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THE PROCEEDINGS is the *Proceedings and Papers of the California Mosquito and Vector Control Association, Inc.* One volume is published each year. Intended coverage by content includes papers and presentations of the Association's Annual Conference, contributions and meritorious reports submitted for the conference year, and a synopsis of actions and achievements by the Association at large during the preceding year.

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California Mosquito and Vector Control Association, Inc.

Volume 57 January 29 thru February 1, 1989

WHY SHOULD YOU CONTINUE TO BE CONCERNED ABOUT ENCEPHALITIS VIRUSES IN CALIFORNIA?

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When Chuck Beesley invited me to talk to you today, he asked me to present essentially the same talk that I gave at the meeting of the Utah Mosquito Control Association last September. At that time, I reviewed the basic cycles of infection of the two mosquito-borne viruses that are of current concern in California, namely western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE). I also discussed the purposes and interpretation of data from state-wide encephalitis surveillance programs. Chuck thought that these topics would be of interest to you as management and trustee personnel for mosquito and vector control agencies. You will find that the theme of my discussion is that the control of these diseases depends upon the continued reduction of vector mosquito populations to levels that will prevent the spread of virus infections from wild birds to humans.

History.

Between 1945 and 1984, 749 cases of WEE and 549 cases of SLE were reported in California. Cases occurred in significant numbers in all counties in the Central Valley, and in lesser numbers in southern California. However, if we look at the year when these cases occurred, we find that there has been a dramatic decrease of cases since 1960. In most recent years, there have been fewer than five cases in the entire state. Why has this decrease occurred? We would all like to say that "Mosquito control solved and eliminated the problem." There is no question that control played a major role in the decrease. However, while the disease problem has largely gone away, the vectors and viruses have not. Both viruses are still here in their invisible cycles between the mosquito *Culex tarsalis* Coquillett and wildlife. *Culex tarsalis* has a very wide distribution that covers all of western North America, except at higher elevations. When WEE was an important disease in horses in the 1930s and 1940s, it had the same geographical distribution as the vector. There is evidence that the viruses and vectors still occur today over most of this region.

I believe there are many reasons why the diseases have decreased, including mosquito control, the widespread use of insecticides on agricultural crops, changes in agricultural practices, vaccination of horses, and changes in the habits of our society, namely the advent of television and air conditioning that decreases outdoor exposure of people to vectors in the evening. We still face difficult problems in that genetic resistance to almost all insecticides is prevalent in vector populations. In addition, you have heard in the program of this meeting that more and more legal and social restrictions are being put on mosquito control practices. It is almost inevitable that vector populations are going to

increase as they already are in some areas. I am concerned that we may experience epidemics in the future.

Virus cycles.

Our concept of the basic cycle of WEE and SLE in 1945 was that *Cx. tarsalis* was the primary vector, and chickens and wild birds were the source of vector infection as the birds had sufficient virus in their blood to infect vectors. In contrast, people that were infected could not be a source of vector infection, and the same was true for most mammals. It is amazing how few changes have occurred in our knowledge of this cycle today. We have elevated wild birds to the central spot in the cycle and classify chickens as incidental hosts that man introduced into the cycle. A few additional mosquito species are now considered to be potential vectors. However, there is no question that the bird-mosquito-bird cycle is central to virus maintenance. I will call this the silent cycle in that we do not see it, as it usually does not produce disease in the birds or vectors. I remind you that while people are clinically susceptible to both viruses, they are accidental hosts that contribute nothing to the maintenance of infection as they do not have sufficient virus in their blood to infect a vector. You also must realize that the absence of diagnosed cases of WEE and SLE in recent years does not mean that no infections are occurring in people. All clinical cases are not reported and in addition, many people who are infected do not become ill. Indeed, hundreds of people can have an inapparent infection for every clinical case that develops and is diagnosed. It is the silent cycle that is still occurring in much of the area depicted in the old epidemic maps and which still may be occurring in your district.

Surveillance.

Let me turn now to the subject of the current state-wide surveillance program for these infections in California. Dr. Emmons and others described current findings on the program earlier this morning. The surveillance program gathers information in the following categories that is relevant to the occurrence of these diseases.

1. Water resources
2. Temperatures
3. Economic resources
4. Vector populations
5. Viral activity
   a. Vectors
   b. Sentinel hosts
   c. Clinical cases

A mosquito control agency readily has first hand knowledge from its own region in the first three categories. Water is necessary to produce *Cx. tarsalis* and an excess of uncontrolled water from natural flooding or from agricultural or urban sources in an area potentially means there will be more *Cx. tarsalis* or other vectors. Temperature data are available to you each day and we know that temperature controls the rate of mosquito development and virus multiplication in the vector. You also know the budget you have each year to control vectors and the limitations in your capacity to effectively reduce larval or adult mosquito populations. You obtain data routinely on vector population levels by running New Jersey light traps or CDC traps to collect adult mosquitoes, and you monitor for the presence of larval populations by water inspections. If you wish, you can focus a significant part of your program on *Cx. tarsalis* control.

Data on virus infection in vectors are obtained when you collect mosquitoes from CDC traps and send them to the State Department of Health or the University laboratories for virus tests. More than three million mosquitoes have now been tested for virus in this program. This is an expensive and labor intensive program, but it provides very specific information on the presence of infection in an area at a particular time and tells us which species are involved as vectors.

Sixty-eight sentinel chicken flocks of 20 to 25 birds each were distributed in California in 1988. The distribution of flocks covers most of the regions where we expect WEE or SLE viruses to occur. These birds potentially are fed upon each night by *Cx. tarsalis*. The birds are bled each month and this results in more than 1,000 blood samples that are tested to see if the chickens have been infected. This year virus activity occurred primarily in southern California, with an extension of SLE into Kern County in late summer.

Information on clinical cases is obtained by veterinarians and physicians and confirmed by laboratory tests.

Interpretation of surveillance data.

The question is, what do you do with the above surveillance data? There is a sequence of events that is being measured through the summer. As a vector population builds up, virus activity can be detected in the vector or in sentinel birds. These events will precede the detection of cases in horses or humans. Virus activity will occur when summer temperatures are rising. You use data from the surveillance system to anticipate an epidemic and to prevent it. You want to intensify and establish vector control before human cases have occurred. Once cases occur, you can be sure that additional people already are infected and in the incubation period. Some of these individuals will become cases. At this point in time it may be too late to intervene with larval control. You can, however, shift or intensify control to reduce the numbers of infected and transmitting adult vectors in the area. Adult control is expensive and may be ineffective unless it can be established over a large area and continued for at least ten days. This is the period necessary to interrupt the continuity of virus transmission between birds and mosquitoes.

Let us turn now to establish an understanding of some of the additional information you can get from a surveillance system and how to interpret it. An intensive surveillance program was carried out in the 1960s in Kern County, where 45 sites were the source of data on mosquito populations and infection rates in mosquito pools and sentinel chickens. At 18 stations, the indices of female *Cx. tarsalis* were less than one per collection, and there was little or no virus activity. It would be difficult to recover virus at such sites by tests on pools of mosquitoes as the population was so low that you could not collect a pool of 50 specimens to test. Yet, an occasional sentinel chicken became infected in such circumstances. It was easy to detect virus in both vectors and sentinel birds at sites where there were more *Cx. tarsalis*. When an intensive control program was established over a large area and further reduced the *Cx. tarsalis* population, virus still remained but was held at a very low level. When *Cx. tarsalis* populations were held at this low level for several years, virus tended to disappear. These findings led to the concept that vector populations could be reduced below a threshold level where virus transmission was very low or even stopped. When this was achieved, obviously there was very little risk of human infection.

To pursue this further, you must realize that the detection of virus in a mosquito pool does not mean that the infected mosquito in the pool can transmit infection if it bites. The mosquito may not have completed virus incubation, and the virus may not be in the salivary glands. To learn this, we exposed susceptible chickens in bait cans for a single night. We then tested both the mosquitoes and chickens for virus infection. We found that only 1 in 4 infected mosquitoes had transmitted virus to the bird.

Clearly, detection of virus in a mosquito pool, or in a sentinel chicken, was a very sensitive measure of virus activity if sampling was intensive enough and covered a sizable area. However, you still must realize that detection of virus activity at a particular site does not mean that you have found the one hot spot in your area and must rush to that site to stamp out the virus. Usually, it means that virus is active over a fairly large area and larval or adult control should be intensified widely.

Studies on mosquito biology.

I want now to emphasize why such detailed field studies and involved techniques must be utilized if we are to understand the biology of mosquitoes and control them. If you need information about mosquitoes, you cannot use the techniques that are so successful in a census or in social studies of people. For example, you cannot go out and ask a female mosquito, "How old are you?" "Where were you last night?" "Where are you going?" "How many children do you have?" To develop such information requires special techniques and tedious hours of work. Let me give you several examples.

Many factors control effectiveness of the maintenance cycle of WEE and SLE viruses and one of these is the feeding habits of *Cx. tarsalis*. It may surprise you that different species of mosquitoes are very selective in what species of animals they feed on. Some species prefer to feed on birds, and others prefer horses, cattle, or even frogs or snakes. Some will feed on almost any blood source in their environment. There also are wide variations within a species and region in host feeding patterns. *Culex tarsalis*, our primary vector, feeds on both birds and mammals, and areas where its highest feeding rates are on birds are generally those with the highest levels of virus activity. It turned out after further study that these differences

were controlled in large part by the size of the vector population and the species of hosts available in the area. To further determine the importance of the abundance of mosquitoes, we again exposed 3-week old chicks in bait cans and examined the mosquitoes collected. We found that as the number of mosquitoes attracted increased, the proportion of mosquitoes that succeeded in feeding decreased. If we did not let the chickens disturb the mosquitoes, they all fed. This reinforced the concept that threshold levels of vector populations were required to increase or decrease virus transmission. At low population densities, *Cx. tarsalis* will feed on its preferred host (birds) and they are a good source of virus infection. As the vector population increases, the birds react and chase some individuals away to feed on alternative sources of blood such as mammals, including people. Most species of mammals are not a good source of virus. This shift in hosts can decrease the efficiency of a virus cycle -- even interrupt it.

Let me turn now to tell you how fragile the virus transmission cycle is. The sequence of blood feeding and egg laying by female mosquitoes is called the gonotrophic cycle. It happens that up to 90% of *Cx. tarsalis* may lay their first eggs without a blood meal. This is called autogeny and can delay the first blood meal after adulthood for four days. Even if a female is not autogenous, she will not feed for up to two days after hatching. The first blood meal may or may not contain virus, and the female will oviposit after four days in the summer and refeed, but she cannot transmit virus at this time as incubation of the virus is not complete. There usually will be an interval of at least one day after oviposition before a second blood meal, and again it requires four days before egg laying. As you can see, a female will be around 8 to 10 days old when she finally takes a third blood meal, has completed virus incubation, and can transmit a virus. If she is autogenous, she may be 10 to 12 days old before she can be a vector.

I needed for you to understand how long a mosquito must live if she is going to transmit infection, as this explains why we found, in earlier studies, that only 1 in 4 mosquitoes infected in nature could transmit infection. The answer is that most mosquitoes will die of natural causes, or a mosquito control activity, in an 8 to 10 day interval. We have determined this by marking, releasing, and recapturing female *Cx. tarsalis*. A paper on this technique is on the program this afternoon. We found that 10 to 35% of a population will die each day and very few females will survive to take three or more blood meals. If the first blood meal is not on a good source of virus, this further decreases the chance of virus persistence. As you can see this is a good reason to try to develop effective ways to kill adult mosquitoes as it can have such an immediate effect on virus transmission.

Other vector species.

Let me turn briefly to a largely unstudied aspect of WEE virus transmission, the role of *Aedes* mosquitoes as vectors. In California, *Aedes melanimon* Dyar, a very common pest mosquito in many of your districts, may become involved as a secondary vector of WEE virus when *Cx. tarsalis* spreads infection to jackrabbits. Again, there is a paper related to this topic by a student on the program late this afternoon. We suspect that when *Aedes* become infected, they can be a very efficient source for spread of WEE to people and horses.

Conclusions.

To finalize this discussion, I would remind you that in 1940 we had three questions to answer when research on WEE and SLE began. These questions were:

1. How are these transmitted?
2. If vector-borne, where do vectors get infected?
3. Is there a promising method for control?

All three of these questions were answered before most of you became involved in vector control. We learned how to control *Cx. tarsalis*, but it is becoming increasingly difficult as they have become resistant to almost all available insecticides and are very tenacious. We are rapidly learning that we cannot put all of our hopes for future control on *Bacillus thuringiensis*, biological control, or some new miracle chemical insecticide. The cost of controlling epidemics is very high, and frankly, I am not certain today that we could control a large population of infected adult *Cx. tarsalis* over a large area. We still are dependent on carrying out larval control beginning early each summer so as to minimize adult populations in the June to August period. The public may not understand how diffi-

cult it is to control mosquitoes and may not agree to our using methods we know will be effective. At the same time, we know the M.A.D.s do not produce mosquitoes, and usually it is the activities of individuals, industries, or even civic agencies that are responsible. They must have a share in the responsibility for abatement of vectors and the diseases they transmit.

In closing, I would emphasize that I do not believe that WEE, SLE, and other mosquito-borne viruses will go away. I would add that the panel in yesterday's program on encephalitis, malaria, and Lyme disease illustrated how old diseases persist and new diseases come into prominence in our communities. We could add plague and heartworm, along with a list of exotic viruses and vectors that could be introduced into California. I can only conclude that vector control agencies still face serious challenges and are essential in the future to protect the health of our people. I hope that you and your Districts will continue to be concerned and innovative in your approach to these problems.

SURVEILLANCE FOR ARTHROPOD-BORNE VIRAL ACTIVITY AND DISEASE IN CALIFORNIA DURING 1988

Richard W. Emmons[1], Dale V. Dondero[1], Cynthia S. Chan[1],
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This 19th report, in a series of annual reports to the California Mosquito and Vector Control Association (CMVCA) since 1969, summarizes the results of cooperative efforts by local mosquito control agencies, local health departments, the California Department of Food and Agriculture, private physicians and veterinarians, and the agencies and programs represented by the authorship. Weekly reports of the surveillance program results (20 during the season, from 6/3/88 to 11/18/88, plus a special Summary Bulletin 1/13/89) were sent to a large mailing list of participants in the program. This summary of the results was prepared for presentation on 1/31/89 at the 57th Annual Conference of the CMVCA, in Los Angeles.

Extensive clinical and laboratory surveillance for human and equine encephalitis cases was conducted throughout the state by physicians, epidemiologists, and by public health and private clinical laboratories. Two laboratory-confirmed cases of St. Louis encephalitis (SLE) were detected: (1) a 69 year old man from San Bernardino County, onset 9/26/88, with source of infection most likely at a location in San Bernardino City which he visited almost daily. This site is within one mile of the residence of the single SLE case documented in 1987, and is very close to a sentinel chicken flock site at which seroconversion to SLE occurred during August, 1988; (2) a 24 year old woman from Imperial County, who became ill 9/30/88 and was subsequently hospitalized in San Diego where the diagnosis was made. The site of exposure was possibly in the Holtville area of Imperial County, or along the Highland and American canals, where she often went fishing, regions where SLE viral activity was detected July through September, 1988.

A total of 25 clinically-suspected cases of western equine encephalomyelitis (WEE) was reported from eleven California counties. Eleven of the cases were tested serologically and 14 cases were tested by virus isolation attempts from brain specimens sent to the State Viral and Rickettsial Disease Laboratory (VRDL) in Berkeley. There were no laboratory-confirmed cases of WEE in equines in California during 1988.

There were 5711 mosquito pools tested during the year by the VRDL, including 246 743 mosquitoes (Table 1), yielding 51 viral isolates (Tables 2 - 3). These included 39 WEE, nine SLE, and three California serogroup viruses. All isolates of SLE and WEE viruses came from pools of *Culex tarsalis* Coquillett, except one isolate each of SLE and WEE from pools of *Culex pipiens quinquefasciatus* Say complex. An additional 1425 pools (47 413 mosquitoes) from southern California were tested by the Arbovirus Laboratory, School of Public Health, University of California at Berkeley, before and after the VRDL testing program was active. These tests resulted in 21 isolations of SLE virus from *Cx. tarsalis* collected in Imperial County in September and October.

To simplify and economize on the surveillance program, cell cultures inoculated with mosquito pools are no longer being screened for Turlock and Hart Park viruses, usually the commonest isolates. There is little or no evidence that these viruses are significant causes of human or equine disease.

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There were 68 sentinel chicken flocks located at selected sites in endemic areas of the state. These were bled monthly for SLE and WEE antibody tests by indirect enzyme immunoassay (EIA). A total of 9445 blood samples were tested. There were 104 SLE, and 65 WEE seroconversions. Except for three seroconversions to SLE virus at Kern County sites, SLE and WEE viral activity was confined to areas in southern California.

WEE infection in chickens was initially detected in Imperial County in June, and no seroconversions occurred after November. The first evidence of SLE infection also was found in Imperial County chickens, but not until July. However, this virus continued to be active through the winter, with seroconversions in several flocks occurring as late as February 1989 (Table 4).

Plans for the 1989 surveillance season include continuation of the 68 sentinel chicken flocks, with better distribution and more frequent bleedings at certain sites to increase the sensitivity of this component of the surveillance system; and limiting viral tests on mosquito pools to more carefully selected sites and only the four vector species of most concern: *Culex tarsalis*, *Culex peus* Speiser (*stigmatosoma*-proposed), *Culex pipiens* complex, and *Aedes melanimon* Dyar. Further attention will also be given to the importance of the wintertime activity in southern California as a possible predictor of summertime epidemic activity.

Table 1.- Numbers of mosquitoes and pools tested during 1988 by the Viral and Rickettsial Disease Laboratory by county and species.

