarely collected during this time and therefore it could be assumed that egg deposition had occurred and cysts had been formed. Water temperatures were on the average higher and dissolved oxygen levels were presumably lower. Nutrient concentrations were at their greatest from the decomposition of thousands of planktonic microcrustaceans. At this point, sometime between early April and early May, these pools became an attractive oviposition site for *Culex* mosquitoes and *Chironomus* midges.

These observations suggest that during a select few weeks in the life of these vernal pools, there existed a window of opportunity during which mosquito control could be performed without serious effects on most of the vernal pool inhabitants. It, of course, cannot be assumed that all vernal pools would behave in a similar manner. However, it seems plausible that, at least among this cluster of pools, healthy undamaged pools generally have such a tightly-knit food web that organic nutrients are mostly tied-up in living organisms. Such an environment probably would not be perceived by gravid female mosquitoes as an optimum oviposition site. As the pool's trophic system begins to collapse during the natural aging process of the pools, organic nutrient concentrations were gradually freed, making the pools more attractive to gravid females.

Acknowledgements.

I extend my appreciation to Glenn Yoshimura for his most valuable help in identification of many of the invertebrates, and to Ken Boyce for his help in sampling. I also thank Dr. Larry Eng and his staff at the Environmental Services Branch of the California Department of Fish and Game for their guidance in evaluating pools and then their prompt issuing of the required permits allowing the sampling and collection of microinvertebrates from vernal pool communities.

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PRELIMINARY TESTING OF THE BLACK-TAILED JACKRABBIT AS A POSSIBLE SENTINEL FOR WESTERN EQUINE ENCEPHALOMYELITIS AND LYME BORRELIOSIS

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ABSTRACT

Black-tailed Jackrabbits were sampled from two areas of the Sacramento Valley as candidates for sentinels of human diseases. Serum was tested by enzyme-linked immunosorbent assay for western equine encephalomyelitis and St. Louis encephalitis antibodies. The samples were also assayed by indirect immunofluorescent antibody for *Borrelia* titers. In Butte County, 12% of the jackrabbits sampled were positive for WEE in 1988 and 15% were positive in 1989. In Sacramento County, 21% of the jackrabbits sampled during 1988 indicated a positive antibody titer for *Borrelia*. Renal cortical tissues from some of the animals were also examined using direct fluorescent antibody technique for *Borrelia* spirochetal isolation. Spirochetes were observed in one sample. Adult mosquito populations were sampled in both areas throughout the 1984-1989 seasons by standard light trapping techniques.

Introduction.

The Black-tailed Jackrabbit, *Lepus californicus*, can be found throughout the Sacramento Valley of California in locally abundant numbers. Under ideal conditions, populations can explode to "plague" numbers (Palmer 1954). Such abundant numbers make these animals an ideal host for a wide variety of hematophagous arthropods. As a result, many of the etiologic agents of a variety of arthropod-borne diseases can be found in these hares (Hardy et al. 1977; Kiorpes and Yuill 1975; Lane and Regnery 1989). In the Sacramento Valley, an endemic transmission cycle of western equine encephalomyelitis (WEE) can occur among the hares and the pasture mosquito, *Aedes melanimon* Dyar (Hardy et al. 1977). In another study conducted in Butte County, sera collected from hares during eruptions of St. Louis encephalitis (SLE) activity indicated that 30 hares of 207 sampled had positive antibody titers for that virus (Beck pers. comm.). Investigations in Alberta, Canada have shown that epizootics of WEE can be detected in the Snowshoe Hare, *Lepus americanus*, two to three weeks prior to epidemics of WEE in humans and epizootics of WEE in horses (Kiorpes and Yuill 1975). It has also been suggested that Lagomorphs may serve as useful sentinels for surveillance of Borreliosis (Lane and Regnery 1989).

Materials and Methods.

During the summer months of 1988 and 1989, Black-tailed Jackrabbits were collected from locations in Butte and Sacramento Counties. The locations in Butte County were widespread, while the Sacramento County collections were made exclusively at McClellan Air Force Base, Sacramento, California. Blood samples of approximately 3 ml were taken from each animal, and the kidneys were removed from many of the animals taken in 1989. Serum was obtained by centrifugation of whole blood samples in separation gel vacutainers (Van Waters & Rogers). Kidneys were placed on dry ice immediately after removal and stored at -70° C prior to examination.

Sera samples were tested for WEE and SLE antibodies by an indirect enzyme-linked immunosorbent assay (ELISA) technique for detecting immunoglobulin M (IgM). These assays were developed for detection of antibodies in chicken and human sera by Dr. T.F. Tsai and R.A.

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Bolin, Division of Vector-Borne Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, Fort Collins, Colorado. Horseradish peroxidase conjugated goat anti-rabbit IgG (Bethyl Laboratories) was substituted during the assay for the conjugated IgG normally used in the Tsai and Bolin method. Similarly, goat anti-rabbit IgM u-chain specific (Bethyl Laboratories) was substituted in the IgM assay. Flat bottom ELISA plates were read by a microplate reading spectrophotometer using a 450 nm filter. A sample was considered positive when the optical density was at least twice that of the optical density of the control well.

In determining antibody titers for *Borrelia*, sera samples were tested by an indirect immunofluorescent antibody assay (IFA). Procedures for this assay have been previously described (Dattwyler et al. 1988b). The procedure was modified only by substituting fluorescein conjugated goat anti-rabbit IgG heavy- and light-chains (Bethyl Laboratories). Fluoroscopy was conducted on a Zeiss fluoroscope. A sample was considered positive if greater than 40% of the spirochetes in the field showed 4+ fluorescence. A positive control serum was produced in a domestic rabbit which had been immunized with heat-killed *Borrelia burgdorferi* Johnson, Schnid, Hyde, Steigerwalt and Brenner (ATCC 35210) as reported by Steere et al. 1983. In order to avoid cross reactivity problems, serum from the test rabbit was assayed for domestic rabbit triponemal infections by the Venereal Disease Research Laboratory slide test prior to inoculations. After a six week period, serum was collected from the rabbit and tested for fluorescence using the procedures previously described. Titer was found to be ≥1:4096. A negative control consisting of pre-immunization serum from the same domestic rabbit was also included on each slide tested.

Renal tissues were tested from those jackrabbits collected in 1989 which were determined to have positive titer for *Borrelia*. Small samples of cortical tissues were thin smeared on fluoro-slides (Van Waters & Rogers). After air drying, smears were fixed with approximately 20 µl of acetone. Fixed slides were then incubated in a humidity chamber for one hour with monoclonal antibody (H9724), kindly provided by Dr. A Barbour. Slides were then treated the same way as described above for the IFA determination except that goat anti-mouse fluorescein conjugated IgG heavy- and light-chains (Bethyl Laboratories) were substituted for the goat anti-rabbit conjugate.

Adult mosquito populations were monitored weekly by sampling with a standard New Jersey Light Trap. Populations from the Sacramento County site were monitored by the Sacramento County-Yolo County Mosquito Abatement District's entomology staff. In all, six light traps were monitored from areas immediately surrounding the area targeted for study. In Butte County, three light traps located near the majority of the jackrabbit collection sites provided the data.

Results.

During the 1988 season, 17 jackrabbits were collected from sites in Butte County. Two (12%) exhibited a positive IgG titer for WEE. Reciprocal IgG titers for both rabbits were determined to be ≥4,096 (Table 1). There were no jackrabbits which showed positive WEE IgM titer of 1:200, nor were there any SLE positives or *Borrelia* positive titers (Table 2).

A total of 109 jackrabbits was collected in 1989. Twenty jackrabbits were collected from ranches in Butte County in areas near the Sacramento River. Three (15%) were positive for WEE IgG antibodies. Reciprocal IgG titers ranged from 256 to ≥4,096 (Table 1). As in the 1988 samples, there were no WEE IgM titers greater than 1:200, SLE positives or *Borrelia* positive titers. There were no WEE or SLE positives from the McClellan AFB collections.

During 1989, 89 jackrabbits were collected from McClellan AFB on two separate occasions. Of the 89, 19 (21%) had a positive titer for *Borrelia* (Table 3). Reciprocal titers ranged from 64 to 1024. Kidney tissues were tested from 13 of the rabbits which exhibited a positive titer. Spirochetes were observed in one sample.

Adult mosquito light trap results were tabulated weekly and totaled to obtain the average number of females per trap-night for the years 1984-1989 (Table 4). Over 10.41 female *Culex*

Table 1. WEE IgG antibody titers.

Rabbit	1:256	1:512	1:1024	1:2048	1:4096
R2-88	+	+	+	+	+
R14-88	+	+	+	+	+
R24-89	+	+	+	-	-
R34-89	+	-	-	-	-
R36-89	+	+	+	+	+

Table 5. Summary of jackrabbit WEE positives by ELISA.

Date	Rabbit	Site	Test results IgM	Test results IgG	Optical density Sample	Optical density Control
7-26-88	R2-88	Honcut	-	+	0.563	0.091
9-08-88	R14-88	Llano Seco	-	+	0.209	0.039
6-14-89	R24-89	M&T Ranch	-	+	0.238	0.030
7-17-89	R34-89	M&T Ranch	-	+	0.446	0.048
7-17-89	R36-89	M&T Ranch	-	+	0.355	0.017

for WEE in Butte County and elsewhere. The IgM class of viral antibodies is usually the primary response to exposure and generally suggests a current infection (Cremer and Riggs 1979). Use of the ELISA for detection of IgM antibodies in sentinel jackrabbits should be a timely method of detecting recent virus transmission. The presence of only IgG antibodies in the samples from Butte County suggests those individuals were exposed during a previous season (Table 5). During the 1984 Southern California SLE epidemic, sentinel chicken flocks and mosquito pools apparently did not provide evidence of widespread virus transmission until a month after the onset of the earliest human case (Work et al. 1985); suggesting the need for better surveillance methods for that virus as well.

Time course information for each immunoglobulin assayed is important in determining the exposure and response time frame. The answers to questions concerning how soon after exposure do IgM and IgG antibodies appear in the blood stream and length of duration can provide significant information. Similarly, determination of a discriminating IgG titer for *Borrelia burgdorferi* by IFA fluoroscopy would add even more significance to serological studies of jackrabbits.

Both the jackrabbit and the mosquito data for Butte County indicate that there is a cycle of WEE virus transmission between jackrabbits and *Ae. melanimon* as described by Hardy et al. 1977. The paucity of female *Ae. melanimon* and lack of positive titers for WEE in the Sacramento County site also seem to support this relationship. Our data also suggests that high early season numbers of *Ae. melanimon* and a high percentage of IgM positive sentinel jackrabbits may signal an active transmission cycle and an increased risk of transmission WEE to other sentinels and possibly humans. Unfortunately, the only confirmation of this hypothesis would be a resurgence of WEE virus activity in humans and sentinel jackrabbits, which the District is committed to prevent, if possible.

Acknowledgements.

The authors thank Drs. T.F. Tsai and C.G. Moore from the Centers for Disease Control, for their assistance with the ELISA test and for providing the test reagents. We also appreciate the assistance and support of Colonel J.E. Wilson, Base Commander of McClellan AFB. We also thank D. Murrill of the Butte County Department of Public Health. The assistance of District employees S. Buckley and J. Shaffer is greatly appreciated. We also appreciate the use of light trap data for Sacramento County provided by G. Yoshimura and K. Boyce of the Sacramento County-Yolo County Mosquito Abatement District.

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PRELIMINARY OBSERVATION REPORT ON

LAWN DRAINS AS URBAN MOSQUITO PRODUCTION SOURCES

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Introduction.

Commercially produced lawn drains have been identified as a source for mosquito production in Santa Barbara, California. Many drains incorporate sediment traps which hold water and trap nutrients which, for their small size, support large numbers of mosquito larvae. The objective of our study was to provide documentation needed to confirm that lawn drains, because of their design, produced enough mosquitoes to be considered a nuisance.

Commercially produced lawn drains are utilized in areas to remove surface water from around buildings and low areas associated with domestic and commercial projects. Typical drains studied included small one-piece, injection molded hi-impact plastic; six-inch "Universal" catch basins utilized in pool areas, lawns and patios; the larger nine-inch and twelve-inch square plastic catch basins used where large amounts of surface water are to be removed such as golf courses, industrial parks and municipal parks; and the twelve-inch square concrete drains for heavy duty service areas.

Materials and Methods.

Sixteen drains representing five locations were sampled for a one-year period from January to December 1989. All locations were sampled once a month with bimonthly samples from June to October because of increased mosquito activity due to longer days and warmer weather conditions. Detailed records were kept for each location and drain noting existing conditions for each sampling. For example, one drain ceased to support mosquito production when it became a convenient location to dump rinse water used to clean paint brushes.

Most of the water was extracted from each drain using a small cup; a common kitchen "baster" was used to remove the remaining water. The sample water from the drain was put through a mosquito larvae concentrator. Since our purpose was to document a drain's ability to produce mosquito larvae, the screened water was placed back into the drain. Otherwise, physical control would have been implemented. At no time did we supplement a drain with additional or new water. The collected larvae were then placed into one-pint containers with water from the source and transported to the laboratory for identification and counting. Since it was common to find large numbers of larvae in each drain, 500 larvae was used as a counting cut-off point to save time.

Results.

Figure 1 represents a summary of the 16 drains and the dates that sampling was completed. On a typical sampling date (August 28) twelve drains were found to contain mosquito larvae with eight drains (two-thirds) containing more than 500 larvae and four drains (one-third) containing less than 500 mosquito larvae each. More than one-half (67.5%) of the drains contained mosquito larvae form June through November, the summer season.

Figure 2 represents precipitation in Santa Barbara during the study period. The highest rainfall was in February (3.5") which created enough runoff to flush out the drains and possibly prevent mosquito breeding during February and March. Rainfall of one inch or less had little effect on a drain's ability to continue producing mosquito larvae (May, October and November). Obviously, a drain located in an area of high impermeability would receive substantially more runoff which would reduce or temporarily eliminate mosquito production in the drain. Examples would be patio drains or roof water run-off drains.

Summary.

Commercial plastic lawn drains with sediment traps are a source that can support tremendous numbers of mosquito larvae. A commonly used six-inch universal catch basin (Fig. 3) with a two-inch sediment trap can contain up to 24 ounces of water. It was not unusual to find 500 - 1,000 larvae in a single drain. During our study, the highest count for a single drain was 1,610 larvae. These drains could be declared a "public nuisance" per the definition of the laws relating to local agencies engaged in mosquito and vector control (Section 2200).

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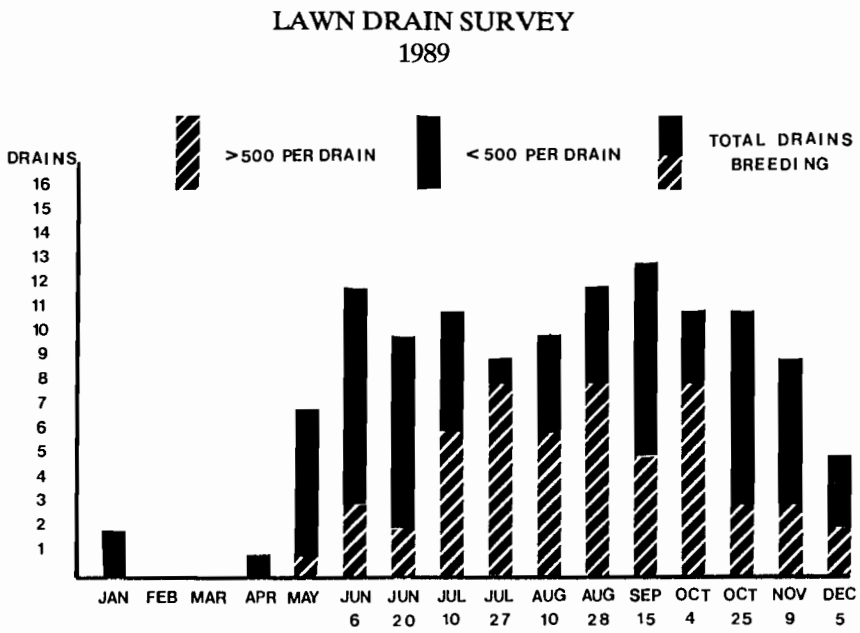

Figure 1. Occurance of lawn drains found breeding mosquitoes during the survey period.

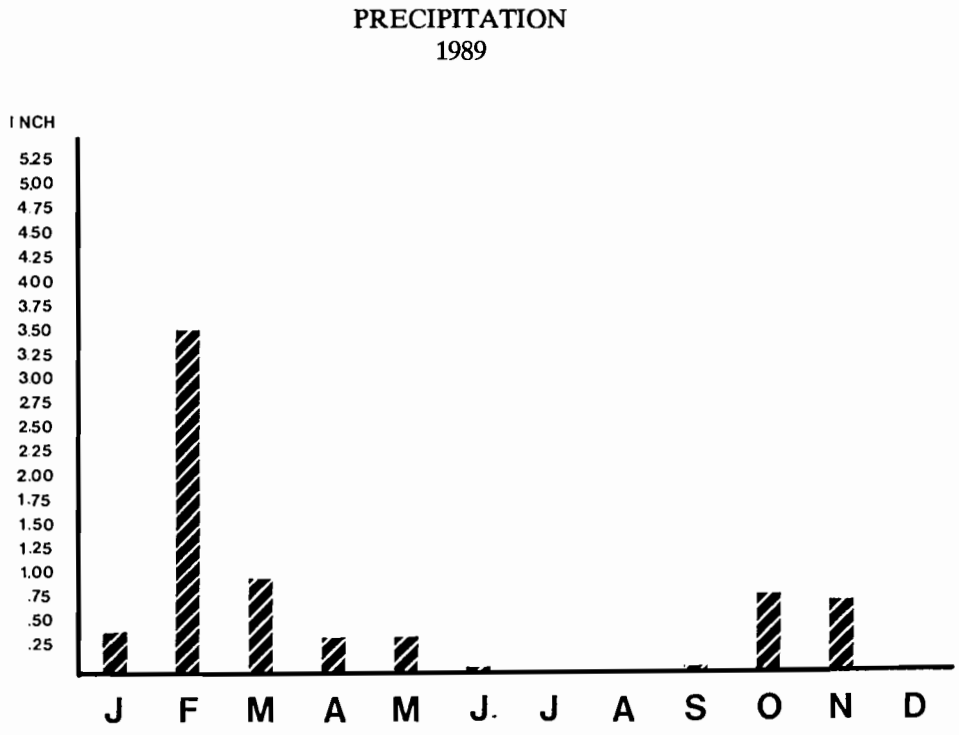

Figure 2. Precipitation (inches of rainfall) in the Santa Barbara area during the survey period.

6" UNIVERSAL CATCH BASIN WITH REMOVABLE UNIVERSAL INSERTS

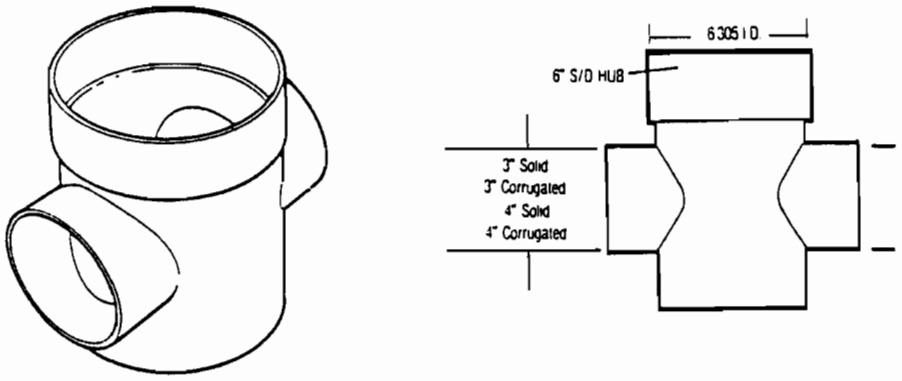

Figure 3. Diagram of 6" universal P.V.C. catch basin and 2" sediment trap frequently found breeding mosquitoes throughout the survey period.

LOW-PROFILE HOUSING ADAPTER - USES SAME
INTERCHANGABLE HUB OUTLETS AS CATCH BASIN

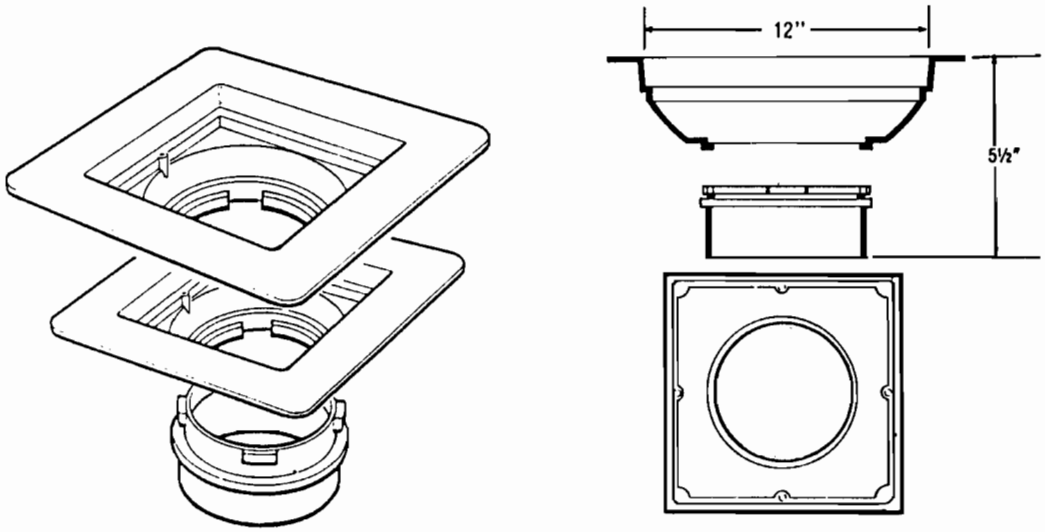

Figure 4. Diagram of P.V.C. low-profile catch basin with no sediment trap rarely found breeding mosquitoes throughout the survey period.

Culex pipiens L., the common house mosquito, was the predominant larval species found in these drains throughout the study period. *Culiseta incidens* (Thompson) was an incidental species found only three times during the study. *Culex pipiens* have been implicated as vectors of St. Louis encephalitis in other areas of the country. They can also transmit the organisms causing bird malaria, fowl pox and heartworm in dogs.

The Uniform Plumbing Code (UPC) as defined by the International Association of Plumbing and Mechanical Officials and the Uniform Building Code (UBC) do not address products or their use related to lawn drainage systems. The manufacture and use of lawn drains with sediment traps is widespread. A leading producer of drainage products indicated that the six-inch universal catch basin (Fig. 3) is one of their most popular products. They sell about 7,000 drains a month or 84,000 drains per year in the United States; the largest market is California. Alternative styles of lawn drains are available but, due to higher costs, are not widely used by homeowners and landscape contractors.

Conclusions.

The Goleta Valley/Carpinteria Mosquito Abatement Districts consider lawn drains or any drainage product which holds water and breeds mosquito larvae to be a public nuisance. Since the Uniform Plumbing Code does not address this problem, the Districts will pursue a local ordinance or code change which will prohibit the use of this type of product. Acceptable alternative products exist which do not promote mosquito production (Fig. 4).

Drains that already exist will be addressed through a public education program which will encourage property owners to implement corrective measures. Where practical, the District will fill the sediment trap of a drain with suitable material which will prevent water retention.

The Districts encourage other vector control agencies to investigate the extent to which this type of drainage product may be serving to promote mosquito problems in their respective areas. Should other agencies confirm similar problems and initiate corrective measures, the Districts would encourage the California Mosquito and Vector Control Association and the California Department of Health Services, Environmental Management Branch to monitor such actions and, if warranted, coordinate a uniform and acceptable approach to this problem.

Acknowledgements.

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EFFICACY OF A MICROBIAL INSECTICIDE AND LARVIVOROUS FISH AGAINST *CULEX TARSALIS* IN DUCK CLUB PONDS IN SOUTHERN CALIFORNIA.

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ABSTRACT

The efficacy of *Bacillus thuringiensis* var. *israelensis* (*Bti*) against *Culex tarsalis* was evaluated in 1.8 ha duck hunting club ponds in the Coachella Valley of southern California. Duck loafing ponds were nearly devoid of interior vegetation and *Cx. tarsalis* larvae were concentrated in the vegetation along the perimeter dikes. Therefore, the inundated perimeter vegetation and 3.1 m of the water surface in from each dike were treated with an aqueous suspension of *Bti* (Vectobac 12AS). The aqueous suspension applied at a per pond rate of 1.2 L/ha reduced mosquito larval populations (3rd-4th instars) by 96-100% those in ponds which were stocked with *Gambusia affinis* at a rate of 1.4 kg/ha. The aqueous suspension applied at a per pond rate of 0.12 L/ha (rate based on the area treated: 1.2 L/ha) was ineffective against *Cx. tarsalis*. Mosquitofish stocked at the current operational rate of 1.4 kg/ha did not significantly reduce *Cx. tarsalis* larval populations. The appropriateness of integrated control and environmental control strategies for *Cx. tarsalis* larval populations in duck club ponds are discussed.

Introduction.

Duck hunting club ponds located on the northern shore of the Salton Sea (in Riverside and Imperial Counties in southern California) are important developmental sites of *Culex tarsalis* Coquillett and pose and a significant vector control problem. In the Coachella Valley, several thousand acres are flooded annually for duck hunting. Large numbers of adult *Cx. tarsalis* (>1,000 individuals/trap-night) are captured in CO_2-baited CDC traps during the spring and autumn (Durso and Burguin 1988) and arbovirus activity is prevalent in the vicinity of duck hunting clubs (Durso and Burguin 1988, Emmons et al. 1988).

Current control methodologies for larval mosquito populations inhabiting duck club habitats consist of stocking ponds with larvivorous fish and spot-treating problem areas with larvicides. Inundation of duck club ponds in the Coachella Valley usually begins in late August-early September and flooding is completed after approximately four to six weeks. As the ponds fill with water, mosquitofish (*Gambusia affinis* (Baird and Girard)) are seined from other sources and stocked into duck club ponds at a rate of 1.4 kg/ha. Abatement measures with fish are often supplemented by treating individual ponds which contain dense larval mosquito populations with larvicides such as Golden Bear 1111 Larviciding Oil (Witco Chemical Co., Oildale, CA).

Mosquito abatement districts are replacing conventional chemical control agents with biological agents (Eldridge 1988). Because it is often necessary to treat many duck club ponds with larvicides, a suitable biological larvicide is needed. A mosquito-specific larvicide such as *Bacillus thuringiensis* var. *israelensis* (*Bti*) used in conjunction with larvivorous fish and naturally-occurring predators may provide cost-effective control of mosquito larvae in duck club ponds.

Previous studies in mesocosms have shown that *Cx. tarsalis* populations declined naturally 2-3 weeks after inundation (Mulla 1990, Walton et al. 1990) and, as compared to either treatment alone, an integrated control strategy which combined mosquito-specific bacterial larvicides with

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mosquitofish was most efficacious against *Cx. tarsalis* (Walton and Mulla 1990b). Duck club ponds differ from the mesocosms in size, extent of vegetation cover, and several physical-chemical factors which influence the effectiveness of microbial and biological control agents (Mulla 1990, Walton and Mulla 1990a). Additionally, duck club ponds can be categorized into two general types: ponds that contain vegetation throughout the interior (foraging ponds) and ponds that lack interior vegetation (loafing ponds).

Bacillus thuringiensis var. *israelensis* reduced *Cx. tarsalis* immature populations by 93-100% of pretreatment levels in unreplicated studies of a Fresno County, California wetland field and a Kern County, California duck club (Mulligan and Schaefer 1981). However, the efficacy of bacterial larvicides against *Cx. tarsalis* has not been examined in the Coachella Valley duck club ponds. In 1989, we studied the effectiveness of *Bti* against *Cx. tarsalis* in loafing ponds in a Coachella Valley duck club and compared the levels of control by *Bti* to that by current mosquito abatement practices using mosquitofish alone.

Materials and Methods.

The effectiveness of *Bti* against *Cx. tarsalis* larvae was studied in six 1.8 ha (4.4 acres) ponds at the Adohr Valley Farms Duck Club, Mecca, California. Water was supplied to the duck club via a single well located on the northwest corner of the duck club (Pond 1; Fig. 1). The predominant directions of water flow through the duck club were to the east and south. With the exception of a few stands of tamarisk (*Tamarix ramosissma*), the interiors of the six ponds were devoid of emergent vegetation. The timing of flooding and the perimeter vegetation were similar for pairs of ponds in each row; therefore, the ponds were grouped by row. The ponds in each row were assigned to two treatments; *Bti* or control. To prevent contamination of the control ponds by *Bti*, ponds assigned to *Bti* treatment (Ponds 4, 8 and 11) were located to the east of the control ponds (Ponds 3, 7 and 10).

Two application rates of *Bti* were tested against *Cx. tarsalis*. An aqueous suspension of Vectobac 12AS (Abbott Laboratories: 1200 ITU/mg) applied to ponds with a pressurized sprayer per label directions. The *Bti* suspension was continuously agitated. Because mosquito larval populations were concentrated in the vegetation along the dikes, the inundated perimeter vegetation and 3.1 m (10 ft) of the water surface from each dike were treated at a rate of 1.2 L/ha (1 pt/acre) on October 18. The area treated was equal to 0.2

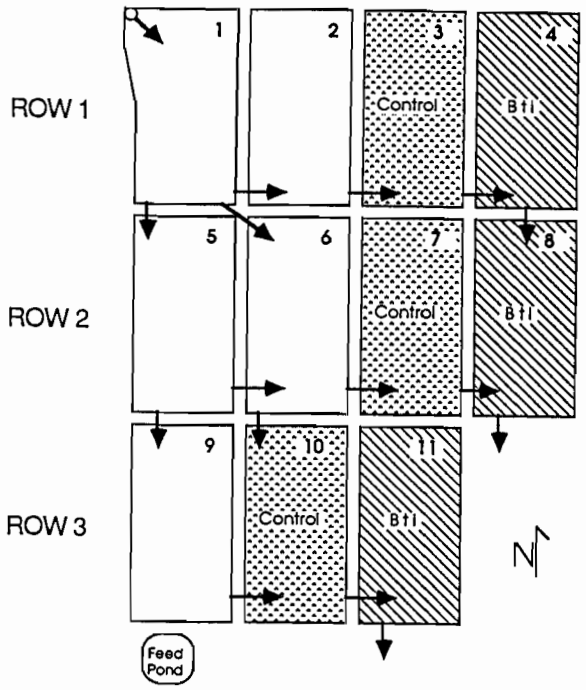

Figure 1. Experimental design in Adohr Valley Farms during 1989. Arrows indicate waterflow directions.

ha; therefore, based on total surface area of the pond, *Bti* was applied at a per pond rate of 0.12 L/ha. This rate was ineffective against *Cx. tarsalis*; hence, on October 25 and November 1, the perimeter vegetation and 3.1 m of the water surface from the dikes were treated at a per pond rate of 1.2 L/ha. Water temperature was measured weekly in Pond 8 with a maximum-minimum recording thermometer.

Mosquito larvae were sampled weekly with a 350 ml dipper at 30 stations in each pond. At each station, five dips were taken along a 2 m region in the perimeter vegetation, combined and preserved in 95% ethanol. The ponds in each row were sampled initially after both ponds had filled with water. The first samples were taken on September 25 (Row 1; Ponds 3, 4), October 2 (Row 2; Ponds 7, 8) and October 10 (Row 3; Ponds 10, 11).

Because these duck club ponds are typically stocked with mosquitofish, we compared mosquito larval abundance in the *Bti*-treated ponds to that in ponds containing *Gambusia affinis*. The rationale for using the current abatement method, instead of a fishless control, is: (1) Stocking mosquitofish into the duck club ponds is common practice; whereas,

leaving ponds unmanipulated is not done routinely. (2) If mosquitofish eventually reduced mosquito larval populations, then we could determine how many bacterial larvicide applications were necessary before fish provided significant levels of control. (3) Both types of controls, with and without mosquitofish, were not possible. Although a fishless control is less preferable than one using mosquitofish for the question which we posed, replicate experimental units (ponds) were not available for a fishless control because the other ponds in the duck club (Ponds 1, 2, 5, 6 and 9) were not disked in 1989, had vegetation throughout the pond interiors and differed markedly from the ponds used in our study.

Mosquitofish were stocked into Ponds 3, 7 and 10 at a rate of 1.4 kg/ha (1.25 lbs/acre) on October 5. *Gambusia* were seined from local ponds, weighed, and a mixture of adult and immature fish were stocked into the control ponds.

Mosquitofish were excluded from the *Bti*-treated ponds by surrounding the dropboxes with doubled-over fiberglass window screen (about seven openings/cm). Because the first pond flooded often develops dense stands of emergent vegetation and contains large numbers of mosquitofish which migrate into other ponds, the dropboxes between the control ponds and ponds not used in this study (Ponds 2, 6 and 9) were not screened.

Mosquitofish populations were sampled weekly with Gee minnow traps (Cuba Specialty Mfg. Co., Fillmore, NY) that were lined with fiberglass window screen. Minnow traps were baited with dog food and placed in the corners of each control pond for 24 hours. To determine the effectiveness of the screens, minnow traps were placed in the *Bti*-treated ponds for a 24 hr period on November 13-14.

Statistical analyses. The efficacy of *Bti* and *G. affinis* against *Cx. tarsalis* larvae were tested statistically by making pairwise comparisons of larval abundance (3rd and 4th instars) in ponds within a row for two of the three rows. Late instar larvae were not collected in dip samples from Pond 4; therefore, this pond was not treated with *Bti*. Our data were not directly amenable to statistical testing by a randomized block analysis of variance because one row (block) was not treated with *Bti*, the movement of water through Adohr Valley Farms duck club precluded randomization of treatments in each row and mosquito larvae either were not collected in some pretreatment samples (Ponds 3 and 4) or were eliminated from the ponds by *Bti* treatments (zero mean and variance for a treatment on some dates).

The effect of each *Bti* treatment on *Cx. tarsalis* larval abundance was tested statistically by a Mann-Whitney U test. Whereas 3rd-4th instar larvae were absent in samples taken five days after the ponds had been treated with *Bti* at a pond rate of 1.2 L/ha, the large numbers of 1st-2nd instar larvae in these samples indicated that the effects of the bacterial larvicide lasted less than one week. Also, at the ambient water temperatures, 3rd and 4th instar larvae in a previous sample would have emerged as adults before the next sample was taken. Therefore, separate comparisons (by date) of 3rd-4th instar larval abundance for each *Bti* treatment are appropriate. For the pair of ponds in each row, the numbers of larvae collected at each station were ranked and the U statistic was corrected for ties. Because this procedure is analogous to making multiple t-tests, we adjusted the probability of Type I error to 0.025.

As do parametric statistics, the Mann-Whitney U test also requires that the treatments are randomized among experimental units. Because we could not randomize the two treatments within each row and larval abundance was determined from combined, random samples at fixed stations, the significance levels of the statistical comparisons are, at best, approximations.

Similar tests were used to examine the efficacy of mosquitofish. However, we set the probability of Type I error to 0.01. Because *G. affinis* was stocked only once, a repeated-measures analysis of variance is more appropriate. Yet, unforseen difficulties in one row (3rd-4th instar larvae were not collected from Ponds 3 and 4) precluded this analysis.

Results and Discussion.

Efficacy of *Bti*. *Bti* applied at a per pond rate of 1.2 L/ha significantly reduced *Cx. tarsalis* abundance (3rd-4th instars) by 96 and 100% relative to those observed in the control ponds (Table 1). *Bti* applied at a per pond rate of 0.12 L/ha was ineffective against *Cx. tarsalis* (Fig. 2). At five days after treatment, the numbers of larvae collected in dip samples from *Bti*-treated ponds did not differ significantly from those of the control ponds.

The aqueous suspension, when applied at a per pond rate of 1.2 L/ha, was effective for nearly one week. Whereas the numbers of 3rd and 4th instar larvae in Ponds 8 and 11 were very low on the last two sampling dates (Fig. 2), 1st and 2nd instar larvae were present in both ponds (Fig. 3).

Efficacy of mosquitofish. Mosquitofish stocked at 1.4 kg/ha did not appreciably reduce *Cx. tarsalis* abundance (Figs. 2 and 3). Mosquito larval abundance in two of the control ponds were greatest on October 10 (total number of larvae/150 dips: Pond 7=538, Pond 10=1141). After October

Table 1. Efficacy of *Bacillus thuringiensis* (Vectobac 12AS) at five days post treatment against *Culex tarsalis* (3rd and 4th instar larvae) in Adohr Valley Farms duck club during 1989. For each comparison, n_1 and n_2 in the Mann-Whitney U test equal 30.

Per pond rate (L/ha)	Date	Water Temp. (° C)	Comparison (ponds)	U	|Z|	P(Z)[a]	Reduction[b] (%)
0.12	October 23	14-29	7 vs 8	438	0.212	ns	0
			10 vs 11	340	1.701	ns	0
1.2	October 30	9-28	7 vs 8	315	3.215	**	100
			10 vs 11	284.5	3.225	**	96
1.2	November 6	9-19	7 vs 8	270	3.812	***	100
			10 vs 11	285	3.617	***	100

[a] ns = P(Z) > 0.025; ** = P(Z) ≤ 0.01; *** = P(Z) ≤ 0.001.
[b] Percent reduction = 100 [1 - (no. of larvae from *Bti*-treated pond/ no. of larvae from control pond)].

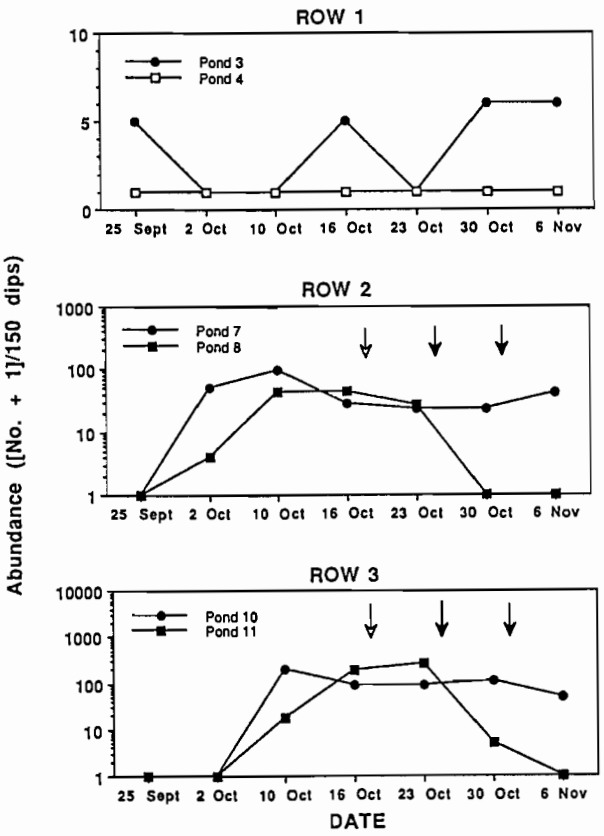

Figure 2. *Culex tarsalis* larval abundance (3rd-4th instars) in duck club ponds at Adohr Valley Farms during 1989. Vectobac treatments at a per pond rate of 0.12 L/ha and 1.2 L/ha are indicated by the open and closed arrows, respectively. • = control pond populations, ■ = *Bti*-treated pond populations (ponds were not treated in row 1).

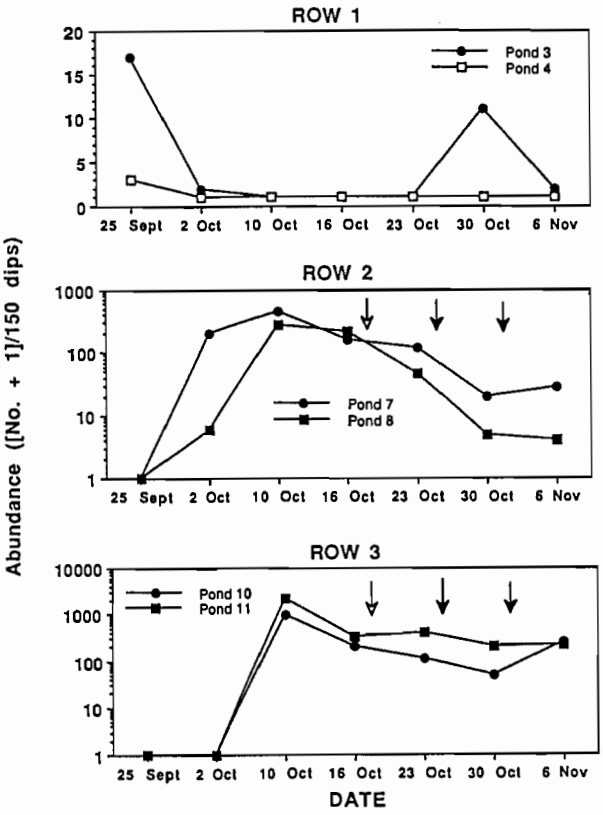

Figure 3. *Culex tarsalis* larval abundance (1st-2nd instars) in duck club ponds at Adohr Valley Farms during 1989. Vectobac treatments at a per pond rate of 0.12 L/ha and 1.2 L/ha are indicated by the open and closed arrows, respectively. • = control pond populations, ■ = *Bti*-treated pond populations (ponds were not treated in row 1).

10, larval abundance (3rd-4th instars) was reduced slightly and was nearly constant through November 6 (Fig. 2).

Larval abundance in ponds that contained mosquitofish did not differ significantly from that observed in ponds without *Gambusia* at 11 and 18 days (October 16 and 23, respectively) after fish were added to Ponds 7 and 10 (Table 2). For the third control pond (Pond 3), the numbers of *Cx. tarsalis* 3rd-4th instars in dip samples were very low (≤ 5 larvae/150 dips) from September 25 to November 6 (Fig. 2).

The failure of mosquitofish stocked at current operational densities (1.4 kg/ha) to reduce *Cx. tarsalis* larval populations in 1989 corroborates our 1988 studies in which mosquitofish stocking rates were manipulated in small enclosures and mesocosms. In 1988, *G. affinis* stocked at 1.4 kg/ha did not significantly reduce mosquito larval populations as compared to fishless controls in 16 m^2 enclosures at Adohr Valley Farms (Walton and Mulla 1990b). During the summer, *Gambusia* significantly reduced *Cx. tarsalis* larval populations when mosquitofish were stocked at the very high rate of 4 kg/ha in 36 m^2 mesocosms at our Coachella Valley research facility. However, a *G. affinis* stocking rate of 1 kg/ha did not significantly reduce mosquito larval populations as compared to fishless controls (Walton and Mulla 1990b, Walton unpubl. data). In both vernal and autumnal studies, the impact of *G. affinis* on *Cx. tarsalis* was apparent 2-3 weeks after fish were stocked into mesocosms. In 1989, *Cx. tarsalis* larval abundance did not decline to nearly zero at 2-3 weeks after stocking mosquitofish or at any time during the one month period after *G. affinis* was added to the ponds.

Mosquitofish catches in minnow traps either declined or were near zero during the three weeks following October 5 (Table 3). For Ponds 7 and 10, the increases in mosquitofish numbers observed on November 6-7 were due to migration of fish from thickly-vegetated ponds into duck loafing ponds as water was being diverted into Ponds 5, 6 and 9. Relative to catches during the previous three weeks, comparatively high numbers of large-sized *G. affinis* were captured in minnow traps adjacent to the dropboxes entering Ponds 7 and 10.

The screens surrounding the dropboxes effectively excluded *G. affinis* from the *Bti*-treated ponds (Ponds 4, 8 and 11). Mosquitofish were not captured by minnow traps in Ponds 4, 10 and 11 on November 13-14.

Implications for mosquito control programs. Service (1983) suggested that integrated control programs which combine biological agents such as mosquito predators and parasites, or utilize biological agents and larvicides, often fail because: 1) larvicide-induced reductions of mosquito populations result in food shortages for the biological control agents, 2) predators or high parasite-induced larval mosquito mortality often interfere with the transmission of mosquito parasites, and 3) the dispersion and aggregation behavior of mosquitoes provide spatial refuges for mosquito larvae. Mosquitoes also colonize a variety of habitats. For example, *Cx. tarsalis* is found in habitats that range in size from small, ornamental containers to large, duck club ponds (Durso and Burguin 1988). Differences in habitat preferences of mosquitoes and their predators, and the enormous number and variety of habitats utilized by this mosquito, make biological control an arduous, if not impossible, task.

In addition to the confounding factors listed above, differences in the life history characteristics of mosquitoes and their predators provide a temporal refuge for mosquitoes (Service 1983). *Culex tarsalis* colonizes newly inundated habitats and develops more rapidly than do many of its predators (Walton et al. 1990).

Last, integrated control programs that combine biological control agents and chemical insecticides often fail because some mosquito predators/parasites are also susceptible to chemical larvicides and their populations often take longer to recover from insecticide-induced mortality than do mosquito larval populations. There are many examples of the enhancement of mosquito larval populations, relative to those in the controls, after treatment with chemical larvicides (e.g. Hoy et al. 1972; Miura et al. 1978).

Integrated control programs which combine larvivorous fishes and bacterial larvicides against *Cx. tarsalis* might, in theory, be successful in duck club ponds because many of these problems are mitigated. Treatment with mosquito-specific, bacterial larvicides will be required during the period when fish populations are small and do not significantly reduce mosquito larval populations. Once mosquitofish populations are large enough to control mosquitoes, repeated applications with the larvicide are no longer necessary. By reducing the number of larvicide applications, this integrated control program also reduces costly larviciding and continued selection for *Bti* resistance in *Cx. tarsalis*.

Will this integrated control strategy succeed in Coachella Valley duck clubs? Our studies demonstrate that bacterial larvicides are very effective against *Cx. tarsalis* populations in duck

Table 2. Efficacy of *G. affinis* against *Cx. tarsalis* (3rd and 4th instar larvae) in Adohr Valley Farms duck club. For each comparison, n_1 and n_2 in the Mann-Whitney U test equal 30.

Date	Days[a]	Comparison (ponds)	U	|Z|	P(Z)[b]
October 16	11	7 vs 8	423.5	0.450	ns
		10 vs 11	423.5	0.408	ns
October 23	18	7 vs 8	438	0.212	ns
		10 vs 11	340	1.701	ns

[a] Days since *G. affinis* was stocked into ponds 7 and 10 at a rate of 1.4 kg/ha.
[b] ns = P(Z) > 0.01.

Table 3. The average number (± 1 SD) of *G. affinis* captured in minnow traps at Adohr Valley Farms duck club. Numbers in parentheses are total number of fish captured/24 hrs.

	average no. captured/minnow trap/24 hrs.		
Date	Pond 3	Pond 7	Pond 10
October 16-17	1.0 ± 2.0 (4)	7.0 ± 5.7 (28)	0 (0)
October 23-24	0.5 ± 0.6 (2)	4.5 ± 8.3 (18)	0 (0)
October 30-31	0.8 ± 1.5 (3)	3.8 ± 3.0 (15)	1.0 ± 1.4 (4)
November 6-7	0.3 ± 0.5 (1)	8.0 ± 10.2 (32)	5.0 ± 6.2 (20)

Table 4. The perimeter vegetation of duck loafing ponds at Adohr Valley Farms duck club.

Family	Species	Abundance
Aizoceae	*Sesuvium varicosum*	common
Chenopodiaceae	*Allenrolfea occidentalis*	common
	Bassia hyssopifolia	rare
Cyperaceae	*Eleocharis macrostachya*	rare
	Scirpus robustus	rare
Poaceae	*Cyndon dactylon*	rare
	Distichlis spicata	common
Tamaicaceae	*Tamarix ramosissma*	common
Typhaceae	*Typha angustifolia*	rare

club ponds. However, at current operational stocking rates, mosquitofish do not significantly reduce mosquito larval populations. Walton and Mulla (1990b) concluded that mosquito abatement in Coachella Valley duck clubs is complicated by the interactions among chronology of pond inundation, seasonal reproduction cycles of mosquitofish, natural sources of mosquitofish mortality, varying degrees of vegetation and water management, and reduced access of MAD personnel to mosquito development sites during duck hunting season.

Typical duck loafing ponds are devoid of interior vegetation and mosquitofish populations are subject to factors that reduce survivorship and reproduction. Thermally-stressful conditions during August and September, large populations of piscivorous birds, and, perhaps, low food abundance in loafing ponds reduce mosquitofish numbers and exacerbate the natural, photoperiodically-induced decline of mosquitofish reproduction during autumn. The size and persistence of piscivorous ardied (herons and egrets) populations in autumn has increased concomitantly with the advent of commercial fish aquaculture in the Coachella Valley (H. Johnson personal communication). While we have shown via mesocosm studies that very high *G. affinis* stocking rates are necessary in the late summer and autumn to significantly reduce *Cx. tarsalis* larval populations, it is unlikely that increasing mosquitofish stocking rates into typical loafing ponds will solve the current *Cx. tarsalis* problem in the Coachella Valley. A large proportion of the enhanced mosquitofish populations would likely succumb to predation by birds in the nearly vegetation-free loafing ponds.

Last, we briefly consider the importance of perimeter vegetation and alternative control strategies for *Cx. tarsalis* populations in duck club ponds. The perimeter vegetation of loafing ponds often provides favorable developmental sites for mosquitoes and can be inhabited by dense larval aggregations (>100 larvae/dip; Walton personal observation). Our 1988 studies verified that removing the perimeter vegetation significantly reduced mosquito larval abundance (Walton and Mulla 1990b). As compared to unmanipulated plots, *Cx. tarsalis* larval populations were reduced significantly in 16 m^2 plots where the perimeter vegetation was removed.

The differences in larval mosquito abundance among the rows in the 1989 study were probably due to the availability of the perimeter vegetation for developmental sites and the extent of cover by grasses. After September 25, water levels in Pond 4 were maintained below the perimeter vegetation and late instar larvae were not collected in dip samples. This was also true for Pond 3 on the same sampling dates. Additionally, the proportion of the pond perimeter covered by grasses, primarily salt grass (*Distichlis spicata*), was smallest for row 1 (Pond 3; 38%, Pond 4; 6%) and increased in rows 2 (Pond 7; 50%, Pond 8; 40%) and 3 (Pond 10; 88%, Pond 11; 50%). Since water levels in row 1 were low, the grasses often were not inundated.

Three other plant species were common along dikes without grasses (Table 4). However, very few mosquito larvae were collected in dip samples within these plants. For the period October 10-23, the total number of larvae collected in dip samples was positively correlated with the abundance of grasses (meters of the pond perimeter covered by grasses: Spearman rank correlation coefficient $=0.823$, $P<0.001$). Rejmánková et al. (1988) also found that *Cx. tarsalis* densities were directly related to graminoid (rice) and sedge (*Cyperus difformis*) abundance in rice fields.

Environmental and insecticidal control are less difficult to achieve than is biological control (Service 1983) and the former may afford a successful cooperation between the duck club ranch managers and the abatement district. Source reduction by the ranch manager should include the removal of perimeter vegetation, particularly salt grass. However, such manipulations are generally contraindicated for waterfowl. Presently, vegetation management differs markedly among the duck clubs. The perimeter vegetation is removed completely in some duck clubs, while in others, perimeter vegetation is encouraged. Not surprisingly, mosquito larval populations are large in the latter duck club group.

Collins and Resh (1989) suggested that manipulating water levels to influence the types of vegetation and reduce the availability of mosquito developmental sites was an alternative ecological control in non-tidal wetlands. For shallow duck club ponds (maximum depth of approximately 30 cm) which are fed by a single well, water management is not very precise and water levels cannot be manipulated to the extent required for ecological control. Water levels in loafing ponds would have to be maintained below the grasses at the interface of the pond bottom and the dikes and, consequently, might be too shallow to attract migrating waterfowl and for hunting purposes (i.e. deployment of decoys). Shallow water depths caused by water diversions or conservation (during the period when hunting ceases in mid-season) also complicate integrated control measures by

increasing mosquitofish mortality (Walton and Mulla 1990b). For ecological control via hydrological regimes, complete drawdown and augmentation should occur within seven to eight days (Collins and Resh 1989). Given the current gravity-fed hydrological systems and soil characteristics, this drawdown/augmentation rate is not possible in the large duck clubs.

Vegetation management via ground disturbance (i.e. disking, harvesting) offers a more promising method of environmental control in duck club ponds. Because of air quality considerations, burning of the perimeter vegetation is typically discouraged. However, the importance of perimeter vegetation in Coachella Valley duck clubs as a food source and attractant for migrating waterfowl needs to be established before implementing vegetation control measures. Unlike wildlife preserves where natural vegetation provides food, cover, and nesting material for waterfowl, southern California duck hunting clubs provide supplemental forage to attract ducks and to prevent damage to commercial agriculture by migrating waterfowl.

The abatement district must continue to monitor larval mosquito abundance and treat problem areas in duck clubs. By reducing the perimeter vegetation in loafing ponds, the number of problem areas should decline and be limited to ponds that develop interior vegetation; usually the first pond flooded. Occasional surveys of a thickly vegetated pond during 1989 revealed that *Cx. tarsalis* larval populations were densest (50-75 3rd-4th instar larvae/dip) in inundated salt grass on the pond perimeter and were much lower in the pond interior (<2 3rd-4th instar larvae/dip). Because larval production is concentrated in the perimeter vegetation, reducing the perimeter vegetation of ponds with interior vegetation will also aid abatement efforts. Although larval mosquito populations are comparatively less abundant in the interior vegetation than in the perimeter vegetation, adult mosquitoes are produced from the entire pond instead of only along the dikes. Therefore, ponds which contain interior vegetation are more difficult to treat with larvicides than are loafing ponds.

An integrated control strategy using bacterial larvicides and enhanced mosquitofish populations may be effective in ponds with interior vegetation. The interior vegetation reduces water temperatures and mosquitofish mortality (Walton and Mulla 1990b). As compared to loafing ponds which lack interior vegetation, ponds with interior vegetation support larger mosquitofish and macroinvertebrate predator populations (Walton and Mulla 1990b). Water management is important because, even in thickly-vegetated ponds, mosquitofish populations can be adversely affected by shallow water depths.

Conversely, this integrated control strategy is ineffective in loafing ponds because mosquitofish populations are never large enough to exert significant levels of mosquito control. For loafing ponds, environmental control by the duck club ranch manager and, if necessary, insecticidal control by the mosquito abatement district are better alternatives to integrated control measures using *Bacillus* and *Gambusia*. Additional studies of the effectiveness of enhanced mosquitofish stocking rates and source reduction via vegetation manipulation are necessary. Our studies in duck club ponds have shown that *Bti* is an efficacious alternative to current insecticides.

Acknowledgements.

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EFFICACY OF *BACILLUS SPHAERICUS* IN CONTRA COSTA COUNTY CREEKS

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ABSTRACT

The efficacy of a liquid formulation of *Bacillus sphaericus* (2362) was evaluated against *Culiseta* and *Culex* mosquitoes in three creeks. Mortality rates ranged from 54 to 90%. Larval numbers were significantly lower ($P < 0.05$) in treated pools than in control pools for up to 30 days.

Introduction.

The bacterial pathogen, *Bacillus sphaericus* Neide, is a highly selective mosquito control agent. Studies have demonstrated that *B. sphaericus* has prolonged larvicidal action in some treated habitats, such as ditches, small ponds, dairy wastewater lagoons and tires (Hertlein et al. 1979, Lacey et al. 1988, Mulla et al. 1988, Kramer 1990). However, no studies have tested the efficacy and persistence of the bacteria in creeks.

In Contra Costa County, slow flowing creeks provide an ideal habitat for several species of *Culiseta* and *Culex* mosquitoes. The purpose of this study was to evaluate the control potential of *B. sphaericus* in a variety of creeks.

Materials and methods.

Three creeks were selected for study in central Contra Costa County. Buttner Creek was steep-sided and narrow with little overhanging vegetation. It flowed slowly through a golf course and formed a string of small (1.5 to 4.5 m^2) primarily sunny pools. Rossmoor Creek formed a series of large (7 to 9 m^2) shady pools and had abundant streamside and overhanging vegetation. Glorietta Creek formed long narrow pools (13.5 to 15 m^2) that were partially shaded by overhanging vegetation. Rossmoor and Glorietta Creeks had greater water flow than Buttner Creek. Water depth in the creeks ranged from 30 to 60 cm.

In each creek, 3 pools were selected for treatment with *B. sphaericus* strain 2362 (ABG-6262, 300 ITU) and 3 pools, located upstream from the treatment pools, were selected as controls. A liquid formulation of *B. sphaericus* was applied to the pools with a handcan at a dosage of 9.35 l/ha (1 gal/acre) in mid-May. A second application at 14.03 l/ha (1.5 gal/acre) was made to Buttner and Rossmoor Creeks 1 week after the initial treatment.

A dipper (400 ml) was used to monitor larval populations before, 2 to 3 days after treatment, and twice per week thereafter. Ten dips per pool were taken at Rossmoor and Glorietta Creeks, and 5 dips per pool were taken at Buttner Creek where the pool size was small. The dips were concentrated in a net and brought to the laboratory for counting and identification. For each creek and for each sampling date, the difference in larval numbers between treated and control pools was analyzed using Student's *t* test ($P < 0.05$).

A maximum/minimum thermometer was placed in one control pool at each creek to monitor water temperature. Water samples were collected from all pools and analyzed for pH, salinity, hardness ($CaCO_3$), phosphate (PO_4) and nitrate-nitrogen (NO_3-N) concentrations.

Results and discussion.

The larval population at Buttner Creek was about 95% *Cs. incidens* (Thomson). The remaining 5% of the larvae were *Cx. pipiens* L., *Cx. stigmatosoma* Dyar and *Cx. tarsalis* Coquillet. The initial *B. sphaericus* application reduced larval numbers by 56% (Fig. 1). This reduction was not considered adequate so the larvae were treated a second time at a higher dosage seven days after the initial treatment. The number of larvae per dip were reduced from about 14 to 3, a reduction of 85%. The larval population remained low for about two weeks before increasing to pretreatment levels. Numbers of larvae were significantly lower in the *B. sphaericus* treated pools than in the controls for approximately three weeks after the initial treatment.

At Rossmoor Creek, the mosquito population was entirely *Cs. incidens*. The initial application resulted in a 54% reduction in larval numbers (Fig. 2). The creek was retreated, resulting in a drop

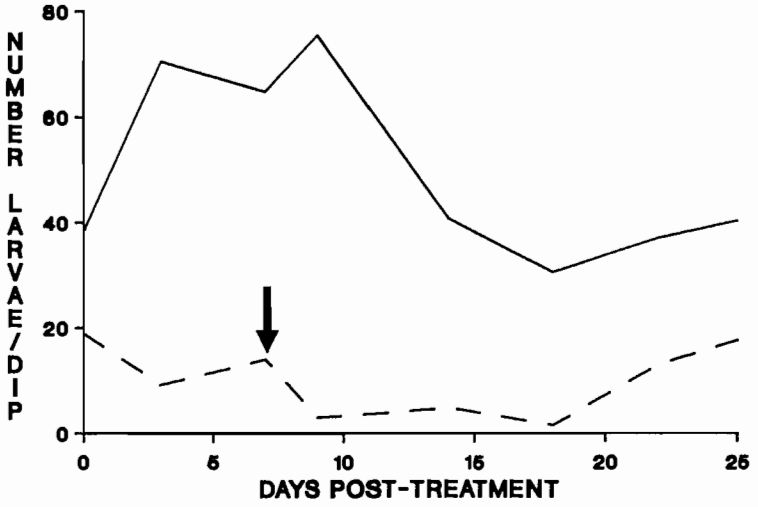

Figure 1. Efficacy of *Bacillus sphaericus* in Buttner Creek (—— control pools; — — — treated pools; arrow indicates second treatment).

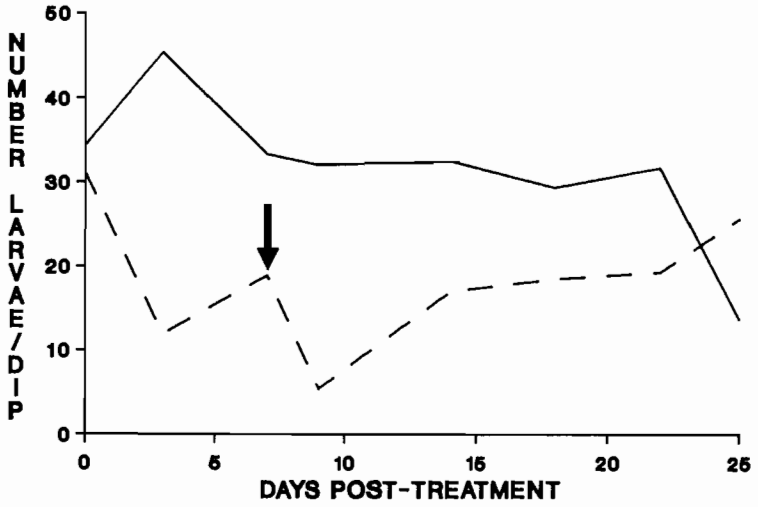

Figure 2. Efficacy of *Bacillus sphaericus* in Rossmoor Creek (—— control pools; — — — treated pools; arrow indicates second treatment).

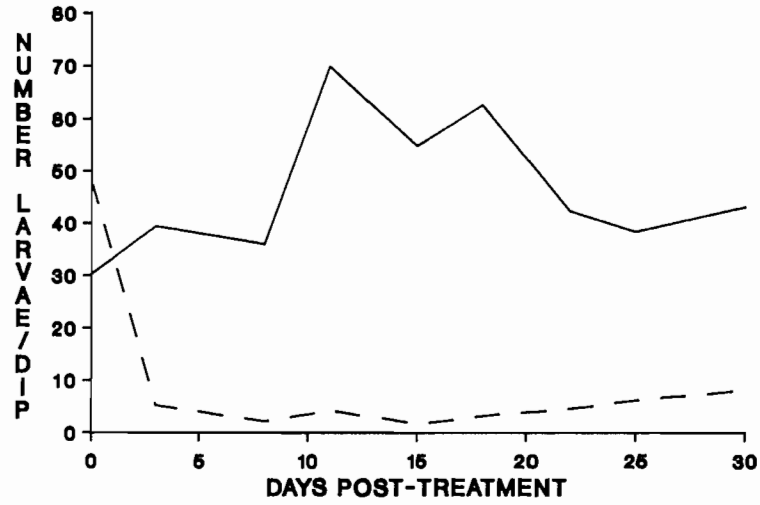

Figure 3. Efficacy of *Bacillus sphaericus* in Glorietaa Creek (—— control pools; — — — treated pools).

Table 1. Water quality parameters of creeks (average of 3 treated pools).

Creek	pH	Salinity (ppt)	Hardness (mg/l)	Phosphate (mg/l)	Nitrate-Nitrogen (mg/l)
Buttner	7.3	0.09	527	0.41	0.37
Rossmore	7.1	0.16	1040	0.47	0.89
Glorietta	7.7	0.22	427	1.18	1.03

from 19 to 6 larvae per dip, a reduction of 57%. Larval numbers increased thereafter, and exceeded the control population about two weeks after the second treatment. Numbers of larvae in the treated pools were significantly lower than in the control pools only on the sampling dates immediately following each treatment (3 and 9 days post initial treatment).

Bacillus sphaericus reduced the *Cs. incidens* population at Glorietta Creek by 90%, from about 48 to 5 larvae per dip (Fig. 3). There were no further treatments and larval numbers remained significantly lower than in the control pools for the 30 days of the study.

Bacillus sphaericus was more effective against mosquito larvae in Glorietta Creek than in either Buttner or Rossmoor Creeks. Water quality parameters did not differ substantially between Glorietta and the other two creeks, except for the phosphate content, which was much higher in Glorietta Creek (Table 1). Water temperatures at the time of treatment and during the course of the study were warmer at Buttner Creek, ranging between 12 and 23° C than at the other two creeks, where temperatures ranged between 11 and 19° C. Water flow at Glorietta Creek was about the same as at Rossmoor Creek. It is unclear why *B. sphaericus* was more effective in Glorietta Creek.

In conclusion, *B. sphaericus* shows the potential to effectively control mosquitoes in creeks for an extended period of time. However, application techniques may need to be further refined and the quality of the formulation tested prior to application in the field.

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TOXICITY OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* (SEROTYPE H-14) AGAINST REPRESENTATIVES OF THREE SUBFAMILIES OF NORTH AMERICAN CHIRONOMIDAE AND OTHER TAXA ASSOCIATED WITH MOSQUITO OR BLACK FLY HABITATS

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ABSTRACT

Fourteen field-collected taxa in three subfamilies of Chironomidae were tested with *Bacillus thuringiensis* var. *israelensis* (*Bti*, Serotype H-14) under laboratory conditions. Predators in the subfamily Tanypodinae (tribes Macropeloplini and Pentaneurini) were about 10-2,000 times less susceptible to *Bti* than were the collectors, filter feeders, and scrapers -represented by the subfamilies Chironominae (tribes Chironomini and Tanytarsini) and Orthocladiinae. Susceptibility between three species of *Tanytarsus* to *Bti* varied by as much as 100 times.