	Aedes melanimon		*Culex peus*		*Culex pipiens*		*Culex tarsalis*		Other Sp*		TOTAL	
	mosq	pool	mosq	pool	mosq	pool	mosq	pool	mosq	pool	mosq	pool
BUTE	735	15					6250	125			6985	140
GLNN							450	9			450	9
IMPR					1498	35	12031	263	2303	58	15832	356
KERN	1839	47					6731	149			8570	196
LAKE	14	1					1169	24			1183	25
L A			976	42	15012	364	6761	165	12777	287	35526	858
MERC	100	2			100	2	1677	36			1877	40
ORAG			970	36	11757	375	6749	199	780	31	20256	641
RIVR			4207	109	12227	295	50291	1090	9177	220	75902	1714
SACR	194	6			146	3	20688	420	19	1	21047	430
SAND			178	5	641	14	1388	29	100	2	2307	50
SBAR					36	3	472	12			508	15
SBND			2905	79	11149	239	10860	238	486	16	25400	572
SHAS							929	19			929	19
STAN	43	2			558	17	916	26	172	5	1689	50
SUTE							2389	53			2389	53
VENT			165	4	343	9	729	20	986	23	2223	56
YOLO	116	3					21979	449			22095	452
YUBA							1575	35			1575	35
Total	3041	76	9401	275	53467	1356	154034	3361	26800	643	246743	5711

*Primarily *Culex erythrothorax* Dyar. Includes *Psorophora columbiae* (Dyar and Knab), *Culiseta incidens* (Thomson), *Culiseta particeps* (Adams), *Aedes vexans* (Meigen), and *Aedes dorsalis* (Meigen).

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Table 2.- Numbers of viral isolates from mosquitoes tested during 1988 by species, county, and agent isolated.

SPECIES	COUNTY	WEE	SLE	CEV	TOTAL
Aedes melanimon	Kern			3	3
Culex tarsalis	Riverside	12	1		13
	Imperial	22	1		23
	San Bernardino	3	1		4
	Los Angeles		5		5
Culex pipiens complex	Imperial	1			1
	Riverside	1			1
	Los Angeles		1		1
	TOTAL	39	9	3	51

Table 3.-Viral isolates from mosquitoes tested during 1988 by the Viral and Rickettsial Disease Laboratory, compiled chronologically and by Mosquito Abatement District.

DIST	POOL	SPECIES	NO MOSQ	VIRUS	DATE COLL	PLACE	COUNTY
CHLV	868	*Cx. tars*	50	WEE	7/20	North Shore	Riverside
	1089	" "	50	SLE	9/12	Mecca	Riverside
COLO	161	" "	15	WEE	7/27	Bard	Imperial
	175	" "	50	WEE	8/22	Needles	San Bernardino
	181	" "	50	WEE	8/22	Needles	San Bernardino
IMPR	784	" "	22	WEE	7/6	Seeley	Imperial
	785	" "	50	WEE	7/6	Seeley	Imperial
	812	" "	50	WEE	7/7	Holtville	Imperial
	831	" "	50	WEE	7/13	Seeley	Imperial
	833	" "	50	WEE	7/13	Seeley	Imperial
	836	" "	50	WEE	7/13	Seeley	Imperial
	838	" "	50	WEE	7/13	Seeley	Imperial
	840	" "	35	WEE	7/14	Holtville	Imperial
	847	" "	50	WEE	7/14	Holtville	Imperial
	848	" "	42	SLE	7/14	Holtville	Imperial
	851	" "	50	WEE	7/20	Seeley	Imperial
	852	" "	49	WEE	7/20	Seeley	Imperial
	870	" "	22	WEE	7/21	Holtville	Imperial
	879	*Cx. pip*	36	WEE	7/27	Seeley	Imperial
KERN	111	*Ae. mel*	50	CEV	8/16	Lost Hills	Kern
	113	" "	50	CEV	8/16	Lost Hills	Kern
	175	" "	50	CEV	9/11	Lost Hills	Kern
NEED	121	*Cx. tars*	50	WEE	9/8	Needles	San Bernardino
	123	" "	50	SLE	9/8	Needles	San Bernardino
PALO	237	" "	50	WEE	5/25	Palo Verde	Imperial
	287	" "	50	WEE	6/15	Palo Verde	Imperial
	292	" "	50	WEE	6/15	Palo Verde	Imperial
	293	" "	50	WEE	6/15	Palo Verde	Imperial
	318	"	50	WEE	6/22	Palo Verde	Imperial
	321	" "	24	WEE	6/22	Palo Verde	Imperial
	307	" "	50	WEE	6/22	Riverside	Blythe
	341	" "	50	WEE	6/30	Palo Verde	Imperial
	347	" "	50	WEE	6/30	Palo Verde	Imperial
	333	" "	50	WEE	6/30	Riverside	Blythe
	337	" "	50	WEE	6/30	Riverside	Blythe
	339	" "	39	WEE	6/30	Riverside	Blythe
	351	" "	50	WEE	6/30	Riverside	Blythe
	374	" "	50	WEE	7/6	Palo Verde	Imperial
	360	*Cx. pip*	28	WEE	7/6	Riverside	Blythe
	361	*Cx. tars*	50	WEE	7/6	Riverside	Blythe
	363	" "	50	WEE	7/6	Riverside	Blythe
	379	" "	32	WEE	7/6	Riverside	Blythe

continued-

Table 3.-continued.

DIST	POOL	SPECIES	NO MOSQ	VIRUS	DATE COLL	PLACE	COUNTY
PALO	381	*Cx. tars*	50	WEE	7/13	Riverside	Blythe
	390	" "	50	WEE	7/20	Riverside	Blythe
	414	" "	50	WEE	8/3	Riverside	Blythe
SOUE	473	" "	27	SLE	8/8	Encino	Los Angeles
	500	" "	42	SLE	8/15	Encino	Los Angeles
	522	" "	50	SLE	8/22	Encino	Los Angeles
	523	" "	18	SLE	8/22	Encino	Los Angeles
	569	*Cx. pip*	35	SLE	8/30	Encino	Los Angeles
	610	*Cx. tars*	07	SLE	9/27	Encino	Los Angeles

Table 4.-WEE and SLE seropositive chickens/number tested (percent positive), California, 1988

Flock location	SLE positive/number tested (percent positive)[a]					
	May 24-Jun 3	Jun 23-30	Jul 20-27	Aug 18-31	Sep 15-26	Oct 17-24
			Northern California			
Shasta, Cottonwood	0/22	0/22	0/21	0/22	0/22	0/22
Tehama, MAD office	0/25	0/25	0/25	0/23	0/25	0/25
Corning, Martin Ranch	0/25	0/25	0/24	0/24	0/23	0/24
Butte, Chico	0/24	0/25	0/25	0/25	0/25	no samp
Butte, Grey Lodge	0/24	0/24	0/23	0/23	0/23	no samp
Butte, Honcut	0/24	0/21	0/21	0/20	0/20	no samp
Glenn, Willows	0/21	0/17	0/19	0/20	0/19	0/18
S-Yuba, P. V. Ranch	0/25	0/25	0/25	0/25	0/23	0/24
S-Yuba, Dean's	0/25	0/25	0/25	0/25	0/25	0/24
S-Yuba, Barker	0/23	0/8	0/7	0/7	0/7	0/7
Sac-Yolo, Merritt	0/23	0/22	0/22	0/21	0/19	0/19
Sac-Yolo, Natomas	0/24	0/24	0/24	0/23	0/24	0/24
Sac-Yolo, Elk Grove	0/25	0/25	0/25	0/25	0/25	0/25
Lake, MAD Office	0/25	0/25	0/25	0/25	0/25	0/24
Marin-Sonoma, W. Santa Rosa	0/23	0/23	0/19	0/23	0/23	0/21
Solano, Dixon	0/25	0/25	0/25	0/25	0/24	0/25
Santa Clara, San Martin	0/16	0/18	0/13	0/16	0/17	0/17
N. CALIFORNIA SLE TOTAL	0/399	0/379	0/368	0/372	0/369	0/299
			San Joaquin Valley			
San Joaquin, Lodi	0/19	0/12	0/19	0/19	0/19	0/19
Eastside, Oakdale	0/20	0/20	0/17	0/19	0/18	0/18
Turlock, Vitoria	0/18	0/20	0/20	0/20	0/19	0/20
Merced, Veldhaus	0/24	0/23	0/23	0/23	0/23	0/23
Merced, Lucky 11	0/26	0/19	0/26	0/26	0/24	0/24
Fresno W'side, Mendota Ref.	0/25	0/25	0/25	0/25	0/25	0/22
Consolidated, Friant Rd.	0/25	0/25	0/24	0/24	0/24	0/24
Kings, MAD Office, Hanford	0/24	0/24	0/24	0/24	0/24	0/24
Delta, Kingsburg GC	0/25	0/25	0/25	0/22	0/21	0/24
Tulare, MAD office	0/25	0/25	0/24	0/23	0/22	0/23
West Side, Lost Hills	0/18	0/18	0/15	0/15	1/11(9)	not bled
West Side, Maricopa	0/20	0/20	0/20	0/18	1/15(7)[b]	not bled
Delano, Teviston	0/24	0/24	0/23	0/22	0/20	0/20
Kern, Wasco	0/25	0/25	0/25	0/24	0/25	0/25
Kern, F.C.Tracy	0/25	0/25	0/24	0/23	0/24	0/22
Kern, Buttonwillow	0/25	0/25	0/25	0/25	1/25(4)	3/25(12)
Kern, John Dale	0/25	0/25	0/25	0/25	0/23	0/23
Kern, Oildale	0/25	0/25	0/24	0/24	0/24	0/24
Kern, Wildlife Refuge	0/24	0/24	0/19	0/19	0/19	0/19
Kern, River Bottom	0/24	0/24	dead[c]	0/20	0/19	0/20
SAN JOAQUIN SLE TOTAL	0/466	0/453	0/427	0/440	3/424(1)	3/399(1)

a. All chickens negative for WEE. b. #3553 negative on Oct 10; Sep 20 sample was positive when retested by EIA and FA. c. Replaced.

Table 4.-Continued. WEE and SLE seropositive chickens/number tested (percent positive), southern California, 1988.

Flock location	WEE positive/number tested (percent positive) - all flocks										
	Jun 24-Jul 7	Jul 22-Aug 1	Aug 19-31	Sep 19-26	Oct 14-20	Nov 14-23	Dec 12-29	Jan 9-19	Feb 7-13	Mar 7-15	Apr 4-5
Goleta, Gray's Ranch	0/18	0/19	0/18	0/19	0/19	not bled	not bled	not bled	not bled	not bled	not bled
Ventura, Pt. Mugu	0/19	0/19	0/19	0/19	0/19	not bled	not bled	not bled	not bled	not bled	not bled
Ventura, Simi Valley	0/20	0/20	0/20	0/20	0/20	not bled	not bled	not bled	not bled	not bled	not bled
Los Angeles, La Brea+	0/23	0/24	0/22	0/23	0/23	0/21	0/22	0/22	0/22	0/22	.
Los Angeles, Cal Poly+	0/23	0/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24
Southeast, Harbor Lake+	0/23	0/23	0/14	killed	.	.	.	.	.	.	.
Long Beach, El Dorado	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
Southeast, Balboa Golf+	0/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24	0/5[a]
Southeast, Norwalk+	0/22	0/22	0/22	0/21	0/21	0/21	0/20	0/20	0/20	0/20	0/20
Orange, Fullerton+	0/21	0/21	0/21	0/21	0/21	0/21	0/21	0/21	0/21	0/21	0/21
Orange, San Mateo Point	0/19	0/19	0/19	0/19	0/19	0/18	0/19	not bled	not bled	not bled	not bled
Orange, Duck Club+	0/23	0/19	0/19	0/19	0/17	0/17	0/18	not bled	0/17	not bled	0/15
San Bernardino, San Bernardino		0/22	0/20	0/24	0/21	not bled	not bled	not bled	0/19	not bled	not bled
West Valley, Chino, Smith	0/24	0/24	0/24	0/24	0/24	0/24	0/24	not bled	not bled	not bled	not bled
West Valley, Briano Bros.	0/15	0/15	0/15	0/9	0/9	0/9	0/9	not bled	not bled	not bled	not bled
Northwest, Corona	0/18	0/18	0/18	0/18	0/17	not bled	not bled	not bled	not bled	not bled	not bled
Coachella Valley, Palm Desert	0/22	0/21	0/21	0/21	0/21	0/21	0/20	0/20	replaced		
Coachella Valley, North Indio	0/24	0/24	0/24	0/24	0/23	0/23	0/23	0/23	replaced		
Coachella Valley, Mecca	0/16	0/16	1/16(6)	1/16(6)	1/16(6)	1/16(6)	1/16(6)	1/16(6)	replaced		
Coachella Valley, Thermal	0/24	0/24	0/24	0/24	0/20	0/20	0/20	0/20	replaced		
Imperial, Finney/Ramer	6/19(32)	15/19(79)	15/18(83)	15/18(83)	15/18(83)	16/18(89)	16/18(89)	16/18(89)	replaced		
Imperial, Keefer Rd., Holtville	3/23(13)	7/23(30)	10/23(43)	10/23(43)	10/23(43)	10/23(43)	10/23(43)	10/23(43)	replaced		
Imperial, Drew Rd., Seeley	1/24(4)	17/23(74)	20/23(87)	20/23(87)	20/23(87)	not bled	not bled	not bled	replaced		
Imperial, Palo Verde	5/24(21)	8/24(33)	11/24(46)	11/24(46)	11/24(46)	11/23(48)	11/24(46)	11/24(46)	replaced		
Imperial, Bard	0/24	5/24(21)	6/24(25)	6/24(25)	6/24(24)	6/24(24)	6/24(24)	6/24(24)	replaced		
San Diego, San Ysidro	0/20	0/20	0/20	0/16	0/16	not bled	not bled	not bled	not bled	not bled	not bled
San Diego, Lakeside	0/17	0/18	0/18	0/18	0/17	not bled	not bled	not bled	not bled	not bled	not bled
San Diego, Vista	0/14	0/14	0/14	0/13	0/13	not bled	not bled	not bled	not bled	not bled	not bled
Colorado River, Needles	0/19	0/17	0/17	0/17	0/17	0/17	0/17	0/17	replaced		
Colorado River, Havasu Refuge	0/23	0/22	0/21	1/21(5)	1/21(5)	1/21(5)	1/21(5)	1/21(5)	replaced		
Colorado River, Blythe	0/22	0/21	0/21	0/21	0/19	0/19	0/19	0/19	replaced		
S. CALIFORNIA WEE TOTAL	15/627(2)	52/643(8)	63/627(10)	64/607(11)	64/593(11)	45/406(11)	45/407(11)	45/356(13)			

Flock location	SLE positive/number tested (percent positive) - SLE positive flocks only										
Long Beach, El Dorado	0/20	0/20	2/20(10)	4/20(20)[b]	10/20(50)	11/20(55)	12/20(60)	12/20(60)	13/20(65)	13/20(65)	13/20(65)
Southeast, Balboa Golf+	0/24	0/24	3/24(13)	10/24(42)	13/24(54)	14/24(58)	14/24(58)	14/24(58)	14/24(58)	14/24(58)	2/5
Orange, Fullerton+	0/21	0/21	0/21	1/21(5)	2/21(10)	2/21(10)	2/21(10)	2/21(10)	2/21(10)	2/21(10)	2/21(10)
Orange, Duck Club+	0/23	0/19	0/19	0/19	2/17(12)	3/17(18)	3/18(17)	not bled	3/17(18)	not bled	3/15(20)
San Bernardino, San Bernardino		0/22	2/20(10)	2/24(8)	1/21(5)	not bled	not bled	not bled	3/19(16)	not bled	not bled
Northwest, Corona	0/18	0/18	0/18	1/18(6)	1/17(6)	not bled	not bled	not bled	not bled	not bled	not bled
Imperial, Finney/Ramer	0/19	1/19(5)	8/18(44)	15/18(83)	15/18(83)	15/18(83)	15/18(83)	15/18(83)	replaced		
Imperial, Keffer Rd., Holtville	0/23	4/23(17)	15/23(65)	22/23(96)	22/23(96)	23/23(100)	23/23/(100)	23/23/(100)	replaced		
Imperial, Drew Rd., Seeley	0/24	0/23	18/23(78)	23/23(100)	23/23/(100)	not bled	not bled	not bled	replaced		
Imperial, Bard	0/24	2/24(8)	3/24(13)	4/24(17)	10/24(42)	10/24(42)	10/24(42)	10/24(42)	replaced		
Colorado River, Needles[c]	0/19	0/17	0/17	0/17	0/17	0/17	0/17	1/17(6)	replaced		
S. CALIFORNIA SLE TOTAL	0/627	7/643(1)	51/627(8)	82/607(13)	99/593(17)	78/406(19)	79/407(19)	77/356(22)			

+ These flocks were tested in Feb, Mar, Apr, early May and late May/early Jun; all chickens were negative except Needles (see below).

a. All other chickens killed in March. b. Three of these were SLE positive on Sep 13 (flock bled twice in Sep).

c. Chicken #981: Feb and Mar sera were negative; Apr was positive (HAI titer for SLE = 1:80); May 2 was SLE positive by EIA in our lab; May 31 was negative by both EIA and FA in VRDL; all subsequent samples were negative in VRDL tests.

CALIFORNIA AND BUNYAMWERA SEROGROUP VIRUSES IN CALIFORNIA[1]

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Introduction.

Although viruses belonging to the California (CAL) and Bunyamwera (BUN) serogroups of the family Bunyaviridae have been known for a long time, they have been studied relatively little in comparison with alphaviruses (e.g. western equine encephalomyelitis) and flaviviruses (e.g. St. Louis encephalitis) which historically have been associated with more disease in humans and domestic animals. This is especially true in California where the prototype virus [California encephalitis (CE)] of the California serogroup was isolated and only three human cases of CE have been documented.

The current status of knowledge of CAL and BUN serogroup viruses in the United States will be summarized in this paper, along with recent serological evidence of CAL and BUN serogroup virus infections in deer in California. Recent evidence of viral infections in California mosquitoes will be presented in a companion paper.

Summary of current knowledge of California and Bunyamwera serogroup bunyaviruses.

Bunyaviruses are classified into serogroups, complexes, viruses, subtypes, and varieties on the basis of antigenic relationships (Calisher and Karabatsos 1988). Five bunyaviruses have been isolated from California mosquitoes, of which two are included in the CAL serogroup, and three in the BUN serogroup (Table 1). There is one representative CAL serogroup virus each in the California encephalitis and Melao complexes: CE and Jamestown Canyon (JC) viruses, respectively. BUN serogroup viruses in California belong in two complexes: Northway (NOR) and Lokern (LOK) viruses in the Bunyamwera Complex, and Main Drain (MD) virus in the Main Drain complex.

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The public health and veterinary importance of CAL and BUN serogroup viruses are only partially known. CE (Hammon and Reeves 1952) and JC (Grimstad et al. 1982) viruses are known to cause encephalitis in humans. JC virus may also cause other symptoms in humans, including pneumonia, abdominal pain, sore throat, and cough (Grimstad et al. 1982, Srihongse et al. 1984). Very little is known about the public health importance of North American BUN serogroup viruses, although there is evidence that humans can be infected with NOR virus (Zarnke et al. 1983) and with LOK virus (Karabatsos 1985). MD virus was isolated from the brain of a horse that died of encephalitis (Emmons et al. 1983).

The ecological setting for these viruses in California ranges from irrigated areas of the Central Valley to high elevation forested areas and high desert areas. JC virus seems to be most prevalent in mountainous areas and in the high eastern plateau of California (Campbell et al. 1989). This is probably a reflection of the northern distribution in North America of these antigenic groups of viruses and their association with arthropods having primarily Nearctic or Holarctic ranges. JC virus is now believed to range from Alaska and Canada into all of the lower 48 continental U.S. states (Grimstad 1988). The isolations of JC virus have come from collections of *Culiseta inornata* Williston in the Central Valley, and in southeastern desert areas of the state (Reeves et al. 1983, Wallace 1972).

CE virus activity has been detected in a number of western U.S. states. In California it is apparently restricted to the Central Valley and Owens Valley (Reeves et al. 1983). NOR virus has up to now been known to occur only in Alaska and northwestern Canada, but recent serologic studies in deer have demonstrated the existence of this virus, or one closely related to it, in California (Campbell et al. 1989). MD and LOK viruses have been isolated from several southwestern states in addition to California (Calisher et al. 1986).

JC virus primarily infects deer, and a number of studies in the Midwest have shown a high degree of correlation between the presence of this virus and population levels of white-tailed deer

Table 1.-California and Bunyamwera serogroup viruses (Genus Bunyavirus, Family Bunyaviridae) known to occur in California.

Serogroup	Complex	Virus
California	California	California encephalitis
	Melao	Jamestown Canyon*
Bunyamwera	Bunyamwera	Lokern Northway
	Main Drain	Main Drain

*The classification by Calisher and Karabatsos (1988) recognizes subtypes and varieties of viruses. In their classification, Jamestown Canyon is considered a variety of Jamestown Canyon subtype of Melao virus. They also recognize Jerry Slough as a variety of Jamestown Canyon subtype. We consider Jerry Slough virus to be synonymous with Jamestown Canyon virus.

Table 2.-Isolations of Jamestown Canyon virus from mosquitoes collected in North America (from various sources).

Aedes:	*Anopheles:*
abserratus	*crucians*
aurifer	*punctipennis*
canadensis	
cantator	*Coquillettidia*
cinereus	*perturbans*
communis	
euedes	
excrucians	*Culiseta*
melanimon	*inornata*
sollicitans	
stimulans	*Psorophora:*
thelcter	*confinnis*
*triseriatus**	*discolor*
trivittatus	
vexans	

*Isolated from adults collected as eggs.

(*Odocoileus virginianus* (Zimmerman)) (Grimstad 1988). CE virus seems to be found mainly in blacktailed jack rabbits (*Lepus californicus* Gray) (Hardy et al. 1977, Reeves et al. 1983). NOR virus appears to infect primarily ungulates. MD virus has been isolated repeatedly from blacktailed jack rabbits, (*Lepus californicus*), and once from a horse brain (Emmons et al. 1983). LOK also is primarily associated with rabbits (Calisher et al. 1986).

All of the CAL and BUN serogroup viruses are apparently associated with mosquitoes. Cold-adapted mosquitoes, such as snow pool *Aedes* and *Cs. inornata*, appear to be involved frequently as vectors. LOK and MD viruses have been isolated often from biting midges (*Culicoides*), and occasionally from mosquitoes. JC virus has been isolated from a number of mosquito species in the genera *Aedes*, *Anopheles, Culiseta*, and *Psorophora* (Table 2). It has been isolated from adult *Aedes triseriatus* (Say) reared from eggs, thus it can be transovarially transmitted in nature (Berry et al. 1977). In Alaska and Canada, NOR virus has been isolated from snow pool *Aedes* mosquitoes and from mosquitoes in the genus *Culiseta* (Calisher et al. 1974).

Taken as a whole, viruses of these serogroups show a number of significant differences from the more extensively studied alphaviruses and flaviviruses. Several of have been shown to be transmitted transovarially in nature at relatively high frequencies. Mammals, rather than birds, are the primary vertebrate hosts. Vectors are usually mosquitoes of the genera *Aedes* and *Culiseta*, rather than *Culex*, the genus of mosquitoes most frequently involved as vectors of alphaviruses and flaviviruses in North America. Forested, mountainous, and desert areas are frequently the ecological setting for these viruses, in addition to irrigated agricultural areas. Finally, the public health and veterinary importance of these viruses as a group is largely unknown.

These viruses have been known since 1943, when the original isolate of CE virus was made from *Aedes melanimon* Dyar in Kern County, California. Three human cases of encephalitis in Kern County in 1945 were later shown to have been caused by this same virus (Hammon and Reeves 1952, Hammon et al. 1952). Subsequent to 1945, there have been no proven human cases of clinical CE virus infection. In 1968, Dr. Richard Emmons reported the presence of JC antibodies in deer in the Yosemite Valley (Emmons 1968).

Recent studies.

No CAL serogroup virus activity had ever been reported from Oregon or Washington prior to the 1980s. Eldridge et al. (1986) reported the first evidence of activity of these viruses in Oregon by demonstrating the presence of antibodies in deer from nine counties in the Cascade and coastal ranges in that state. Mule deer, blacktailed deer, and elk sera showed evidence of antibodies to JC, CE, snowshoe hare (SSH), Cache Valley (CV), and Klamath viruses. SSH virus is a CAL serogroup virus in the California encephalitis subtype; CV is a BUN serogroup virus. Klamath virus is in the family Rhabdoviridae, which contains the viruses causing rabies and vesicular stomatitis.

Campbell et al. (1989) used serum-dilution neutralization tests to detect viral antibodies in California deer sera from both high elevation mountainous areas in the Sierra Nevada, Modoc Plateau, Great Basin, and Klamath and Cascade ranges, and from low elevation coastal ranges. For JC virus, they found the level of seropositivity in deer to be about 23% in samples from high mountain areas, but only 7% from low coastal areas. They also found antibodies to NOR virus (previously known to occur only in Alaska and Canada), but antibodies had about the same frequency in samples collected at both high and low elevations. The results with JC antibody tests were consistent with an association with snow pool *Aedes* as vectors. The NOR results suggested relationships to widespread vectors such as *Cs. inornata* or *Aedes increpitus* Dyar.

We intend to broaden our serology testing to look for evidence of infection of other vertebrates with these viruses, including human beings. We already have obtained a number of human sera. We plan to continue testing arthropods, especially mosquitoes, for viruses, and to carry out vector competence studies with different mosquito species for the viruses of interest in this study. As we gain a better understanding of the vector associations of these viruses, we will intensify our studies of the ecology and biosystematics of vector populations. We hope to have more to report on these studies

next year.

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PRELIMINARY STUDIES OF CALIFORNIA AND BUNYAMWERA SEROGROUP BUNYAVIRUSES IN CALIFORNIA MOSQUITOES[1]

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ABSTRACT

More than 14,000 mosquitoes in four genera were collected as adults and larvae from the Sierra Nevada range, Modoc Plateau, and the northern coast of California during the spring and summer of 1988. Species in the *Aedes communis* Group, including *Ae. communis* (sensu lato),[4] *Aedes cataphylla*, and *Aedes hexodontus*, accounted for more than 87% of the total collection. Five virus strains were isolated, all from mosquitoes collected in or adjacent to Faith Valley in Alpine County at approximately 2,300 m elevation where nearly 2/3 of the total collection of mosquitoes was made. The first strain was recovered from a pool of male *Ae. cataphylla* collected as larvae in early May and reared to the adult stage in the laboratory. The remaining four strains were from pools of female *Ae. communis* (s.l.) collected as adults. All five strains were shown to be bunyaviruses in the California serogroup and closely related to Jamestown Canyon (JC) virus. The isolation of virus from males reared from larvae is evidence for vertical transmission in mosquitoes, which has been reported for JC virus elsewhere in North America.

A recent serological study of deer in California revealed widespread evidence of the presence of a virus similar or identical to Northway (NOR) virus of the Bunyamwera serogroup of bunyaviruses.[5] Prior to that study the known geographic distribution of NOR virus was limited to Alaska and northwestern Canada, where it has been shown to infect ungulates and other large mammals, including humans. Neutralization tests done with three strains of unidentified bunyaviruses that had been isolated from mosquitoes in the Central Valley of California during the past 20 years showed a close antigenic relationship to NOR virus. Two of these NOR-like viruses were isolated from *Anopheles freeborni* and one was from *Aedes sierrensis* collected in Butte County during 1970-1971. Another unidentified bunyavirus isolated from *Aedes taeniorhynchus* collected in San Diego County in 1985 was shown to be a strain of Main Drain virus of the Bunyamwera serogroup, the occurrence of which has been well documented in California.

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[4]*Aedes communis* is known to be a complex of species in North America. Until detailed studies are made of the geographic ranges of the species making up the complex, we cannot say with certainty species occur(s) in Alpine County.

[5]Campbell, G. L., B. F. Eldridge, J. L. Hardy, W. C. Reeves, D. A. Jessup, and S. B. Presser. Prevalence of neutralizing antibodies against California and Bunyamwera serogroup viruses in deer from mountainous areas of California. Am. J. Trop. Med. Hyg. 40: 428-437.

EVALUATION OF MOSQUITO AND ARBOVIRUS ACTIVITY IN ORANGE COUNTY, 1988

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In 1988, the Orange County Vector Control District (OCVCD) increased its encephalitis virus surveillance (EVS) in the suburban backyard habitats. Mosquito collections were made at least once each week with CDC/CO_2 light traps from May through October. More than 21 000 mosquitoes were collected, identified, and pooled (883 pools) for arbovirus (SLE and WEE) testing. This collection included 549 pools of *Culex quinquefasciatus* Say, 217 pools of *Culex tarsalis* Coquillet, 59 pools of *Culex stigmatosoma* Dyar (= *Culex peus* Speiser), 42 pools of *Culiseta incidens* (Thomson), and 16 pools of *Culiseta inornata* (Williston). Most of the *Cx. tarsalis* specimens were collected from rural habitats. None of the pools yielded positive SLE or WEE results.

Culex quinquefasciatus was the most frequent and most prevalent mosquito species found in the immediate environs around the Orange County homes studied. Occasionally *Culex erythrothorax* Dyar, *Cx. tarsalis, Cx. stigmatosoma*, and/or *Cs. incidens* were collected. Landing/biting studies on humans conducted by one of us (ETS) have shown that *Cs. incidens* was the most commonly collected species, although the total number of landing mosquitoes was small.

Sentinel chicken data from Orange County include two SLE seroconversions in Fullerton (one seropositive chicken was bled in early September and the other in the middle of October), and three at the 20 Ranch Duck Club in Irvine (two were bled in the middle of October and the other in November).

Four sentinel pigeons from the 20 Ranch Duck Club (Irvine) bled in the middle of August also seroconverted for SLE antibodies, as did a sentinel pigeon from Fullerton bled in the middle of September.

In the Small Bird Sera Collection Program for 1988, conducted by John A. Gruwell and

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Table 1. SLE/WEE seroconversions in small bird sera samples in Orange County, 1988.

Species	SLE	WEE	No. Blood Samples	Percent SLE	Percent WEE
Rock Dove	31	16	1448	2.1	1.10
House Sparrow	7	2	2097	0.3	0.10
Scrub Jay	1	0	9	11.1	0.00
House Finch	21	1	4185	0.5	0.02
Total	60	19	7739		

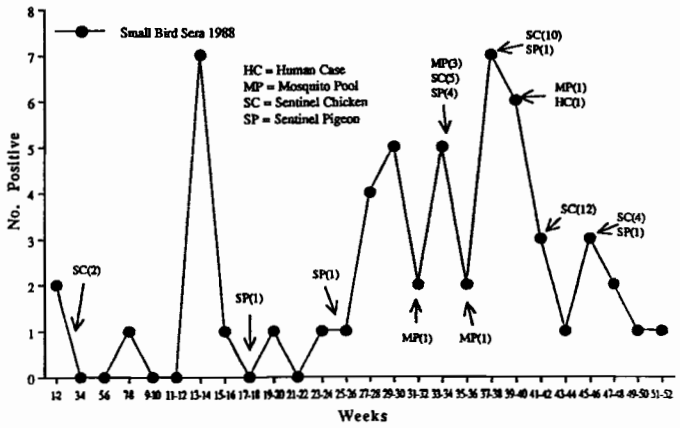

Figure 1.-SLE virus activity in the Los Angeles Basin, 1988.

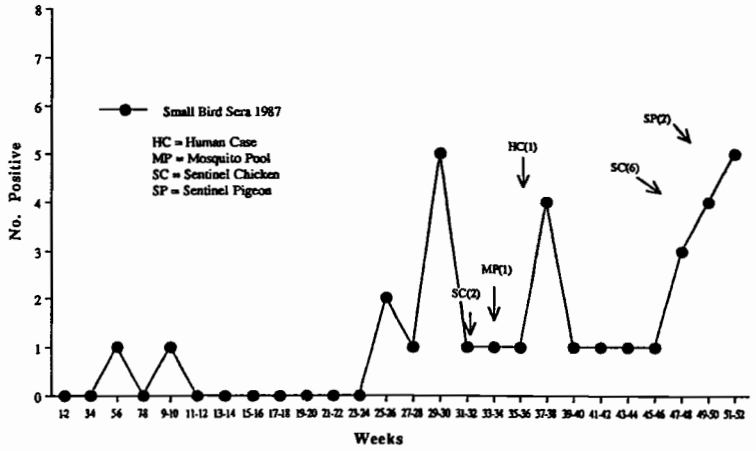

Figure 2.-SLE virus activity in the Los Angeles Basin, 1987.

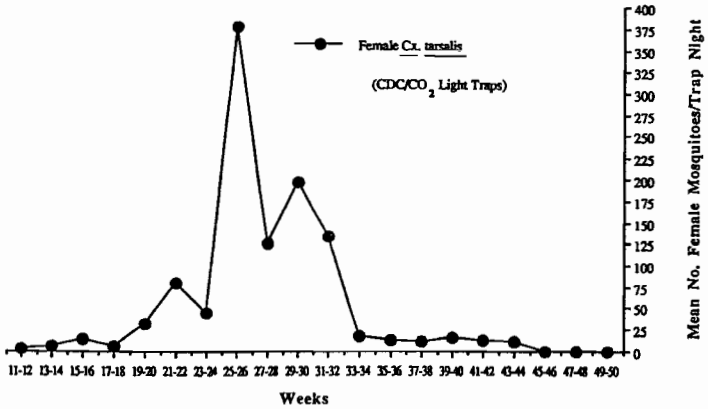

Figure 3.-Female *Cx. tarsalis* activity in the San Joaquin Marsh, Irvine, 1988 - CDC/CO_2 Light Traps.

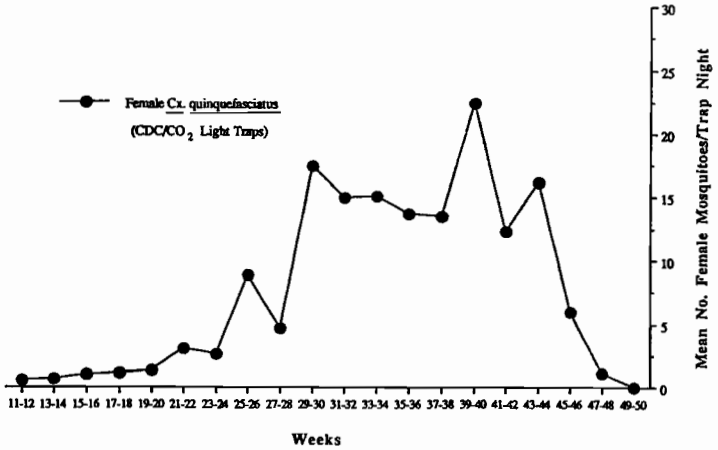

Figure 4.-Female *Cx. quinquefasciatus* activity in the San Joaquin Marsh, Irvine, 1988 - CDC/CO_2 Light Traps.

assisted by Ms. Becky Brown, a total of more than 20000 birds was trapped from which 8501 serum samples were recovered and analyzed for SLE and WEE antibodies. Table 1 depicts the results. Free-ranging rock doves (*Columba livia*) exhibited the highest number of positive results for both SLE and WEE (if you ignore the small number of scrub jay (*Aphelocoma coerulescens*) sera). Pigeons, house finches (*Carpodacus mexicanus*), and house sparrows (*Passer domesticus*) have consistently yielded seropositive individuals each year since Orange County began its surveillance program in 1985.