Similarly, two predatory species of Diptera other than the Chironomidae (the snail killer, *Sepedon praemiosa* (Sciomyzidae), and a biting midge, *Palpomyia* sp. (Ceratopogonidae)) were unaffected at 100 μg/ml. However, two non-predatory species, *Dixa californica* (Dixidae) and *Hedriodiscus* sp. (Stratiomyidae) were susceptible, (LC_{50} = 71 and 8 μg/ml, respectively).

Twenty-three non-target taxa in seven other orders showed no effects to *Bti* at levels approximately 200-4,000 times greater than comparable LC_{50} levels for late third to early fourth instar larvae of a laboratory strain of *Culex pipiens* and early fourth instar larvae of *Aedes aegypti* (Bora Bora strain, Pasteur Institute, France).

Introduction.

Numerous studies have shown that *Bacillus thuringiensis* var. *israelensis* (*Bti*) is an effective control agent against many species of aquatic insects. Justifiably, most research attention has focused on its mode of action and pathogenicity against potential target vectors of disease, particularly mosquitoes and blackflies (Margalit and Dean 1985; Qiu and Lei 1986). Representatives of these families have been tested under both laboratory and field conditions (Goldberg and Margalit 1977; de Barjac 1978; Undeen and Nagel 1978; Garcia and DesRochers 1979; Undeen and Berl 1979; Undeen and Colbo 1980; Garcia et al. 1981; Wraight et al. 1987).

However, there has been an equally active concern by a number of authors to test for any potential adverse impact that this "microbial pesticide" might have on other organisms that occur in the same aquatic environment, but which are not known vectors of human disease (Garcia et al. 1980; Gharib and Hilsenhoff 1988). Few studies have investigated its effects against one of the more abundant functional groups (the Chironomidae) and for those that are available, rarely have test individuals been identified at the species level (Garcia et al. 1981; Miura et al. 1980; Ali 1981). This is unfortunate, since the Chironomidae contains not only many nuisance species (Grodhaus 1963, 1975), but an enormously abundant and

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ecologically rich, diverse fauna (Oliver 1971).

Therefore, the focus of the present study was to expand the informational database for representatives of some of the more commonly occurring chironomid subfamilies of North America -primarily Tanypodinae, Chironominae and Orthocladiinae (Oliver 1971). More specifically, this paper presents results of the activity of *Bti* against 14 taxa within these subfamilies and for 27 additional taxa in eight orders.

Materials and Methods.

During 1981, samples of larval chironomids were collected from the field and immediately returned to the laboratory in thermally insulated containers. Subsamples were removed for initial larval identifications and for reared immature adult associations. Water obtained from each collection site was placed in 1-liter glass cylinders, held in a Percival Temperature Cabinet, and allowed to acclimate to a uniform thermal regime of 12-15° C by aerating the water. Late instar larvae were then added. A wettable powder formulation of *Bti*, Bactimos®, rated at 3500 international *Aedes aegypti* (L.) units per milligram was suspended in water and applied by pipette to the water surface of test containers. Most test and control individuals were held for 1-3 days. Both dead and live specimens were counted (Table 1) and then mounted on slides for taxonomic confirmation.

Using this same *Bti* formulation, bioassays were conducted on all other semiaquatic/aquatic taxa. Except as indicated (Tables 2, 3, 4), only later immature growth stages were selected for testing. Late third to early fourth instar larvae of our laboratory strain of *Culex pipiens* Say and early fourth instar larvae of *Aedes aegypti* (Bora Bora strain, Pasteur Institute, France) were assayed to establish standardized baseline reference levels.

All collections of Chironomidae were made from water sources in the San Francisco Bay area at the following locations during 1981:

-*Paralauterborniella elaschista* (Townes) and a *Corynoneura* sp. were collected from a sun-exposed pond in Marin County (122° 32'W, 38° 07'N) in early autumn. Both species were consistently found associated with *Anopheles freeborni* Aitken and *Culex tarsalis* Coquillett larvae in dense surface growths of aquatic vegetation.

-*Macropelopia venusta* (Coquillett) and *Zavrelimyia thryptica* (Sublette) were sampled from clear shaded shallow pools in Dimond Canyon Creek, Alameda County (122° 14'W, 37° 49'N)) during summer. A species of *Rheocricotopus*, and an unidentified species in the same tribe (Orthocladiini) were recovered from shaded riffles at the Dimond Canyon Site during spring.

-Two unidentified species in the genus *Tanytarsus*, designated Species A and B, were collected during spring and autumn, respectively, from shaded pools in the inlet stream to Briones Reservoir, Contra Costa County (122° 09'W, 37° 56'N). A third species, Species C, was obtained from sun-exposed overflow pools of a drinking fountain in Tilden Park, Contra Costa County (122° 16'W, 37° 53'N).

-*Brundiniella eumorpha* (Sublette) was collected from shaded pools and riffles of Strawberry Canyon Creek and *Micropsectra* sp. from Claremont Creek, Alameda County (122° 15'W, 37° 52'N) during summer and fall.

-Larval samples of *Chironomus maturus* Johannsen were recovered from a sun-exposed, rain-filled redwood tank in Alameda County (122° 19'W, 37° 53'N) and a freshwater drainage ditch in Contra Costa County (122° 22'W, 37° 56'N).

Collections of other taxa were made from the following sampling sites during 1981:

-*Hydropsyche oslari* Banks, was sampled from Lauterwasser Creek, ca. 1 km east of the inlet to San Pablo Reservoir and at the intersection of Wildcat Canyon Road, Contra Costa County (122° 12'W, 37° 54'N) during April.

-*Ironodes californicus* (Banks) and *Paraleptophlebia* sp., were sampled from the inflow of Wildcat Canyon Creek above Lake Anza, in Tilden Park, Contra Costa County (122° 15'W, 37° 53'N) during February. *Gerris remigis* Say, *Malenka depressa* (Banks), *Neophylax rickeri* Milne, and *Hydropsyche* sp. were collected from the same site during October.

-*Calineuria californica* (Banks), *Soliperla* sp., *Eubrianax* sp., and *Ephemerella* sp. were collected from a fast flowing mountain stream in Gates Canyon, Solano County (122° 04'W, 38° 23'N) during February.

-*Gumaga nigricula* (McLachlan) and *Psychoglypha* sp. (Banks) were found in pool areas in Lagunitas Creek, Marin County (122° 43'W, 38° 00'N) in late February.

-*Dixa californica* Johannsen was found in a small pool of water fed by a leaky drinking fountain adjacent to Jewel Lake in Tilden Park, Contra Costa County (122° 16'W, 37° 54'N), during early March.

-*Dytiscus* sp., *Berosus* sp. and *Hedriodiscus* sp. were collected from a saltwater marsh adjacent to Oakland Airport, Alameda County (122° 13'W, 37° 43'N) in early March.

-*Ophidonais* species A was sampled from a sun-exposed rain-filled redwood tank in Alameda

County (122° 19'W, 37° 53'N) in early April. *Ophidonais* species B was found in an adjacent stream during the same time period.

-*Endalus disgregus* Burke and *Sepedon praemiosa* Giglio-Tos were collected from a small pond near the intersection of American Canyon Road and Interstate-80, Napa County (122° 12'W, 38° 10'N) during mid-April. *Malenka californica* (Claassen) was sampled from an adjacent stream during the same period.

-*Lepidostoma cantha* Ross was collected from a shallow sun-exposed pool in Franklin Canyon Creek, Contra Costa County (122° 14'W, 38° 01'N) during late May.

-*Palpomyia* sp. was obtained from a deep shaded pool area in Strawberry Canyon Creek, Alameda County (122° 15'W, 37° 52'N) in mid-May.

-*Isoperla mormona* (Banks), *Deronectes* sp., and *Krendowskia* sp. were sampled from Dimond Canyon Creek, Alameda County (122° 14'W, 37° 49'N) during late August.

Results and Discussion.

Of the 14 taxa of Chironomidae tested in this study, all three species of midges in the subfamily Tanypodinae (tribes Macropelopliini and Pentaneurini) were recovered from stream pools (*Brundiniella eumorpha* was collected from both a pool and riffle habitat) and were actively swimming predators under laboratory conditions. In the field, they are believed to consume small crustaceans, oligochaetes and chironomids. These same tanypodinid species showed the greatest tolerances to *Bti* of all taxa tested (Table 1). Mortality was not observed until levels of 100-200 µg/ml were reached. Results are in general agreement with Colbo and Undeen (1980), who observed no effect on some "Tanypodinae and other Chironomidae" at 1 x 10^5 cells/ml in a small stream in New Foundland. Ali (1981) noted a slight increase in mortality at 0.5 µg/ml over a 14-day period when mixed larval populations of two tanypodinid tribes, Coelotanypodini and Macropelopiini, were tested.

In the subfamily Chironominae, tests were conducted on representatives from two tribes, Tanytarsini and Chironomini. Of four species tested in the tribe Tanytarsini, all were detritivores found only in the calmer waters of ponds and stream pools. Widest range of susceptibility measured within any genus challenged in this study was between three *Tanytarsus* species of this same tribe. Specifically, *Tanytarsus* species B was the most susceptible, showing 90% mortality at only 0.01 µg/ml, while species A required 100 times the amount necessary to induce similar mortality. Ali (1981) observed a 19-88% increase in larval mortality of mixed populations of *Tanytarsus* spp. and *Rheotanytarsus* spp. at increased concentrations of 0.5-2.5 µg/ml.

In the tribe Chironomini, *C. maturus* and *P. elaschista* were highly susceptible to levels that did not produce 100% mortality in the majority of other species tested in this study; no end point concentration was established because of the low number of test animals available from the sampling site.

Previous reports have indicated that *Chironomus decorus* Johannsen may be much more tolerant than *C. maturus* to *Bti*, although water quality may have influenced differences in susceptibility (Garcia et al. 1981). Both *C. maturus* and *P. elaschista* are detritivores and tube builders; the former in depositional sediments of ponds (Grodhaus 1968) and the latter on submerged aquatic vegetation such as the pondweed, *Potomogeton* (Magy et al. 1969).

In the subfamily Orthocladiinae, tests on four species revealed a moderate degree of tolerance to *Bti*. However, one unidentified orthoclad was susceptible to a dosage of only 0.1 µg/ml.

Several laboratory-tested chironomid species reported in this study revealed susceptibilities equivalent to those found for commonly occurring *Culex* and *Aedes* mosquito species. It is projected that, under field conditions, chironomid mortality rates would approach dosages equivalent to those applied for mosquito or black fly control.

The effect of water quality did not appear to influence the pathogen's activity during these studies. Using water from each chironomid collection site, bioassay tests conducted on our laboratory strain of 3rd instar *Culex pipiens* larvae revealed no differences from expected values for demineralized tap water at test chamber temperatures of 12-15° C and at room temperatures. However, for species inhabiting sewage oxidation ponds, water depth and benthic sediment adsorption might prove important as factors that could potentially attenuate the pathogenicity of the bacterium. Evidence for physical influences such as temperature and pH on the microbial efficacy of *Bti*, has recently been shown by Lacoursiere and Charpentier (1988).

Reasons for susceptibility differences among the chironomids examined in this study might be surmised. Pre-test consideration was given to the possibility that altered feeding responses may seriously affect test results. Consequently, individual larvae were closely observed during testing. Appropriately normal behavioral patterns

Table 1. Toxicity of *Bacillus thuringiensis* var. *israelensis* to various taxa of Chironomidae.

Taxon	Habitat	Trophic[a] Relationship	Number Tested	Dosage[b] (μg/ml)	Exposure (Days)	Percent Mortality
TANYPODINAE						
MACROPELOPIINI						
Brundiniella	Lotic (pool)	Engulfers	10	200.00	3	100
eumorpha	Stream A	(predators)	10	100.00	3	40
Brundinella	Lotic (riffle)	Engulfers	10	100.00	3	80
eumorpha	Stream B	(predators)	10	10.00	3	0
Macropelopia	Lotic	Engulfers	10	200.00	3	50
venusta	(pool)	(predators)	10	100.00	3	60
PENTANEURINI						
Zavrelimyia	Lotic	Engulfers	20	200.00	3	25
thryptica	(pool)	(predators)	10	10.00	3	0
CHIRONOMINAE						
CHIRONOMINI						
Chironomus	Lotic	Collectors	10	0.10	2	100
maturus	(pool)	gatherers	10	0.01	2	100
Paralauterborniella	Lentic	Collectors	10	100.00	3	100
elaschista	(pond)	gatherers	10	10.00	3	100
TANYTARSINI			10	100.00	2	100
Tanytarsus	Lotic	Collectors/filterers	10	10.00	2	90
species A	(pool)	gatherers	10	1.00	2	10
Tanytarsus	Lotic	Collectors/filterers	20	0.10	3	100
species B	(pool)	gatherers	10	0.01	3	90
Tanytarsus	Lentic	Collectors/filterers	10	0.10	3	100
species C	(pond)	gatherers	10	0.01	3	10
Microspectra	Lotic	Collectors	10	100.00	3	100
sp.	(pool)	gatherers	10	10.00	3	90
			10	1.00	3	0
ORTHOCLADIINAE						
ORTHOCLADIINI	Lotic	Collectors/scrapers	10	0.100	1	100
	(riffle)	gatherers	10	0.010	1	40
			10	0.001	1	0
Cricotopus	Lotic	Collectors/scrapers	10	10.00	4	100
sp.	(riffle)	gatherers	10	5.00	4	90
			10	1.00	4	0
Rheocricotopus	Lotic	Collectors/gatherers	10	10.00	3	100
sp.	(riffle)					
CORYNONEURINI						
Corynoneura	Lentic	Collectors/gatherers	10	10.00	3	100
sp.	(pond)					

[a] After Merritt and Cummins (1978).
[b] Bactimos® (Biochem Co. wettable powder formulation of *Bti*).

Table 2. Toxicity of *Bacillus thuringiensis* var. *israelensis* to non-Chironomid Diptera.

Taxon	Habitat	Trophic[a] Relationship	Number Tested	Exposure (Days)	Slope ± SE	LC_{50} (μg/ml)[b] (95% CL)
NEMATOCERA						
DIXIDAE						
Dixa californica	Lentic (pond)	Collectors gatheres	120	2	2.45 ± 0.47	70.83 (47.03 - 95.5)
BRACHYCERA						
STRATIOMYIDAE						
Hedriodiscus sp.	Lentic (<1% saline)	Scrapers	120	1	1.09 ± 0.20	8.32 (4.00 - 15.17)

[1] After Merritt and Cummins (1978).
[2] Bactimos® (Biochem Co. wettable powder formulation of *Bti*).

Table 3. Toxicity of *Bacillus thuringiensis* var. *israelensis* to non-Chironomid Diptera.

Taxon	Habitat	Trophic[1] Relationship	Number Tested	Dosage[2] (μg/ml)	Exposure (Days)	Percent Mortality
NEMATOCERA						
CERATOPOGONIDAE						
Palpomyia sp.	Lotic (pool)	Engulfers (predators)	10	100.0	3	No effect
BRACHYCERA						
SCIOMYZIDAE						
Sepedon praemiosa	Lentic (pond)	Engulfers (predators)	10	100.0	1	No effect

[a] After Merritt and Cummins (1978).
[b] Bactimos® (Biochem Co. wettable powder formulation of *Bti*).

Table 4. Toxicity of *Bacillus thuringiensis* var. *israelensis* to associated non-Dipteran taxa.

Taxon	Habitat	Trophic[a] Relationship	Number Tested	Dosage[b] (μg/ml)	Exposure (Days)	Percent Mortality
TRICHOPTERA						
SERICOSTIMATIDAE						
Gumaga nigricula	Lotic (pool)	Shredders	30	10.0	3	No effect
LIMNEPHILIDAE						
Neophylax rickeri	Lotic (riffle)	Scrapers	10 Egg Masses	10.0	3	No effect (Hatched)
Neophylax rickeri	Lotic (riffle)	Scrapers	30	10.0	3	No effect
Psychoglypha sp.	Lotic (pool)	Collectors/shredders detritivores	25	10.0	3	No effect
HYDROPSYCHIDAE						
Hydropsyche species A	Lotic (pool)	Collectors/filterers	10	200.0	21	No effect
			10	100.0	21	No effect
			10	10.0	21	No effect
Hydropsyche oslari	Lotic (riffle)	Collectors/filterers	10	100.0	1	No effect
			10	10.0	1	No effect
LEPIDOSTOMATIDAE						
Lepidostoma cantha	Lotic (pool)	Shredders detritivores	10	10.0	14	No effect (Emerged)
PLECOPTERA						
PELTOPERLIDAE						
Soliperla sp.	Lotic (riffle)	Shredders/scrapers detritivores	6	10.0	10	No effect
PERLIDAE						
Calineuria californica	Lotic (riffle)	Engulfers (predators)	6	10.0	10	No effect
NEMOURIDAE						
Malenka depressa	Lotic (riffle)	Shredders detritivores	5	10.0	3	No effect
Malenka californica	Lotic (riffle)	Shredders detritivores	5	10.0	3	No effect
PERLODIDAE						
Isoperla mormona	Lotic (riffle)	Engulfers (predators)	5	10.0	2	No effect

[a] After Merritt and Cummins (1978).
[b] Bactimos® (Biochem Co. wettable powder formulation of *Bti*).

Table 4. Continued.

Taxon	Habitat	Trophic[a] Relationship	Number Tested	Dosage[b] (μg/ml)	Exposure (Days)	Percent Mortality
EPHEMEROPTERA						
HEPTAGENIIDAE						
Ironodes californicus	Lotic (riffle)	Collectors/scrapers gatherers	30	10.0	6	No effect (Molted)
EPHEMERELLIDAE						
Ephemerella sp.	Lotic (riffle)	Collectors/shredders herbivores	5	100.0	2	No effect
LEPTOPHLEBIIDAE						
Paraleptophlebia sp.	Lotic (riffle)	Collectors/shredders detritivores	30	10.0	6	No effect (Molted)
COLEOPTERA						
HYDROPHILIDAE						
Berosus sp.	Lentic (<1% saline)	Collectors/piercers herbivores	15	200.0	3	No effect
			15	100.0	3	No effect
			15	10.0	3	No effect
DYTISCIDAE						
Deronectes sp.	Lotic (pool)	Piercers carnivores	10 Adults	10.0	3	No effect
Dyticus sp.	Lwntic (<1% saline)	Piercers carnivores	10	100.0	2	No effect
PSEPHENIDAE						
Eubrinax sp.	Lotic (riffle)	Scrapers	10	100.0	1	No effect
			10	10.0	1	No effect
CURCULIONIDAE						
Endalus disgregus	Lentic (pond)	Shredders herbivores	15 Adults	100.0	1	No effect
			15 Adults	10.0	1	No effect
HEMIPTERA						
GERRIDAE						
Gerris remigis	Lotic (pool)	Piercers carnivores	10	100.0	5	No effect
OLIGOCHAETA						
NAIDIDAE						
Ophidonais species A	Artificial Containers	Shredders detritivores	30	100.0	1	No effect
Ophidonais species B	Lotic	Shredders detritivores	30	100.0	3	No effect
HYDRACARINA						
Krendowskia sp.		Piercers/shredders carnivores/parasites detritivores	5	200.0	3	No effect

[a] After Merritt and Cummins (1978).
[b] Bactimos® (Biochem Co. wettable powder formulation of *Bti*).

such as filter feeding, predation, or tube building were recorded for each species considered here. Based on these observations, it appears that reduced feeding response would not have accounted for such extreme differences in species specific mortality rates.

Instead, differential tolerance responses to *Bti* within the Chironomidae most likely reflect physiological differences between the various species. Similar trends have been reported for lepidopteran larvae tested against strains of *Bacillus thuringiensis*. Such studies have established that high pH and corresponding proteolytic enzyme activity of the fore and midgut of caterpillars are important factors that determine susceptibility levels of these species (Burgerjon and Martouret 1971). It is speculated that corresponding studies on chironomid larvae may reveal a similar relationship.

Thus far, the primary activity of *Bti* against immature and adult aquatic fauna appears restricted to Diptera in the families Culicidae, Simuliidae and Chironomidae (Garcia et al. 1980; Garcia et al. 1981; Miura et al. 1980; Ali 1981). While taxa in two other non-predatory dipteran families, *Dixa californica* (Nematocera: Dixidae) and *Hedriodiscus* sp. (Brachycera: Stratiomyidae) appeared unaffected at *Bti* levels commonly used against mosquitoes and black flies, mortality was, nevertheless, observed at higher dosage rates (Table 2). However, two predatory dipterans, the snail killer, *Sepedon praemiosa* (Brachycera: Sciomyzidae) and a biting midge, *Palpomyia* sp. (Nematocera: Ceratopogonidae) were unaffected at levels as high as 100 μg/ml (Table 3).

For the other numerous non-dipteran and non-target organisms tested at *Bti* levels 200-4,000 times greater than a comparable LC_{50} level of 0.05 μg/ml for *Culex pipiens* and *Aedes aegypti*, no mortality was observed (Table 4). These data provide further support to restrict specificity of *Bti* to the order, Diptera, and additionally contribute towards delineation of *Bti* as a relatively narrow-spectrum "microbial insecticide."

Chironomid midges are unique from mosquitoes and black flies, since the Chironomidae contains not only susceptible target organisms, but also beneficial non-target fauna. While our data are not extensive, the importance of apparent suspected differences in susceptibility between Chironomidae and other dipterans should warrant continued and renewed efforts by other investigators to provide additional information on this taxonomically, medically, and ecologically important insect order and for non-target fauna concurrently exposed to this pathogen.

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FIELD EVALUATION OF BENDIOCARB, CHLORPYRIFOS, DIAZINON, AND PERMETHRIN FOR CONTROL OF PLAGUE VECTORS IN THE NORTHERN SIERRA NEVADA OF CALIFORNIA

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Introduction.

California's Sierra Nevada Mountains are heavily used for recreational purposes. At many recreational sites, populations of ground squirrels and chipmunks have become extremely dense. High densities of susceptible rodent hosts and vector fleas are pre-requisites to epizootics of bubonic plague in endemic areas. Plague is endemic in the Sierra Nevada Mountains, first documented in 1937, when an epizootic and associated human case occurred on the Nevada side of Lake Tahoe (Meyer 1942). A resurgence of plague among wild rodents began in the Sierra Nevada Range in the mid-1970's, and has continued to the present time.

Forty-six percent (14/30) of California's human plague cases since 1970 have been contracted during recreational pursuits. Fifty percent of these cases have been associated with recreational sites in the Sierra Nevada Mountains (California Department of Health Services' records). All cases have been either directly, or indirectly, associated with epizootics among wild rodents and fleas. Transmission to humans has been primarily by bite of infected rodent fleas. One case, however, a fatality, involved direct primary pneumonic transmission from a pet cat to its owner. The cat had fed upon infected chipmunks in the area of the home at South Lake Tahoe (Werner et al. 1984).

In the Sierra Nevada Range of California plague has been isolated from the following sciurid rodent species: ground squirrels (*Spermophilus beecheyi, S. lateralis, S. beldingi*), chipmunks (*Tamias amoenus, T. senex, T. quadrimaculatus, T. minimus, T. speciosus*), pine squirrel (*Tamiasciurus douglasii*), and flying squirrel (*Glaucomys sabrinus*) (California Department of Health Services' records).

Oropsylla montana a ground squirrel flea, is a proven plague vector. *Oropsylla idahoensis*, found on ground squirrels, has been described as a poor individual vector but may be involved in mass transmission of plague among rodents. Chipmunk fleas, *Monopsyllus eumolpi* and *M. ciliatus,* are proven plague vectors. *M. ciliatus*, however, may be less of a vector than the former species (Eskey and Haas 1940). Chipmunk fleas readily bite humans and may be of considerable importance in human plague transmission in the Sierra Nevada (Barnes and Kartman 1960). Plague positive chipmunk fleas have been recorded in recent years in human plague case investigations in the Sierra Nevada by the California Department of Health Services (CDHS).

Control of vector fleas with insecticide is warranted in known or latent epizootic periods in the Sierra Nevada to lessen plague transmission potential to humans. Control efficacy data for the various insecticides available for wild rodent flea control is needed for this important plague endemic region. Consequently, we made field comparisons of beniocarb, chlorpyrifos, diazinon, and permethrin dusts for control of fleas on plague hosts in the northern Sierra Nevada Mountains. The present paper summarizes the results of these trials using present application methods, and compares the effectiveness of each material. This study was also designed to emphasize the need for an integrated control approach to plague management at recreational sites in plague endemic areas, integrating flea control with rodent control methods.

Materials and Methods.

The trials presented were a portion of a three year study towards development of a plague management strategy for two state parks in the Sierra Nevada Mountains by the California Department of Health Services, Enviromental

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Management Branch (CDHS-EMB), in cooperation with the California Department of Parks and Recreation (CDPR).

Trap station grids (1,000 foot square with station points at 100 foot intervals) were used for rodent trapping at Upper Jamison Creek campground, Plumas-Eureka State Park, Graeagle, California, and at Creek campground, Donner Memorial State Park, Truckee, California. Alternative sites within the two state parks, and a campground in a similar habitat at Berger Creek, Sierra County, California, were used for comparison. One Tomahawk and two Sherman live traps baited with peanut butter and crimped oats were used at each grid trapping station. Traps were set overnight from 1600 to 1000 hours, and were opened for three consectutive nights in the spring and fall months (May-June and September-October). In summer months (July-August), traps were set overnight on two alternate nights. Grid trapping continued on a monthly basis, May through October; the activity season for hibernating rodent species present in the study areas. Captured rodents were anesthesized using ethyl ether, marked with ear tags (fish fingerling tags), combed for fleas, sexed, weighed, measured, and released again at the point of capture. Fleas were placed in a 2% saline solution for transport to the laboratory for identification.

Bendiocarb (Ficam®) 1% is the only insecticide currently registered for wild rodent flea control in California. The use of diazinon 3% dust was under a special local needs use registration issued to the CDHS by the California Department of Food and Agriculture (CDFA). Research authorizations were issued by the CDFA to the CDHS for the use of chlorpyrifos 1% and permethrin 0.5% dusts for these trials.

Pre- and post-treatment flea indices were used to evaluate control success in the field trials. Sample size followed the criteria established by Schwan (1984) using 20 as the minimum sample size for each host species to establish the flea index.

Trials using diazinon, bendiocarb, chlorpyrifos, and permethrin dust formulations were conducted at Plumas-Eureka State Park and aimed at control of *Monopsyllus* spp. fleas occurring on the shadow chipmunk, *Tamias senex* (*Eutamias townsendii senex*). An additional trial using permethrin was conducted at Donner Memorial State Park aimed at control of *Oropsylla montana* and *Oropysslla idahoensis* fleas on the golden-mantled ground squirrel, *Spermophilus lateralis*.

In all trials, insecticide was applied in 4 inch diameter by 18 inch long PVC pipe bait stations. One-third pipe caps were used at each end of the bait tubes to contain bait and insecticide (Beard et al. 1988). Non-toxic bait blocks of peanut butter, oats, and paraffin were suspended above the insecticide dust as bait. Bait and insecticide were checked on an every other day basis and replenished as necessary. Insecticide was applied at the rate of 2-4 ounces per bait station in all trials. Bait stations were placed at established grid points at 100 foot intervals for grid trials and at 50 foot intervals in lines for non-grid trials.

Evaluation of effectiveness was based on the criteria of a mean number of one or less flea per host, and on the percentage of rodents infested before and after treatment. Since a plague epizootic was in progress at the time of the bendiocarb and diazinon trials, no control plot could be established for comparison, and these materials were compared directly under usage at Plumas-Eureka State Park.

Evaluation of effectiveness of chlorpyrifos and permethrin was based on the above criteria of one or less flea per host, and on comparison with an untreated control site sampled concurrently at Berger Creek. Evaluation of permethrin for control of fleas on golden-mantled squirrels was based on the above criteria of none or less flea per host, and on comparison with an untreated site within Donner Memorial State Park, sampled concurrently.

Studies on the need for integrated plague management were conducted in campgrounds at Donner Memorial State Park. The predominant species involved in these studies were the golden-mantled ground squirrel (*Spermophilus lateralis*) and the fleas, *Oropsylla montana* and *Oropsylla idahoensis*. Ground squirrels were live-trapped, marked and released again on the study grid at Creek campground on a monthly basis, May through October, for three years (1986-1988). Flea indices were established for each monthly sampling period. Fifty ground squirrels were removed from the grid in June, 1986 preceeded by flea control using chlorpyrifos 1% dust in bait stations operated for 18 days. A total of 70 ground squirrels were removed from the grid in October, 1987 with no flea control integrated into the rodent removal. The results of the rodent removal with no flea control as shown in the 1988 monthly flea indices were then compared to the 1986-1987 integrated removal and flea control.

Results.

Efficacy of beniocarb and diazinon: Bendiocarb (Ficam®) 1% and diazinon 3% dusts were compared directly under usage for control of a

plague epizootic among shadow chipmunks at Plumas-Eureka State Park in May, 1986. The bait stations used for each trial were operated for 23 days. Areas of flea control were designated as "non-grid" for the bendiocarb trial, and "grid" for the diazinon trial. Both locations were in the campground at the state park, and were separated by Jamison Creek. Pre-treatment level was 3.62 fleas per host for shadow chipmunks.

Figure 1 shows results of control of fleas on shadow chipmunks using bendiocarb 1% dust in bait stations for 23 days at Plumas-Eureka State Park. Post-treatment evaluations were made at 10, 26, and 81 days after initial deployment. Control criteria of one or less flea per host was not achieved for fleas on shadow chipmunks at 10, 26, or 81 days with bendiocarb.

Figure 2 gives results of control of fleas on shadow chipmunks using diazinon 3% dust in bait stations for 23 days at Plumas-Eureka State Park. Post-treatment evaluations were made throughout the diazinon site at 10, 26, 53, 81, 108, 134, and 164 days after initial deployment. Fleas were reduced from the pre-treatment level of 3.62 per host to a level of 0.04 per host within 10 days with diazinon. The average number of fleas per chipmunk stayed at less than one per host for at least 53 days.

Efficacy of chlorpyrifos and permethrin: Chlorpyrifos (Dursban®) 1% and permethrin 0.5% dust formulations were compared for control of *Monopsylla* spp. fleas occurring on shadow chipmunks at Plumas-Eureka State Park one year after the bendiocarb-diazinon trials. Insecticides were applied in bait stations operated for 30 days. Post-treatment evaluations were made 33, 56 and 84 days after initial deployment for each trial. Treatment evaluations were compared with monthly flea indices taken at the control site at Berger Creek campground, sampled concurrently. Figure 3 gives the monthly flea indices for shadow chipmunks, May through October, at Berger Creek campground.

Figure 4 shows results of flea control using chlorpyrifos 1% dust in bait stations at Plumas-Eureka State Park. Fleas on shadow chipmunks were reduced from a pre-treatment level of 2.34 to a level of 0.4 per host for at least 34 days with chlorpyrifos 1% dust.

Figure 5 gives results of flea control using permethrin 0.5% dust in bait stations at Plumas-Eureka State Park. Pre-treatment flea index for this trial was 1.91 fleas per host. Post-treatment evaluations at 33, 56, and 81 days after initial deployment showed no significant reduction in fleas using permethrin 0.5% dust formulation on shadow chipmunks. Close observations were made of bait station usage at each post-treatment check. Shadow chipmunks showed an apparent, unexplained, aversion to the permethrin formulation and did not readily enter bait stations. Eighty percent of bait

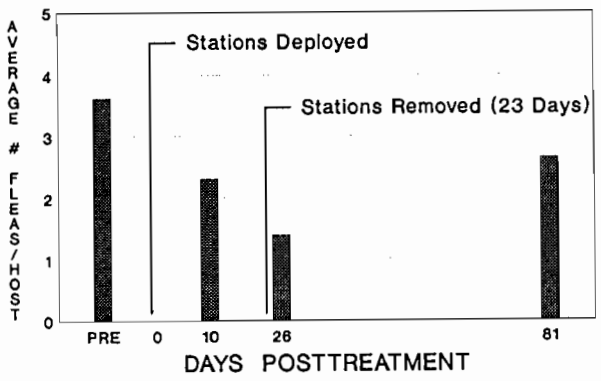

Figure 1. Flea control using bendiocarb 1% (Ficam® D) in bait stations at Plumas-Eureka State Park (non-grid), May, 1986, for control of fleas on the Shadow Chipmunk, *Tamias senex*.