The temporal distribution of the small bird seroconversions is shown in Figure 1. These data are compared to other virus activity detection systems, including sentinel chickens (SC), sentinel pigeons (SP), mosquito pools (MP), and human cases (HC). The only human case recorded in 1988 involved a 69 year old San Bernardino man. Peridomestic bird (house finches and house sparrows) seroconversion activity began by weeks 7-8 (February 11-24) and accelerated during weeks 13-14 (March 25-April 7). The two sentinel chickens (SC) that converted in January (weeks 3-4) probably represent the end of a period of peaking activity that began in 1987 (see Figure 2). Data accrued during weeks 47-52 (November 20-December 31) in 1987 indicated SLE seroconversion acceleration during the remainder of the year and into early 1988 (see Figure 1). Although reported 2-4 weeks behind the small bird data, sentinel chickens (SC) and sentinel pigeons (SP) also demonstrated positive SLE seroconversions during this same time period.

Mosquito activity was monitored intensively in Orange County suburban habitats in addition to the usual surveillance operations in the traditional rural habitats. There were large numbers of *Cx. tarsalis* in the San Joaquin Marsh with the greatest amount of activity occurring between weeks 23-32 (June 6-August 11) as shown in Figure 3. Peak numbers were reached during the period between middle to late June (weeks 25-26). A low level of *Cx. tarsalis* activity continued in the marsh through late October (weeks 43-44). This peak and the range of activity from May through October correlate with data obtained each year since 1983.

Host-seeking *Culex quinquefasciatus* were also collected from the same rural environs of the San Joaquin Marsh (Figure 4). Comparison with collection records from past years indicated that the numbers of *Cx. quinquefasciatus* for 1988 were essentially unchanged. Host-seeking activity began in mid April and ended in early December (weeks 47-48). Egg laying females exhibited significant levels of activity in the fall and winter months (Figure 5). Gravid female traps collected *Cx. quinquefasciatus* in greater numbers throughout the fall and winter months in the rural habitat with peak activity (as many as 40 gravid females per trap night) during late September and early October.

Gravid female traps collected even larger numbers of *Cx. quinquefasciatus* in suburban habitats. As many as 140 females per trap night were collected during peak activity as seen in Figure 6. Females were also collected in relatively large numbers throughout the fall and winter months in the suburban environs.

Culex tarsalis host-seeking females were also monitored in backyards. Although this species is not found around homes as frequently or in as great numbers as *Cx. quinquefasciatus* (Webb et al. 1987, Webb et al. 1988, Reisen et al. 1988), it still may be significant as a factor in peridomestic mosquito/bird and/or mosquito/human interactions.

When comparing the mean number of *Cx. tarsalis* females trapped per night and the small bird seroconversions (SLE) from the entire county, there seems to be a correlation between the peaks of bird seroconversions and the peaks of *Cx. tarsalis* activity (Figure 7). This situation is apparent until weeks 35-36 (late August-early September), when a rapid decline of *Cx. tarsalis* numbers occurred. Small bird seroconversions for SLE continued on through the remainder of the year.

Host-seeking *Cx. quinquefasciatus* on the other hand, maintained measurable numbers throughout the year (Figure 8), and unlike *Cx. tarsalis* (Figure 7), *Cx. quinquefasciatus* peaks and profile roughly correlate with those of the small bird seroconversion records throughout the year, including the late fall and winter months.

Discussion and Conclusions.

The data accrued in 1988 support the 1987 observations (Gruwell et al. 1988) that SLE virus is being transmitted during all months of the year. Seroconversion information from house finches, house sparrows, and free-ranging pigeons provided the earliest evidence of SLE and WEE virus trans-

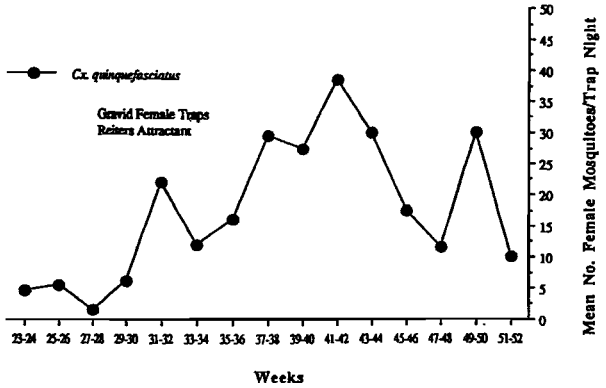

Figure 5.-Gravid female *Cx. quinquefasciatus* activity at the 20 Ranch Duck Club, Irvine, 1988 - Reiter Traps.

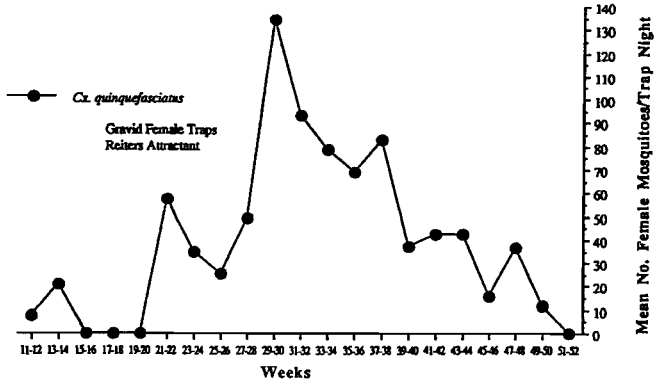

Figure 6.-Gravid female *Cx. quinquefasciatus* activity at a suburban residence, Irvine, 1988 - Reiter Trap.

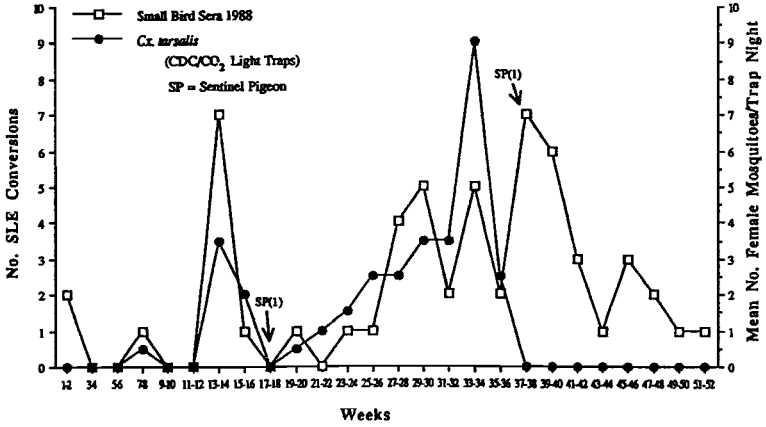

Figure 7.-Host-seeking *Cx. tarsalis* collection records from Fullerton and small bird seroconversions (SLE) from Orange County - Suburban Habitats, 1988.

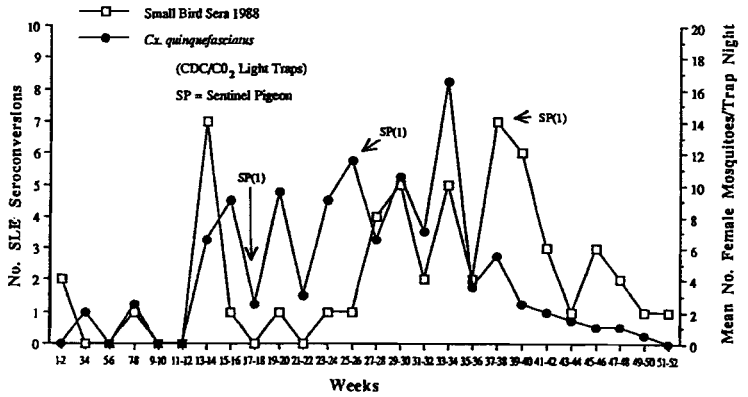

Figure 8.-Host-seeking *Cx. quinquefasciatus* collection records from Fullerton and small bird seroconversions (SLE) from Orange County - Suburban Habitats, 1988.

mission in the Los Angeles Basin. Information from the monitoring of these three species was available four to six weeks before the results were obtained from the sentinel chicken, sentinel pigeon, and human case detection systems.

The numbers of both host-seeking and gravid female *Cx. quinquefasciatus* were consistently and significantly higher than those of *Cx. tarsalis, Cx. stigmatosoma*, and *Cs. incidens* in the suburban peridomestic environs. Present methods (Reiter Gravid Female traps and alfalfa attractant) of collecting gravid mosquitoes are biased toward the acquisition of *Cx. quinquefasciatus*; however, the collection studies by one of us (ETS, unpub. data) using an "AFS Sweeper" (Meyer et al. 1983) indicates similar incidence and frequency of species found in the present study.

St. Louis encephalitis and western equine encephalitis virus transmission to the human population from a vector mosquito species in the peridomestic suburban environment still remains to be demonstrated. One or more of the several mosquito species found in these habitats may be important as vectors of the encephalitis causing arboviruses. Continuation of studies of the backyard mosquito ecology and virus transmitting capability of potential vectors is strongly encouraged.

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ANALYSIS OF ARBOVIRUS ISOLATION: *CULEX TARSALIS* AND *CULEX QUINQUEFASCIATUS* COLLECTED IN THE IMPERIAL VALLEY, 1967-1987

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A long-term ecological study[3], involving collection of mosquitoes for isolation and identification of viruses, was undertaken from 1967 to 1987 in several aquatic habitats of the Imperial Valley (Figure 1) (Work 1975, 1977). The objective was to search for interepidemic sources of arboviruses causing St. Louis encephalitis (SLE) and other encephalitides, and to test the hypothesis that the viruses might be maintained and active throughout the year in an arid irrigated habitat virtually free of freezing.

More than 20 000 pools of mosquitoes were processed for arboviral isolation during a three-phase investigation beginning at Wister (1967-1971), with a brief interlude along the Alamo River at the border during the VEE epidemic in Mexico in 1971 and 1972, then at Finney Lake (1970-1978), and finally on the New River (1976-1978 and 1987).

Table 1 presents a summary of 654 448 *Culex tarsalis* Coquillett and *Culex quinquefasciatus* Say collected during the investigation. A total of 608 922 *Cx. tarsalis* was processed in 14 116 pools, which yielded 774 strains of SLE virus; there were 45 432 *Cx. quinquefasciatus* processed in 1843 pools, which yielded 19 strains of SLE virus in a comparable period. Table 2 summarizes the findings for these two species at Finney Lake and on the New River.

Table 1 also shows that 516 isolates of WEE came from the same collection of *Cx. tarsalis*, but for the purpose of this presentation they are excluded as being unimportant in the causation of human infection in the area (Jozan, 1977).

As the field study advanced, collections were intensified at Finney Lake, beginning in 1970, to study the dynamics of *Cx. tarsalis* activity which have been published previously (Berlin et al. 1976, Clark et al. 1976, Work et al. 1977). The cumulative data at Finney Lake cover 51 out of 52 weeks (Figure 2). The percentages of SLE virus in the second level of Figure 2 show that SLE activity did not occur from weeks 1-15 and 46-52, and indicate that in spite of continued activity of *Cx. tarsalis* throughout the winter, there was no evidence of SLE transmission by *Cx. tarsalis* in December, January, February, and March.

Recognition of this phenomenon in 1975 resulted in a transfer of investigative effort to the New River transect, which was established in 1976. Collections were carried out routinely at 1-mile intervals from the border to a point 15 miles downriver. The purpose of this new effort was to elucidate any evidence that transmission followed the New River as a conduit into the Imperial Valley, with subsequent introduction into the Alamo drainage, of which Finney Lake is the key focus.

Because WEE is more damaging to infants and small children than SLE, researchers in the west have focused on *Cx. tarsalis* as the principal vector of arboviral encephalitis (Reeves and Hammon 1962). As a result, *Cx. quinquefasciatus* has been somewhat neglected as a potential vector of SLE to human population. The accumulation of *Cx. quinquefasciatus* during this study did not reflect the actual activity of this species because CDC light traps do not attract the species readily (Magy et al. 1976). Special collections conducted on the Alamo River at the border in 1972 (Bown and Work 1973) produced 30 079 *Cx. quinquefasciatus*. The specimens were processed in 652 pools which yielded six strains of SLE virus.

When the New River studies were initiated, special effort was directed toward collecting *Cx. quinquefasciatus* in baited traps with a resulting yield of 12 strains of SLE virus from 873 pools of *Cx. quinquefasciatus*.

The comparable year-around results from three years research on the New River are shown in Figures 3 and 4. Half-logarithm values were used on the first level of Figure 4 because the logarithmic scale tended to blunt the contrast between the actual numbers of pools collected for both species. The graphs show that although *Cx. tarsalis* and *Cx. quinquefasciatus* were active throughout the year, SLE virus was detectable only from week 21 through week 44, and there was no evidence of transmission in December, January, February, and March north of the border. These

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[3]Partially supported by NIAID Grant 032.

Table 1. Imperial Valley Mosquito Data, 1967-1987. *Culex tarsalis* and *Culex pipiens quinquefasciatus* number of pools, mosquitoes, and virus isolation.

YEAR LOCATION:	TOTAL; POOLS;	TOTAL NB; MOSQUITO;	VIRUS; ISOL;	NB POOLS; TARSALIS;	TOTAL NB; TARSALIS;	SLE IN; TARSALIS;	WEE IN; TARSALIS;	NB POOLS; QUINQU;	TOTAL NB; QUINQU;	SLE IN; QUINQU;	WEE IN; QUINQU;
1967 WISTER	153	8781	1	152	8780	0	1	1	1	0	0
1968 WISTER	532	24185	45	531	24184	13	11	1	1	0	0
1969 WISTER	104	3979	7	103	3977	1	1	1	2	0	0
1970 WISTER	252	10326	13	245	10308	4	3	7	18	0	0
1971 WISTER	882	38789	8	785	35842	0	7	97	2947	0	0
1971 WISTER-ALAMO	180	6411	8	163	6293	0	6	17	118	0	0
1971 ALAMO-BORDER	22	189	0	18	160	0	0	4	29	0	0
1972 ALAMO-BORDER	1335	60407	52	682	30328	26	10	653	30079	6	2
1974 FINNEY-ALAMO	69	2607	0	63	2592	0	0	6	15	0	0
1970 FINNEY-LAKE	467	21078	123	463	21074	85	5	4	4	0	0
1971 FINNEY-LAKE	717	31234	66	694	31028	1	47	23	206	0	0
1972 FINNEY-LAKE	303	12759	1	297	12742	1	0	6	17	0	0
1973 FINNEY-LAKE	2655	120577	219	2605	120288	61	14	50	289	0	0
1974 FINNEY-LAKE	2487	116585	304	2419	114958	290	3	68	1627	0	0
1975 FINNEY-LAKE	1460	69857	299	1460	69857	26	270	0	0	0	0
1976 FINNEY-LAKE	539	18042	27	519	17895	25	2	20	147	0	0
1977 FINNEY-LAKE	344	15529	116	339	15498	95	20	5	31	1	0
1978 FINNEY-LAKE	164	5713	39	157	5703	16	22	7	10	0	0
1976 NEW RIVER	1487	38425	87	1050	32920	71	0	437	5505	10	0
1977 NEW RIVER	1276	33032	117	952	29664	39	69	324	3368	2	3
1978 NEW RIVER	414	10791	56	311	9850	13	25	103	847	0	0
1987 NEW RIVER	117	5152	9	108	4981	7	0	9	171	0	0
TOTAL	15959	654448	1597	14116	608922	774	516	1843	45432	19	5

Table 2. Finney Lake and New River, 1970-1987. *Culex tarsalis* and *Culex pipiens quinquefasciatus* number of pools, mosquitoes, and virus isolation.

YEAR LOCATION:	TOTAL; POOLS;	TOTAL NB; MOSQUITO;	VIRUS; ISOL;	NB POOLS; TARSALIS;	TOTAL NB; TARSALIS;	SLE IN; TARSALIS;	WEE IN; TARSALIS;	NB POOLS; QUINQU;	TOTAL NB; QUINQU;	SLE IN; QUINQU;	WEE IN; QUINQU;
FINNEY LAKE											
1970 FINNEY LAKE	467	21078	123	463	21074	85	5	4	4	0	0
1971 FINNEY LAKE	717	31234	66	694	31028	1	47	23	206	0	0
1972 FINNEY LAKE	303	12759	1	297	12742	1	0	6	17	0	0
1973 FINNEY LAKE	2655	120577	219	2605	120288	61	14	50	289	0	0
1974 FINNEY LAKE	2487	116585	304	2419	114958	290	3	68	1627	0	0
1975 FINNEY LAKE	1460	69857	299	1460	69857	26	270	0	0	0	0
1976 FINNEY LAKE	539	18042	27	519	17895	25	2	20	147	0	0
1977 FINNEY LAKE	344	15529	116	339	15498	95	20	5	31	1	0
1978 FINNEY LAKE	164	5713	39	157	5703	16	22	7	10	0	0
Subtotal	9136	411374	1194	8953	409043	600	383	183	2331	1	0
NEW RIVER											
1976 NEW RIVER	1487	38425	87	1050	32920	71	0	437	5505	10	0
1977 NEW RIVER	1276	33032	117	952	29664	39	69	324	3368	2	3
1978 NEW RIVER	414	10791	56	311	9850	13	25	103	847	0	0
1987 NEW RIVER	117	5152	9	108	4981	7	0	9	171	0	0
Subtotal	3294	87400	269	2421	77415	130	94	873	9891	12	3
TOTAL	12430	498774	1463	11374	486458	730	477	1056	12222	13	3

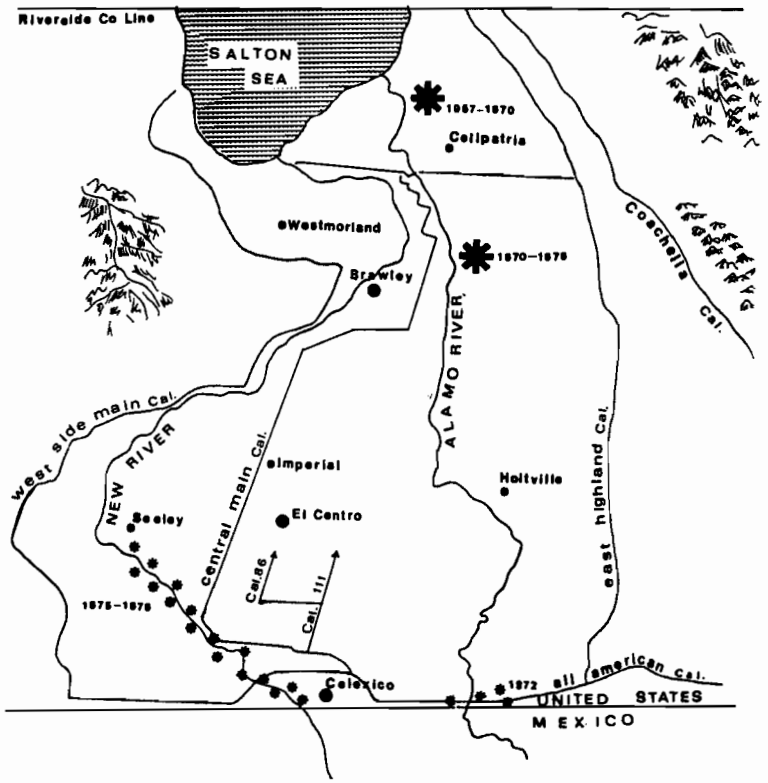

Figure 1. Imperial Valley, California - Field Study Areas.