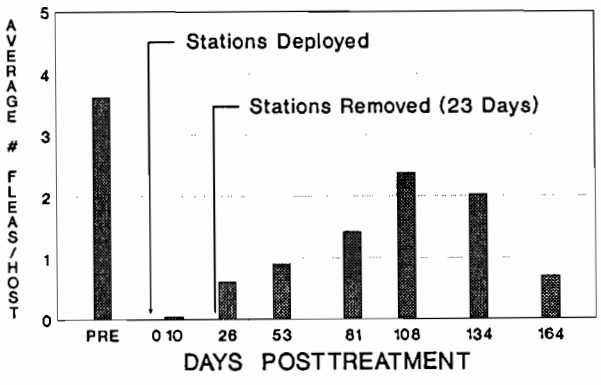

Figure 2. Flea control using diazinon 3% in bait stations at Plumas-Eureka State Park (grid), May, 1986, for control of fleas on the Shadow Chipmunk, *Tamias senex*.

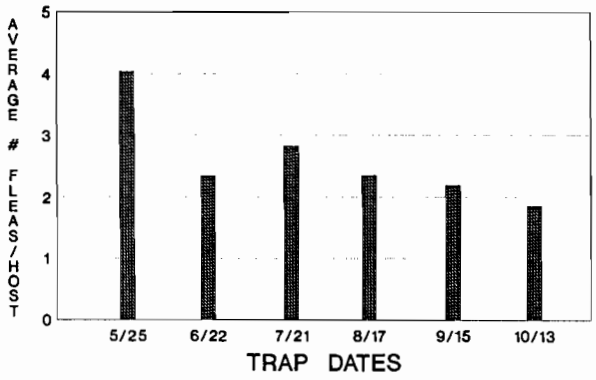

Figure 3. Monthly flea indices for the Shadow Chipmunk, *Tamias senex*, at Berger Creek Campground, Tahoe National Forest, California, based on live-trapping and fur-combing.

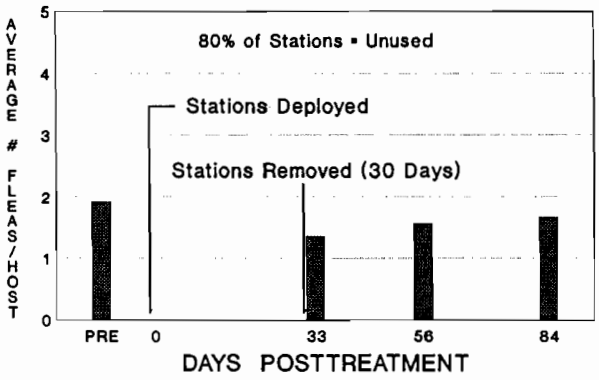

Figure 5. Flea control using permethrin 0.5% in bait stations at Plumas-Eureka State Park (grid), July, 1987, for control of fleas on the Shadow Chipmunk, *Tamias senex*.

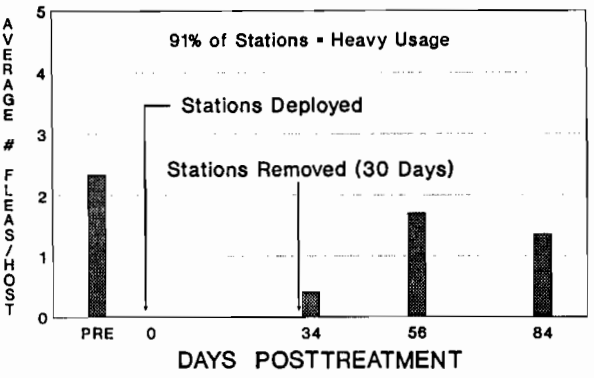

Figure 4. Flea control using chlorpyrifos 1% (Dursban®) in bait stations at Plumas-Eureka State Park (non-grid), July, 1987, for control of fleas on the Shadow Chipmunk, *Tamias senex*.

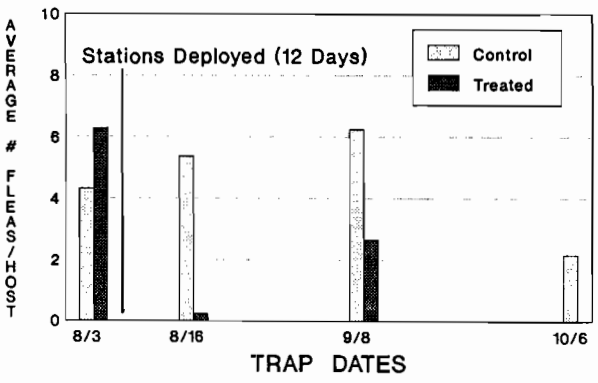

Figure 6. Flea control using permethrin 0.5% in bait stations at Donner Memorial State Park, August, 1988, for control of fleas on the Golden-mantled Ground Squirrel, *Spermophilus lateralis*.

stations in the permethrin trial were unused by this chipmunk species. In contrast, 91% of bait stations containing chlorpyrifos 1% dust in the other portion of the same campground showed heavy usage by this chipmunk species.

Figure 6 shows results of flea control using permethrin 0.5% dust in bait stations for control of *Oropsylla montana* and *Oropsylla idahoensis* fleas occurring on golden-mantled ground squirrels at Donner Memorial State Park. *Spermophilus lateralis*, the golden-mantled ground squirrel, showed no aversion to the permethrin formulation. Treatment area in this trial was compared directly to a control area within the park sampled concurrently, as shown in Figure 6. Daily observations were made of bait station usage by rodent species. Both golden-mantled ground squirrels and yellow pine chipmunks (*Tamias amoenus*) readily entered bait stations containing the 0.5% permethrin formulation. The pre-treatment flea index was 6.82 per host for the golden-mantled ground squirrel. This was reduced to a level of 0.2 in 13 days (8/16 sample date) using permethrin 0.5% dust in bait stations. At 34 days (9/8 sample date) the flea index for golden-mantled ground squirrels was 2.6 per host; higher than the control criteria of one or less flea per host, but significantly lower than the concurrent flea index at the control area for golden-mantled ground squirrels of 6.25 fleas per host.

Integrated plague management studies: Figure 7 shows the monthly average number of fleas, May through October, 1986, for golden-mantled ground squirrels at Donner Memorial State Park for the integrated plague management studies. A trapping grid at Creek campground within the park was used for these studies. Fifty ground squirrels were removed from the grid in June as shown in Figure 7, preceeded by flea control using chlorpyrifos 1% dust in bait stations for 18 days. The monthly average number of fleas per host for golden-mantled ground squirrels remained at less than 2.5 for the remainder of the trapping season following the integrated rodent removal and flea control.

Figure 8 shows the monthly average number of fleas, May through October, 1987 for golden-mantled ground squirrels on the trapping grid at Donner Memorial State Park. Flea indices are also shown for the golden-mantled ground squirrel at additional sites within the state park at East Ridge and Ridge campgrounds. Monthly flea indices remained below 4 per host at all sites for the 1987 trapping season. Seventy ground squirrels were removed from the grid and the two additional sites following the October sampling. No flea control was integrated with the rodent removal.

Figure 9 gives the monthly average number of fleas, May through October, 1988 for golden-mantled ground squirrels on the trapping grid at Donner Memorial State Park. Flea indices at East

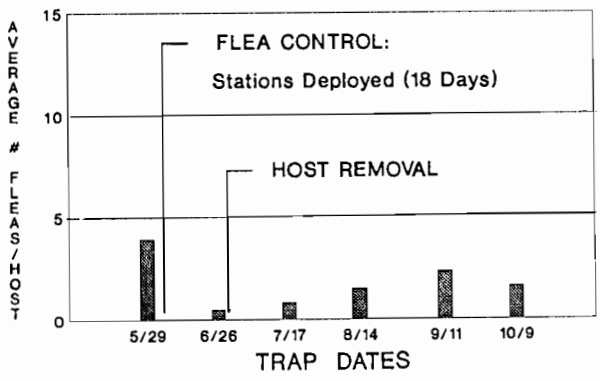

Figure 7. Monthly flea indices for the Golden-mantled Ground Squirrel, *Spermophilus lateralis*, at Donner Memorial State Park (grid), 1986, showing integrated flea control (chlorpyrifos 1%) and host removal.

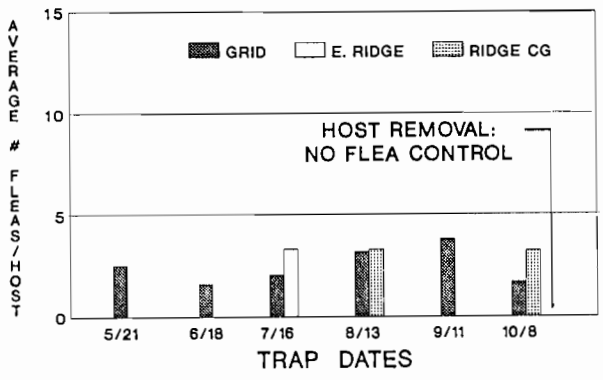

Figure 8. Monthly flea indices for the Golden-mantled Ground Squirrel, *Spermophilus lateralis*, at Donner Memorial State Park, 1987, showing host removal without flea control.

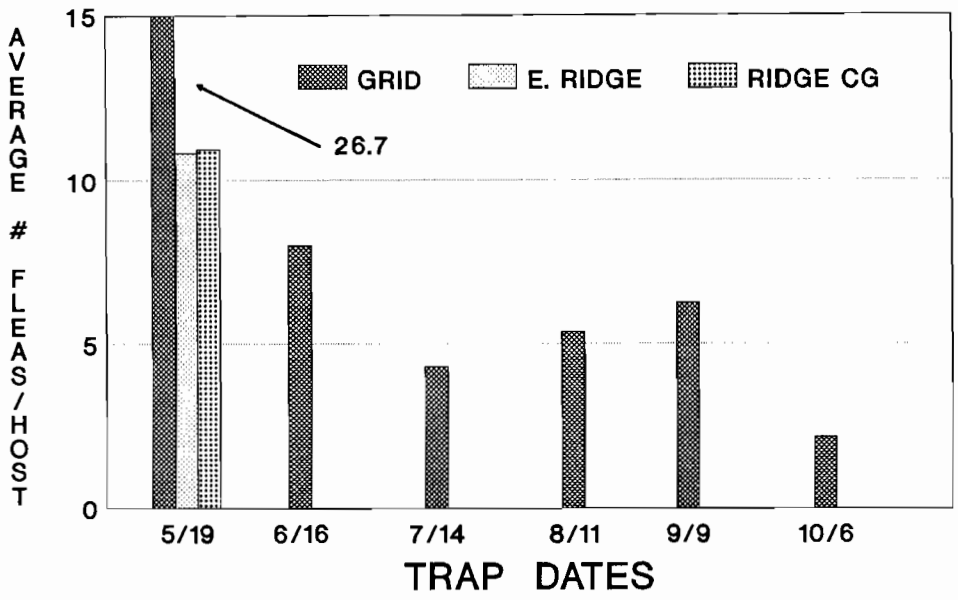

Figure 9. Monthly flea indices for the Golden-mantled Ground Squirrel, *Spermophilus lateralis*, at Donner Memorial State Park, 1988, showing overwintering survival and concentration of fleas in the spring on remaining and new migrant hosts, following host removal without flea control in the fall of the previous year.

Ridge and Ridge campgrounds for golden-mantled ground squirrels in May are also shown in Figure 9. Flea indices in May were 26.7, 10.83, and 10.95 per host, respectively at the grid, East Ridge and Ridge campgrounds. Rodent removal in the previous fall with no flea control resulted in overwintering survival and concentration of fleas on surviving rodent hosts, and on emigrating rodent hosts which entered the study areas the following spring. Flea indices remained higher in 1988 on the study grid following rodent removal with no flea control, than in either of the previous years when flea control was integrated into the management program.

Discussion.

Control measures in epizootic plague are necessarily under emergency conditions aimed at lessening the transmission potential of infection to humans by reducing flea vectors. Control of vector fleas interrupts the natural transmission cycle of the disease. Ideal control materials should provide a rapid reduction of fleas and a significant residual effect on the flea population.

Barnes (1982) listed criteria which precipitate control:

1. Presence of susceptible rodents and vector fleas in areas of human activity and exposure potential.
2. Observation of plague epizootics in such areas.
3. Detection of residual activity where human cases have originated.

Based on Barnes' criteria, plague control in the Sierra Nevada Mountains in our program has been aimed primarily at areas of greatest human transmission risk where wild rodents, fleas and humans closely intermingle.

Application techniques for control of wild rodent fleas have been described by Barnes et al. (1974), Barnes and Kartman (1960) and Nelson and Smith (1974). Methods include hand dusting of rodent burrows and the use of bait stations. Where mixed rodent species are involved in plague epizootics in the Sierra Nevada, we have found a combination of both systems may be necessary for successful control. Ground squirrel burrows may be easily located and reached by hand dusters. Chipmunk burrow entrances, on the other hand, are not easily located. In our studies at Plumas-Eureka State Park, shadow chipmunks (*T. senex*) were observed by radio-telemetry to use ground burrows for nesting, nests in stumps and hollow logs, nests in hollow snags, and even nests high in large cedar trees. Due to this variation in nesting habitats, bait stations are more effective for control of chipmunk fleas than burrow dusting.

In our trial at Plumas-Eureka State Park, bendiocarb 1% dust applied in bait stations did not prove effective in reducing fleas on shadow chipmunks to one or less per host. Bendiocarb has, however, proven effective elsewhere in California (CDHS records).

Permethrin 0.5% dust gave mixed results in flea control trials at Plumas-Eureka and Donner Memorial State Parks. Shadow chipmunks at Plumas-Eureka State Park demonstrated an apparent aversion to the permethrin formulation and would not readily enter bait stations. Golden-mantled ground squirrels and yellow pine chipmunks, on the other hand, showed no such aversion at Donner Memorial State Park. Fleas on these species were siginificantly reduced at Donner Memorial State Park using permethrin 0.5% dust.

Diazinon 3% and chlorpyrifos 1% dusts provided effective control of wild rodent fleas in the Sierra Nevada Mountains in our trials. Chlorpyrifos 1% gave a rapid knockdown of fleas on golden-mantled ground squirrels at Donner Memorial State Park and a reduction to a level of less than one per host for up to 47 days. Fleas occurring on shadow chipmunks at Plumas-Eureka State Park were rapidly reduced and maintained at a level of less than one per host for up to 34 days with chlorpyrifos 1% in bait stations.

Diazinon 3% dust was the material best meeting our control criteria. Diazinon provided a rapid knockdown of fleas on shadow chipmunks and a reduction to a level of less than one flea per host for at least 54 days at Plumas-Eureka State Park. Diazinon 3% dust has shown similar effectiveness for control of fleas on ground squirrels and chipmunks elsewhere in California in recent control of plague epizootics (CDHS records).

Arthropod vector control using safe, effective, insecticides remains the best solution for short-term control of plague epizootics in areas of human transmission risk. Long-term solutions in the control of bubonic plague in endemic areas involve an intergrated approach utilizing education, rodent exclusion and possible habitat modifications, and rodent and flea control.

Acknowledgements.

Acknowledgement is given to K. Townzen, R. Doty, K. Hansgen, J. Clover, L. Hui, F. Ennik, J. Bradley, P. Gillies, B. Wilson, E. Jacobo, J. Tucker, and M. Ramirez of the Environmental Management Branch, California Department of Health Services for assistance in the field studies. M. Thompson of the Branch is acknowledged for assistance with the computer graphics. The late B.C. Nelson and R.E. Yescott of the Branch are recognized for their contributions to plague ecology and management studies in the Sierra Nevadas. The staff of the California Department of Parks and Recreation at Plumas-Eureka State Park and Donner Memorial State Park are acknowledged for their excellent cooperaiton during the field studies. These studies were made possible under a contract from the California Department of Parks and Recreation to the Department of Health Services.

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C.M.V.C.A. YEAR-END COMMITTEE REPORTS: 1989

Charles Beesley

CMVCA Vice President
Contra Costa Mosquito Abatement District
155 Mason Circle
Concord, California 94520

The following reports reflect the activities of the California Mosquito and Vector Control Association's standing and ad hoc committees for the year 1989 as submitted by the chairperson(s) of each committee:

LEGISLATIVE COMMITTEE

Jack V. Fiori (Chair)
William E. Hazeltine, Allen R. Hubbard, Suzanne Ingram, L. Lino Luna, John R. Panzak, Fred C. Roberts, Michael J. Wargo, and Douglas C. White

The Legislative Committee met on three occasions during the 1989 year. The CMVCA's Legislative Advocate, Mr. Ralph Heim, and the CMVCA's Legal Counsel, Dale Bacigalupi, attended part of the sessions and each contributed a great deal of information.

Very few legislative bills were presented during 1989 that would have a significant impact on mosquito abatement districts. Don Womeldorf of the State Department of Health Services reported on the Department's 1989-90 budget allocation. John Combs discussed the role of the Legislative Committee.

The following items were discussed during the 1989 year:

1. S.B. 344 (McCorquodale) State Wetlands.
2. F.I.F.R.A. Bill 1989.
3. S.B. 2126 (Rogers) Pest Control Advisors.
4. Section 2200(d) Health and Safety Code definition of "property".
5. Withdrawal of District funds from County Auditor, Northwest Mosquito Abatement District (L. Lino Luna).
6. Redevelopment (Mike Wargo).
7. Section 4011 Fish and Game Code (Bill Hazeltine).

CONTINUING EDUCATION COMMITTEE

B. Fred Beams (Chair)
Glenn E. Bissell, James R. Caton, Charles P. Hansen, Susan Critchfield-Maggy, and John Stroh

The Continuing Education Committee held four regularly scheduled meetings in 1989. During the year the committee accomplished the following:

1. Reviewed 36 programs submitted for approval of continuing education credit.
2. Developed, in cooperation with the Environmental Management Branch of the State Department of Health Services, regulations which will be adopted to carry out AB-4114 (which became law July 1, 1989).
3. Developed and distributed the California Mosquito and Vector Control Association publication "Certified Vector Control Technician Certification and Continuing Education Guide for Managers and Training Officers".
4. Adopted standards for instruction time conversion to CEU.
5. Began development of a statewide computer program to be used for recording continuing education credit for member agencies' Certified Vector Control Technicians.
6. Continued assistance in the development of a series of certification videotapes and texts which are being produced by Chico State University under the guidance of the California Mosquito and Vector Control Association and the Environmental Management Branch of the State Department of Health Services.

DISEASE CONTROL (AD HOC) COMMITTEE

William C. Hazeleur (Chair)
James R. Caton, Major S. Dhillon, Allen R. Hubbard, William C. Reeves, and Robert K. Washino

The assignment of this committee was: "To foster inter-agency cooperation in the development of procedures to protect the public health from vector-borne disease for 1989, specifically to develop a model malaria control plan."

Although considerable amount of progress was made, this assignment was not able to be completed within one year. The effort should continue in the coming year, and I will be happy to assist in the future with this endeavor.

BIOLOGICAL CONTROL COMMITTEE

Craig W. Downs (Chair)
Arthur E. Colwell, Robert L. Coykendall, David G. Farley, Vicki L. Kramer, Marc R. Pittman, and Werner P. Schon

The Biological Control Committee met three times in 1989. In addition to the business meetings, we toured the fish production facilities of the Sacramento County-Yolo County Mosquito Abatement District and the Contra Costa Mosquito Abatement District as well as The Fishery, a commercial fish farm in Galt.

The main focus at the meetings was the revision of "Fishes in California Mosquito Control". We assigned sections of the publication to members for rewrites to be completed by the year's end. We hope to have the new edition published by mid-1990.

Vicki Kramer was appointed the editor of BioBriefs. Items to be included in BioBriefs should be directed to her at Contra Costa Mosquito Abatement District.

Lagenidium giganteum was added to the list of BioNotes published. This edition was co-written by Ken Boyce and Susan Maggy of the Sacramento County-Yolo County Mosquito Abatement District.

We also compiled a listing of BioBriefs and BioNotes to be published in an upcoming issue of BioBriefs. The central office will be given a complete complement of both publications.

RESEARCH COMMITTEE

Gilbert L. Challet (Chair)
Charles Beesley, James R. Caton, Arthur R. Colwell, William C. Hazeleur, and Douglas C. White

The Research Committee met several times during the year for committee work and reviewing mosquito research proposals. On March 7, 1989, the committee met at Contra Costa Mosquito Abatement District to review 36 research proposals. The committee met again in Monterey on August 3, and decided to hold its research proposal review at the same time as the MRTC formal presentations. The following is a summary of the University-wide Mosquito Research Program grants for the year provided by Dr. Bruce Eldridge, committee consultant:

Proposals:	
Proposals reviewed	36
Proposals funded	27
Total funds granted	$442,690
Breakdown of funded grants:	
Biological Control	38%
Chemical Control	19%
Epidemiology & Surveillance	36%
Ecology	7%
	100%

PHYSICAL CONTROL COMMITTEE

John R. Stroh (Chair)
Peter B. Ghormley, Ronald L. McBride, Melvin L. Oldham, Kevin Pinion,
James R. Stannard, and Jim Wanderscheid

The Physical Control Committee met three times in 1989. The committee's goal was to develop a plan for implementation of a document entitled "Vector Prevention in Proposed Developments: Guidelines, Standards, and Checklist". Although this goal was not reached, the committee did make available to all districts and agencies copies of the document and provided consultation regarding its intended use.

Other items brought before the committee for review included:

1. Urban lawn drains; their design and ability to act as a mosquito breeding source.
2. Vegetation management as a means of source reduction in non-crop areas.
3. The future of the committee.

COMPUTER APPLICATIONS (AD HOC) COMMITTEE

Bruce F. Eldridge (Chair)
David A. Brown, Craig W. Downs, Jack E. Hazelrigg, Barbara A. Kozusko, Marilyn M. Milby
Allan R. Pfuntner, and Fred C. Roberts

We have furnished a draft of our final report to the Ways and Means Committee, and asked them to support it in concept (as follows):

1. To establish a computerized information system for mosquito and vector control operations.
2. To locate the system at the CMVCA headquarters.
3. To hire a computer specialist to work under the supervision of a full-time Executive Director. The specialist would maintain the software, keep the data bases current, and assist users in accessing the system.
4. Information updating to be done by specialists in CMVCA, UC, DHS, and other agencies.

At our meeting held November 30, 1989, we fine-tuned the final report. I will submit it to the board in January. We contemplate no need for funding any of the system until 1991.

In the meantime, we would like to use 1990 to familiarize CMVCA members with the proposed system and its capabilities. To do this, we recommend holding an open forum just before the quarterly CMVCA Board of Directors meeting in April. We will invite, in advance, members to pose questions about the system to the committee. We realize that a number of potential users have questions about compatibility, computer requirements, etc. We will answer any such questions at any time, of course, but we believe a forum in April would be advisable.

Finally, the committee believes that we have designed a system with excellent potential to become a "class act" and which will be unique in the area of mosquito abatement. It should provide the "expanded service" mosquito abatement district managers and their boards will want for any dues increase, and will also have users in other states (at a fee).

ENVIRONMENTAL AND LIAISON COMMITTEE

Fred C. Roberts (Chair)
John R. Anderson, Gilbert L. Challet, Charles H. Dill, Eugene E. Kaufman,
Harvey I. Scudder, and Douglas C. White

The major efforts of the Environmental and Liaison Committee were directed toward insuring that our proposed Public Health Exemption be appropriately incorporated into the Environmental Protection Agency's (EPA) Endangered Species Program.

The EPA issued a FEDERAL REGISTER NOTICE (FRN) on Monday, July 3, 1989, describing their revised program. The committee reviewed the program, developed a set of recommendations and formulated a letter to be sent by our President, Claude Watson. The letter was approved by the Board of Directors at their meeting on August 2, 1989. It was sent by Claude Watson on August 17, 1989. The letter was referenced by many California agencies responding to the FRN and was referred to by the American Mosquito Control Association in their formal response to the EPA.

The final "negotiations" concerning the Public Health Exemption (PHE) are now occurring between public health interests and the EPA. Dr. John Kliewer of the EPA has sent flowcharts of the proposed PHE to our committee for review. The committee is reviewing it to insure that it is consistent with the recommendations made by President Watson.

The EPA indicated that 39 responses were received from public health agencies in response to the FRN on the Endangered Species Program. Those agencies that expressed their points of view should be commended.

WAYS AND MEANS COMMITTEE

Charles P. Hansen (Chair)
J. Warren Cook, Harmon L. Clement, Charles H. Dill, Allen R. Hubbard,
Michael J. Wargo, and Douglas C. White.

The charge given to the committee was to "Evaluate the CMVCA administrative structure and make recommendations leading to the year 1991. Examine dues structure to assure adequacy." To accomplish these goals the committee chose to hold a Management Workshop at the Granlibakken Conference Center on the western shores of Lake Tahoe on October 23-25, 1989. The workshop format was selected because we felt it was the correct forum for our membership to be given an opportunity to express themselves about CMVCA goals and objectives guiding the Association in the 21st century.

One integral part of our association's future, discussed by many, was the position of a part-time versus full-time Executive Director. However, there are many important issues that need to be addressed and the workshop identified seven (7) that were significant and critical to our association over the ensuing years. This report outlines the seven specific issues and identifies key dates, action planning, conflicts, etc., and summarizes these areas for the Board of Directors without restating the entire report. It will be extremely important for the Board to evaluate the report, prioritize the issues, assign committees, outline specific duties and implement an "Action Plan" with realistic goals. Where appropriate, recommendations are submitted by the Ways and Means Committee for specific issues and this report concludes with a list of additional recommendations. If the Committee had no additional recommendations on the report, we simply stated none.

URBANIZATION

Need for strengthening our association through planning, policy, publications and legislation.

Recommendation: None.

MANPOWER

Evaluate the need for and future availability of trained professionals entering vector control programs. Will there be medical entomology professors to provide the training in the academic institutions?

Target Date: 1990.

Recommendation: The shortage of public health professors and the need for new medical entomology textbooks, etc. should be coordinated with other organizations with similar interests (i.e., AMCA, SOVE, ESA, etc.).

LEGISLATION

Utilize proactive environmental legislation to promote and support public health programs. Involve Trustees at the local level.

Target Date: 1990.

Recommendation: None.

COMMUNICATION

Basically, a three-fold approach. Interacting with the general public, other related agencies and improving communications within our association - particularly between Trustees and Managers. Utilize a statewide computerized informational system to dispense this information to our general membership.

Actions:

1. Conduct regional surveys to identify specific State and Federal agencies that Mosquito and Vector Control Districts need to network with in the future (1990).
2. Board of Directors to delegate to Regional Representatives the responsibility of the survey by January 1, 1990.

When: Survey results to be presented at the Board of Directors meeting in the fall of 1990.

Recommendation: None.

ENVIRONMENTAL ISSUES

Coordinate our vector control programs with appropriate legislation and other regulatory activities to insure public health receives the proper recognition and support.

Recommendation: Establish target dates for action plan.

FUNDING

The three basic areas of discussion were the funds associated with: local vector control agencies, the association and research.

Actions:

1. Develop a Marketing Plan which outlines what the CMVCA provides to its membership for their dues.
2. Ways and Means Committee research the costs associated with hiring an Executive Director by June, 1990.
3. Increase association dues. First increase to be in 1991 and the second in 1992 with future increases via some indexing plan.

Recommendations:

1. The Marketing Plan needs to be completed by April 30, 1990. This data will be necessary since it will provide important support information to convince Board of Trustees of the need to increase dues.
2. Appoint an ad hoc committee to research the expense of hiring an Executive Director. This review committee will serve to interview applicants for the full Board of Directors. This committee, which should not be the Ways and Means Committee, should include the current part-time Executive Director, Treasurer, CMVCA Officers and a Trustee Corporate Board representative.
3. Request the Executive Director and Treasurer to generate estimated dollar figures if membership dues doubled or tripled. Utilizing a 5% inflation figure for both the CMVCA budget and the operational budget of each district.

STRUCTURE

Four specific areas were identified:

1. The overwhelming conclusion was that the CMVCA needs a full-time Executive Director. Most, if not all of the seven major issues would require the guidance of a full-time person.
2. The role of the Trustees surfaced many times during the workshop.
3. Restructuring the regional boundaries to balance representation.
4. Corporate versus Associate membership.

Actions:

1. Acquire a full-time Executive Director by September 1, 1990. To be handled by the Ways and Means Committee. Projected cost; a minimum of $100,000 impact on the association.
2. Improve communications between Trustees and Managers, beginning with the 1990 annual conference program.
3. Appoint and ad hoc committee to prepare and present a report on regional boundaries at the spring Board of Directors meeting (April/May, 1990).
4. Assign the Ways and Means Committee to evaluate the role of "associate members" within the association and report at the spring Board of Directors meeting (April/May, 1990).

Recommendations:

1. The CMVCA Board of Directors appropriate a line item in the 1990 budget for a full-time Executive Director.

2. Four out of the six small groups addressed specifically the position of an Executive Director with target dates varying from June, 1990 to 1992. The Ways and Means Committee would concur with the $100,000 estimate to "bring on board" a full-time Executive Director and recommend the target date be October 1, 1990.
3. Reports on Actions 3 and 4 be extended to the summer Board of Directors meeting.

COMMITTEE RECOMMENDATIONS

1. Do not ask Mr. Robert Rauch to make a presentation at the conference January 29, 1990.
2. Put in a line item on the 1990 budget for a full-time Executive Director by December 28, 1989.
3. For continuity between the workshop and the 1990 CMVCA officers, request Charles Beesley to appear on the Trustee conference program as a speaker covering the Management Workshop by December 1, 1989.
4. Because of the limited time left on President Watson's term of office, we suggest that President-elect Bob Washino, after taking over as President, be responsible for appointing the necessary committees and implementing the "Action Plans" after the appropriate board review. Process to begin immediately following the 1990 conference.
5. The 1990 officers will, with the input from the full Board, prioritize the issues, establish target dates, define the specific duties of the committees and implement programs in a reasonable and realistic time frame.
6. Direct the committee charged with the selection of a full-time Executive Director to incorporate the recommendations of the Computer Application Committee report into a search for the qualified persons to fill the position. Again we state, that this responsibility be given to an appointed committee and not be charged to the Ways and Means Committee.
7. Dues increase effective January 1, 1990. Raise the cap to $4,750 (exempting Alameda County Vector Control and Santa Clara Vector Control) and increase the rate from .0033 to .0045.

COMPUTER APPLICATIONS COMMITTEE REPORT:

A PLAN FOR A STATEWIDE COMPUTER INFORMATION NETWORK

Bruce F. Eldridge (Chair)

Committee Members
David A. Brown, Craig W. Downs, Jack E. Hazelrigg, Barbara A. Kuzusko, Marilyn M. Milby, Allan R. Pfuntner, and Fred C. Roberts

Introduction.

In 1987 then California Mosquito and Vector Control Association (CMVCA) President, Chuck Hansen, appointed the Computer Applications Committee with the following charge: "Examine present computer information systems and describe the short and long term options. These options should also be accompanied by cost figures and suggestions for funding. In exploring options, the Committee should not be constrained by previous assumptions as to existing equipment and systems but should, of course, take such items into consideration in describing the range of options available."

Since that time the Committee has considered a number of configurations, software programs and management schemes to bring such a system into being. We also conducted a survey of microcomputer usage among corporate members of CMVCA (Table 1). In late 1988, the Committee felt it had enough information to enable it to proceed with detailed implementation of a system. What follows are these recommendations, including a budget for equipment and software purchase and annual maintenance costs. We feel that such a system would be a benefit to every member agency of CMVCA and recommend adoption and implementation of these recommendations by the Board of Directors.

What The System Will Do.

The CMVCA computer system would be the hub of a statewide computerized information system linking all vector control agencies with information services vital to vector control. The system would become the primary database for vector control in California, as well as other cooperating agencies. The comprehensive database would provide essential data to decision makers at all levels of vector control in California. Specifically:

1. The CMVCA system will access and store from a variety of existing databases. This information will then be accessible to local mosquito control agencies through their microcomputers. Databases currently accessible are:
   a. Mosquito-borne disease data (human and animal cases, sentinel chicken flock conversions, mosquito isolations of arboviruses) from the State Health Department (VECTOR BYTES). These data could be expanded to include insecticide resistance information, mosquito biology, and pesticide labeling.
   b. Data on endangered species from the computer database developed by the State Department of Fish and Game.
   c. IPM information available from a University of California database (IMPACT). The information includes mosquito biology, pesticides, weather and other data important to IPM.
2. The CMVCA office staff will create and update file systems unique to vector control in the State of California. These files would also be accessible to the vector control agencies through their microcomputers and could include:
   a. Update of pertinent legislation.
   b. Calendar of upcoming events.
   c. Yearbook and other CMVCA data.
   d. Pesticide label information.
   e. Catalog information from suppliers of mosquito control materials and equipment.
   f. Other files as deemed necessary.
3. Finally, the CMVCA system will receive reports from local vector control agencies by telephone transmission. These data could then be processed and used to update the vector control database and then transmitted to other agencies as needed. The following types of information would be handled in this manner:
   a. Mosquito abundance information (light trap data, larval surveillance data, etc.).
   b. Local weather data vital to prediction of vector-borne disease.
   c. Insecticide usage data.
   d. Other data as the need arises.

Table 1. Results of a survey of California mosquito abatement agencies concerning microcomputer usage.

	Number	%
Survey forms mailed	48	100
Agencies responding	42	88
Own at least 1 microcomputer	29	69
Own no microcomputer	13	31
Agencies owning at least 1 microcomputer		
IBM-PC compatible	20	69
Pre-IBM PC	8	28
Apple	1	3
Own a modem	16	55

Table 2. Specifications for a host system for a statewide computer information network for mosquito control information.

Hardware (Base Unit)	
CPU:	80386 or 80486
Math coprocessor:	80387 or 80487 (as needed)
Operating speed:	20 Mhz or faster
RAM:	8
Hard disk:	300 MB (or 2-150 MB)
Floppy disks:	1-5.25" 720 KB (R&W 360 KB)
	1-3.50" 1.44 MB (R&W 720 KB)
Expansion slots:	10 or more
Serial ports:	8
Parallel ports:	2
Graphics:	EGA or better
Clock-calendar:	Internal
Power supply:	265 watts or more
Hardware (Additional)	
Pointing Device:	Mouse
Modems:	6 at 2400 baud each
Back-up device:	Tape - 60 MB
Printer:	24-pin dot matrix
Monitor:	Color - high resolution
Software	
UNIX System V (or equivalent)	
Database program for UNIX	
Communications program for UNIX	
Windows/386	
MS-DOS version 4.0 or higher	
OS/2 with presentation manager	

System Description.

The general specifications of the system, as deemed by the committee, would be as listed in Table 2. All the specified hardware and software are currently available from a number of retail and wholesale sources.

The committee reviewed a number of systems from vendors, including IBM, AT&T, Sun Microsystems, and Digital Equipment Corporation. We selected as a recommended system, which fulfilled the specifications, the AT&T PC 6386E Mdel 75. Details of the system we selected with pricing and accessories appear in Table 3. We also list recommended software for this unit, including the UNIX operating system. We stress that this is the operating system for the host microcomputer, and it will not be necessary for users to use UNIX. If they are currently using MS-DOS, for example, the may continue to do so.

System Operation.

Management: We recommend that the system be installed at the CMVCA headquarters and that the management of the system be under the overall direction of the Executive Director of the CMVCA. In general terms, the more complex the system, with the more services offered, the greater the amount of day-to-day management the system will require. Under certain circumstances, with a minimal system, management could be done with relatively little input from additional personnel.

Installation: During the start-up period of approximately one year, a full-time Computer Operations Manager would be needed to get the system set up, install the software and the various databases, prepare documentation and provide training for users either on-site or at their home offices. See Table 4 for a sample job description for this position.

Maintenance: Once established, the system would require a part-time person working under the direction of the Executive Director to maintain software and hardware and to provide consulting and additional training for users. For hardware maintenance, we recommend an annual maintenance contract with the vendor.

Organization: The system would be organized into modules. One module, for example, would be the vector-borne disease surveillance information (similar to the present VECTOR BYTES system). As at present, this module would be maintained by personnel of the Environmental Management Branch, CDHS. Information on mosquito research would be maintained by the UC Mosquito Research

Table 3. Sample hardware configurations and pricing for a host system for a statewide computer information network for mosquito control information.

Hardware (Base Unit)	
AT&T PC 6386E Model 75 (floor standing model)	
101-key AT-compatible keyboard	
Model 75 CPU with 20 MHz clock	
EGA-compatible monochrome monitor	
6 MB RAM	
9 serial ports and 3 parallel ports (includes 1 expansion board with 8 serial and 2 parallel ports)	
5.25" (1.2 MB) floppy disk drive (3.5" floppy drive also available)	
8 available expansion slots (3 32-bit, 3 16-bit, 2 8-bit)	Price: $14,804.36*
Tape Back-up Units (includes controller and software)	
60 MB tape back-up unit	Price: $ 1,555.00*
125 MB tape back-up unit	Price: $ 1,844.50*
Modems	
110 - 2400 baud per second modem	Price: $ 773.00**
Software	
Recommend the "bundling" offering of AT&T UNIX System V including the Foundation Set and the Software Development Set	Price: $ 995.00*

* Reflects full retail price. Approximate 30% discount to state and local governments available.
** Reflects full retail price. Approximate 22% discount to state and local governments available.

Table 4. Sample job description for the position of Computer Operations Manager.

COMPUTER OPERATIONS MANAGER

Duties:

Oversee the initial phase of the electronic computer installation; operate a main computer console, multiple on-line telecommunications systems, auxilary consoles, and peripheral equipment on electronic computer systems and assist users in the performance of computer operating duties.

Minimum Qualifications:

Completion of at least two years at a recognized college or university and successful completion of a computer operation or programming curriculum which includes actual computer operation experience and at least 200 hours of classroom instruction, and experience and versatility using UNIX System V Foundation Set and Software Development Set.

Knowledge of:

Principles, capabilities and operation of electronic computer systems and related peripheral equipment.

Ability to:

Recognize an work out solutions for operational problems; speak and write effectively; analyze data; work cooperatively with others and gain their respect and confidence.

Program. Harvey Scudder has volunteered to maintain a pesticide label information module. Modifications of the databases could be either by modem or by using media sent through the mails to the Computer Operations Manager.

Communications: Users would access the system through dial-up modems. In order to achieve uniformity in communications packages, suitable communication software would be purchased in bulk lots and distributed to users so that all users will be using the same communication system. Most modern PC's and remote terminals should work with this system. The survey which we conducted (Table 1) showed that IBM PC-compatible computers predominate. The problems with incompatible hardware can be handled on a case-by-case basis. Generally speaking, we see few problems.

Costs.

Initial (first year) costs for the recommended system would be approximately $51,000 as outlined in Table 5. Subsequent year costs would be for the Computer Operations Manager ($33,429 plus periodic cost-of-living increases) plus costs for supplies and software and equipment maintenance (estimated at $5,000 per year).

Table 5. Initial (first year) costs for a typical system for a statewide computer information network for mosquito control information.

Hardware		
AT&T PC 6386E Model 75	$14,803	
125 MB tape back-up unit	$ 1,845	
		$16,648
Software		
AT&T UNIX System V Foundation and Software Development Sets		$ 995
Personnel		
Operations Manager*	$26,124	
Benefits @ 28%	$ 7,315	
		$33,439
TOTAL COSTS		$51,082

* Based on monthly salary of $2,177 - $2,617.

REPORT ON THE MANAGEMENT WORKSHOP AT GRANLIBAKKEN

OCTOBER 23 - 25, 1989

Charles Beesley

CMVCA Vice President
Contra Costa Mosquito Abatement District
150 Mason Circle
Concord, California 94520

Introduction.

This workshop was conducted to review the future goals and objectives of the California Mosquito and Vector Control Association (CMVCA) and to ensure the continued success of both member districts and the Association, to effectively meet future challenges.

Background.

The workshop was the result of many events over the last 10-20 years. The need for improved services and increasing pressures on our association and its participating districts, has been continuous. For instance, in 1967 Fred De Benedetti on behalf of the Trustee Corporate Board recommended a full-time Executive Secretary for the CMVCA. In 1975, Ronald Wolf of Goleta Valley/Carpinteria MADs wrote a report by the Ways and Means Committee on the need for a full-time Executive Director. He noted at that time that it would require reevaluating and restructuring CMVCA goals and objectives to successfully incorporate an Executive Director. Then in 1984, Gil Challet chaired an ad hoc Central Office Committee to review the feasibility of merging the activities of the CMVCA and Vector Control Joint Powers Agency (VCJPA). Instead, the committee recommended a full-time Executive Director for the CMVCA. Finally, last January, the Trustee Corporate Board of Directors unanimously recommended a full-time Executive Director which was reiterated in detail at the May Board of Directors meeting by our current part-time Executive Director, John Combs.

While the demand for services was very apparent to those close to the front lines, the need for, and impact of, such a change was still uncertain by most of the membership. This, coupled with the financial difficulties of the last 11 years since the passage of Proposition 13, is the reason it's taken so long to get to where we are today.

To pull everyone together, and objectively review our association, we sought out a facilitator to conduct the workshop. We selected Mr. Robert Rauch from Santa Barbara, who has a strong background in working with special districts. Tables were set up with group leaders to report to the facilitator and the full audience. All ideas were posted in discussion and Action Plans were developed for all key items.

There were 43 participants from 29 Districts (30 management and 11 trustees) and 2 outside agencies (the University of California and California Department of Health Services, Environmental Management Branch). The group leaders were Charley Dill, Mike Wargo, Jack Fiori, Herbert Marsh, Mel Oldham and Fred Beams. There were three facets for all the groups and participants to consider:

(1) Current issues facing the Association.
(2) Future issues facing the Association.
(3) Action Plans to effectively deal with these issues.

Results.

There were seven key issues identified.

A. Urbanization: California is going through continued growth and urbanization, and will do so for the foreseeable future. The state is expected to grow from 29 million to almost 40 million people in the next ten years, almost a 38% increase in population.

What needs to be done: We need increased public and community awareness through education, public relations programs and the development of preventative planning guidelines for interacting with cities/counties.

Who: The central office can produce these programs, pamphlets and guidelines.

When: They should be released on a regular basis, not just on an emergency basis as is currently done.

How: The Executive Director, in conjunction with the Publications and Legislative Committees, could prepare pamphlets as well as planning guidelines for the public,

legislators and cities respectively. Another expressed concern with the continued statewide urbanization was the need for our association to make a policy statement encouraging the establishment of vector control districts in uncontrolled areas. Paradoxically, most districts present at the workshop still only provide mosquito control services.

B. Manpower: There is a shortage of professionals in vector control.

What is the problem: At both the district and academic level both medical entomology and public health are losing ground to other disciplines in both professional training, technology and recruitment. Retiring professors of medical entomology are not always being replaced. This, coupled with the reported shortage of manpower in the workforce over the next decade, will become a difficult problem to overcome and will affect our programs. We may not have anyone to teach us and no one to hire.

Who is responsible: The universities, colleges, community colleges, districts and county health departments are all involved in teaching and training of vector control as a public health profession.

When: We need to respond now, before more vacancies exist. Districts trying to recruit personnel are already seeing the impact of these problems with the limited number of qualified applicants.

How can we turn this around: We will need an increased commitment from our Association to reinforce the need for our work and our personnel. We can better promote our profession by doing things like:

- Participating in local Career Day activities at schools.
- Continued development of Association programs in vector control for use by districts as a promotional tool.
- Promote increased funding of research at the academic level to support our training programs.
- Coordinating our concerns at the national level with other organizations such as AMCA and SOVE.

C. Legislation: Both state and federal laws affect us:

What: We must be able to better influence present and future legislation to incorporate vector control concerns before laws are passed. We must also improve legislative support for research funding by the University.

Who: The Legislative Committee of the CMVCA in coordination with trustees, district boards and management, under the leadership of an Executive Director could do this.

When: We need to step up our efforts in 1990.

How: We must increase our legislative impact through broader lobbying activities as discussed at the August Board of Directors meeting by our legislative advocate, Ralph Heim.

D. Communications: There were three separate concerns within this topic; interacting networks, public relations and communications within the CMVCA.

1. Interacting Networks: Between agencies and districts.

What is the problem: Districts work too independently for their own good. There is no association plan to deal with state or federal regulatory agencies. We're all acting on our own. There is no automatic process or mechanism for us to use comparable to that of other regulatory processes such as the California Environmental Quality Act (CEQA) which regulates the environmental review process.

What should be done: We should initiate regional surveys of districts to see who they interact with and what their operational needs are with these agencies. Then we must develop a state-wide standard procedure for the Association and districts so they can implement their procedures and more effectively deal with outside agencies.

Who: The Board of Directors should assign this to the Regional Representatives.

When: They should conduct this survey over the next eight to nine months and report back to the Board of Directors at the November 1990 meeting.

2. Public Relations:

What: We need to develop a statewide program.

Who: Ideally the Executive Director or his/her designee(s) could outline and coordinate the program.

When: We need a public relations program now and it should be completed as soon as possible, either as a tool for a new Executive Director to work with or one which they could develop on the basis of past experience.

How: We could produce "canned" videos like the

AMCA film on mosquito control or on other public health topics for use by either districts, the Executive Director or, perhaps, the development of a Speakers Bureau. Funds to support this could be a line item in the budget as we develop a three-fold program (informational, educational and emergency use), to be supplemented with occasional releases by the Executive Director. The Association should also consider distributing a newsletter for general release.

3. Communications Within the Association:

What: There were two concerns. (1) Inadequate communications to trustees, with a resultant lack of trust by them in the membership and the Board of Directors and (2) inconsistent communication between committees, the Board of Directors and the Executive Director.

Who: Both trustees and committees are at fault for not consistently following up on their responsibilities. The Board of Directors is at fault for not maintaining consistency in communications regarding board policy and Association procedures.

When: This was to have been addressed immediately, starting at the November 1989 Board of Directors meeting, with the report by the Ways and Means Committee to the Board of Directors. But you saw some of the problems created when the Board of Directors did not follow the recommendation of the Trustee Corporate Board of Directors on raising the ceiling of corporate members' dues to $5,000/year.

How should this be handled: By periodically reviewing our organizational process, such as at the beginning of each term, and implementing needed changes to the bylaws, when necessary.

E. Environment

What: There is increasing public concern over the "misuse" of the environment and use, abuse or perceived misuse of pesticides. This has resulted in more and more federal and state regulations which increasingly restrict our abilities to operate effectively. For instance, the new Federal Endangered Species Act (FESA) will further restrict pesticide usage for mosquito control in sensitive habitats. This compounds an already difficult task because of past conflicts between MAD's, the California Department of Fish and Game and the U.S. Department of Fish and Wildlife over jurisdictional differences of public health (mosquito control) vs. wildlife habitat management.

Who: These events are far reaching and committees such as Environmental and Legislative should ensure we are consistent and thorough in our responses to legislators in this process. We need to improve public health considerations by the legislators as legislative bills are being developed.

When: These committees and our Association need to improve our capabilities as soon as possible.

How: We must better influence state and federal legislation. To do so we must improve university research funding, the quality and type of research projects, and further our ties with peripheral associations such as the County Health Officers' to encourage continued research and development and product availability by pesticide manufacturers. We need to develop a list of pesticides we want to use for regular or emergency use, and insist upon their acceptance by these agencies and their availability by manufacturers. We should institute workshops with these regulatory agencies to ensure our success.

F. Funding: It takes money to hire people in order to have a program, whether locally, state-wide, or nationally. There are two separate concerns: the CMVCA and UC research funding.

1. CMVCA:

What: We need to increase our operating funds to expand our efforts, keeping in mind that our primary source of revenue is Association dues.

Who: The Board of Directors should direct the Ways and Means Committee to review and make recommendations for dues changes and subsequent approval by the Board of Directors and the Trustee Corporate Board of Directors.

When: Dues must be increased immediately, beginning in January 1990, with the understanding it will take more than one increase to achieve all our goals.

How: Because the primary source of dues is from corporate members, we would like to see the Executive Director better promote the Association, market it for increased membership and at the same time retain existing members. This could be done through advertisements, newsletters or other means.

2. Research:

What: Actual research dollars in available spending funds has continued to diminish

through inflation and increased administrative overhead by the University. Funding needs to be increased to both offset this and expand the number, quality and type of research projects.

Who: Because of the many facets of approving research funding, the Executive Director would be the appropriate person to coordinate our efforts with the University and legislators.

When: Our efforts need to be improved as soon as possible.

How: We could achieve this by establishing an Advisory Committee for legislators and by reestablishing a line in the state budget for expanded university research; and perhaps by creating a research foundation within the Association to promote grants. Also, we could enhance dues regionally or by common goals for specific research projects and/or solicit help from vendors and manufacturers for project support.

G. Structure: All organizations must have a structure or framework to work effectively. There were four items of concern: the central office, the role of trustees, regional representation and levels of membership.

1. The Central Office: This office is the core of our organization.

What: It lacks centralized, consistent leadership. It was almost unanimously agreed upon that this will not be accomplished without a full-time Executive Director. The Association needs to appoint a committee to define the position, advertise, screen candidates and make a recommendation to the Board of Directors for hiring.

Who: The Ways and Means Committee could be charged with this responsibility.

When: The person should be in place by September 1, 1990.

How: To do so we must increase the Association budget by $100,000 to cover salary, benefits and operational expenses.

2. Trustee Role: There was considerable discussion on the role of trustees.

What: It was quite apparent the trustee role has changed somewhat and needs redefining, for many feel they need to be more involved.

Who: The leadership of this Association, including the Board of Directors, Managers, and the Trustee Corporate Board of Directors should review this as soon as possible.

When: Starting in January 1990, at the conference with this session.

How: Trustees want better communications from the Association. This can be done with better conference programs, regional workshops, video tapes for trustees, newsletters for trustees, pre-appointment sheets, and even greater participation by trustees in the California Special Districts Association (CSDA) Board Management Institute workshops, and by revising the Trustee Manual.

3. Regional Representation:

What: Because of the disproportionate size and growth of some regions within the state, it is questionable whether the regional divisions still provide for effective representation.

Who: An ad hoc committee needs to be appointed to review the current regional structure.

When: A first draft should be presented to the Board of Directors by May 1990.

How: The committee should review existing regions and membership, and if necessary, restructure the boundaries and/or change the number of regions to balance the overall representation.

4. Levels of Membership: There was concern expressed about the different levels of membership and ability to vote in Association matters.

What: There are 49 Corporate vs. 79 Associate members in the CMVCA.

Who: The Ways and Means Committee should review levels of membership and dues structures.

When: The committee should report to the Board of Directors by May 1990.

How: It was suggested that an at-large appointment (with voting rights) could be made to the Board of Directors to represent Associate and/or other members.

Ways and Means Committee Recommendations.

Taking all of the above into consideration, it was obvious there was much to be done by both the Association and the districts. Realizing we could not do it all at once, the Ways and Means Committee recommends the following priorities and actions to be taken on these seven key issues:

A. Urbanization: Strengthen our Association through advanced planning, development of policies, publications and legislative actions.

B. Manpower: Evaluate the need for and future availability of trained professionals entering

vector control programs. We must further influence our universities, colleges and community colleges to continue public health and medical entomology programs to prevent a professional void.

C. **Legislation:** We need to utilize a more proactive approach towards legislation to promote and support public health programs. We must involve trustees at the local and state level where appropriate.

D. **Communications:** We need to enhance our interaction with the general public and other related agencies. We must improve communications within the Association, particularly between committees, the Board of Directors and the Trustee Corporate Board of Directors. A regional survey also needs to be conducted this year by the Regional Representatives to develop statewide planning guidelines for interacting with outside agencies.

E. **Environment:** It is essential that we promote our vector control programs to insure that public health receives proper recognition, public support, legislative exemptions and other relevant considerations.

F. **Funding:** You cannot have programs without money! We need to develop a marketing plan for the Association, determine the necessary costs for an Executive Director, and increase our dues accordingly. The first increase would be in 1990, followed by a second increase in 1991 to provide adequate long-term funding.

G. **Structure:** The overwhelming conclusion from our current obstacles and those ahead was that our Association needs strong leadership and a continued commitment to coordinate all these efforts by members and trustees. This can best be done with a full-time Executive Director.

We need to review regional representation and make changes, if necessary. At the same time, we should review the role of Associate members giving them more influence in Association activities it it's wanted and appropriate.

Although the Ways and Means Committee doesn't always agree with who should be assigned these tasks, they fully understand that it's critical for our Association to get our priorities lined up before further actions are taken. These seven key items, in their report form will be presented to the Board of Directors on the morning of Wednesday, January, 31, 1990.

A last thought by the Ways and Means Committee was that they also recognize the importance of continued improvements in computer capabilities at the Central Office and the need to incorporate the recommendations of the Computer Applications Committee. Very briefly, this would establish a computerized information system which would expand our capabilities and, perhaps, generate revenue for Association services.

On a Personal Note.

As your outgoing Vice President, I'd like to finish by saying that the amount of work to be done and the sense of urgency certainly shouldn't come as a complete surprise to us. These things have been brewing for a long time and members have repeatedly pointing out these trends and expected problems. Unfortunately, ever since Proposition 13, many agencies have been busy trying to get back to where they were in 1978. Meanwhile state and federal regulations coupled with statewide growth have literally surrounded and restricted our capabilities while the need for our services has continued to grow. In many ways, this is how it all started years ago with the creation of the CMVCA in 1930; funding was tight, obstacles were numerous but the need for services was growing and we responded to the challenge.

Well, we have responded to some of these new problems by improving our Health and Safety Code to let us generate additional revenues. Several Districts have done this to date, while others have held back. It is up to each district to ensure that they are adequately funded if they are to survive and be effective. For the sum total of the input of the workshop, by all the participants was that we must better promote our mission at the local, state and federal level, and at a more effective level than in recent years. California is a dynamic state slated for continued urbanization, the public expects and deserves our services and we must grow accordingly. If we do not respond in kind, we do ourselves a disservice.

To most effectively promote our profession, enhance our communications and our statewide image, will require a full-time Executive Director. Your 1990 Board of Directors is about to be handed the torch of change to fund for and hire a full-time Executive Director, but it cannot be done without your support.

I encourage each and everyone of you to work with the incoming Board, to support them at your districts, through committee or legislators and above all pull together during this time of change. We need a full-time Executive Director and we need your help to fund the position and enable the Association to become more professional and better recognized and, once again, follow the footsteps of leadership and recognition we deserve.

Acknowledgements.

I'd like to express sincere appreciation to the 1989 Ways and Means Committee for recognizing the need for a review of our Association. Also, to Bob Washino, John Combs, Claude Watson and Chuck Hansen for their specific input in the objectives, goals and format of the workshop. And lastly, Linda Sandoval, for transcribing the workshop posterboards or "minutes". For without her efforts, I would have been unable to make this report.

WILLIAM C. REEVES NEW INVESTIGATOR AWARD

The William C. Reeves New Investigator Award is given annually by the California Mosquito and Vector Control Association in honor of the long and productive scientific career of Dr. William C. Reeves, Professor Emeritus, School of Public Health, University of California at Berkeley.

The award is presented to the outstanding research paper delivered by a new investigator based on quality of the study, the written report, and presentation at the annual conference.

Gary N. Fritz was the recipient of the 1990 award at the 58th Annual Conference held in Sparks. The other finalists were John Fry, Bruce K. Orr, Noor S. Tietze. The four finalists' papers are printed on pages 193-227.

Previous William C. Reeves New Investigator Award Winners:
1988 - Vicki L. Kramer
1989 - Truls Jensen

1990 WINNER

WILLIAM C. REEVES NEW INVESTIGATOR AWARD

POLYTENES, ISOZYMES AND HYBRIDS: DECIPHERING GENETIC VARIABILITY IN *ANOPHELES FREEBORNI.*

Gary N. Fritz

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Introduction.

Population genetics is not only useful in understanding the genetic basis of evolution and in establishing phylogenetic relationships, but can also have more practical implications. Studies on population genetic structure, for example, can be useful in understanding the epidemiology of vector-borne diseases, can be useful in identifying vector versus non-vector populations, and are also necessary first steps in certain types of control programs (e.g. population replacement).

Though considerable information on the cytogenetics of anophelines has accumulated during the past three decades (Kitzmiller 1976; Narang and Seawright 1990), studies on population genetics have been few. It is clear from past studies of mosquitoes, particularly the anophelines of S.E. Asia (Reid 1968) and Africa (Coluzzi et al. 1979), that wide-ranging species vary geographically. This variation may take the form of morphological differences (often enough to require region-specific keys), ecological differences, and vector capacity differences (Reid 1970).

The little that is known about the genetics of *Anopheles freeborni* Aitken includes mapping of the salivary polytene chromosomes by Kitzmiller and Baker (1963), somatic chromosome size comparisons by Mukherjee et al. (1966), interspecific hybridization studies (Kitzmiller et al. 1967), the frequency of a chromosome inversion at three sites in California (Smithson 1970), and a comparison of alkaline phosphatases (Bianchi and Piroda 1968). This work has provided some baseline information on the genetics of *An. freeborni*, but there is no information on the population genetic structure and divergence of this species throughout its distribution. The purpose of this investigation was to study the population genetic variability of *An. freeborni* through an analysis of polytene chromosome banding patterns, enzyme electrophoresis, crossing studies, and rDNA restriction enzyme fragment differences. The following questions were addressed:

1. Are all populations of mosquitoes, presently identified as *An. freeborni*, really conspecific?
2. Are there diagnostic loci or chromosome inversions that distinguish sibling species or conspecific populations?
3. Is there genetic homogeneity among conspecific populations and what are the genetic distances?
4. How much genetic polymorphism (polymorphic loci, mean heterozygocity, chromosome inversion frequency and distribution) exists in natural populations and are there any discernable patterns?

Materials and Methods.

Collections: *Anopheles freeborni* larvae and adults were collected in California, Oregon and Washington throughout July-October, 1988 and in Utah in 1987 (Fig. 1, Table 1).

Electrophoretic Technique: The electrophoretic techniques, enzyme system recipes, and materials

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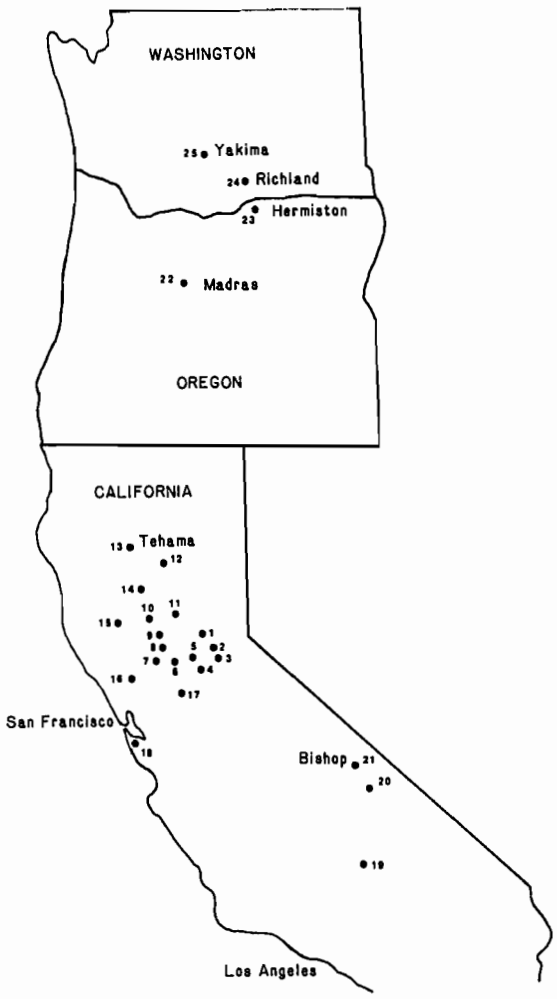

Figure 1. Collection sites in California, Oregon and Washington.

employed in this study were those described by Steiner and Joslyn (1979). Gels were prepared as 12.5% solutions (w/v) of hydrolysed starch (Connaught Laboratories, Ontario, Canada) in an appropriate buffer. Only adult mosquitoes were electrophoresed. The RF values (relative mobilities) of electromorphs on gels were computed by using, as a standard, an isoline that was homozygous for most enzymes of interest. An updated version of the Biosys-1 computer program developed by Swofford and Selander (1981) was used to analyze allozyme variation within and between populations.

Cytogenetics: The technique used to prepare the ovarian nurse cell polytene chromosomes was generally as described by French et al. (1962). Chromosomes, banding patterns and inversions were identified by using the description and map prepared by Faran (1981), and by using a photographic map. Polytene chromosome inversion frequencies and associations were analyzed by Pearson Chi-Square and tested for homogeneity by using statistical analysis software (SAS). The sample of mosquitoes collected in Utah was not included in this statistical analysis.

Hybridization: Crosses were made between several purported conspecific populations of *An. freeborni*. One aim was to cross two widely separated geographic strains that also differed chromosomally. For this purpose, a strain was obtained from Richland, Washington (WASH strain) and crossed to a strain from the Sacramento Valley, California (DAVIS strain). The WASH strain was already known to possess a polymorphic inversion on the X chromosome (In(X)A, hitherto unknown in *An. freeborni*), whereas the DAVIS strain lacked this inversion (hereafter referred to as the standard homokaryotype).

Three strains from California, which were fixed for one or the other form of the inversion on the X chromosome, were also crossed. These crosses were deemed necessary when several samples collected in California were found to have relatively high frequencies of inversion heterokaryotypes for an inversion on the X chromosome. One of the homokaryotypes was that described by Kitzmiller and Baker (1963), Faran (1981) and Menchaca (1986) as being specific to *An. freeborni* (standard

Table 1. Site number and collection location for samples of *Anopheles freeborni* collected in California, Oregon, Washington and Utah.

#	STATE	COUNTY	LOCATION
1	California	Nevada	Wolf Creek & Hwy 49
2		El Dorado	Camino, Carson Road
3		El Dorado	Pleasant Valley Road
4		Sacramento	Sloughouse
5		Sacramento	Folsom
6		Sutter	Hwy 99 & Howsley Road
7		Yolo	Capay Valley, Guinda
8		Yolo	Knights Landing
9		Colusa	Millers Landing
10		Colusa	Hwy 20 near Williams
11		Sutter	Yuba City, Butte House Road
12		Butte	Chico
13		Tehama	Tehama, Gyle Road
14		Glenn	Willows, Hwy 162
15		Lake	Clear Lake
16		Sonoma	Sonoma
17		Sacramento	Hwy 99 & Twin Cities Road
18		San Mateo	Jasper Ridge Preserve
19		Kern	Onyx, Canebrake Creek
20		Inyo	Big Pine
21		Inyo	Bishop
22	Oregon	Jefferson	Madras
23		Umatilla	Hermiston
24	Washington	Benton	Richland
25		Yakima	Yakima
26	Utah	Uintah	

homokaryotype), whereas the other homokaryotype was that described as specific to a new sibling species, *Anopheles hermsi* Barr and Guptavanij (Baker 1965, Morrison 1985, Menchaca 1986, Barr and Guptavanij 1988).

One of the strains used in the crosses was collected at the Jasper Ridge Preserve (JASP strain) just west of Palo Alto and approximately 10 miles from the coast. This strain was fixed for In(X)A. Another strain, also fixed for In(X)A, was collected at the north end of Clear Lake (LAKE strain) in Lake County. The third strain was the DAVIS strain mentioned above.

All crossing methods and rearing techniques were standardized and are described in detail by Fritz (1989). All parental crosses and the backcrosses were compared with each other and with controls for fertility and fecundity. Hybrid polytene chromosomes were checked for banding pattern homology and the degree of synapsis.

Species Identification: The discovery, in this study, of mosquitoes that were polymorphic for the inversion on the X chromosome cast doubt on the use of this chromosome as a reliable character for distinguishing *An. freeborni* from *An. hermsi*. Thus, species identity was determined independently by analysis of rDNA restriction enzyme fragment pattern differences between both species (courtesy of Frank Collins and Chuck Porter), as described by Collins et al. (1990). Restriction fragments of rDNA were generated, in a blind test, for samples from populations that were polymorphic for the inversion on the X chromosome, as well as populations fixed for either homokaryotype. Samples of mosquitoes from three populations of *An. hermsi* (collected in southern California by Stan Cope) were also analyzed.

Results.

Allozyme Analysis: A total of 17 enzyme systems including 24 loci were used in the electrophoretic analysis of *An. freeborni*. Only six loci accounted for most of the polymorphism observed: Acon-1, Est-2, Got-1, Mpi-1, Pgi-1 and Pgm-1. Consequently, measures of genetic variability, including mean heterozygocity values and the percentage of polymorphic loci, were quite low (Table 2).

Although there were no diagnostic loci (see Ayala and Powell 1972) that distinguished any two samples, differences in electromorph frequencies were obvious between geographically distant samples. Mean heterozygocity values for samples from California, for example, were essentially due to two loci, Mpi-1 and Got-1. Samples from Washington and Oregon, however, were essentially fixed for Got-1 and mean heterozygocity values were due primarily to polymorphism at Mpi-1 and Pgm-1. Except for Yakima (site 25), all samples from Washington and Oregon were monomorphic for Est-2, whereas in California Est-2 was polymorphic at most sites. The sample from Jasper Ridge was distinctive in being monomorphic for Est-2, Got-1, and Pgm-1, but polymorphic for Pep-4.