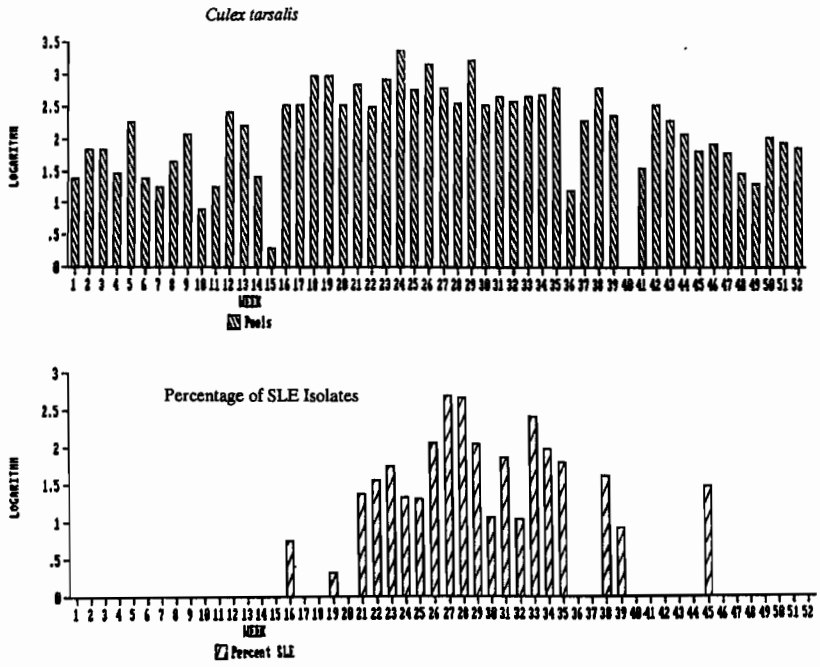

Figure 2. Finney Mosquito Summary, 1970-1978.

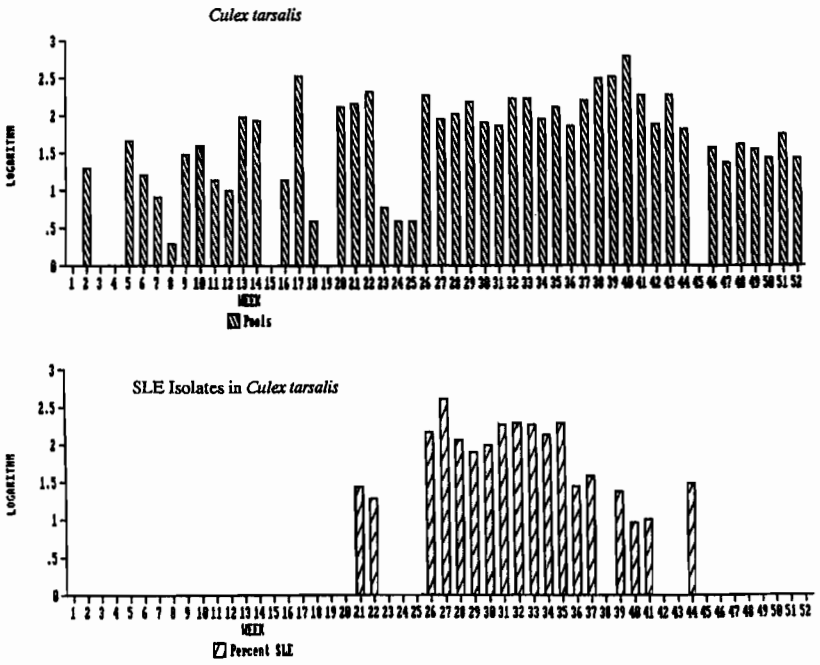

Figure 3. New River Summary, 1976-1987.

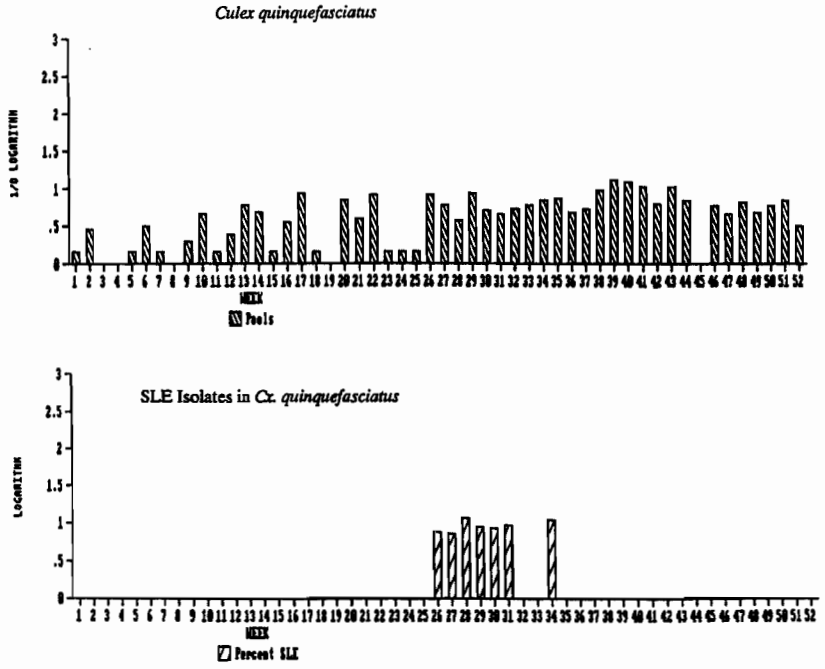

Figure 4. New River Summary, 1976-1987.

findings support the conclusion that winter transmission of SLE virus does not occur in the Imperial Valley.

The consistent reappearance every spring, year after year, of the virus in *Cx. tarsalis* mosquitoes, which are clearly the most sensitive detectors of SLE transmission, indicates that SLE virus must be reintroduced annually from elsewhere; possibly a tropical maintenance focus in Mexico. The persistent absence of any virus isolation from an otherwise active *Cx. tarsalis* population during the winter months for many years also militates against the concept of transovarial transmission in this situation.

The different patterns of the numerous isolates of WEE virus recovered from these collections reflect previous evidence that WEE activity is supported by a different cycle (Jozan 1977).

Other pools, such as those of *Culex erythrothorax* Dyar, were excluded from this analysis because of the negligible results obtained from processing pools at random in similar fashion (Berlin et al. 1976, Ryan et al. 1987).

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ARBOVIRUS ACTIVITY IN PASSERIFORM BIRDS IN ORANGE COUNTY, CALIFORNIA, 1988

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ABSTRACT

In the second year of a study to determine the prevalence of St. Louis encephalitis and western equine encephalomyelitis arboviruses in the peridomestic and rural wild bird populations of Orange County, 20 311 specimens were captured and 8501 were sampled for evidence of virus antibody. Seventy-eight (.92%) tested positive for virus. Including the data from 1987, virus activity occurs at a low level year-round in Orange County.

Introduction.

A study to determine the prevalence of St. Louis encephlalitis (SLE) and western equine encephalomyelitis (WEE) arboviruses in the peridomestic and rural wild bird populations of Orange County was initiated in 1987. Following the suggestion of a CDC field research team (McLean 1988) and our own experience in 1987 (Gruwell et al. 1988), the authors concluded that the study should concentrate on the house finch (*Carpodacus mexicanus*), the house sparrow (*Passer domesticus*), and the rock dove (*Columba livia*). They are not only the most numerous species in the county, but also due to their behavior tend to be more closely associated with the human population, facilitating a bird-mosquito-human transmission of an arbovirus. These three bird species have also been strongly implicated as being excellent host reservoirs both in the wild and in the laboratory (Hardy 1988, McLean 1988).

Materials and Methods.

Passerine birds were collected in modified crow traps, and columbines (rock doves) were obtained by shooting. Detailed descriptions of collecting and bleeding techniques are provided by Gruwell et al. (1988).

The blood sera samples were analyzed with HAI tests conducted by Paul Stanford at the Orange County Health Department (OCPHD). The elapsed time from submission of samples to completed testing and results averaged about six days; results were received within 48 hours when necessary.

Locations of the twelve crow traps placed around the county included five residences, four park maintenance yards, one fire station, one duck club, and one ex-Coast Guard Station near the Former Western White House. (Figure 1, Table 1). Rock doves were collected by shotgun at Bonita Canyon (Coyote Canyon) County Landfill in Irvine (Location XIII, Figure 1).

Results.

The most frequently captured bird was the house finch (*C. mexicanus*) at 49.23 percent of total samples, 64.19 percent of total recaptures, 58.79 percent of total resamples, and 57.96 percent of all live captures. The house sparrow (*P. domesticus*) was the second most frequent at 24.67 percent of total samples, 27.34 percent of total recaptures, 28.56 percent of total resamples, and 26.24 percent of all live captures. Rock doves accounted for 17.03 percent, white crowned sparrows (*Zonotrichia leucophrys*) 4.12 percent, and other species 4.95 percent of the total samples (Tables 2-5).

Positive birds were found throughout the county (Figure 1, Table 6) with a majority 58 (73.36%) collected from the City of Irvine south to the San Diego County line. Forty-seven positives (60.25%) were from Bonita Canyon (Coyoté Canyon) County Landfill (Location XIII, Figure 1) and all were rock doves.

Positive birds were collected every month of the year (Figure 2, Table 6) indicating that the virus was present at low levels year-round. Peak activity

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in 1988 was in April (3.33%) compared to peak level in December 1987 (3.27%). Rock doves represented 47 of the total positives (60.26%) (Figure 3) with house finches accounting for 21 positives (27%) (Figure 4). See Table 7 for a complete summary.

Discussion.

A very low level of HAI positive birds has been detected every month for the last 18 months. The number of positive versus total samples in one month has not exceeded 4 percent, and averaged less than 1 percent overall. Because there were no positive mosquito pools collected during this time span and very few sentinel chicken and pigeon seroconversions, it seems that the SLE virus cycled in the avian fauna at very low levels. More research is needed into all the facets of these arbovirus cycles in the Los Angeles Basin.

Acknowledgments.

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

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Table 1.-SLE/WEE Positives by Site, Orange County, 1988.

No.	Address	No. Positive Birds
I	Byers Residence, East Oak Lane, Santiago Oaks	1
II	Fire Station 19, El Toro Road and Jeronimo, El Toro	1
III	Cole Residence, 31501 Ave. Los Cerritos, San Juan Capistrano	1
IV	Challet Residence, 5262 Royale Ave., Irvine	1
V	Mason Park Maintenance Yard, Irvine	1
VI	20 Ranch Duck Club, Irvine	5
VII	Pett Residence, 608 Jana Circle, Huntington Beach	2
VIII	Central Park Maintenance Yard, Huntington Beach	6
IX	Modjeska Park Maintenance Yard, Anaheim	3
X	Carbon Canyon Region Park Maintenance Yard, Brea	2
XI	Fine Residence, 337 Las Riendas, Fullerton	7
XII	San Mateo Point, San Clemente	1
XIII	Bonita Canyon Sanitary Landfill, Irvine	47
	Total	78

Table 2.-Small Bird Species Collected in Crow Traps by Month, 1988.

Common Name	Scientific Name	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	% Total Samples
House Finch	*Carpodacus mexicanus*	91	75	73	147	379	783	840	766	323	371	151	186	4,185	49.23
House Sparrow	*Passer domesticus*	33	9	40	52	220	412	435	377	212	173	86	48	2,097	24.67
Rock Dove	*Columba livia*	-	-	37	89	167	201	165	170	204	217	125	73	1,448	17.03
White-Crowned Sparrow	*Zonotrichia leucophrys*	63	26	60	62	7	-	-	-	1	15	18	98	350	4.12
Red-Winged Blackbird	*Agelaius phoeniceus*	37	4	3	1	36	13	2	-	-	-	3	1	100	1.18
Oregon Junco	*Junco oreganus*	6	2	-	3	-	-	-	-	-	-	1	2	14	0.17
Brown-Headed Cowbird	*Molothrus ater*	1	1	-	5	26	23	11	3	2	1	-	-	73	0.86
Mourning Dove	*Zenaidura macroura*	1	3	3	-	1	-	-	-	-	-	1	-	9	0.11
Ground Dove	*Columbigallina passerina*	3	1	-	7	-	1	5	1	3	3	4	8	36	0.42
Song Sparrow	*Melospiza melodia*	1	2	11	14	19	14	5	9	3	3	5	11	97	1.14
Say's Phoebe	*Sayornis saya*	3	3	1	3	10	3	1	1	-	1	-	2	28	0.33
Scrub Jay	*Aphelocoma coerulescens*	1	5	2	-	-	-	-	-	-	1	-	-	9	0.11
Western Gull	*Larus occidentalis*	-	-	1	1	-	-	-	-	-	-	-	-	2	0.02
California Gull	*Larus californicus*	-	-	2	1	-	-	-	-	-	-	-	-	3	0.03
Black-Headed Grosbeak	*Pheucticus malanocephalus*	-	-	-	2	-	-	-	-	-	-	-	-	2	0.02
Bush Tit	*Psaltriparus minimus*	-	-	-	1	-	-	-	-	-	-	-	-	1	0.01
Common Raven	*Corvus corax*	-	-	-	2	-	-	-	-	-	-	-	-	2	0.02
Starling	*Sturnus vulgaris*	-	-	-	-	3	4	-	-	-	-	-	-	7	0.08
Mockingbird	*Mimus polyglottos*	-	-	-	-	-	-	2	4	1	-	1	-	8	0.09
Common Crow	*Corvus brachyrhynchos*	-	-	-	-	-	-	1	-	12	5	-	-	18	0.22
Rufous Sided Towhee	*Pipilo erythrophthalmus*	-	-	-	-	-	-	-	-	1	1	-	2	4	0.05
Loggerhead Shrike	*Lanius ludovicianus*	-	-	-	-	-	-	-	-	-	-	1	3	4	0.05
Audubon's Warbler	*Dendroica auduboni*	-	-	-	-	-	-	-	-	-	-	2	-	2	0.02
Lark Sparrow	*Chondestes grammacus*	-	-	-	-	-	-	-	-	-	-	-	1	1	0.01
Golden-Crowned Sparrow	*Zonotrichia atricapilla*	-	-	-	-	-	-	-	-	-	1	-	-	1	0.01
	TOTAL ALL BIRDS	240	131	233	390	868	1454	1467	1331	762	792	398	435	8,501	100.00

Table 3.-Recaptured Samll Bird Species Taken in Crow Traps by Month, 1988.

Common Name	Scientific Name	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	% of all Recaptures
House Finch	*Carpodacus mexicanus*	197	51	89	94	418	950	1038	1477	1160	1039	726	342	7,581	64.19
House Sparrow	*Passer domesticus*	40	8	18	33	168	529	686	630	521	400	131	65	3229	27.34
White-Crowned Sparrow	*Zonotrichia leucophrys*	197	40	78	103	25	4	-	-	-	2	8	95	552	4.67
Say's Phoebe	*Sayornis saya*	22	7	3	2	6	8	8	1	-	1	-	-	58	0.50
Mourning Dove	*Zenaidura macroura*	1	-	2	-	-	-	-	-	-	-	-	-	3	0.02
Red-Winged Blackbird	*Agelaius phoeniceus*	100	3	4	5	15	16	4	-	-	-	-	2	149	1.26
Oregon Junco	*Junco oreganus*	13	8	12	4	-	-	-	-	-	-	4	3	44	0.37
Song Sparrow	*Melospiza melodia*	9	1	4	18	14	15	11	15	14	7	12	8	128	1.09
Ground Dove	*Columbigallina passerina*	3	1	2	2	-	-	1	3	2	1	2	12	29	0.25
Brown-Headed Cowbird	*Molothrus ater*	-	-	-	1	17	10	3	-	-	-	-	-	31	0.26
Rufous Sided Towhee	*Pipilo erythrophthalmus*	-	-	-	-	-	-	-	-	-	-	-	1	1	0.01
Loggerhead Shrike	*Lanius ludovicianus*	-	-	-	-	-	-	-	-	-	-	-	1	1	0.01
Scrub Jay	*Aphelocoma coerulescens*	-	4	-	-	-	-	-	-	-	-	-	-	4	0.03
	TOTAL ALL BIRDS	582	123	212	262	663	1532	1751	2126	1697	1450	883	529	11,810	100.00

Table 4.-Resampled (blood) Small Bird Species by Month, 1988.