F-Statistic Analysis: This analysis is a procedure for quantifying the genetic differentiation of populations by F-statistics (see Wright 1965, 1978; Nei 1977). Wright's Fst value, or fixation index, provides a measure of the genetic variation in the population that is attributable to sub-populations; i.e., departure from panmictic expectations of allele frequencies within sub-populations relative to those of the entire population. When Fst values are found to be significant (by chi-square analysis), this indicates significant genetic separation for these subpopulations. The chi-square test for significance of gene frequency differences at each locus among subpopulations is:

$$X^2 = 2NFst\ (K-1)$$

with (K-1) (s-1) degrees of freedom, where N is the total sample size, K is the number of alleles for the locus, and s is the number of populations (Workman and Niswander 1970).

Although differences in electromorph frequencies were obvious between geographically distant areas, it was not clear whether significant differences existed among proximate sites. The four most proximate sampling sites within a relatively homogenous ecological zone (central Sacramento Valley) were Sacramento (site 6), Knights Landing (site 8), Millers Landing (site 9) and Williams (site 10). All sites were within a 50 mile radius from each other and had the most similar electromorph frequencies observed in this study. Fst values were calculated for three of the most polymorphic loci among these four samples and combinations of samples from other locations (Table 3). Both Pgm-1 and Mpi-1 had Fst values that were highly significant in all cases ($P = 0.001$).

Genetic Distance and Identity Values: One of the most widely used measures of genetic distance is the D value of Nei (1978). This value expresses the probability that a randomly chosen allele from each of two different populations will be identical, relative to the probability that two randomly chosen alleles from the same population will be identical. When two populations are identical, the identity value $I = 1$ and the genetic distance $D = 0$. The

Table 2. Percentage of polymorphic loci, mean heterozygocity, mean number of alleles per locus, and mean sample size per locus for populations of *Anopheles freeborni*.

	SITE	Mean sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic*	Mean heterozygosity Direct-count	Mean heterozygosity HdyWbg expected
24	RICHLAND, WA	58.9 (6.2)	1.9 (.2)	25.9	.050 (.023)	.084 (.032)
23	HERMISTON, OR	28.7 (3.3)	1.5 (.2)	14.8	.037 (.021)	.069 (.034)
25	YAKIMA, WA	39.3 (3.7)	1.9 (.2)	18.5	.039 (.015)	.062 (.019)
22	MADRAS, OR	9.5 (1.5)	1.4 (.2)	22.2	.087 (.045)	.099 (.040)
18	JASPER, CA	34.0 (3.5)	1.8 (.3)	22.2	.070 (.027)	.106 (.038)
6	SACRAMENTO, CA	40.7 (4.9)	2.7 (.4)	29.6	.080 (.033)	.125 (.042)
9	MILLERS LANDING, CA	34.9 (3.0)	2.4 (.4)	25.9	.078 (.031)	.121 (.044)
10	WILLIAMS, CA	35.2 (3.4)	2.2 (.4)	22.2	.078 (.034)	.098 (.039)
8	KNIGHTS LANDING, CA	34.2 (2.9)	2.0 (.3)	25.9	.091 (.040)	.115 (.044)
13	TEHAMA, CA	21.5 (2.2)	2.0 (.3)	18.5	.090 (.035)	.119 (.045)
12	CHICO, CA	21.2 (1.8)	1.9 (.3)	25.9	.081 (.030)	.110 (.040)
15	CLEAR LAKE, CA	49.0 (4.3)	2.5 (.4)	29.6	.079 (.028)	.112 (.039)
3	PLEASANT, CA	9.0 (1.1)	1.6 (.2)	25.9	.059 (.026)	.096 (.039)
2	CAMINO, CA	19.4 (2.6)	1.7 (.2)	22.2	.036 (.015)	.065 (.027)

*A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

Table 3. Summary of F-statistics for genotypic frequency distributions at the Got-1, Mpi-1 and Pgm-1 loci in: All samples from Oregon and Washington (Sites 22 - 25); all samples from California (Sites 1 - 21); four proximate samples within the Sacramento Valley (Sites 6, 8, 9 10); a sample from the Sacramento Valley (Site 6) with one from Utah (Site 26); and a sample from Yakima, Washington (Site 25) with one from Utah (Site 26).

Locus		Oregon + Washington	California	Sacramento Valley	Sac. Valley + Utah	Washington + Utah
	Fst	---	.049	.004	.014	---
Got-1	X^2	---	401.31	13.63	23.52	---
	P	---	.001	.700	.001	---
	Fst	.232	.119	.014	.246	.030
Mpi-1	X^2	544.02	765.41	50.60	503.81	25.68
	P	.001	.001	.001	.001	.001
	Fst	.031	.026	.015	.123	.051
Pgm-1	X^2	48.50	271.91	56.07	109.22	31.21
	P	.001	.001	.001	.001	.001

BIOSYS-1 program calculates D and I values for all possible combinations of pairs of populations.

In this study, D and I values generated for all pairs of samples shows that the genetic distance between them is low (Table 4). Even the sample from Jasper Ridge, which was found to be *An. hermsi* (explanation below), had I and D values that were indistinguishable from the intraspecific I and D values for *An. freeborni*. Overall, the sample from Yakima had the greatest genetic distance values when paired with all other samples.

Cytogenetics: Certain areas of the autosomes, particularly those near the centromere, had a propensity for asynapsis, but the degree of asynapsis varied within and between individuals of a given sample; in effect, asynapsis was not specific to any sample. Furthermore, all samples, except that collected at Jasper Ridge (site 18), were polymorphic for one or two inversions on chromosome 3. Some samples were also polymorphic for an inversion on the X chromosome.

X chromosome: All mosquitoes collected in the Sacramento Valley and in the Owens Valley had the X chromosome described by Kitzmiller and

Table 4. Genetic distance and identity values for populations of *Anopheles freeborni*. Below diagonal: Nei (1978) unbiased genetic distance; above diagonal: Nei (1978) unbiased genetic identity.

SITE		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Richland, WA	****	.993	.995	.970	.974	.983	.985	.981	.981	.982	.978	.980	.975	.965
2	Hermiston, OR	.007	****	.979	.985	.983	.988	.990	.982	.985	.988	.981	.986	.986	.973
3	Yakima, WA	.005	.021	****	.950	.954	.967	.970	.966	.964	.966	.962	.961	.958	.950
4	Madras, OR	.031	.015	.051	****	.972	.975	.979	.968	.975	.978	.972	.976	.978	.965
5	Jasper, CA	.026	.017	.047	.028	****	.995	.993	.994	.993	.993	.992	.995	.984	.970
6	Sacramento, CA	.017	.013	.033	.025	.005	****	1.000	.997	1.000	.999	.996	.997	.988	.977
7	Millers L., CA	.015	.010	.030	.021	.007	.000	****	.995	.999	1.000	.996	.997	.992	.978
8	Williams, CA	.019	.018	.035	.032	.006	.003	.005	****	.999	.995	.999	.997	.983	.974
9	Knights L., CA	.019	.015	.036	.025	.007	.000	.001	.001	****	1.000	.998	.998	.989	.979
10	Tehama, CA	.018	.012	.035	.022	.007	.001	.000	.005	.000	****	.995	1.000	.992	.979
11	Chico, CA	.023	.019	.039	.028	.008	.004	.004	.001	.002	.005	****	.996	.984	.972
12	Clear Lake, CA	.021	.014	.040	.024	.005	.003	.003	.003	.002	.000	.004	****	.986	.974
13	Pleasant, CA	.026	.014	.043	.022	.017	.012	.008	.017	.011	.008	.017	.014	****	.990
14	Camino, CA	.035	.027	.051	.036	.031	.024	.022	.027	.021	.022	.029	.027	.010	****

Baker (1963) as that of *An. freeborni* (Fig. 2, Table 5). Samples from the coastal region or the foothills of the Sierra Nevada, however, were either fixed for the inversion homokaryotype (type found in *An. hermsi*) or were polymorphic for the inversion (included heterokaryotypes). At Clear Lake (site 15), for example, 8% of the mosquitoes sampled were heterokaryptypes. Jasper Ridge (site 18) and Camino (site 2), on the other hand, were fixed for the inversion karyotype.

All mosquitoes collected in Madras, Oregon (site 22) were inversion homokaryotypes (Fig. 3, Table 5). As one proceeded north into Washington, the frequency of the standard homokaryotype increased to as high as 0.90 in Yakima (site 25). The observed frequencies of the homokaryotypes and heterokaryotypes in Hermiston (site 23), Richland (site 24), and Yakima (site 25) did not differ significantly from those expected under Hardy-Weinberg equilibrium (Table 5). A test of homogeneity was done to determine whether the separate samples were sufficiently uniform to express a common over-all ratio of the three karyotypes; no two samples were found to be homogenous ($P \leq 0.05$).

Chromosome Arm 3L: An inversion on 3L was by

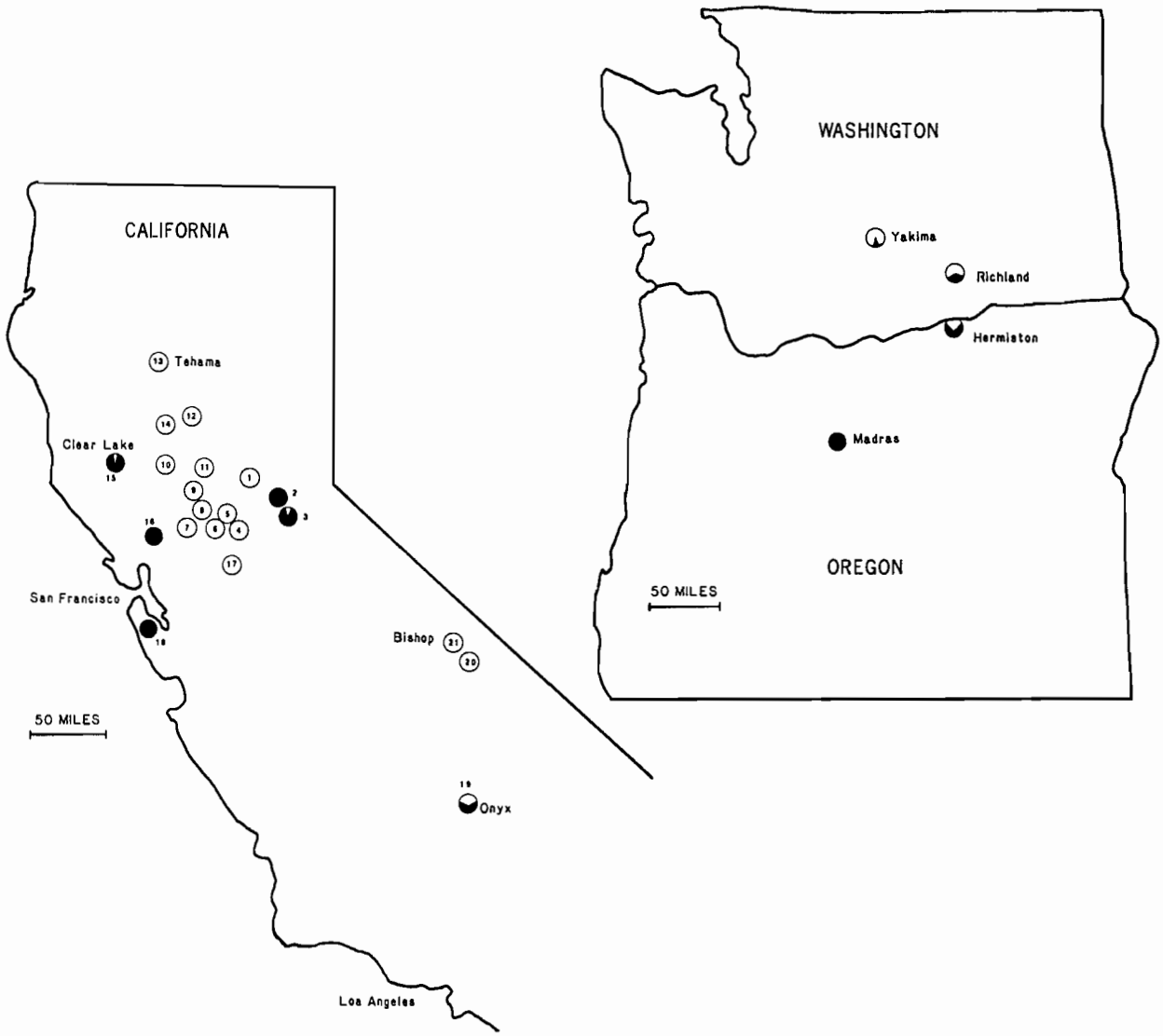

Figure 2. Frequency of the standard (white) and inversion (black) karyotype for the X chromosome at various collection sites in California, Oregon, and Washington.

Table 5. The observed (o) and expected (e) numbers of standard homokaryotypes (S/S), inversion homokaryotypes (I/I) and heterokaryotypes (S/I) for an inversion on the X chromosome of *Anopheles freeborni* collected from various sites in California, Oregon and Washington.

		Chromosome X								
		S/S		S/I		I/I			Frequency	
Site*	n	o	e	o	e	o	e	X^2	S	I
1	1	1	-	0	-	0	-	-	1.00	0.00
2ab	27	0	0	0	0	27	0	0	0.00	1.00
3ac	13	1	0.08	0	1.85	12	11.08	12.51	0.08	0.92
4	9	9	9	0	0	0	0	0	1.00	0.00
5	2	2	-	0	-	0	-	-	1.00	0.00
6	50	50	50	0	0	0	0	0	1.00	0.00
7	3	3	-	0	-	0	-	-	1.00	0.00
8	22	22	0	0	0	0	0	0	1.00	0.00
9	35	35	35	0	0	0	0	0	1.00	0.00
10	50	50	50	0	0	0	0	0	1.00	0.00
11	22	22	22	0	0	0	0	0	1.00	0.00
12	27	27	27	0	0	0	0	0	1.00	0.00
13	44	44	44	0	0	0	0	0	1.00	0.00
14	50	50	50	0	0	0	0	0	1.00	0.00
15b	60	0	0.01	5	4.56	55	55.20	0.04	0.04	0.96
16	1	-	-	-	-	1	-	-	0.00	1.00
17	16	16	16	0	0	0	0	0	1.00	0.00
18a	41	0	0	0	0	41	41	0	0.00	1.00
19	4**	0	-	3	-	1	-	-	0.38	0.62
20	9	9	9	0	0	0	0	0	1.00	0.00
21	2	2	-	0	-	0	-	-	1.00	0.00
22ab	15	0	0	0	0	15	15	0	0.00	1.00
23c	47	6	3.61	14	18.83	27	24.57	3.06	0.28	0.72
24d	73	26	27.73	38	34.46	9	10.73	0.75	0.62	0.38
25e	50	41	40.50	8	9.00	1	0.50	0.62	0.90	0.10

* Sample sites followed by the same letter do not differ significantly in frequencies of S/S, S/I and I/I (homogeneity X^2 $P \geq 0.05$).

** Includes two females collected by Stan Cope.

Table 6. The observed (o) and expected (e) numbers of standard homokaryotypes (S/S), inversion homokaryotypes (I/I) and heterokaryotypes (S/I) for an inversion on chromosome arm 3L of *Anopheles freeborni* collected from various sites in California, Oregon and Washington.

		Chromosome arm 3L								
		S/S		S/I		I/I			Frequency	
Site*	n	o	e	o	e	o	e	X^2	S	I
1	1	0	-	1	-	0	-	-	0.50	0.50
2a	27	13	12.67	11	11.65	3	2.68	0.08	0.67	0.33
3a	13	8	8.48	5	4.05	0	0.48	0.74	0.78	0.22
4a	7	4	3.56	2	2.86	1	0.57	0.64	0.71	0.29
5	2	1	-	1	-	0	-	-	0.75	0.25
6a	49	31	27.94	12	18.30	6	2.94	5.59	0.76	0.24
7	3	1	-	2	-	0	-	-	0.67	0.33
8a	22	10	10.23	10	9.57	2	2.23	0.05	0.68	0.32
9a	35	23	23.20	11	10.60	1	1.21	0.05	0.81	0.19
10a	50	27	25.92	18	20.16	5	3.92	0.54	0.72	0.28
11a	22	14	13.92	7	7.17	1	0.92	0.01	0.80	0.20
12a	23	15	14.08	6	7.81	2	1.09	1.24	0.78	0.22
13a	44	28	26.29	12	15.44	4	2.27	2.21	0.77	0.23
14a	50	31	32.00	18	16.00	1	2.00	0.78	0.80	0.20
15ab	58	45	44.83	12	12.34	1	0.84	0.04	0.88	0.12
16	-	-	-	-	-	-	-	-	-	-
17a	16	10	9.00	4	6.00	2	1.00	1.78	0.75	0.25
18c	41	41	41.00	0	0	0	0	-	1.00	0.00
19	2	0	-	2	-	0	-	-	0.50	0.50
20a	9	6	6.25	3	2.49	0	0.25	0.36	0.83	0.17
21	2	2	-	0	-	0	-	-	1.00	0.00
22bc	15	15	15.00	0	0	0	0	-	1.00	0.00
23c	46	45	44.99	1	1.00	0	0.01	0.01	0.99	0.01
24c	70	69	69.02	1	0.97	0	0.003	0.00	0.99	0.01
25bc	48	45	45.05	3	2.91	0	0.05	0.05	0.97	0.03

* Sample sites followed by the same letter do not differ significantly in frequencies of S/S, S/I and I/I (homogeneity X^2 $P \geq 0.05$).

far the most common inversion in samples collected in California (Table 6). This inversion is the same as that described by Frizzi and DeCarli (1954) and Kitzmiller and Baker (1963). Except for Jasper Ridge (site 18), Clear Lake (site 15), and several locations with small sample size, the frequency of In(3L)A was remarkably similar throughout California and ranged from 0.19-0.33. None of the observed frequencies of homokaryotypes and heterokaryotypes differed significantly from those expected under Hardy-Weinberg equilibrium (Table 6). Furthermore, none of the samples collected in California, except for Jasper Ridge (site 18), differed significantly from each other in the frequency of the inversion (test for homogeneity, $P \geq 0.05$). In Oregon and Washington the frequency of In(3L)A was very low (0.03 or less) and all samples were homogenous for this inversion ($P \geq 0.05$).

Chromosome Arm 3R: Although Menchaca (1986) reported three inversions on chromosome arm 3R, In(3R)A was essentially the only inversion found on this arm in this study. In(3R)C was observed from samples taken within the Sacramento Valley, but the frequency was too low to be included in any analysis.

The standard karyotype for 3R was most common in all samples from Oregon and Washington and there were no significant frequency differences

Table 7. Numbers of standard homokaryotypes (S/S/), inversion homokaryotypes (I/I) and heterokaryotypes(S/I) for an inversion on chromosome arm 3R of *Anopheles freeborni* collected from various sites in California. The percentage of heterokaryotypes for In(3R)A and/or In(3L)A is also given.

					Frequency		% Heterokaryotypes
Site	n	S/S	S/I	I/I	S	I	In3L + In(3R)A
2a	27	27	0	0	1.00	0.00	40.7
3ab	13	13	0	0	1.00	0.00	38.5
4	6	0	3	3	0.25	0.75	66.7
5	2	0	0	2	0.00	1.00	50.0
6d	49	3	11	35	0.17	0.83	42.9
7	3	0	1	2	0.17	0.83	66.7
8e	22	0	0	22	0.00	1.00	45.5
9e	35	1	3	31	0.07	0.93	40.0
10e	50	0	1	49	0.01	0.99	38.0
11d	22	0	5	17	0.11	0.89	54.5
12e	23	0	2	21	0.04	0.96	31.8
13d	44	0	8	36	0.09	0.91	45.5
14e	50	0	0	50	0.00	1.00	36.0
15a	58	56	2	0	1.00	0.00	20.7
17e	16	0	0	16	0.00	1.00	25.0
18a	41	41	0	0	1.00	0.00	0.0
19	3	2	1	0	0.83	0.17	33.3
20e	9	0	0	9	0.00	1.00	33.3
21	2	0	1	1	0.00	0.75	50.0
22c	15	11	4	0	0.87	0.13	26.7
23c	46	37	9	0	0.90	0.10	19.6
24bc	70	57	13	0	0.91	0.09	21.4
25c	48	37	11	0	0.89	0.11	27.1

* Samples followed by the same letter do not differ significantly in frequencies of S/S, S/I and I/I (homogeneity X^2 $P \geq 0.05$).

between sites (homogeneity test, $P \geq 0.05$) (Table 7). In the Sacramento Valley, California the reverse was true. The inversion was either fixed or was the most common karyotype, and the frequency of heterokaryotypes varied dramatically between sites, even when these were in close proximity. At site 10, for example, 2% of all individuals sampled were heterokaryotypes, whereas, at a site 30 miles away (site 11) the frequency was 22.7%.

In California no inversion heterokaryotypes were found in half of the collection sites in which the sample size was at least 13 individuals. These sites appeared to be fixed for the standard or inversion karyotype. Those sites at high elevation or on the coast (sites 2, 3, 15, 18) were fixed for the standard karyotype or had a very low frequency of In(3R)A. Alternatively, samples from sites within the Sacramento Valley (sites 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 17) were fixed for the inversion karyotype or had it in high frequency.

rDNA Probe: Samples of mosquitoes from 11 of 12 collection sites in California, Washington and Oregon had the same restriction enzyme fragment pattern regardless of their type of X chromosome (Table 8). Mosquitoes from Jasper Ridge (site 18), and those from samples of *An. hermsi* collected in southern California were the only individuals with a restriction fragment pattern specific to *An. hermsi*. No individuals were found to a have pattern that was a hybrid of that found in *An. hermsi* and *An. freeborni*.

Hybridization: In the parental crosses, all strains mated freely with each other and produced viable offspring. The F_1 hybrid males from crosses between the LAKE and DAVIS strains had genitalia and quantities of sperm that were similar to those observed in the controls (Table 9). However, hybrid males from crosses between the LAKE or DAVIS strains with the JASP strain were completely or partially sterile. When the male parent was from the DAVIS or LAKE strain and the female was from the JASP strain, the hybrid male progeny had no sperm in their testes. Although the genitalia appeared to be normal, the testes were often translucent and smaller than the controls. Hybrid males, from crosses in which DAVIS or LAKE was the female parent, had varying amounts of sperm in their testes. The amount varied from none to quantities that looked near normal. In general, the testes were filled with what appeared to be globular spermatocytes and partially developed spermatozoa.

The results of the backcross series substantiated the results obtained from the dissection of hybrid males. All crosses involving

Table 8. Sample site, type of polytene X chromosome, and the rDNA probe determination of species. F = *freeborni* type; H = *hermsi* type; HF = heterokaryotype; - = Not scored.

Site	X Chromosome	rDNA Probe	Site*	X Chromosome	rDNA Probe
2	H	F	20	F	F
2	H	F	20	F	F
2	H	F	22	H	F
6	F	F	22	H	F
6	F	F	23	HF	F
6	F	F	23	F	F
12	F	F	23	H	F
12	F	F	24	HF	F
12	F	F	24	F	F
13	F	F	24	H	F
13	F	F	25	HF	F
13	F	F	25	F	F
15	HF	-	25	H	F
15	H	F	1003	H	H
15	H	F	1003	H	H
18	H	H	1063	H	H
18	H	H	1063	H	H
18	H	H	1063	H	H
19	HF	F	1074	H	H
19	-	F	1074	H	H
19	-	F	1074	H	H

* Collections of *An. hermsi* from southern California.
1003: Riverside County, Rubidoux, Carlson Park
1063: Ventura County, Piru Creek
1074: San Luis Obispo County, Santa Margarita

Table 9. Fertility of progeny from crosses between four strains of *Anopheles freeborni*.

Parental Crosses			Fertility of Progeny	
Male		Female	Male	Female
DAVIS	X	DAVIS	Normal	Normal
LAKE	X	LAKE	Normal	Normal
JASPER	X	JASPER	Normal	Normal
WASH	X	WASH	Normal	Normal
DAVIS	X	LAKE	Normal	Normal
LAKE	X	DAVIS	Normal	Normal
DAVIS	X	WASH	Normal	Normal
WASH	X	DAVIS	Normal	Normal
DAVIS	X	JASPER	Sterile	Normal
JASPER	X	DAVIS	Low	Normal
LAKE	X	JASPER	Sterile	Normal
JASPER	X	LAKE	Low	Normal

hybrid males, in which DAVIS or LAKE had been the parental male, produced eggs that did not hatch. Nor did the eggs contain any stage of embryonic development. In the reciprocal crosses, the percentage hatch was significantly less than the control hatch, and unhatched eggs also contained no embryos. There were no significant differences between controls and backcrosses for the number of eggs laid/female. All hybrid females were fertile and had a similar percentage hatch as controls when backcrossed to either parental strain. Many of the backcrosses had a percent hatch that was higher than the controls, indicating possible heterosis, but none of these differences were significant.

The ovarian polytene chromosomes of hybrid progeny did not differ from controls in the amount or degree of synapsis, or in banding pattern.

Discussion.

Since, according to Barr and Guptavanij (1988), *An. hermsi* and *An. freeborni* differ by a fixed inversion in the X chromosome, it was unexpected to find heterokaryotypes for this inversion at so many sampling sites. The large number of heterokaryotypes raised some important questions:

1. Were *An. freeborni* and *An. hermsi* really distinct species?
2. Are *An. freeborni* and *An. hermsi* hybridizing in sympatric populations?
3. Are both or one species polymorphic for an inversion on the X chromosome?
4. Are populations with a polymorphic inversion on the X chromosome a new sibling species?

Samples from Yakima (site 25), Hermiston (site 23) and Richland (site 24) have frequencies of homokaryotypes and heterokaryotypes that are consistent with those expected under Hardy-Weinberg equilibrium. In effect, mosquitoes at these locations are mating randomly with respect to X chromosome type - not an indication of the presence of two or more species.

The results of the rDNA probe indicate that all samples, except Jasper Ridge (site 18), are conspecific. It is noteworthy that the population at Jasper Ridge was the only one that did not have any polymorphic inversions whatsoever. This was also true for the only other population of *An. hermsi* that was ever sampled for the presence of inversions (Menchaca 1986).

Hybridization of strains from various collection sites confirmed the results obtained with the rDNA probe; only crosses that included the Jasper Ridge strain produced sterile hybrids. The pattern of male sterility was the same as that reported by Fujioka (1988) when he crossed *An. hermsi* to *An. freeborni*.

The results from the cytogenetic survey, laboratory hybridizations, and the rDNA probe are all consistent in assigning only the sample from Jasper Ridge to the species *An. hermsi*. It is also clear now that the X chromosome is not a reliable character for distinguishing *An. hermsi* from *An. freeborni*. In fact, there are no differences in any of the polytene chromosomes between these two species; *An. hermsi* has merely a subset of the variation found in *An. freeborni* (this conclusion is also substantiated by observations of hybrid polytene chromosomes).

Prior to this study, *An. hermsi* was known only as far north as Santa Maria, San Luis Obispo County and no further inland than 75 km from the coast (Barr et al. 1987, Cope et al. 1988). It is now clear that this species extends up the California coast as far north as San Mateo County, and probably further. Bailey et al. (1972) reported collecting *An. freeborni* near San Pablo Bay and along the Russian River near Healdsburg (Sonoma County). Since both sites are near the coast, it is probable that these mosquitoes were actually *An. hermsi*.

There is no indication from our results that all other sampling sites (aside from Jasper Ridge) include two or more cryptic species. The compliance of chromosome inversion frequencies with Hardy-Weinberg expectations, and the absence of sterility in the hybrids (regardless of X chromosome type) support the hypothesis that these samples represent a single species. Apparently, populations of *An. freeborni* can have three different karyotypes with respect to the X chromosome: Some are fixed for the inversion homokaryotype (e.g. Madras, Oregon), others are fixed for the standard homokaryotype (e.g. Sacramento Valley), and some are polymorphic (e.g. Clear Lake, Onyx, Hermiston, Richland, and Yakima). Ethological barriers to mating may certainly exist between these purported conspecific populations, but unless diagnostic characters are found along with sites where two or more mating types are sympatric, it is not possible, at present, to distinguish these specifically. The only unusual collection site, with respect to the X chromosome, was site 3 in the foothills of the Sierra Nevada. At this site, one standard homokaryotype was found among 12 inversion homokaryotypes. The expected frequency of the standard homokaryotype in Hardy-Weinberg equilibrium would be 0.08.

Anopheles freeborni appears to have relatively low levels of enzyme polymorphism, particularly for

a species that is so widespread and inhabits such a variety of ecological zones. The majority of loci analyzed in this study are monomorphic, but even of those loci that are polymorphic only two or three have many alleles. Some polymorphic loci (eg. some of the esterases) were not included in the analysis of variability because the zymograms of these enzymes were too smeary and could not be read with any confidence. This may explain the relatively low heterozygocity values observed for this species.

Like Fujioka (1986), this study did not find any loci that were diagnostic for distinguishing *An. freeborni* from *An. hermsi*. In addition, the genetic differentiation (genetic distance) between both species is no greater than that found intraspecifically for *An. freeborni*. At present, *An. hermsi* and *An. freeborni* can only be distinguished by hybridization or rDNA analysis. This is the first example, in the culicids, of sibling species that cannot be distinguished by both polytene chromosome banding pattern and allozyme frequencies.

There is obvious genetic substructuring of *An. freeborni* throughout its range, both in the frequencies of chromosome inversions and allozymes. In general, the genetic variation between populations reflects differences in ecological zones (e.g. Sierra Nevada vs Sacramento Valley) or geographic distance. In California, for example, X chromosome karyotype frequencies seem to have a pattern of sorts. In valleys, whether at high or low altitude (e.g. Owens and Sacramento Valleys), only the standard homokaryotype is present. Samples in hilly or mountanous zones are either polymorphic for the X inversion or may be fixed for the inversion homokaryotype (Camino, Pleasant Valley Rd., Clear Lake and Onyx; sites 2, 3, 15, 19, respectively). The presence of the inversion homokaryotype does not seem to be solely correlated with altitude, since mosquitoes collected in the Owens Valley (highest sites in this study) had the standard X chromosome only. This digression from a purely altitudinal correlation was also observed in samples from Washington and Oregon.

Differences in the frequencies of chromosome inversions and allozymes are not unexpected between relatively distant or isolated populations of a species. Such populations could have minimal or no genetic exchange, and selection and genetic drift may generate interpopulation variation. There are, however, significant microgeographical differences in the frequency of In(3R)A and electromorphs at some enzyme loci even within the Sacramento Valley. As Coluzzi et al. (1979) point out, attempts to explain such variations involve testing various hypotheses that are not mutually exclusive: 1. population bottlenecks with subsequent genetic drift; 2. different selective pressures depending on different adult environments and/or larval breeding places not uniformly distributed in space and/or time; 3. non-random distribution of the genetic variants at the adult stage due to different behavioral responses in a heterogenous environment. Although Coluzzi et al. (1979) suggested that non-random distribution of adults was an important contributing factor in their study, this effect would not seem to be important in this investigation. Within the Sacramento Valley, nearly all adults were collected under similar conditions. The collection sites were near irrigated rice fields and adults were obtained from under bridges. In many areas, bridges appeared to offer the only shelter from the sun.

Population bottlenecks with subsequent genetic drift seem more plausible explanations for the variation in the frequencies of allozymes and of In(3R)A. Most breeding sites for *An. freeborni* in the Sacramento Valley disappear during the late summer and early fall when rice fields are drained. All or most adult females are then presumed to migrate to overwintering sites within the foothills of the surrounding mountains (Bailey and Baerg 1966; Bailey et al. 1972). How newly-irrigated fields are re-populated with *An. freeborni* in the spring and summer is not known. Perhaps some females migrate back into the Sacramento Valley, or flooded fields are merely re-populated by the few females that may have overwintered locally. Either scenario, it is possible that newly established populations originate from few overwintering females.

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MOSQUITO CONTROL SIMULATION ON THE CONNECTION MACHINE[1]

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ABSTRACT

We have developed a computer model of *Culex pipiens* mosquitoes which attempts to capture, through simulation, the complex interplay of environmental, geographical and human factors which affect mosquito populations. We recently implemented this model on the Connection Machine, a massively parallel supercomputer, which can simulate the development of thousands of breeding sites in parallel, providing a substantial speed-up over conventional, serial computers. A built-in expert system, or rule-based decision-making program, is used to manage insecticide treatments on the breeding sites. The program is used to predict the probable effect of complex mosquito control strategies. The Connection Machine provides sufficient speed-up that it should now be possible to simulate the evolution of resistance to insecticides in realistic settings.