Common Name	Scientific Name	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	% of all Resamples
House Finch	*Carpodacus mexicanus*	40	27	12	32	60	123	132	201	164	206	54	120	1,171	58.79
House Sparrow	*Passer domesticus*	8	8	8	6	29	87	101	90	97	85	20	30	569	28.56
Say's Phoebe	*Sayornis saya*	3	3	-	2	1	1	-	-	-	1	-	-	11	0.55
Oregon Junco	*Junco oreganus*	2	2	-	3	-	-	-	-	-	-	1	2	10	0.50
Song Sparrow	*Melospiza melodia*	1	1	4	5	4	3	-	2	-	1	2	5	28	1.41
Red-Winged Blackbird	*Agelaius phoeniceus*	9	3	2	1	2	-	-	-	-	-	-	-	17	0.85
White-Crowned Sparrow	*Zonotrichia leucophrys*	29	15	33	43	7	-	-	-	-	1	-	42	170	8.53
Ground Dove	*Columbigallina passerina*	1	1	-	-	-	-	1	-	-	1	-	3	7	0.35
Scrub Jay	*Aphelocoma coerulescens*	-	2	-	-	-	-	-	-	-	-	-	-	2	0.10
Brown-Headed Cowbird	*Molothrus ater*	-	-	-	1	4	1	-	-	-	-	-	-	6	0.30
Rufous Sided Towhee	*Pipilo erythrophthalmus*	-	-	-	-	-	-	-	-	-	-	-	1	1	0.06
	TOTAL ALL BIRDS	93	62	59	93	107	215	234	293	261	295	77	203	1,992	100.00

Table 5.-Summary of Total Numbers and SLE (and WEE) Seroconversions of Small Birds, 1987-1988.

Species	1987 Total Samples	Percent 1987 Total Samples	Percent 1987 Ttl Live Captures	1987 Number SLE Positive Seroconversions	Percent 1987 SLE Positive Seroconversions	1988 Total Samples	Percent 1988 Total Samples	Percent 1988 Ttl Live Captures	1988 Number SLE Positive Seroconversions	Percent 1988 SLE Positive Seroconversions	1987 & 1988 Total Samples	Percent 1987 & 1988 Total Samples	Percent 1987 & 1988 Ttl Live Captures	1987 & 1988 Number SLE Positive Seroconversions	Percent 1987 & 1988 SLE Positive Seroconversions
House Finch *Carpodacus mexicanus*	2,224	40.04	40.74	9	22.50	4,185	49.23	57.96	20 (1)	33.90 (5.3)	6,409	45.60	51.60	29 (1)	25.40
House Sparrow *Passer domesticus*	1,656	29.81	33.09	11	27.50	2,097	24.67	26.24	7 (2)	11.90 (10.5)	3,753	26.70	28.80	18 (2)	17.00
White Crowned Sparrow *Zonotrichia leucophrys*	297	5.35	8.20	6	15.00	350	4.12	4.44	0	0.00	647	4.60	5.80	6	5.10
Rock Dove *Columba livia*	383	6.89	3.23	5	12.50	1,448	17.03	7.13	31 (16)	52.50 (84.20)	1,831	13.00	5.70	36 (16)	44.00
Other Species	995	17.91	14.74	6 (3)*	22.50 (100)	421	4.95	4.23	1	1.70	1,416	10.10	8.10	7 (3)	8.50
TOTALS	5,555	100.00	100%	40	.73 100%	8,501	100%	100%	.92 78	100%	14,056	100%	100%	.84 118	100%

*WEE Seroconversions

Table 6.-Sites of SLE Positive Birds, Orange County, 1988.

Catalog No.	Species	Sex	Age	Locality	Date Collected	Titer
JAG 88-43	House Finch	♂	Ad	X	Jan. 8	1:40
-70	House Finch	♀	Ad	X	Jan. 12	1:20
	January - 240 Samples, 2 positives (0.83%)					
JAG 88-259	House Finch	♂	Ad	VI	Feb. 12	1:80
	February - 131 Samples, 1 positive (0.76%)					
JAG 88-433	Scrub Jay		Ad	XI	Mar. 11	1:40
-525°	Rock Dove		Ad	XIII	Mar. 23	1:20
-558	House Finch	♂	Ad	VII	Mar. 25	1:20
	March - 233 Samples, 3 positives (1.29%)					
JAG 88-654°	Rock Dove		Ad	XIII	Apr. 6	1:40
-655°	Rock Dove		Ad	XIII	Apr. 6	1:40
-659°	Rock Dove		Ad	XIII	Apr. 6	1:40
-660°	Rock Dove		Ad	XIII	Apr. 6	1:40
-661°	Rock Dove		Ad	XIII	Apr. 6	1:40
-662°	Rock Dove		Ad	XIII	Apr. 6	1:20
-663	Rock Dove		Ad	XIII	Apr. 6	1:40
-664	Rock Dove		Juv	XIII	Apr. 6	1:40
-665	Rock Dove		Ad	XIII	Apr. 6	1:40
-666	Rock Dove		Juv	XIII	Apr. 6	1:40
-667	Rock Dove		Ad	XIII	Apr. 6	1:40
-668	Rock Dove		Ad	XIII	Apr. 6	1:40
-779°	House Finch	♀	Ad	VII	Apr. 15	1:40
	April - 390 Samples, 13 positives (3.33%)					
JAG 88-1085°	Rock Dove		Juv	XIII	May 4	1:80
-1089°	Rock Dove		Juv	XIII	May 4	1:80
-1093°	Rock Dove		Juv	XIII	May 4	1:40
-1098°	Rock Dove		Ad	XIII	May 4	1:40
-1127°	House Sparrow		Fleg	II	May 5	1:80
-1242°	Rock Dove		Ad	XIII	May 11	1:80
-1277	House Finch	♀	Ad	IV	May 12	1:40
-1367°	Rock Dove		Ad	XIII	May 18	1:20
	May - 868 Samples, 9 positives (0.92%)					
JAG 88-2199	Rock Dove		Ad	XIII	June 8	1:40
-3198	Rock Dove		Ad	XIII	June 29	1:40
	June - 1,454 Samples, 2 positives (0.14%)					
JAG 88-3520°	Rock Dove		Ad	XIII	July 6	1:40
-3741	Rock Dove		Ad	XIII	July 13	1:20
-3743	Rock Dove		Juv	XIII	July 13	1:40
-3744°	Rock Dove		Juv	XIII	July 13	1:20
						1:20

Table 6.-Sites of SLE Positive Birds, Orange County, 1988 – Continued.

Catalog No.	Species	Sex	Age	Locality	Date Collected	Titer
-4126	Rock Dove		Ad	XIII	July 20	1:40
-4247*	House Finch		Imm	V	July 21	1:20
-4353*	House Sparrow		Imm	VIII	July 22	1:20
-4615	Rock Dove		Ad	XIII	July 27	1:20
-4618	Rock Dove		Ad	XIII	July 27	1:20
-4625	Rock Dove		Ad	XIII	July 27	1:20
-4633	Rock Dove		Ad	XIII	July 27	1:20
-4753	House Finch		Imm	XI	July 29	1:20
	July - 1,467 Samples, 12 positives (0.82%)					
JAG 88-5186	Rock Dove		Ad	XIII	Aug. 10	1:20
-5391°	House Sparrow		Imm	IX	Aug. 16	1:160
						1:160
-5625*	House Finch		Imm	III	Aug. 22	1:20
-5787*	House Finch		Imm	VII	Aug. 23	1:320
-5805*	House Sparrow	♂	Imm	VII	Aug. 23	1:20
-5812*	House Sparrow		Imm	VIII	Aug. 23	1:20
-5987	House Finch		Imm	XI	Aug. 26	1:80
	August - 1,331 Samples, 7 positives (0.53%)					
JAG 88-6143	House Finch	♂	Imm	XII	Sept. 5	1:20
-6176	Rock Dove		Juv	XIII	Sept. 7	1:20
-6275*	House Finch		Imm	XI	Sept. 13	1:40
-6341	Rock Dove		Juv	XIII	Sept. 14	1:20
-6358	Rock Dove		Juv	XIII	Sept. 14	1:40
-6370	House Finch	♂	Imm	I	Sept. 15	1:40
-6384*	House Finch		Imm	XI	Sept. 16	1:40
-6543°	Rock Dove		Ad	XIII	Sept. 21	1:40
						1:40
-6628	House Sparrow		Imm	XI	Sept. 23	1:20
-6742	Rock Dove		Ad	XIII	Sept. 28	1:40
-6744	Rock Dove		Ad	XIII	Sept. 28	1:20
-6824*	House Finch		Ad	Vi	Sept. 29	1:80
-6874	House Sparrow		Imm	IX	Sept. 30	1:20
	September - 762 Samples, 13 positives (1.71%)					
JAG 88-7021	Rock Dove		Juv	XIII	Oct. 5	1:20
-7025	Rock Dove		Juv	XIII	Oct. 5	1:20
-7041*	House Finch		Ad	VI	Oct. 6	1:20
-7223	Rock Dove		Ad	XIII	Oct. 12	1:80
-7237	Rock Dove		Juv	XIII	Oct. 12	1:80
-7495*	House Finch		Ad	VI	Oct. 20	1:20
-7586	Rock Dove		Ad	XIII	Oct. 26	1:40
	October - 792 Samples, 7 positives (0.88%)					
JAG 88-7830	Rock Dove		Juv	XIII	Nov. 9	1:20
-7890	Rock Dove		Juv	XIII	Nov. 16	1:20
-7892	Rock Dove		Ad	XIII	Nov. 16	1:80
-8046	House Sparrow	♀	Ad	XI	Nov. 25	1:20
-8058*	House Finch	♀	Ad	VIII	Nov. 25	1:20

Table 6.-Sites of SLE Positive Birds, Orange County, 1988 – Continued.

Catalog No.	Species	Sex	Age	Locality	Date Collected	Titer
	November - 398 Samples, 5 positives (1.26%)					
JAG 88-8123*	House Finch	♀	Ad	VI	Dec. 5	1:40
-8269*	House Finch	♂	Ad	VIII	Dec. 20	1:20
	December - 435 Samples, 2 positives (0.46%)					
TOTAL for 1988 =	8,501 Samples, 78 positives (19 WEE, 59 SLE) (0.92%)					

° Wee Positive
* Recaptured, Resampled

Table 7.-SLE/WEE Positives by species, Orange County, 1988.

	SLE	WEE	Total
Rock Dove	31	16	47
1,448 samples, 47 positives, 3.25% of all Rock Doves samples			
House Finch	20	1	21
4,185 samples, 21 positives, 0.5% of all House Finch samples.			
House Sparrow	7	2	9
2,097 samples, 9 positives, 0.43% of all House Sparrow samples.			
Scrub Jay	1	0	1
9 samples, 1 positive, 11% of all Scrub Jay samples.			

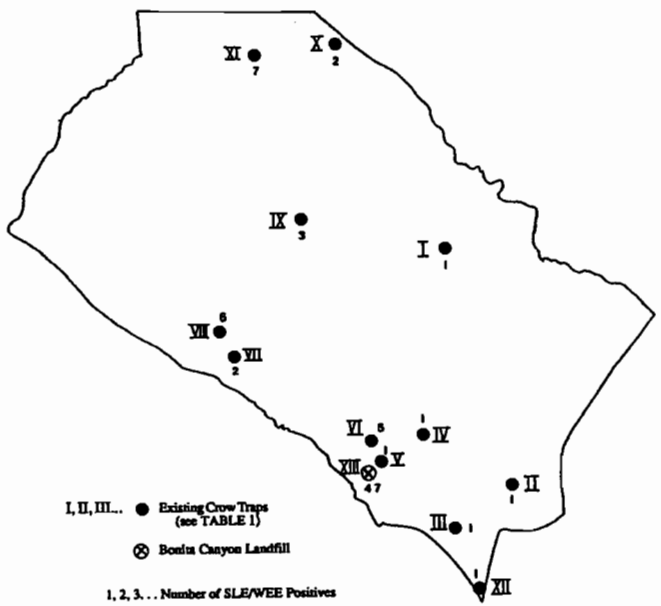

Figure 1.-Positive SLE/WEE Localities in Orange County, California, 1988.

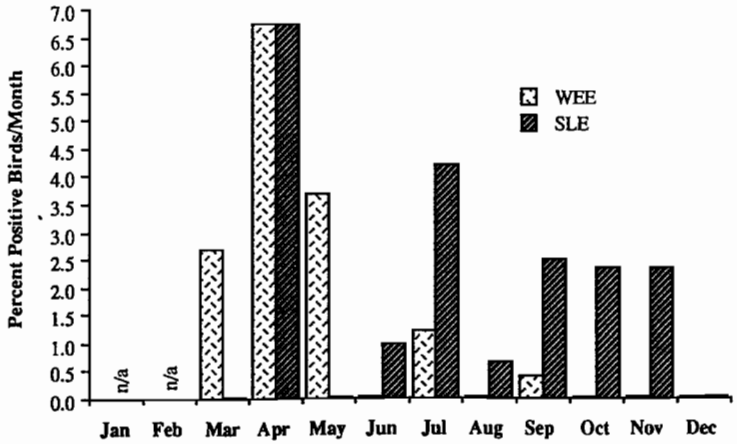

Figure 3.-Positive Seroconversions of Rock Dove (pigeon) Sera for SLE/WEE in Orange County, 1988.

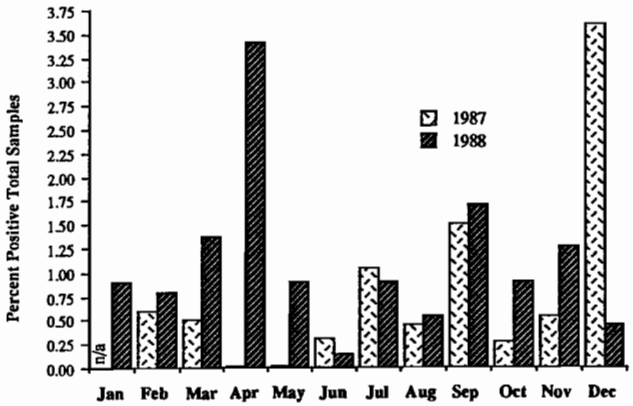

Figure 2.-Positive Seroconversions of Wild Bird Sera for SLE/WEE in Orange County, 1987 and 1988.

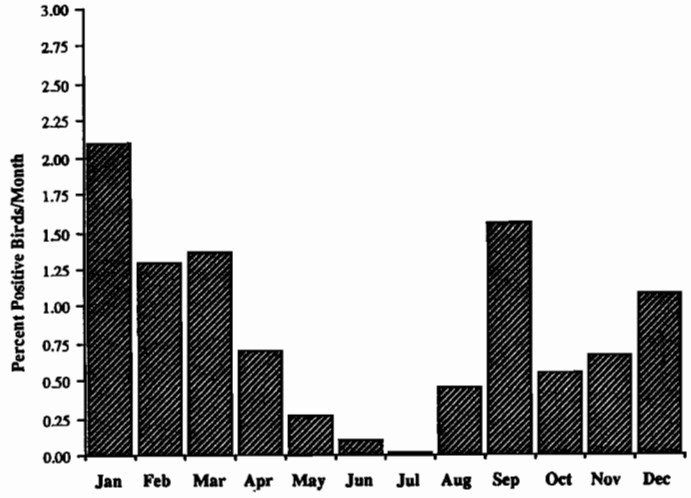

Figure 4.-Positive Seroconversions of House Finch Sera for SLE in Orange County, 1988.

EPIZOOTIOLOGY OF CANINE CARDIOVASCULAR DIROFILARIASIS IN SIX NORTHERN CALIFORNIA COUNTIES

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Introduction.

Cardiovascular dirofilariasis, caused by the filarial nematode, *Dirofilaria immitis* (Leidy), was described 150 years ago in dogs by Dr. Joseph Leidy, who was working with parasites. Initially, the disease was apparently limited to the warmer coastal areas in the tropics of Central and South America and Africa. Existence of the disease has been found on every continent since its discovery. The United Kingdom is the only major landmass currently known that has escaped its presence. Murdock (1984) suggests that the primary reason for the world-wide spread of dog heartworm is the unrestricted movement of infected dogs from enzootic areas. The UK has long practiced a restriction on immigration of dogs from other countries. Another popular suggestion (Grieve et al. 1983) is that the increase in vector resistance to insecticides in enzootic areas has allowed an increase in vector numbers. However, the spread of the disease has been going on longer than the use of chemical insecticides. A more likely answer was suggested by Otto (1975), who said that a genetic change in an offshoot parasite population with the ability to develop at the lower temperatures common in temperate regions was the root of the expansion into the new regions. This may also have led to an increase in the number of potential vector species.

North America was first invaded by *D. immitis* from the south through the gulf coast region. The nematode then migrated north along the Atlantic sea-board, slowly expanding its range westward into the central US and Canada. Most recently, the parasite has shown up in the extreme west, California, and Hawaii. The disease established itself in the coastal, valley, and foothill regions of northern California within the last 20 years.

Northern California has become enzootic for the disease in recent years. Filariasis was first discovered in native California dogs in 1946 (Roberts 1946), but there were very few cases documented until 1970. During the time since the first cases were diagnosed, the disease spread rapidly throughout northern and southern California. It was learned in the late 1970s that the native coyote population in northern California was harboring the parasite (Weinmann and Garcia 1980), and was probably acting as a reservoir of infection for domestic dogs. In 1982, Walters and Lavoipierre isolated advanced stages of filarial larvae from vector mosquitoes captured in Northern California, thereby establishing that the disease had become enzootic.