Introduction.

Vector and disease control efforts would be greatly aided by a good simulation system for mosquito populations. Such a system would help in evaluating different control strategies and assist efforts to direct limited resources to those places where they will be most effective. Our laboratory has been developing such a system, first for the mosquitoes and management practices of Orange County, in Southern California, and more recently for Alameda County, in Northern California (Taylor et al. 1988; Fry et al. 1989). This model reproduces past records of mosquito abundance fairly accurately and is robust to changes in management practices. It thus represents an important improvement over past efforts such as those of (Haile 1986) and (Greever and Georghiou 1979). However, the simulations are slow, taking 30-40 hours on Apollo DN4000 workstations to simulate one year. This slowness seriously compromises the model's ability to quickly and efficiently explore large numbers of alternative practices for control and to incorporate additional complications, such as insecticide resistance.

Recent developments in computer hardware offer the opportunity to speed up these simulations significantly. For example, the Connection Machine, a parallel computer with as many as 65,536 processors, is capable of sustained operation at 5,000 million floating point operations per second (Anonymous 1987). The architecture of the Connection Machine is such that a conventional front-end computer broadcasts single instructions, and the thousands of parallel processors simultaneously carry out its instructions on the data in their own memories (Hillis 1985). Not all problems will give substantial speedup from this approach, but many of them, termed data-parallel problems, will (Hillis and Barnes 1987).

The mosquito simulation that we have developed is highly data-parallel. That is, it involves many breeding sites acting according to more or less the same program, but with data peculiar to their own situation. With a Connection Machine, thousands of breeding sites can be represented by separate processors, each with its own memory storing data peculiar to it its own circumstances, executing in concert. The speedup in execution time over a serial computer can be on the order of thousands, because of the large number of processors. This offers the exciting prospect that many problems that were once too large can now be practically addressed. Programming such highly parallel devices requires a radically different computational approach. Below we describe how we have written our simulation program to run on

[1] Connection Machine is a registered trademark of Thinking Machines Corporation.

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such a machine and present some of our first results.

Overview of the simulation.

In the past we developed programs which were capable of simulating *Culex pipiens* mosquito populations over a large geographical area. The immatures are grouped into breeding sites, which develop in daily increments depending on the type of site, air and water temperature, precipitation, and mosquito control measures.

The region to be simulated is divided into a rectangular grid of locations. Each breeding site is assumed to exist in a specific location on the grid, and is influenced by the environmental conditions there. Each breeding site is considered to be of a certain type, such as a swimming pool, marsh, storm drain, flood control channel or container. In principle, the model can simulate any desired region, provided the user specify the types and distribution of breeding sites, as well as provide weather data files for the locations in that region. We downloaded weather data files for Orange County and Alameda County from the University of California Statewide Integrated Pest Management Project (IMPACT), which offers data files for hundreds of weather stations throughout California. Daily temperature and precipitation data are available for specific past years, as well as 30-year average data.

An expert system, or rule-based decision-making program, is used to make treatment decisions for mosquito control, using rules specified by the user. This approach gives the flexibility necessary to model the effects of a wide variety of treatment strategies. As the program executes, the adult mosquito population is monitored at regular intervals and recorded in an output file. Thus, the simulated populations can be compared to actual trap data for specific past years. In the future, we plan to add a genetic model of insecticide resistance to the program, in order to determine when resistance might occur and how to prevent it.

Non-parallel versions of this program are described in (Fry 1989) and in (Fry et al. 1989). These versions use the RAM simulation system (Taylor et al. 1988; Goldman 1990) to achieve parallel-like data abstraction for the breeding sites. They run on ordinary sequential computers such as Apollo and Sun workstations, as well as higher-end Macintosh and IBM-compatible microcomputers.

Population modeling within processors.

The UCLA Connection Machine has 16,384 processors, each with 8K bytes of memory. The simulation program described below is written in *Lisp (pronounced "star Lisp"), a superset of the Common Lisp programming language which includes parallel processing commands and data structures (Anonymous 1988; Steele 1990). Our program makes use of this parallel structure by modeling each breeding side on a separate processor. Each breeding site is a separate, simple compartment model of its population, where survival and development time depend on the local weather variables and on insecticide applications. In the serial version each breeding site stores the adult population in an array and the immature population in a dynamic linked list of cohorts. On the Connection Machine, where data structures must be of uniform size across processors, we represent both adult and immature populations as static arrays.

Figure 1 shows how data are stored in a sample processor during the simulation. Each active processor is initialized with arrays which hold several cohorts of mosquitoes in a particular breeding site at various stages of development. We refer to each compartment or element of a life stage array as a tier. A tier holds the number of mosquitoes at a discrete level of development. They will typically not correspond directly to larval stages, but rather to the time spent in that stage of development. As mosquitoes develop to maturity, they move up through these life stage arrays by advancing through the tiers.

A mosquito population, contained in one tier, will "jump forward" n tiers on a given day according to the formula $n=td$, where t is the number of tiers holding the population and d is the development rate. We set the adult female development rate to a constant value of 0.2. Immature development, in contrast, is based on water temperature at the location of the breeding site. We use the following immature development rates, developed in a field study by Madder et al. (1983), where d is the development rate and t is the water temperature in degrees celsius:

For egg stage: $d = 0.0325\,t - 0.2394$
For larval and pupal stages: $d = 0.00795\,t - 0.0749$

The number of tiers in each life stage can be set at the beginning of the program, according to the desired processor configuration and complexity of the model. We generally use four tiers for the egg stage, twelve tiers for the larval stages, four tiers for the pupal stage, and twelve tiers for the adult females (Fig. 1). Males can optionally be ignored by the simulation to improve processing

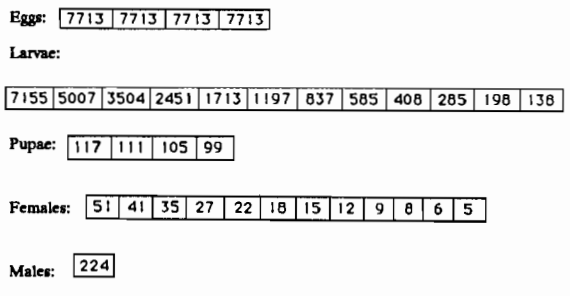

Figure 1. Information stored in a sample processor of the Connection Machine during the simulation. Each active processor simulates one breeding site at a specific location on the environment grid. Adult female and immature populations advance through the arrays as they age, usually decreasing due to imperfect survival. The number of adult males is stored as a single integer, the other stages are divided into tiers. Larval carrying capacity and chances of being known about by the MAD depend on type of source. The nearest weather station determines the temperature and precipitation for each site.

speed.

Each simulation day, the active processors execute the following algorithm in parallel:

1. Calculate immature development rates, based on water temperature at the nearest weather station.
2. Calculate adult survival rate, based on air temperature at the nearest weather station.
3. Calculate larval survival rate, based on larval density.
4. Advance populations through their life stages, based on development and survival rates calculated above. Only the surviving percentage can advance; the others are removed from the array. Those that grow out of their life stage array go into the first tier of the next one.
5. Those females about to exit their life cycle (generally on day 6) will lay eggs, either at the current site, a nearby site, or a new site. If they survive, they can oviposit again, typically after three days. New eggs go into the first tier of the eggs array at the site at which they are oviposited.

Weather stations provide only air temperature and precipitation. We calculate water temperature t_w from air temperature t_a at each weather station using the following formula, derived from a southern California field study (Fujioka 1981):

$$t_w = 22.183 \ln t_a - 47.332$$

We also calculate the standing water at a weather station as r/d where r is the total number of inches of the last period of rainfall, and d is the number of days since that period. By period of rainfall we mean contiguous days in which precipitation occurs.

Our model of day-to-day immature survival is taken from a field study of *Culex pipiens* in Los Angeles (Wilmot 1986). Survivorship is assumed to depend on life stage: all eggs survive from day to day; the daily survival rate for pupae is set to a constant value of 0.95; daily larval survival depends on population density. We use the following formula from Wilmot's model to implement density dependence:

$$S = \begin{cases} S_{max} - N/K\,(S_{max} - S_{min}) & \text{if } N <= K \\ S_{min} & \text{if } N > K \end{cases}$$

where S is larval survival rate, S_{max} and S_{min} are the maximum and minimum survival rates possible, N is the total number of larvae present, and K is the carrying capacity of the system. When N is greater than K, survival is set to S_{min} in order to avoid a negative value. In our model, S_{min} is set to 0.01, S_{max} to 0.95, and the larval carrying capacity depends on the source type. The larval carrying capacity of each source type must be specified by the user.

The daily survival rate of adults is based on air temperature in that zone, and implemented according to the following formula from Haile (1986):

$$S = \begin{cases} 0.85 + 0.005\,T_{adj} & \text{if } T_{adj} < 0 \\ 0.85 - 0.015\,T_{adj} & \text{if } T_{adj} >= 0 \end{cases}$$

where S is adult survival rate, T_{air} is air temperature, and $T_{adj} = T_{air} - 24$, which is the adjusted air temperature. This equation is based on a maximum survival rate of 0.85, which occurs at the optimal temperature of 24° C. Survival decreases as the temperature deviates from the optimal value. Adult survival rate is calculated each day for each zone.

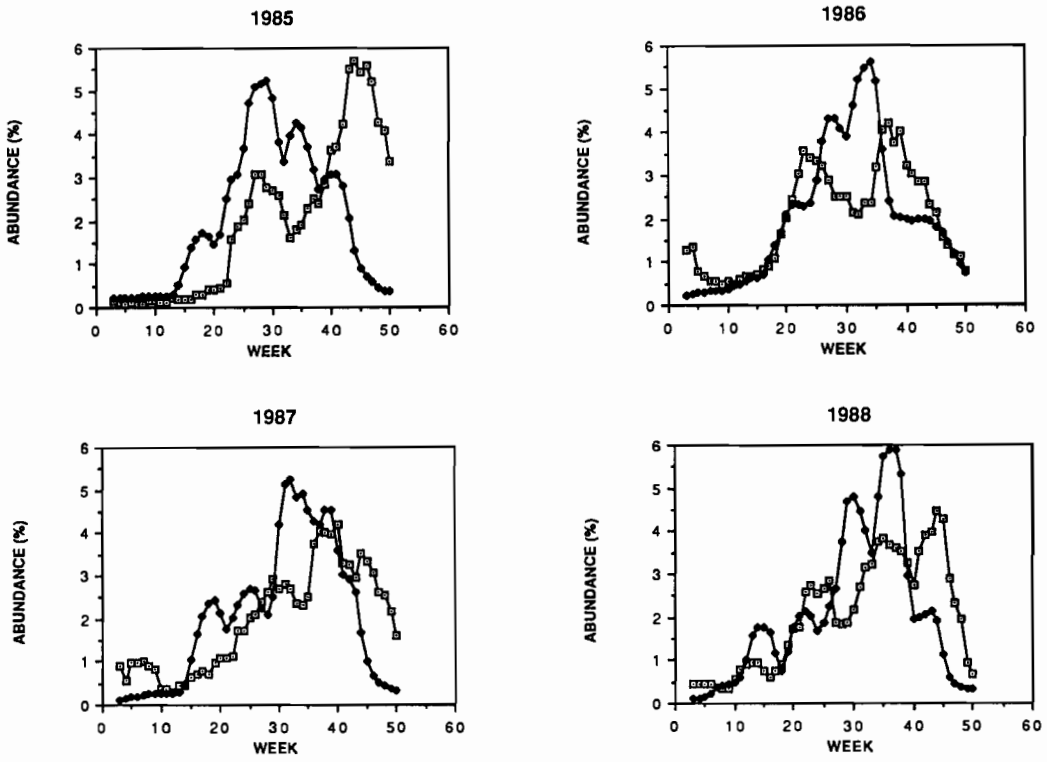

Figure 2. Simulated (open boxes) and observed (closed boxes) abundances of female *Culex pipiens* for Orange County, California during 1985-1988.

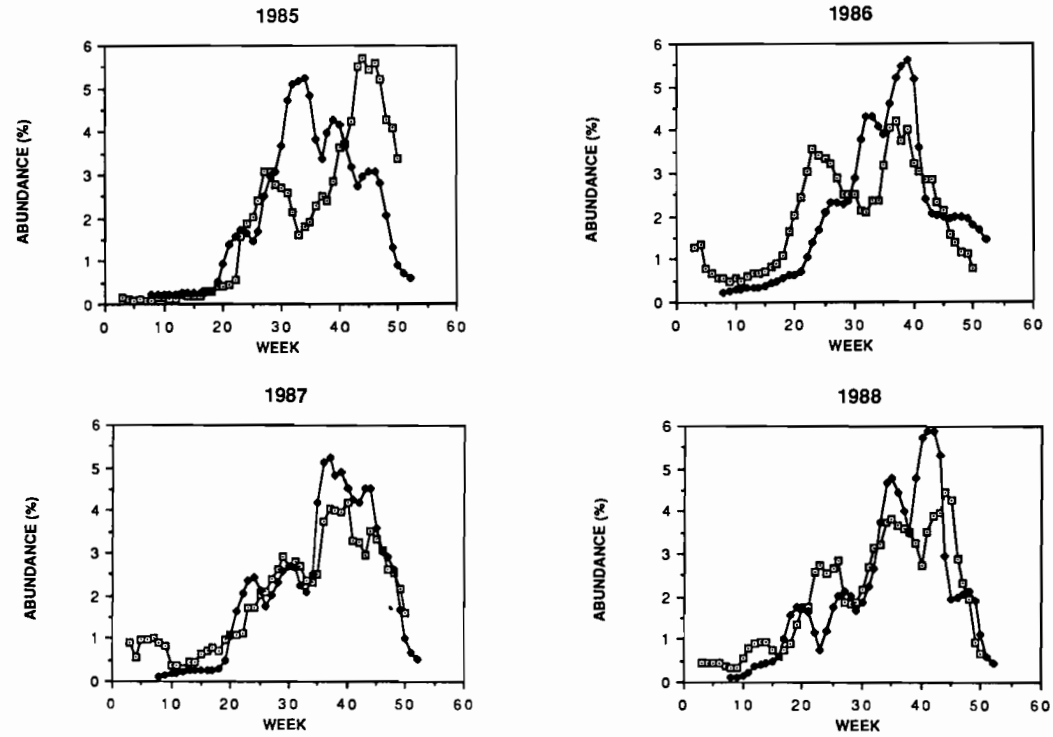

Figure 3. Simulated (open boxes) and observed (closed boxes) abundances of female *Culex pipiens* for Orange County, California during 1985-1988. In this case the simulated abundances were shifted 5 weeks to the right. See text for discussion.

A female in the egg-laying stage can oviposit in one of three ways: (1) in the breeding site from which she emerged; (2) in a random breeding site in the same location; or (3) in a new breeding site in the same location, thus activating a new processor on the Connection Machine. The model assumes that females never migrate out of their grid location. It has proven difficult to measure adult movement by mosquitoes in this area precisely (Palca 1988), but existing evidence suggests that movement between the 4 mile-square locations is sufficiently small that it can be ignored (J. Webb, personal communication). At the beginning of the program, the user can specify the likelihood of each egg-laying behavior as a function of standing water at the nearest weather station.

Expert system for mosquito control.

Our program simulates the effects of mosquito control, and can be used to test different control strategies. The user can define treatment types, corresponding to pesticides, predators, or other forms of mosquito control, and specify the effectiveness of the treatment on each mosquito life stage at the sites where the treatment is applied. The user must also specify the likelihood that each source type will be "known about" by the local mosquito abatement district, and the likelihood that new sites of that source type will be discovered. Only "known about" breeding sites are affected by treatments (Fig. 1).

A major goal of the program is to take into account the decision-making process of mosquito abatement; that is, when to take control measures and when not to. To this end, a technique from artificial intelligence known as expert systems or rule-based decision-making is used (Winston 1984; Hayes-Roth et al 1983). This approach allows a certain amount of flexibility in specifying control strategies.

The user specifies the treatment rules in the following format:

RULE <*rule - name*>
IF<*condition 1*> <*condition 2*>...< $condition_n$>
THEN <*consequent*>

If all the conditions in a rule are met in the simulation, then the rule is said to be activated and the consequent is executed. This process of testing conditions to arrive at conclusions is called *forward chaining*. For this program, the consequent can be an assertion to be matched by the condition of different rule, or else a command to treat. A consequent can specify that a certain treatment type is used against certain source types in a certain area.

Here, for example, are two treatment rules recognized by the system:

```
RULE 1
IF MONTH > APRIL
   MONTH < OCTOBER
THEN POOLS ARE BEING USED

RULE 2
IF
  POOLS ARE BEING USED
  DAYS SINCE LAST TREATMENT OF
   POOLS > 12
  PRECIPITATION IN (? REGION) < 0.1
THEN TREAT POOLS IN (? REGION) WITH
   GB-OIL
```

Note that a conclusion from the Rule 1 is used by Rule 2 in making its decision. Also, a variable was used in Rule 2 in order to make the rule apply to any region for which the rule is true. Thus, every 12 days in the summer months, Rule 2 will treat pools in all regions for which the day's precipitation is less than 0.1 inches. Variables can be used in this way to make the same rule apply to any number of treatments, source types, or regions.

Evaluation and discussion.

The model we have developed to simulate mosquito populations under various control strategies has been described elsewhere (Fry et al. 1989), and has been found to fit such field data as exist reasonably well. A detailed analysis of the fit is currently in preparation. Simulations on the most widely available computers take enough time that the model's use for comparing alternative strategies is limited and extending it to insecticide resistance on those machines is not feasible at this time. The primary goals of this study were to see whether that model could be adopted to the parallel Connection Machine and, if so, to get an idea of its performance there.

The model was modified from its serial version to run on the UCLA Connection Machine without much difficulty. There were a number of small differences between implementations, the most significant of which was the calculation of development rates as a function of temperature. The serial model used a table look-up, based on empirical measurements of Shelton (1973). This technique is inefficient to implement in a straightforward manner on the Connection Machine, so we used a formula for development that was empirically derived by Madder et al. (1983). The

estimates from the two methods are similar, but not identical. The Madder estimates tend to be a little bit shorter, on average. Which of the two methods is more accurate for Orange County is difficult to assess, because that would involve measuring the density and temperature of a wide variety of breeding sites, and then weighing them according to the contribution of each to the total adult mosquito abundance. While it is desirable to have a correspondence between all phases of the simulations and the population to be simulated, until more knowledge is available the best measure of how well the model works is simply how well the abundance predicted by the model fits the available data on actual abundance.

The predicted and measured densities of mosquitoes for 1985-1988 are shown in Figures 2 and 3. In each case these have been smoothed by taking 3-week running averages and normalizing so that comparisons are made within the year. Changes in personnel, trap location and techniques make comparisons across years of questionable value.

The fits between observed and simulated are unlike any we have observed in our previous simulations. From Figure 2 it is apparent that the fits are not especially good for the direct simulations themselves. (For 1985-1988 the correlations are 0.28, 0.64, 0.64, and 0.63, respectively). However, when the abundances predicted by the model are shifted 5 weeks to the right, then the correlations are quite good (0.55, 0.74, 0.95, and 0.79). That for 1987 is the highest we have ever observed in any of our simulations, even in the laboratory. We do not understand why this 5 week shift occurs, especially since it is so consistent across years. We are currently looking at several possibilities, including the manner that development rates are calculated.

An especially important outcome of this study has been that the Connection Machine permits a very large speed-up in our calculations. Our past simulations required an average of 38 hours on an Apollo DN4000 for about 1,000 breeding sites. On the Connection Machine we typically simulate 16,000 breeding sites in about two hours. This is a speed-up of about 300-fold. While we made some effort to streamline the code on the Apollo workstations, no such effort has been made for the Connection Machine. For practical application we could probably obtain another 10-20% speed-up without much effort.

This difference in performance is very significant. Because the turnaround is so much faster on the Connection Machine, we anticipate that it will be much more valuable for comparing strategies by mosquito abatement district personnel. Especially important is the ability to incorporate additional complexity. Our long-term objective has been to better control the evolution of resistance to insecticides. That, of course, will require even more calculation, and will impose even more computational demands on the computer. It is our expectation that the Connection Machine will permit studies that were heretofore not practical.

There are some disadvantages to the Connection Machine. First, it requires greater knowledge of Computer Science and greater programming skills than do the serial versions of our model. It has been our experience, however, that *Lisp is not very different from regular Common Lisp. Second, the cost, while much less than that of a comparable serial machine, such as a Cray, is still substantially greater than that of ordinary workstations. However, the speedup is a factor of 300 for a machine that costs only 30 times as much, so at current prices, the cost per calculation is very much less on the Connection Machine. Finally, the programming tools necessary for stopping and peering into the various breeding locations, to see where aberrations are occurring and where additional empirical observations would be most helpful, are less developed and less available with the Connection Machine than on, say, the Apple Macintosh version of RAM (Goldman 1990). None of these problems are, however, insurmountable, and we expect that in a few years all of them will be solved.

In conclusion, this study has shown that the problem of simulating mosquito populations using artificial life programs is highly data-parallel and is well-suited to the Connection Machine; speed-ups of 300-400 times over conventional methods are easily achieved. This will permit much better use of computers for comparing experimental and proposed methods of mosquito control, and will allow us to attack problems that were, in the past, computationally too demanding for us to study.

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INTERACTIONS AMONG AQUATIC VEGETATION, PREDATORS AND MOSQUITOES: IMPLICATIONS FOR MANAGEMENT OF *ANOPHELES* MOSQUITOES IN A FRESHWATER MARSH

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ABSTRACT

Aquatic macrophytes strongly influence the distribution and abundance of *Anopheles* mosquitoes. Spatial variability in *Anopheles* abundance at Searsville Lake (San Mateo County, California) was related to the type (i.e. species) and amount of aquatic macrophyte cover present. Field experiments demonstrated that macrophyte species differed in their attractiveness to ovipositing *Anopheles* females and in the amount of refuge from predation provided for *Anopheles* larvae. Extensive beds of parrotfeather (*Myriophyllum aquaticum*) provided the most productive habitat for *Anopheles* at this site. Parrotfeather (1) was strongly preferred as an oviposition substrate; (2) provided larvae with the best microhabitat and refuge from predation; and (3) was the most abundant and consistently available vegetative cover type. Mechanical harvesting can temporarily remove the surface canopy of parrotfeather and reduce *Anopheles* abundance. Ecological strategies for control of *Anopheles* mosquitoes should exploit the natural patchiness of aquatic plants and mosquitoes by focusing control efforts on patches with the greatest potential for production of *Anopheles*.

Introduction.

Populations of *Anopheles* mosquitoes occur in a variety of freshwater wetlands throughout California. Encroachment of industrial and residential development upon many of these wetlands has dramatically increased the need for reliable and effective control of anopheline mosquitoes. It has also created new problems for vector control agencies because many of these marshes must be managed for a variety of uses, including such diverse functions as flood control, wildlife habitat, and environmental education. Conventional chemical control is often precluded due to conflicts with other management objectives. Development of an alternative ecological (i.e. non-chemical) vector control strategy may thus be required. Such a control strategy must be based on a detailed understanding of the ecological relationships that influence the recruitment and survivorship of *Anopheles* in these freshwater wetland systems.

It is well known that anopheline mosquitoes typically prefer habitats with dense beds of floating, submergent, or emergent macrophytes (Hess and Hall 1943; Aitken 1945; Hall 1972; Collins and Resh 1989). Within a given site, the amount of vegetative cover at the water surface is a primary habitat characteristic. The positive association between *Anopheles* larval density and amount of aquatic plant cover present has been documented in a number of studies (e.g. Hess and Hall 1943; Rozeboom and Hess 1944; Balling and Resh 1984; Walker et al. 1988; Collins and Resh 1989). It appears from these studies that the amount and type of intersection line (i.e. air-water-plant interface) present is more important than total plant biomass or cover in terms of microhabitat suitability for production of *Anopheles*. A number of hypotheses concerning the beneficial effects of aquatic vegetation on *Anopheles* have been proposed. The major hypotheses include protection from predators and physical disturbance, enhanced food resources, favorable oviposition substrate, and optimal thermal conditions for rapid larval development (see references in Orr and Resh 1989; Collins et al. 1988). Critical experimental tests, especially under field conditions, are needed to evaluate the relative importance of these alternative, but not mutually exclusive, hypotheses.

In 1987 we began studies of *Anopheles* and

aquatic macrophytes at Searsville Lake, a man-made lake located on Stanford University's Jasper Ridge Biological Preserve (San Mateo County, California). Our primary objective was to elucidate the roles of aquatic vegetation type (i.e. species) and quantity in determining the local patterns of distribution and abundance of *Anopheles* larvae in the wetlands associated with Searsville Lake. An understanding of the mechanisms that produce spatial variation in *Anopheles* abundance will allow more accurate prediction of anopheline population dynamics. This, in turn, will lead to implementation of more effective ecological control strategies.

This paper summarizes the results of our field studies at Searsville Lake and discusses the management implications of our findings.

Study Site Description.

We used the 40 ha complex of open water and vegetated wetlands at Searsville Lake as a model system for exploring the influence of macrophytes on *Anopheles*. The shallow waters of the littoral zone of the main lake and adjacent marshes support a variety of aquatic macrophytes. We identified four major aquatic vegetation zones at this site (Table 1). The dominant species of *Anopheles* at Searsville Lake was previously identified as *An. freeborni* Aitken but has recently been determined to be *An. hermsi* Barr and Guptavanij (G. Fritz, personal communication). The mosquitofish, *Gambusia affinis* (Baird and Girard), is the major mosquito predator in this system. More detailed site descriptions are given in Smith (1963) and Otieno (1977).

Influence of Aquatic Macrophytes on the Distribution and Abundance of Immature *Anopheles*.

Our first research objective was to document the relationship of macrophyte patchiness to observed spatial variability in the abundance of *Anopheles* eggs and larvae. We hypothesized that macrophyte species differ in the suitability of anopheline microhabitat they provide (these differences may be in either the quality or quantity of microhabitat provided by vegetative cover) and that interspecific differences in potential for anopheline production can be predicted given our understanding of anopheline microhabitat requirements and plant architecture (Collins and Resh 1989). We also hypothesized that within monospecific beds of macrophytes the amount of anopheline microhabitat will vary in relation to plant density (i.e. stems/m^2), which should be an easily measured correlate of the amount of surface cover and intersection line present. The spatial distribution and abundance of immature *Anopheles* should reflect these differences in microhabitat availability and suitability. We predicted, based on the amount of intersection line and subsurface structure provided by each macrophyte species (Table 1), that parrotfeather should have the highest densities of *Anopheles*, followed (in order of decreasing larval abundance) by pondweed, smartweed, and open water. In addition, larval abundance within patches of a given vegetation type

Table 1. Characteristics of the four major cover types at Searsville Lake, listed in decreasing order by amount of total surface area covered.

Cover Type	Dominant Macrophyte Species	Amount of Intersection Line	Complexity of Subsurface Canopy	Predicted Value for Anopheline Production
Open Water	None	None	None	Very Low
Parrotfeather	*Myriophyllum aquaticum*	High	High	High
Smartweed	*Polygonum coccineum*	Low to Intermediate	Low to Intermediate	Low to Intermediate
Pondweed[a]	*Potamogeton pectinatus*	Intermediate to High	Intermediate to High	Intermediate to High

[a] Pondweed zone includes understory of hornwort (*Ceratophyllum demersum*).

should be related to the amount of surface cover present.

Results of three years of sampling (1987-1989) support our first hypothesis: there were consistent differences among macrophyte species in *Anopheles* egg and larval abundance. Our predicted ranking of anopheline production versus vegetation type held, with the exception that *Anopheles* density in pondweed was not consistently higher than in smartweed. Total anopheline density was generally several times greater in parrotfeather than in other vegetation types. The open water zone always had the lowest *Anopheles* density (usually 0 per dip). Pondweed and smartweed beds consistently had intermediate levels of *Anopheles*. Larval counts in pondweed were especially variable from one sampling date to the next, probably because of the more ephemeral nature of the surface canopy of pondweed compared to the canopies of other macrophytes at this site.

The second hypothesis was tested in 1989. Intensive sampling within parrotfeather beds was conducted on two dates to test the correlation between plant surface cover and *Anopheles* abundance using the following method. A 0.25 m^2 quadrat was placed randomly within monospecific parrotfeather beds. *Anopheles* eggs and larvae were then sampled by taking five standard dips within the quadrat. The amount of surface cover present was quantified as total number of emergent parrotfeather stems within the quadrat. There was a significant positive relationship between stem density and total anopheline abundance (Fig. 1). Similar results were obtained when egg and larval abundance data were analyzed separately. Simple linear regression models accounted for 25-60% of the measured variance in *Anopheles* density. Parrotfeather density at Searville Lake varied from 0 to 1,400 stems/m^2, with most undisturbed patches having densities in the range of 500 to 1,000 stems/m^2 during peak anopheline season (July-September).

Influence of Aquatic Macrophytes on *Anopheles* Oviposition.

Aquatic vegetation can influence oviposition by mosquitoes. This may be due to physical factors such as the amount of shade, intersection line, or above-water physical obstructions created by floating or emergent vegetation (e.g. Russell and Rao 1942; Rejmánková et al. 1988). Chemical cues, which can be either attractory or inhibitory, that are produced by plants may also be important (e.g. Bates 1949; Angerelli 1980; Bentley and Day 1989). Preliminary laboratory experiments indicated that *Anopheles* preferred to oviposit in tubs containing one of the common macrophytes from Searsville Lake versus tubs containing only deionized water. We then moved to field experiments to test whether preference for aquatic macrophyte patches as oviposition sites was important under more natural conditions.

Floating baskets, lined with screen and polyethylene plastic sheet, were used in tests of oviposition site selection among the common vegetation types. The initial test indicated a strong preference for parrotfeather over five other cover types (smartweed, pondweed, hornwort, water-primrose (*Ludwigia peploides*), and open water). The number of *Anopheles* recovered in the parrotfeather treatment was an order of magnitude higher than pondweed, the treatment with the next highest oviposition rate. None of the other macrophyte treatments had oviposition levels that were significantly higher than that in the open water treatment. These results were so striking that we suspected that placement of the experimental baskets adjacent to a parrotfeather bed might have biased our results by including only eggs laid by females that had already cued in on parrotfeather.

To test for the effect of surrounding vegetation, or position, on oviposition site selection we repeated the above experiment with several changes. Two macrophyte treatments were dropped (hornwort and water-primrose) because oviposition rates were so low for these treatments and because of the limited importance of these two species in the overall matrix of vegetation at the site. Blocks of the remaining four treatments were set out, simultaneously, in three different macrophyte (pondweed, smartweed, and parrotfeather) beds. The results were almost identical to the first experiment. Parrotfeather was strongly preferred as an oviposition substrate, regardless of what type of vegetation surrounded the blocks of experimental baskets.

Variation in oviposition rates within monospecific patches of vegetation is also important in determining the pattern of larval distribution and abundance in wetlands. We tested the response of *Anopheles* oviposition rate to the density of parrotfeather in basket experiments during 1988 and 1989. In both years there was little oviposition at the lowest stem densities, a pronounced peak at 800-1,200 stems/m^2, and then a drop in oviposition as stem density approached 2,000 stems/m^2 (Fig. 2). These results are similar to those obtained with *An. freeborni* ovipositing in parrotfeather in the Central Valley (Rejmánková et al. 1988). The drop in oviposition at higher densities was probably caused

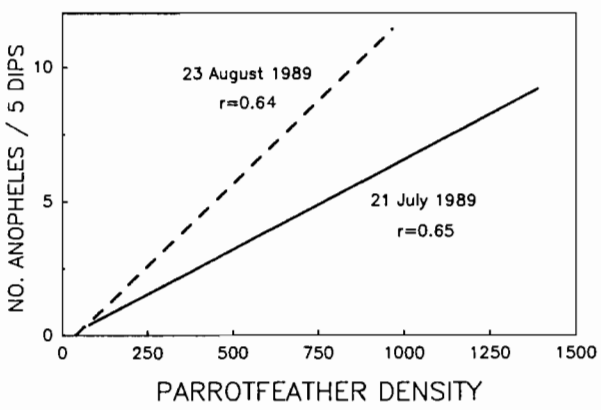

Figure 1. Simple linear regression models demonstrating the positive relationship between parrotfeather density (stems/m^2) and *Anopheles* abundance (no. eggs + larvae/5 dips) on two different sampling dates.