Soon after the confirmation that an epizootic was in progress, northern California coastal region veterinary hospitals began working together to establish infection rates and geographical distribution as determined from the number of dogs positive out of all dogs tested by the veterinary hospitals (Acevedo and Theis 1980). The area of infection covered Alameda, Contra Costa, Napa, Solano, Sonoma, Stanislaus, Merced, San Mateo, and Marin Counties. The eastern valley and foothill counties were not included in the study. Four years later, the Sacramento/Yolo Mosquito Abatement District, in cooperation with the Sacramento Veterinary and Medical Association, started a similar survey in the eastern area of the Sacramento Valley and adjacent foothills. The survey covered two valley counties, Sacramento and Yolo, and four foothill counties, Placer, El Dorado, Amador, and Calaveras. The data presented here summarize the results of a five-year collection.

Methods.

The survey area covers a diverse array of contrasting geographical and vegetational habitats. Two major biotopes are represented; the oak-grassland biotope in the valley, and the oak-pine woodland biotope of the foothills and higher elevations in the Sierra Nevada. The mountain communities are generally considered a single biotope. The valley counties, Sacramento and Yolo, represent the open grassland and river delta riparian woodlands around the Sacramento and American Rivers, with elevations ranging from 10 to 300 feet.

The dominant flora are the valley oak (*Quercus lobata*), and the blue oak (*Q. douglasii*). The area is primarily utilized for agricultural activities and urban development. The counties of Placer, El Dorado, Amador, and Calaveras represent the foothill woodlands and mountain forests in the north central portion of the Sierra Nevada range, with the elevations between 200 and 4000 feet. The dominant flora consist of digger pine (*Pinus sabiniana*), ponderosa pine (*P. ponderosa*), incense cedar (*Calocedrus decurrens*), douglas fir (*Pseudotsuga menziesii*), and the black oak (*Quercus kellogii*). The primary mosquito vector in northern California, *Aedes sierrensis* (Ludlow), is abundant throughout both biotopes.

A total of 125 regional veterinary hospitals was contacted by letter and phone at the beginning of each year and asked to participate in the survey. Letters urging cooperation in the survey followed from the president of their veterinary association. Each hospital and clinic was given a set of 12 monthly survey forms for the upcoming year. The completed survey form was sent to the Sacramento/Yolo MAD laboratory, where the data were entered into a computer, analyzed, and compiled by county at the close of each month. The veterinary hospitals provided information on breed, sex, age, sleeping patterns, residence, and travel history of *Dirofilaria* infected dogs. Other information was provided on the number of dogs tested and percentage of dogs on preventive medication, and the specific diagnostic test used was included for each dog. Host characteristics, incidence rate, and geographical distribution of canine heartworm infections were then calculated for the region.

Results and Discussion.

Incidence Rate.-A total of 45 443 dogs was examined for *D. immitis*: 28 923 (63.6%) from the valley counties, and 16 520 (36.3%) in the foothill counties. The combined infection rate in 1984 was 5.10 percent, which was the highest during the survey. The infection rate declined to 3.44 percent in 1985 and then leveled off at 1.99, 1.89, and 1.64 percent for the last three years respectively (Figure 1). The decline of the incidence rate was possibly due to two factors; first, in 1984, only dogs that demonstrated some signs of illness were tested for heartworm. Most veterinary hospitals were routinely testing all dogs under their care by 1988, regardless of condition. This initial selectivity in testing dogs biased the sample in the direction of increased incidence rate. Second, only 4430 dogs were sampled during 1984. The sample size increased each consecutive year of the survey, and 12 565 dogs were tested in 1988. Statistically, the smaller sample could contain more built in bias than the larger samples, and therefore the later samples probably portrayed the more realistic incidence level.

Looking at the various counties individually, several different patterns can be observed, which likely are a reflection of the number of veterinary hospitals that participated in the survey in each county. Sacramento County, with the greatest number of participating veterinary hospitals, showed a pattern similar to that of the overall trend (Figure 3). The infection rate in 1984 was 2.91 percent of 1819 dogs tested. In 1985, it fell to 2.22 percent with 3947 dogs tested. The infection rate declined during the next three years as the numbers of dogs tested increased to more than 7000 per year. Yolo county, having one of the smallest numbers of participating veterinary hospitals, had an incidence rate which fluctuated substantially from year to year (Figure 2). It had an incidence rate of 2.59 percent in 1984, while the rate fell to 0.61 percent in 1985. In the next three years, it rose to half the total of the first year, then declined during each following year.

The foothill communities had an overall higher incidence rate than the valley communities. This suggested a longer association with the parasite and, probably, a larger population of vectors. Placer County's incidence rate fluctuated from year to year (Figure 3). Initially, the incidence rate was 2.99 percent, then in 1985 it increased to 3.92 percent. The lowest rate was recorded at 1.45 percent during 1986. In 1987 and 1988, the rate increased to 2.07 percent and 2.86 percent, respectively. El Dorado county showed a steady decline throughout the survey period (Figure 3). It had the highest infection rate (13.11%) encountered to date within the survey area in 1984. The incidence rates fell to 8.53 in 1985, 8.10 in 1986, 4.61 in 1987, and 4.96 in 1988. Amador County's incidence rates fluctuated throughout the survey period (Figure 4). An incidence rate of 10.91 percent was recorded in 1984. The following year the rate had dropped by more than half to 5.6 percent. The rate hit its lowest level at 4.4 percent in 1986. The rate went back up to 6.4 percent in 1987. No data were received during the

1988 period. The incidence rate in Calaveras County declined each year throughout the survey except in 1988. We found an incidence rate of 12.5 percent for the first year of the survey. In the following years the infection rate was 6.6 percent, 3.3 percent, 1.9 percent, and 2.6 percent, respectively (Figure 5).

The overall reduction in incidence rate over the last five years may be the result of several factors. An intensive educational campaign was conducted by veterinary hospitals regarding canine heartworm and its prevention during the same time period in which the survey was taken. The number of dogs tested and put on preventive medication increased substantially each year. The effect of this precipitous increase in testing and prevention caused a reduction in the observed incidence rate, as the incidence rate is calculated from those dogs

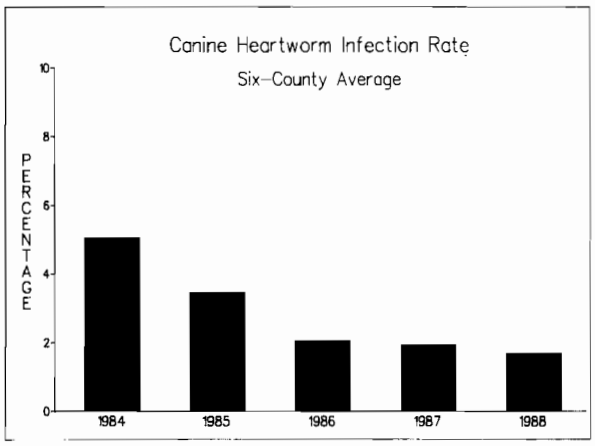

Figure 1.-Five-year average infection rate of *Dirofilaria immitis* in dogs sampled from all six counties.

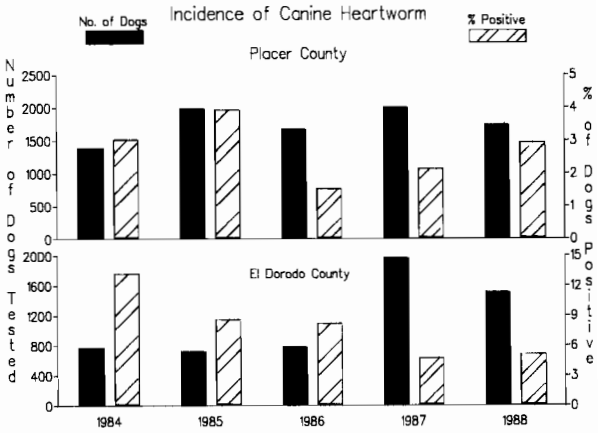

Figure 3.-Incidence rate and number of dogs sampled in Placer and El Dorado Counties.

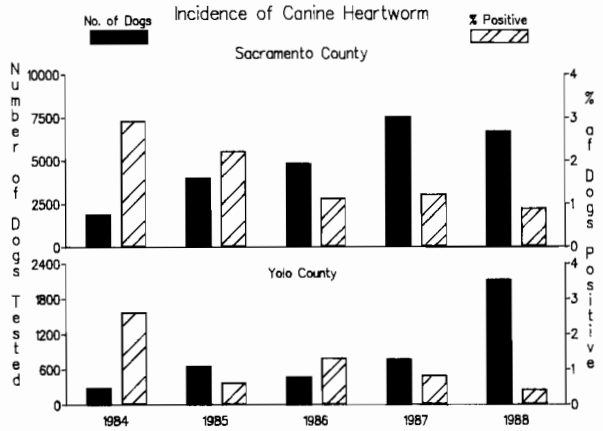

Figure 2.-Incidence rate and number of dogs sampled in Sacramento and Yolo Counties.

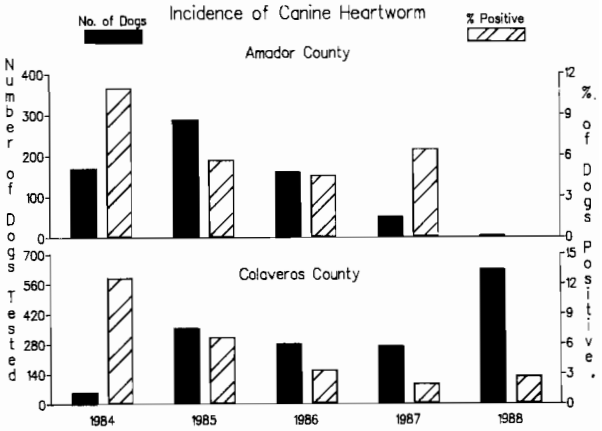

Figure 4.-Incidence rate and number of dogs sampled in Amador and Calaveras Counties.

tested rather than a random sample. In selected phone conversations, most veterinary doctors interviewed felt that this apparent reduction was only an artifact of the sampling technique, and might not reflect the real incidence rates. This seems to be substantiated by the distribution data.

Geographic Distribution.-Despite the obvious reduction in incidence rate, the geographic distribution of the disease has increased within the survey area during the five-year period. The majority of cases were distributed among the larger towns and cities located along the major roadways and freeways in the initial years. The heaviest populated and fastest growing communities were the foci of the epizootic in valley and mountain counties alike (Figure 5). The foothill counties had a wider distribution of cases than did the valley counties. Cases became more evenly distributed throughout the counties in 1988. The same clusters of cases existed, but more cases were occurring in smaller towns and outlying communities (Figure 6). Sacramento County demonstrated this trend most obviously. In 1984, 97 percent of all cases fell within an area between Interstate 80 in the north, the city of Sacramento in the west, Highway 50 in the south, and the city of Orangevale in the eastern portion of the county. Concentrations were found in Sacramento, Carmichael, and Orangevale, the largest cities within the area. The situation had changed significantly in 1988. Cases had dispersed north and south from the larger cities and moved into the small rural communities. Clusters were found in Elk Grove and Wilton, fifteen miles south of Sacramento, and individual cases were found in Courtland and Walnut Grove, twenty and twenty-five miles southwest of Sacramento.

These observations fit well with the suspected channel for the spread of the disease. People living in enzootic disease areas who move into disease free areas will most commonly choose the newer, faster growing communities. Such communities had the first reported cases. Once the disease is established there, it can spread to the smaller outlying communities.

Risk Factors.-Four different risk factors were monitored during the survey: canine breed, age, sex, and sleeping pattern. The only factor that showed any discernible risk for contacting the parasite was sleeping habits. Sleeping habits of dogs were listed as either sleeping inside, sleeping outside, or sleeping in both locations. Those dogs that slept outside and were exposed to the vector during optimum feeding times were infected over 50 percent more frequently than dogs with the other

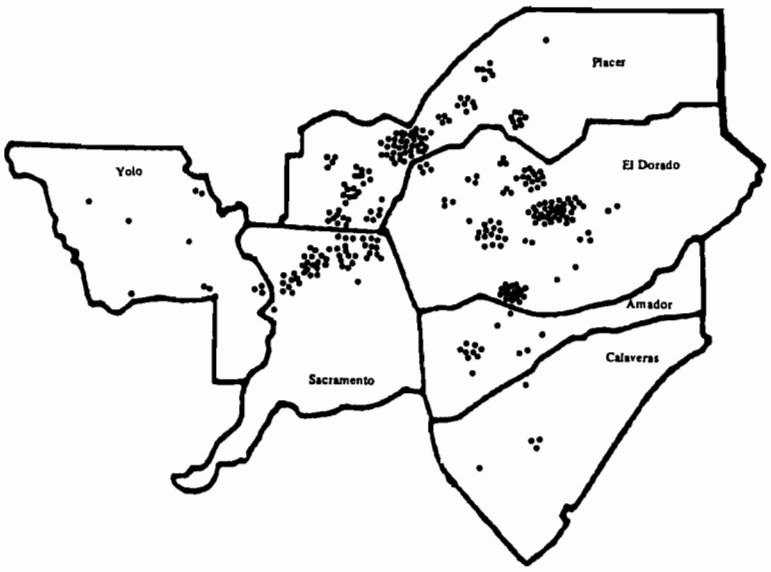

Figure 5.-Approximate geographic distribution of canine heartworm cases in 1984 within the six county survey region.

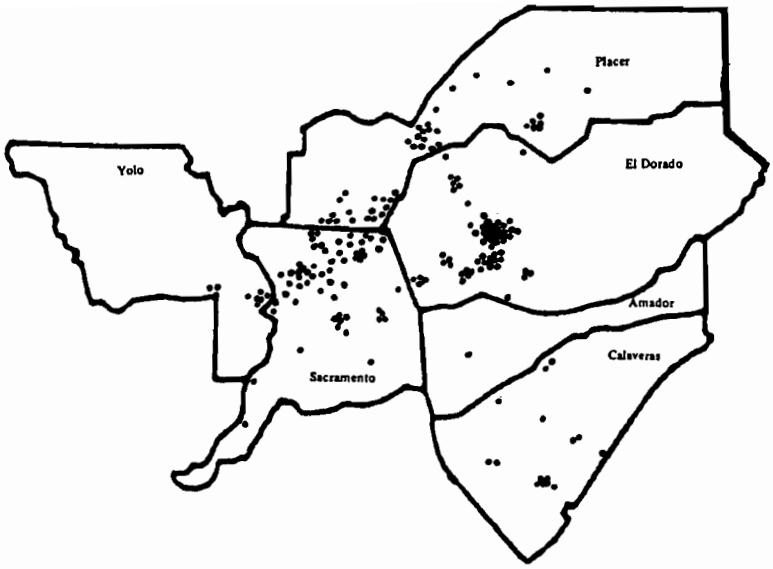

Figure 6.-Approximate geographic distribution of canine heartworm cases in 1988 within the six county survey region.

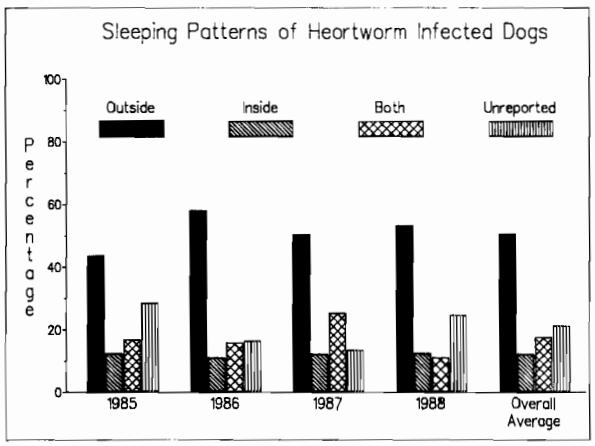

Figure 7.-Percentage of sleeping behavior patterns of dogs infected with *Dirofilaria immitis* during the survey period.

sleeping patterns. Dogs that slept in both locations had an infection rate of approximately 20 percent, which was between those that slept either inside or outside. Surprising, dogs that slept inside comprised 18 percent of all infected dogs (Figure 7).

Each year, male dogs were found to be more frequently infected than were female dogs. Over the the five-year survey, 16 percent more male dogs than female dogs were infected with heartworm, and the annual data closely reflected this five-year trend. Regional behavioral patterns of dogs showed that more male dogs are out in the evenings and wander farther than females (Yolo County Animal Control, personal communication). This behavior may have enhanced the opportunity for male dogs to become infected with the heartworm if it increased exposure to the local vectors. *Aedes sierrensis* is considered the major vector within the survey area, however other mosquitoes have also been implicated (Walters and Lavoipierre 1982).

The average age of infected dogs sampled during the survey is 6.09 years. The age range of infected dogs started at six months and continued through 16 years. The annual average age remained nearly constant at about six years throughout the survey.

German shepherd crosses were the most commonly infected breed, making up 18.4 percent of all infected dogs. The Australian shepherd followed closely at 13.1 percent. These two breeds, plus eight other common breeds: black labrador, golden retriever, doberman pinscher, border collie, springer spaniel, poodle, Irish setter, and shelty registered 74.4 percent of all infected dogs. The

remaining 25.6 percent was distributed among 26 other breeds. This infection frequency corresponds almost exactly with a list of the most popular dogs in the study area (Yolo County animal control, personal communication), and suggests that there is little or no correlation between breed of dog and the risk of contacting heartworm within the study area.

Veterinary Participation.-An average of 38.6 percent veterinary hospitals participated on an annual basis throughout the five-year survey. This represents only 31 percent of the active veterinary hospitals within the survey area. Such a response was comparable to that attained by other veterinary hospital surveys (Schlotthauer et al. 1979, Noyes 1980), where a range of 21 percent to 36 percent participation was achieved.

There was greater veterinary interest in the valley counties than in the foothill communities. Two foothill counties, Amador and Calaveras, were represented by only a few hospitals, and in some years by only one. The most accurate representation and the most responsive hospitals were in Sacramento, Placer, and El Dorado counties.