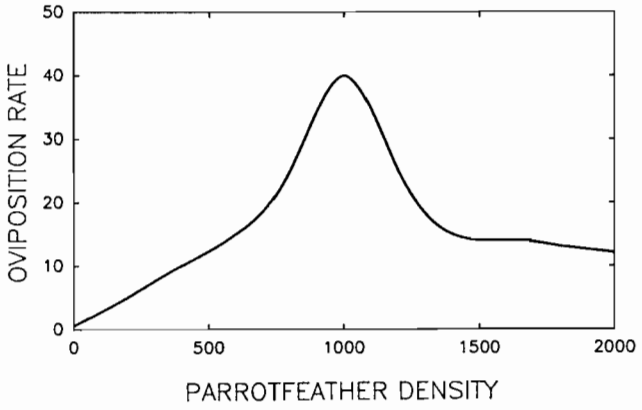

Figure 2. General model of the response of *Anopheles* oviposition rate (no. eggs laid/day/m^2) to parrotfeather stem density (no. stems/m^2). The response curve is based on the results of experiments conducted in 1988 and 1989.

by increased physical obstruction of the water surface created by the emergent stems at the highest densities. Ovipositing females may simply have been unable to reach the water surface. Further research is needed to determine what factors associated with parrotfeather serve as the attractive cues.

Influence of Aquatic Macrophytes on Predator-Prey Interactions and *Anopheles* Larval Survivorship.

Our early work at Searville Lake and other sites demonstrated (for parrotfeather and two other macrophyte species) that surface plant cover creates favorable microhabitat and provides a partial refuge from predation for *Anopheles* larvae (Orr and Resh 1989). Larval survivorship was positively related to amount of plant cover and negatively related to density of *Gambusia*. The general response of larval survivorship to *Gambusia* density and plant cover level is shown in Figure 3. The trend towards increased larval survivorship at higher cover levels in the absence of predators indicates a beneficial effect of plant cover beyond its role in providing a refuge from predation.

Subsequent work focused on the differences among the dominant macrophytes in the amount of microhabitat and refuge they provide anopheline larvae. A factorial design experiment was used to examine the influence of cover type and predator density on larval survivorship. Four cover treatments, representing the major cover types associated with *Anopheles* at Searsville Lake (parrotfeather, smartweed, pondweed, and open water), were combined with two predator treatments (*Gambusia* present or absent). As in our previous experiments (Orr and Resh 1989), 100 newly hatched *Anopheles* larvae were added to each experimental cage initially. After ten days, all surviving larvae were removed and counted. Parrotfeather and pondweed treatments had the highest larval survivorship, as expected, both in the presence (18 and 14% survivorship) and absence (58 and 44% survivorship) of *Gambusia*. Smartweed provided better habitat than open water when predators were absent (38% vs. 26% survivorship), but both of these cover treatments had close to 100% mortality when *Gambusia* were present. Larval development tended to be faster in the vegetated treatments than in the open water treatment.

Based on the above results, we ranked the cover types in terms of suitability for production of anopheline larvae: parrotfeather > pondweed > smartweed > open water. This corresponds to observed pattern of larval abundance at Searsville Lake during the periods when the three vegetation types are well developed. The actual production of *Anopheles* in pondweed patches is greatly limited by the short duration (usually only 2-4 weeks during late July and early August) of well-developed pondweed surface canopy at this site (cf. Coyote Hills Marsh - Balling and Resh 1984; Feminella and Resh 1989). Smartweed beds are common from May-July, but by mid- to late August most smartweed beds are left dry and exposed above the waterline because of declining water levels in the lake and marshland. Parrotfeather, on the other hand, is the dominant aquatic macrophyte

throughout the anopheline breeding period. Thus, parrotfeather is clearly the most productive vegetation type for *Anopheles* in this system: parrotfeather (1) provides larvae with the best microhabitat and refuge; (2) is strongly preferred as an oviposition substrate; and (3) is the most abundant and consistently available vegetative cover type in this system.

Mechanical Harvesting of Parrotfeather and its Impact on *Anopheles*.

Mechanical harvesting of parrotfeather is one means of directly reducing, at least temporarily, the amount of *Anopheles* habitat present and indirectly reducing the abundance of *Anopheles* larvae. There are several drawbacks to this approach: (1) it is labor intensive; (2) it may need to be repeated several times each season and (3) it may facilitate the spread of parrotfeather by dispersing plant fragments that are capable of reproducing vegetatively (A. Grundmann, personal communication). The only alternative to harvesting that is acceptable to preserve managers at present is the use of *Bacillus thuringiensis* var. *israelensis* (*Bti*). The efficacy of *Bti* at Searsville Lake is reduced by limited penetration and dispersal of *Bti* formulations in dense stands of parrotfeather, the need for multiple application during the peak anopheline season, and the difficulty of getting boats and spraying equipment into heavily vegetated areas (R. Schoeppner, personal communication).

We examined the impact of mechanical harvesting on plant regrowth and anopheline abundance during the summer of 1989. Several swaths of parrotfeather were harvested in early June. Harvesting removed all parrotfeather biomass from the upper 1 m of the water column, but left roots and stems below 1 m intact. Regrowth of parrotfeather stems was rapid: mean stem density at the water surface in the harvested areas was 210 stems/m^2 within six weeks of harvesting, and aproximately 360 stems/m^2 after 12 weeks (versus 600-800 stems/m^2 in adjacent unharvested areas). *An. hermsi* eggs and larvae were absent immediately following harvesting, but soon began to increase as the parrotfeather surface canopy regenerated. Larval counts in previously harvested zones were about 40% of the counts in unharvested areas six weeks after harvesting (Fig. 4). After 12 weeks, larval counts had increased in the previously harvested areas, but still were only 40% of counts in unharvested parrotfeather due to a general increase in anopheline abundance throughout the site. These results are consistent with what would be predicted by the empirically derived relationships between parrotfeather surface cover (i.e. stem density) and anopheline abundance, oviposition rate, and larval survivorship discussed above. Rates of plant regrowth and, more importantly, surface canopy regeneration will be influenced by a variety of factors, including water turbidity and nutrient loadings, water temperature, and plant physiological status. The rate at which the lake water level declines will also affect plant regrowth and rate of surface canopy regeneration. The rate at which *Anopheles* recolonize the regenerated parrotfeather surface canopy will also depend upon a variety of factors, such as the availability of alternative habitat (especially more preferred oviposition subtrate such as parrotfeather beds with 1,000 stems/m^2), the size of the reproductive adult population at the site, and time of year.

Management Implications.

Plants can provide favorable microhabitat for anopheline production by providing protection

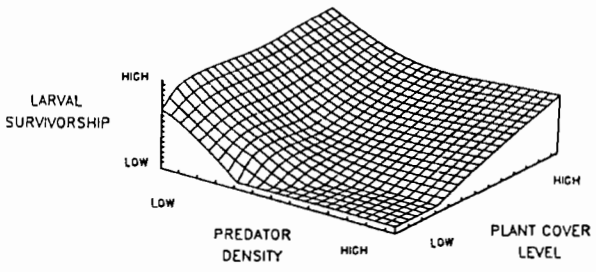

Figure 3. A general model showing the influence of plant cover and predator (*Gambusia*) density on *Anopheles* larval survivorship. Based on data presented in Orr and Resh (1989).

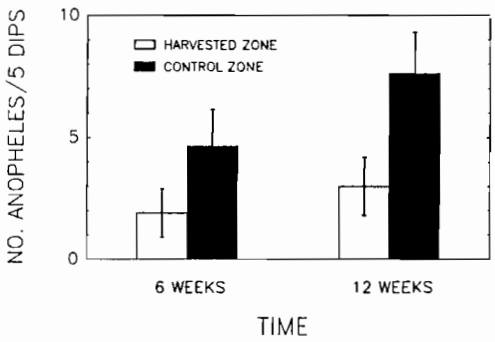

Figure 4. The impact of mechanical harvesting of parrotfeather on *Anopheles* abundance 6 and 12 weeks after harvesting.

from predators and physical disturbance, enhancing larval food resources, and creating optimal thermal conditions for rapid larval development. In addition, macrophyte beds may provide attractive oviposition sites for *Anopheles* females. Macrophyte species differ in the amount and quality of anopheline microhabitat they provide. Thus, within a given site there may be a hierarchical pattern of spatial variability in *Anopheles* abundance, with variation occurring both among and within macrophyte patches because of variability in oviposition and subsequent larval survivorship. Egg distribution should be affected primarily by within and among macrophyte species variability in substrate suitability or attractiveness to ovipositing females. Larval distribution will be affected by this spatial variability in oviposition and by differential larval survivorship resulting from variability in microhabitat conditions and predator density both within and among macrophyte patches.

Wetland managers and mosquito abatement districts can exploit certain aspects of the natural patchiness of plants and mosquitoes when designing and implementing control strategies for shallow macrophyte-dominated wetlands. Specific macrophyte patches can be assigned management or control priority ranking based upon comparison of the positive resource value (e.g. value for fish or waterfowl) of a patch with its potential for anopheline production (i.e. Collins and Resh 1989, appendix I). The three main options for management of macrophyte patches would be the maintenance, reduction, or elimination of specific patches. Mechanical harvesting, water level regulation, herbivore regulation or, in some cases, herbicides might be chosen to manage macrophytes (and mosquitoes), depending upon site-specific environmental and economic considerations. Our research has improved our understanding of the mechanisms governing interactions among macrophytes, predators, and mosquitoes. Such knowledge is an essential component in the development of ecologically based control strategies for freshwater wetlands.

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EFFICACY OF TADPOLE SHRIMP, *TRIOPS LONGICAUDATUS*, AS A BIOLOGICAL CONTROL AGENT OF *CULEX* AND *PSOROPHORA* MOSQUITOES

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ABSTRACT

Natural populations of the tadpole shrimp (TPS), *Triops longicaudatus* produced excellent control of immature *Culex* mosquitoes in experimental field ponds in Oasis, California due to TPS-induced mosquito oviposition deterrence and active predation on larvae and pupae. Ponds containing TPS were found to have significantly ($P<0.05$) fewer mosquito egg rafts than control ponds during the second to fourth weeks postflooding; a time when TPS were abundant. This was in accordance with other field and laboratory studies that provided evidence of the role of TPS in deterring *Culex* oviposition. In ponds with TPS, first, second and third instar larval *Culex* abundance were reduced 95% by the first week postflooding, while fourth instar larvae and pupae were reduced 95% within the second and third weeks, respectively.

The efficacy of TPS in reducing the floodwater mosquito, *Ps. columbiae*, was studied in a flooded date garden in the Coachella Valley. In contrast to predation on *Culex* spp., TPS predation upon synchronously developing *Ps. columbiae* was generally low but increased during the first four days postflooding. No reduction in *Ps. columbiae* populations was detected when comparing rows containing TPS to rows where TPS were eliminated by a single cypermethrin treatment (1.21 g/ha). It was hypothesized that, due to rapid development, this mosquito species temporally escapes TPS predation by developing to fourth instar by the third day postflooding when TPS were still too small (average carapace length =2.5 mm) to catch them.

Introduction.

In temporary waters, establishment of a spatio-temporal overlap between immature mosquito prey and their predators must be achieved for successful biological control. Due to lack in a spatio-temporal overlap and difficulty in mass-rearing, insect predators of mosquito larvae have not become operational biological control agents (Bay 1974, Collins and Washino 1985). Similarly, larvivorous fish such as *Gambusia affinis* (Baird and Girard) are known to be less effective in ephemeral waters (Meisch 1985) and costly inundative releases are necessary to achieve adequate control of floodwater mosquitoes (Davey and Meisch 1977).

Tadpole shrimp (Notostraca: Triopsidae) are Crustaceans uniquely adapted to survival in ephemeral freshwater habitats (Longhurst 1955) as well as being predators of larval Diptera (Mail 1934, Maffi 1962, Dodson 1986). Tadpole shrimp (TPS) produce desiccation-resistant eggs (Hempel-Zawitkowska and Klekowski 1968) that remain dormant when the habitat is dry and hatch soon after rehydration; thus ensuring their survival between floodings. This attribute, coupled with a rapid development to reproductive maturity, make TPS prime candidates as mosquito control agents in temporary waters.

The role of TPS as predators of mosquitoes was recognized as early as 1934 by Mail in Montana. In the laboratory, he noted a single TPS consumed 475 second instar larvae within 98 hours or less. In Somalia, Maffi (1962) reported *Triops granarius* (Lucas) as a predator of the malaria mosquito, *Anopheles gambiae* Giles.

In the laboratory, prey-size selection tests indicated that the TPS, *Triops longicaudatus* (LeConte) was a size-dependent predator of *Culex quinquefasciatus* Say larvae (Tietze and Mulla 1989). As TPS grew larger, their prey-size preference among larval instars changed; smaller TPS fed

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exclusively on first and second instar larvae while larger TPS (carapace length >4.0 mm) consumed all instars and pupae (Tietze and Mulla 1989). Thus, relative developmental rates of TPS and immature mosquitoes may dictate the efficacy of this predator in reducing mosquito populations.

The goal of this study was to assess the efficacy of TPS as biological control agents of *Culex* populations in experimental field ponds and *Psorophora columbiae* (Dyar and Knab) populations in a flood-irrigated date garden.

Materials and Methods.

Efficacy of TPS against *Culex*: To study the impact of TPS on immature *Culex* mosquitoes, dry field ponds located at the Aquatic and Vector Control Research Facility in Oasis, California, were mowed and 0.91 kg of dry chicken mash was added per pond to promote mosquito oviposition. Each pond was equipped with a float-valve system to maintain the proper water depth of about 25 cm. Pond vegetation consisted of nutgrass, Bermuda grass and crab grass.

Four days postflooding, four ponds containing TPS were chosen as "TPS (treatment) ponds" and four ponds lacking TPS were chosen as controls.

Tadpole shrimp abundance was assessed using an aquatic "D"-net (BioQuip, Santa Monica, CA) with an opening area of 0.05 m^2, nylon mesh with 0.8 mm pore size and a 1.5 m wooden handle. The "D"-net was dragged 11 m along the pond bank, twice per pond. The sample was transferred to a white enamel pan and the TPS were counted and measured in carapace length before returning them to the pond.

Larval and pupal mosquitoes were sampled weekly by taking four, 400 ml dips per pond, combining them using a concentrator cup (Mulla et al. 1982) and preserving them in 70% ethanol. In the laboratory, numbers of each immature stage were counted and species identifications were made. Mosquito egg rafts were sampled by placing a yard stick along the pond bank and counting the number of unhatched rafts along the transect. Egg rafts were thus sampled three times per pond on each sampling day from the second to seventh weeks postflooding. Numbers of egg rafts in treatment and control ponds were compared using a paired t-test for each day sampled.

The reduction of larval and pupal mosquitoes was calculated using the formula:

$$R = 100[(X_c - X_t)/Xc]$$

Where R represents the larval or pupal reduction effect of the tadpole shrimp (expressed as a percentage), X_c is the mean number of larvae or pupae in the control ponds (those where tadpole shrimp were initially eliminated) and X_t is the mean number of larvae or pupae in the treatment ponds (those with tadpole shrimp). The percent reduction of each larval instar and pupae was calculated for each day sampled.

Water temperature was measured using a maximum-minimum thermometer (Taylor Instrument Co., Arden, NC).

Efficacy of TPS against *Ps. columbiae*: To assess the impact of TPS on the floodwater mosquito, *Ps. columbiae*, coexisting populations of these species were studied in a flood-irrigated date garden in the Coachella Valley, California during two years. The date garden was located about six kilometers west of the Thermal airport. The rows of date palms were about nine meters apart and extended 91 meters in length. When dry, the area between rows was routinely disked and fallen palm fronds were buried into the soil. The irrigation schedule was about once every two to three weeks during the summer and fall. Livestock were present in the date garden during both years of the study.

In September, 1988, *Ps. columbiae* and *T. longicaudatus* bionomics were studied in a single flood-irrigated row. Density and growth of TPS and *Ps. columbiae* were monitored during the flooding period. A circular quadrate sampler (0.2 m^2) was randomly placed into the irrigated row and the contents transferred to a white enamel pan using an aquarium fish net (mesh size =1.35 mm). The sample was then concentrated using a larval concentrator (Mulla et al. 1982) and preserved in 50% ethanol. Three samples were taken on each day postflooding. In the laboratory, mosquito and TPS size and density were determined using a dissecting microscope with a calibrated ocular micrometer. Tadpole shrimp size was measured as the length of the carinal suture that longitudinally divides the carapace. Larval mosquito size was defined as the distance from the anterior end of the head capsule to the base of the air siphon. Pupal mosquito size was measured as the distance from the anterior end of the head to the base of the paddles.

Tadpole shrimp predation upon immature *Ps. columbiae* was studied using floating cages (Mulla et al. 1974) which had screened openings on the sides to allow water flow and were then placed into a styrofoam ring for buoyancy. The containers held about 500 ml when immersed in water and had a surface area of 90 cm^2. Three immature mosquito densities (5, 10, and 20 larvae or pupae per cage)

were exposed to single TPS for one hour each day postflooding. Each mosquito density was replicated three times. All mosquitoes and TPS used in this study were collected from the same row and were thus synchronously developing. Percent consumption was calculated for each larval density and each day postflooding.

The capacity of TPS to feed upon various *Ps. columbiae* larval instars and pupae was studied on the fourth day postflooding using the floating containers as described above. In this test, sets of ten second, third and fourth instar larvae and pupae were each exposed to single TPS for one hour. Four replicates were made for each prey category. In this test, second and third instar larvae were collected from the same row as the tadpole shrimp. The percent consumption was calculated for each prey category.

In September of 1989, further tests were performed in the same date garden. In this study, the effect of excluding TPS from rows was compared to rows where TPS were present. Tadpole shrimp populations were maintained in four rows and eliminated from four rows by a single cypermethrin treatment per row at 1.21 g/ha using a 1-gallon sprayer (B&G Equipment Co., Plumsteadville, PA) and Teejet nozzle (#0006). The eight rows used in this study were flooded in series and thus at approximately the same time. *Ps. columbiae* abundance was assessed in each row by taking ten dips using a 400 ml dipper. The approximate number of immature mosquitoes per dip was recorded before replacement. The rows were thus sampled each morning of the fifth day postflooding. Water depth and width of each flooded row was measured in the afternoon of the second to fifth days postflooding. Water temperature was measured using a maximum-minimum thermometer.

Results and Discussion.

Efficacy of TPS against *Culex*: In the Oasis ponds, *Cx. tarsalis* was the dominant (>95%) mosquito species, but *Ps. columbiae* and *Cx. quinquefasciatus* were also detected. Larval and pupal *Culex* populations in ponds containing TPS were much lower than that of control ponds. This difference was in part due to significantly ($P<0.05$) more egg rafts in control ponds than in TPS ponds during the second to fourth weeks postflooding (Fig. 1). During the fifth to seventh weeks postflooding when there was no significant ($P>0.05$) difference in mosquito egg raft abundance between TPS ponds and controls, depressed larval and pupal *Culex* abundance in TPS ponds may have been primarily due to TPS predation, assuming other natural enemies are evenly distributed in the two treatments.

Larval and pupal abundance was greatly reduced in ponds with TPS compared to control ponds. The reduction of first, second and third instar *Culex* larvae was >95% during the first week postflooding (Table 1). Fourth instar larvae and pupae were reduced >95% during the second and third weeks postflooding, respectively (Table 1).

The maximum abundance of TPS was 52 TPS/2 "D"-net samples on the second week postflooding, rapidly decreasing to 10 TPS/2 "D"-net samples during the fourth and fifth weeks and about two TPS/2 "D"-net samples on the sixth and seventh weeks postflooding. Tadpole shrimp grew to about 4.6 mm carapace length during the first week postflooding and gradually increased to 6.00 mm in size during the following six weeks.

Water temperature of the ponds varied from 17 to 30° C during the seven weeks postflooding (Table 2).

Efficacy of TPS against *Ps. columbiae*: The densities of *Ps. columbiae* and TPS were found to have dramatic increases during the course of the flooding period. Since water levels of the aqueous habitat receded at rates of 10 cm/day during the 1988 study and 7 cm/day during the 1989 study, increases in synchronously developing mosquito and TPS densities can be attributed to declining water volume. In 1988, on the second, third and fourth days postflooding, *Ps. columbiae* densities averaged 249, 590 and 1088 larvae and pupae/m^2, respectively. During the same flooding, TPS densities averaged 46, 44 and 149 TPS/m^2 on the second, third and fourth days postflooding, respectively. In 1988, on the fifth day postflooding, remaining water

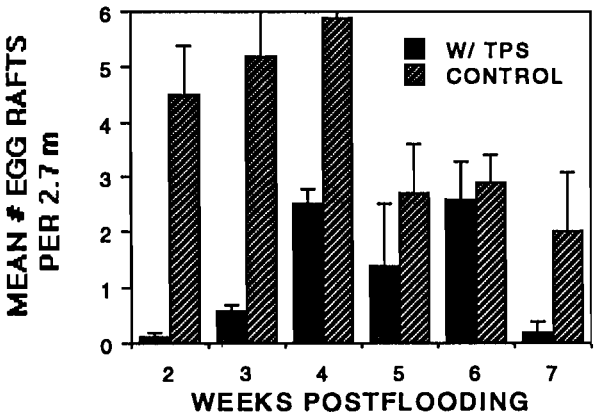

Figure 1. *Culex* egg raft abundance (± SE) in ponds with and without (n=4, each) tadpole shrimp during seven weeks postflooding in Oasis, California.

Table 1. Mean weekly reduction (%) of immature *Culex* mosquitoes by tadpole shrimp as sampled from four field ponds in Oasis, California.

Stage	Weekly reduction (percent)						
	1	2	3	4	5	6	7
L1	100	100	100	97	94	84	100
L2	99	100	98	94	92	86	90
L3	96	100	99	92	85	92	93
L4	74	99	99	87	66	83	92
P	--[a]	86	98	100	0[b]	82	100

[a] treatment and control equal zero.
[b] treatment equals zero.

was confined to small puddles in the cattle hoofprints and human tracks where *Ps. columbiae* and TPS were highly concentrated. After several hours these puddles had disappeared and large numbers of pupae and TPS were observed in the soft mud. On the fifth day postflooding of the 1989 study, only one row lacked water while the other seven rows remained flooded.

In both years, *Ps. columbiae* progressed to third instar larvae on the second day, fourth instar larvae on the third day and both fourth instar larvae and pupae on the fourth day postflooding (Fig. 2). The larvae grew slightly larger during the second year perhaps due to slightly higher water temperatures. Water temperatures ranged from 20 to 31° C during the 1988 study and 22 to 32° C during the 1989 study.

Tadpole shrimp growth rates were similar during both years of the study (Fig. 2). During the first year, mean TPS size (±SE) was 1.2 (0.09), 2.5 (0.18) and 4.0 (0.15) mm on the second, third and fourth days postflooding, respectively. Daily size during the second year averaged (±SE) 2.2 (0.32), 2.5 (0.23) and 4.6 (0.34) mm on the second, third and fourth days postflooding, respectively.

Predation by single *T. longicaudatus* on larval *Ps. columbiae* in floating cages for one hour were initially low but increased slightly with the progression of the flooding period (Fig. 3). The highest consumption was for pupae on the fourth day postflooding (Fig. 3). Overall, the consumption was very low, especially during the second and third days postflooding when the TPS averaged less than 3.0 mm in carapace length.

Table 2. Minimum and maximum weekly water temperatures (°C) of four field ponds (30 m^2) in Oasis, California. Ponds were initially flooded on September 26, 1988.

	Weekly water temperature (°C)						
	1	2	3	4	5	6	7
Minimum	21	19	18	21	19	18	17
Maximum	30	29	29	29	29	28	22

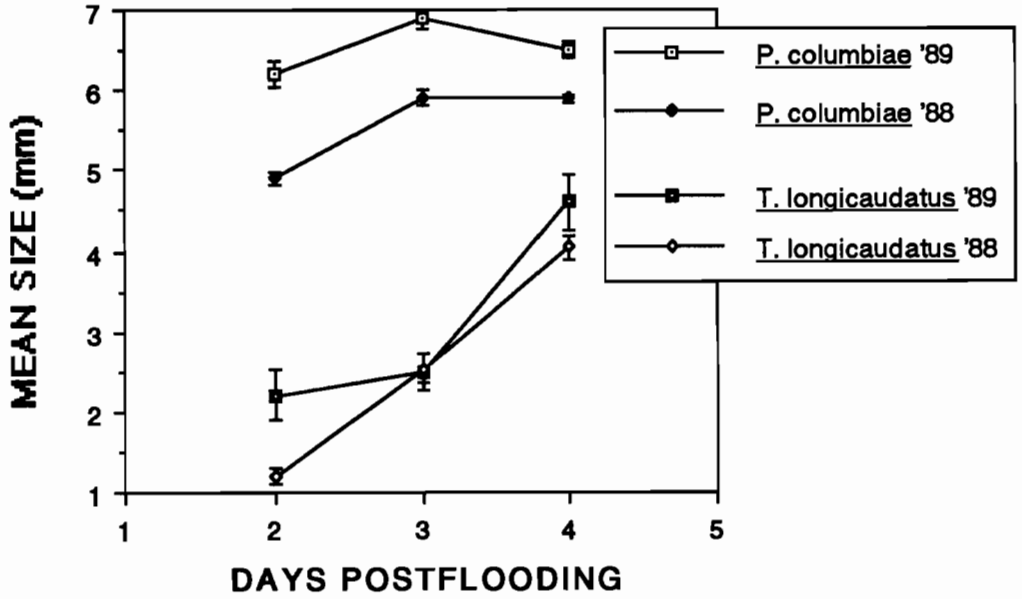

Figure 2. Growth of larval *Ps. columbiae* (± SE) and *T. longicaudatus* (± SE) during the second to fourth days postflooding in a date garden in the Coachella Valley, California.

When different *Ps. columbiae* instars and pupae were exposed to TPS, much higher predation rates were recorded. Consumption rates on second and third instar larvae were much higher than that of fourth instar and pupae (Fig. 4). Consumption of second and third instar larvae, not normally accessible to TPS on the fourth day postflooding, averaged 92 and 62%, respectively, whereas that of fourth instar larvae and pupae developing synchronously to the TPS averaged 15 and 5%, respectively. Thus it is possible that the majority of *Ps. columbiae* larvae escape TPS predation by rapid development before TPS were large enough to effectively capture and handle them.

No reduction in *Ps. columbiae* populations was detected when comparing their abundance in rows with TPS to rows where tadpole shrimp were eliminated (Fig. 5). These data suggest tadpole shrimp may be ineffectual biological control agents of *Ps. columbiae*, but further testing is warranted.

Reproductive strategy and immature developmental rates of *Culex* and *Ps. columbiae* both influence the degree of temporal overlap between mosquito and TPS, and subsequently, the efficacy of TPS in reducing their populations. Since *Culex* must deposit egg rafts in flooded areas to initiate larval development, time is needed for locating oviposition sites and for hatching of eggs; during this time, TPS achieved larger sizes (>4.0 mm carapace length) that are known to be more effective predators of *Culex* mosquitoes (Tietze and Mulla 1989). In contrast, *Ps. columbiae* eggs were oviposited prior to flooding and hatched upon hydration; the larvae developed to size much too large for this predator (Fig. 4).

This study detected large differences in the rate of development between *Culex* and *Ps. columbiae* larvae: The time needed for initial *Culex* pupation to occur was nine days postflooding, while that of *Ps. columbiae* occurred four days after flooding. The differences in relative size of predator and prey were partly responsible for differences in larval reduction. Lack of oviposition deterrence may have contributed to the failure of *Ps. columbiae* reduction by TPS in the test plots.

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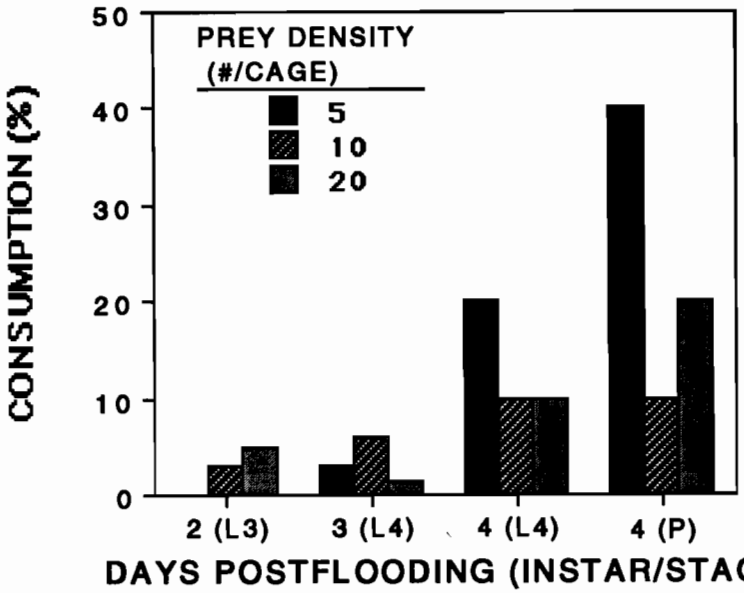

Figure 3. Consumption (%) of larval and pupal *Ps. columbiae* at three prey densities by synchronously developing tadpole shrimp in a flooded date garden in the Coachella Valley, California.

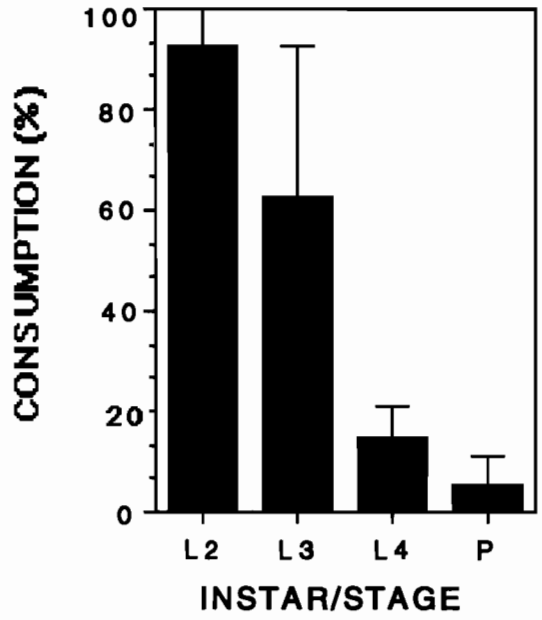

Figure 4. Consumption (%) of *Ps. columbiae* immatures by tadpole shrimp (carapace length =4.0 (± 0.15) mm) on the fourth day postflooding in a date garden in the Coachella Valley, California.

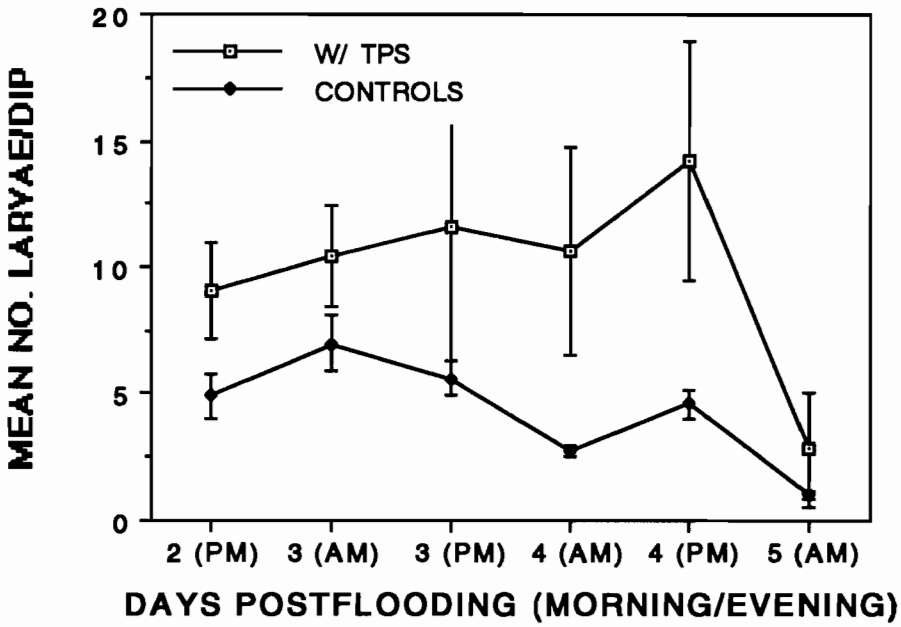

Figure 5. Abundance (± SE) of immature *Ps. columbiae* in flooded rows with and without (n=4, each) tadpole shrimp as sampled twice daily during the second through fifth days of postflooding.

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