In diagnosing dog heartworm, 82 percent of the participating veterinary hospitals and clinics used the difil filter test. It was also the most commonly used test in association with other diagnostic techniques, such as the antibody, smear, radiography, or the Knott stain technique. The Knott stain was used exclusively as a diagnostic test 8 percent of the time. Exclusive use of the Knott stain was reported in only a few of the hospitals located in the smaller foothill communities.

Conclusion.

Despite the increased use of preventives, expanding public awareness, education, and ongoing vector surveillance and control, the geographic distribution of dog heartworm continues to spread within our sample area of northern California. These control efforts appear to have slowed the distribution, as evidenced by the reduction in incidence rate, but have not stopped it. Such results are encouraging but increased effort is needed.

Male dogs seem to be at a greater risk of infection, which may be correlated with their nocturnal roaming behavior. Dogs roughly six years of age are more frequently diagnosed positive than any other age cohort. There seemed to be no significant correlation between heartworm infection and dog breed.

It is important to keep in mind that data collected in a survey such as this are inherently incomplete due to its built-in selectivity, which makes it inappropriate to speculate much beyond the obvious. Many canine pets within the survey area are not under the care of a veterinarian, and therefore were not part of the sample. Still others were transient and not accounted for, yet those are possibly the most frequently infected. Finally, wild canines such as the coyote and fox are likely to be the reservoir for the disease, and those animals may more accurately represent the incidence and distribution of a filarial epizootic.

Acknowledgments.

We would like to extend special thanks to Dr. Richard Pollack for composing, initiating, actively promoting, and consistently participating in the survey. Thanks also are due to Annett Eiffert for developing the computer program and coordinating the statistics. Final thanks are extended to the veterinary hospitals and clinics that worked with us in this program.

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VECTOR CONTROL AS COEVOLUTION

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Preamble.

I am a manager of a mosquito abatement district. We managers work real hard. We are always upgrading our programs. It doesn't do any good. The districts do not give us sabbatical leave to prevent burnout. Instead of a sabbatical, I present a paper every decade or so, to step back from the trees to look at the forest. This is such a paper.

Introduction.

I would like to discuss vector control from the point of view of evolutionary theory. The paper will provide no new information. It will, however, focus on areas not usually stressed. Hopefully it will offer a fresh perspective and maybe even some insight. As an aside, I need to warn you that contrary to the teachings of my English teachers, I believe mixing metaphores can be expressive.

The paper is not a scientific paper, but it is somewhat logically driven. The process has led to some suprising conclusions. The premise of the paper suggests that a vector control program, when suppressing a vector population, operates in a manner similar to coevolution. If this is true, then we should expect to learn something by looking at what is known about coevolution.

Coevolution and the Red Queen.

Coevolution is when two or more species interact in a reciprocal fashion. Species A, perhaps a predator, applies selective pressure to species B, its prey, which adapts to avoid predation, causing reciprocal selective pressure on species A. Dawkins (1987), a modern evolutionist, describes the process as an "arms race." It is characterized by escalating, reciprocal adaptations. He considers coevolution to be the major mechanism by which progressiveness is injected into evolution. Both sides will continue to improve their survival advantage as long as neither side gains a decisive advantage (De Angelis et al. 1986). Evolutionsists refer to this as the "Red Queen" effect. The term refers to when Alice, in *Through the Looking Glass*, is holding hands with the Red Queen and running faster and faster, yet staying in the same place.

I would like to extract the salient features of coevolution and use them to test the analogy with vector control. The first test would be to see if two or more organisms are interacting. The second would determine if each side causes selective pressure upon the other, resulting in reciprocal adaptations. The third would ask if there is an increase in complexity on both sides. The final test would determine whether either side gains a long term adavantage, or, in other words, whether the "Red Queen" effect is operating.

The Elegant Proof.

In the first test, we ask whether there is an interaction of two species in vector control. On the one side we have a vector population, obviously a species. On the other, we have the vector control organization. One is an organism. One is an organization. Organism, organization; close enough for me. The first test passes.

The second test is not so easy. It asks whether both sides apply selective pressure to the other resulting in adaptations. Looking at the phenomenon of insecticide resistance gives a clear picture here. The vector control organization applies insecticide A. The vector population develops resistance to insecticide A, creating a control failure. The vector control organization adapts by applying insecticide B.

The best reason to use resistance as an example, of course, is that there is ample evidence to suggest this kind of process of reciprocal adaptation is not uncommon (Brown and Pal 1971). It may also be the case that our vector control programs are pressuring the vector population and creating other less evident adaptations. A mosquito population under larviciding pressures may adapt by seeking more cryptic larval habitats, developing pesticide avoidance behavior, increasing autogeny rates, or shifting to alternate hosts for a blood meal.

There are some discrepancies in the analogy with test two. The time frame in which vector control occurs is measured in decades. Evolution occurs in a geologic time frame. How then could it be possible that true evolution could be going on in such a short period of time? The answer lies in the fact that most vectors are r-pests (Conway 1981). They produce great numbers of individuals, preadapted forms, that are widely distributed into the environment. The environment then selects the

fittest. From this perspective, the vector population could be viewed as a machine generating hypotheses to be tested. The natural environment operates as the selection system; only those hypotheses that pass the test survive.

It is well known that random mutations of genes supply the basis for change in a species. Mutations create the new ideas for survival if we can be teleological for a moment. But, it is unlikely that mutation is occurring at a significant rate, if at all, in the time frame of vector control (Brown and Pal 1971). Nevertheless, the vector population has possibly stored a heavy load of historical mutations over geologic time. These mutations are distributed in individual prototypes which are generated in great numbers and variety conferring genetic variability to the population. The selective pressures applied by our recently arrived vector control programs are simply changing the environmental test to select individual vectors that have pre-adapted in a geologic time frame.

Another discrepancy is evident in the anology when we realize that a different kind of selection and change is occurring on the vector control side. Our vector control programs acquire characteristics through our rational processes, and they are passed to the future as updated vector control programs. This is a Lamarkian-like evolutionary process that can result in almost instantaneous changes, while the vector population's most elemental changes, mutations, require a geologic time frame. Korzybinski (Postman, 1988) recognized this as an enormous advantage that man has over plants and animals when he described man as a time binder. In spite of discrepancies of time and method of evolution, however, the prevailing relationship between the control organization and the target organism, as demonstrated by the example of insecticide resistance, is that of two sides interacting reciprocally. Test two passes.

Test three asks whether both sides increase in complexity. Again we can use the example of insecticide resistance. Insecticide resistance occurs when individuals in the vector population are selected that possess a physiologic mechanism to detoxify pesticides. The physiological complexity of these individuals is greater than that of those individuals who cannot detoxify the insecticide. The increased complexity is expressed at the population level as the selection process creates a higher frequency of resistant genes in the population. On the vector control side, we have been forced to introduce another pesticide. An obvious increase in complexity of the program. Test three passes!

I would like to digress for a moment on this matter of the complexity of a vector control program. Our agency is currently using computer simulations. They formalize our decision making and quantify the complexity of the decision. Alan Berryman, at our recent AMCA Conference in Seattle, extolled the benefits of bottom up modelling. He felt this approach would lead to practical models that were no more complex than they have to be. He said to start simple and add one change at a time. Each change is then validated or discarded. The validation process, of course, is to test the model in the real world. Does this procedure sound hauntingly similar to evolution? From this perspective, the vector control organization could be viewed as a machine generating hypotheses to be tested, or validated. The "real world" operates as the selection system. Only those hypotheses that pass the test survive.

The final test may be made by simply asking if all of you in the audience are holding hands with the Red Queen. Are you working harder and harder and not making any progress? Yes, I know that some of the changes we make are associated with safety, environmental, or efficiency considerations, but could some of the changes be adjustments to adaptations made by the vector population? I need only gently remind you of the 60s and 70s when the term "insecticide treadmill" was used so often to describe our programs. The term of course is the infamous "Red Queen" in disquise.

Case closed! Test four has passed.

Darkness Descends on Vector Control.

If we accept the logic of the preceding argument, then must we not also accept the following?

Unless we know the adaptive capacity of a vector population, and whether our control efforts exceed that capacity, we are doomed to ever increasing demands to upgrade our program while gaining no significant long term control benefits from the changes. We are doomed to running in place with the Red Queen.

Oh what darkness has descended upon vector control.

Science to the Rescue.

There is hope of course. A review of the scientific literature shows that scientists in our field, as well as other fields, are working on the problem. Charles Taylor (1986) is approaching the problem from our point of view. He has investigated, by way of computer simulations, whether using several insecticides at once creates a survival hurdle too high

for a population to develop resistance. He has chosen the term "survival hurdle" to describe a quantity of control effort in relation to the ability of the vector population to survive.

Geneticists are also looking at the problem. The ability of a population to survive is a function of its genetic composition. Evolutionary change, is at the basic level, a change in gene frequency distribution. Geneticists are developing techniques that will enable them to determine the amount of genetic variation present in populations of vectors (Cockburn and Seawright, 1988). These scientists appear to be moving toward a time when we will be able to determine an "adaptive capacity" of the population. The very practical and timely work of Dr. Georghiou and other researchers (1989) at the University of California at Riverside deals specifically with providing us with the tools to determine gene frequencies for insecticide resistance in vector populations.

Evolutionists are currently looking at the same problem from their perspective. A term "ecological load" has been developed and mathematically defined (Stenseth, 1985). The term is related to the concept of "survival hurdle." The magnitude of the ecological load on a species determines its probability of survival or extinction.

Wildlife biologists, coming from the opposite point of view, that of species preservation, have developed another related mathematical concept called "minimum viable population size" (Reed et al. 1986). The tool would be used to determine how large a population would have to be able to maintain sufficient genetic variability over time to insure survival.

This most cursory review of the literature has indicated that wildlife biologists, evolutionists as well as vector biologists, in spite of their different perspectives and objectives, are all interested in developing the tools to measure the "adaptive capacity" of a population to environmental change. It appears that the advancing field of population genetics holds the key. Some wildlife biologists have made a direct plea to population geneticists to study the problem (Reed et al. 1986). I feel that we should do the same. We should make every effort to support and understand their work.

By requesting so much of geneticists and other scientists, we place a tremendous demand on science; but science is a unique and powerful process. The late Heinz Pagels expressed it well when he likened the scientific process to a selective system. He believed that scientific ideas, because of their vulnerability to failure imposed by the actual order of nature, are subject to a unique, self-imposed selective pressure. From this perspective, scientists collectively could be viewed as a machine generating hypotheses to be tested. The scientific process, linked to nature, operates as the selective system. Only those hypotheses that pass survive.

Conclusions.

I believe the paper has shown the existence of a significant correspondence between coevolution and the process of vector control. I believe it leads to these conclusions:

1. Our programs may suffer from the "Red Queen" effect, exhibiting ever increasing complexity while gaining little or no long term advantage over the vector population.

2. It is incumbent upon us to learn more about the "adaptive capacity" of the vector population.

3. We need a means to measure our control effort in relation to the "survival hurdle" that we impose on the target population.

4. We need to encourage and support the efforts of population geneticists and other researchers to pursue these matters.

5. Wildlife biologists could benefit from as well as contribute to similar research.

6. We may also benefit from the research by utilizing information about the "adaptive capacity" of wildlife species to design control programs with less adverse impact on wildlife.

7. Most vector populations have at least one significant advantage over us. They can afford, by virtue of their high reproductive rate and genetic diversity, to create many "cheap" survival models to be tested in the environment. We cannot afford this kind of approach.

8. We have our own advantages in our struggle with vector populations:

   A. We, as time binders, have time on our side. By making many changes in a short period of time, we are robbing the vector population of a geologic time frame. There is, therefore, little time for mutations, the basic mechanism of change. The vector population

must depend upon historical mutations stored as genetic variability in the population. In the near future we may be able to determine the "adaptive capacity" that their store of mutations represents, and use the information in designing our control programs.

B. The control agency has an enormous advantage over the vectors by evolving through Lamarkian methods. The characteristics of the programs are "acquired" through rational processes and passed to the future. The vector population is limited to bottom-up, small incremental changes while the control agency can make large top-down or bottom-up changes. We are limited only by the extent of our knowledge, resources, and creativity.

C. Finally, we have science on our side. Science is a most powerful selective system. Pagels (1988) believed that the power of science is derived from the fact that the selective process is ultimately linked to natural order.

The conference which we now attend is an arm of science. We are here to exchange ideas and to discuss new technology. We are able to select those approaches and tools that are most appropriate for our individual programs. From this perspective, we speakers at the conference are part of a CMVCA machine, generating hypotheses to be tested. You, the audience, operate as a selective system. Those hypotheses that pass your test will survive.

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MANAGEMENT: THE KEY TO FUTURE MOSQUITO CONTROL

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The theme for this year's Trustee Session is "management and administration." This presentation is being given for two reasons: as a result of the two years activities by the CMVCA'S 21st Century Committee, and as a prelude to todays speakers on "future issues" facing each and every one of us at Mosquito Abatement Districts.

The speakers will address the question, "What can we expect in two, five, ten, and more years from now?" The majority of the presentations are administrative in content, not technical. Why? Because, as managers and trustees, we administer public agencies that are responsible for mosquito control. The laws which govern us, local and state politics, and changing levels of revenue, directly influence our ability to accomplish our job, which involves far more than merely swatting mosquitoes, as is commonly perceived.

Mosquito control agencies in California employ approximately 800 people on a permanent and part-time basis, and spend over $33,000,000 per year. This is supplemented by $550,000 per year in supportive research by the University of California. However, continuous changes in administrative laws, pesticide regulations, and reduced revenues make our work far more difficult than in the past. Yet, most districts still rely on a small staff of one to five people to administer these increasingly complex programs.

The manner in which MADs conduct business in California has not really changed in the 45 years since the end of World War II. At that time, MADs were required to hire entomologists who could establish mosquito control programs that could prevent outbreaks of malaria caused by the military veteran population that had been infected with the disease overseas, and provide the capabilities to qualify for state subvention in the late 1940s. Few changes in technical personnel have been made since that time.

Unfortunately for the management of mosquito control programs, the rest of the State has rapidly changed the size and complexity of public businesses. With the proliferation of cities, creation of redevelopment agencies, formation of special assessment districts to utilize vector control legislation, local and regional transportation and planning agencies, and environmental regulations with mandatory permit review, the MADs are no longer the "independent special agencies" they once were.

We, as managers and trustees, need to know more about running public agencies, like our counterparts in the California Special Districts Association (CSDA), in order to keep pace. We are just as responsible to the communities we serve as are the administrators of these other public agencies. Our MADs are comparable to small cities or counties because of our large geographical jurisdictions and the multitude of responsibilities we have. We have open meetings, fiscal responsibilities, labor problems, insurance programs, contracts, and environmental interactions; these are all elements shared with any city, county, or other public agency. Each of these subjects requires special management and administrative skills at both the district and association level, yet we have not responded to these challenges by hiring appropriate technical staff to deal with these issues.

Perhaps one of the best examples of our collective management foresight was the formation of the Vector Control Joint Powers Agency (VCJPA). It started off small and has grown to a full-fledged, multi-million dollar, statewide self-funded insurance agency, comparable to the JPAs of many cities and counties. We have come a long, long way in a few short years. Why? Because we planned and developed the program one step at a time. The VCJPA, through effective management and administrative programs, has become the real leadership of our association. We set and attained specific goals allowing us to expand further should that be desired.

You may ask, "How does this apply to the District level, where the real operational problems reside?" Let me remind you, the VCJPA was formed because of two state-wide political pressures affecting our ability to conduct these "real operations." Proposition 13 drastically cut our revenues, which affected our personnel and opera-

tional policies, while insurance costs skyrocketed. We felt helpless in 1978 and many thought we, like some cities, would have to go without insurance. But we worked together at the association level and formed the VCJPA. This organization has allowed us to become more financially independent through resolution of many of our concerns. We should be proud of our results!

However, the next series of problems will be more vague and broader in scope, involving both old and new subjects such as changing labor laws, continued legislative mandates, future direction and leadership of the CMVCA, funding for research, and viral surveillance. We will be increasingly tested, far from our field operations, in legislative offices and board rooms. We must further develop and enhance our capabilities at both the district and association level in the fundamentals of public administration: leadership, laws and regulations, planning, personnel management, community relations, and financial management to make these adjustments. Increased administrative capabilities at the district and association level are essential if we are to continue to succeed in the public sector. People with these administrative skills need to be employed to meet these demands.

We should have the ability to deal effectively with our counterparts within those political agencies influencing our programs. Coincidently, the CSDA is holding its semi-annual management seminar addressing all of the above topics on February 24-26, 1989, in Newport Beach, California. That's how important these subjects are to our sister agencies, and we should be participating with them to stay competitive with them. These agencies are well aware that California is a dynamic state with projected long term growth and changing demographics which will impact all mosquito abatement districts. We can not ignore this growth and change it any more than we can stop it. These pressures will continue to influence our programs as the communities grow. Consequently, continued success of our programs demands that we look ahead and develop a strong planning component. We can achieve this by continuing our management workshops, utilizing outside sources like the CSDA for staff and board training, and hiring appropriate management, technical, and administrative personnel.

Summary.

Our past successes were no accident. They came from the collective and individual efforts of many people who understood that effective management and administration comes from advanced planning. The issues were understood and we had a willingness to work together to lead ourselves through the inevitable changes resulting from growth in California. The increasingly rapid changes and the greater complexity of our task in mosquito control mandate that we be even better prepared now than in the past. We must be more willing to adjust to these changes in order to continue our success. The process should begin with managers, trustees, and the CMVCA leaders. Some trends are evident and others are not, as will be shown by the following speakers. But, the one thing upon which we can all rely is that changes will occur. We must not take things for granted.

A LARGE, ECONOMICAL HOLDING TANK DESIGN FOR MOSQUITOFISH

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Introduction.

One of the problems becoming increasingly important to mosquito abatement districts is that of continuously maintaining a readily available stock of fish for its technicians and the public. The demand for fish to stock mosquito breeding sources varies considerably from day to day throughout a typical mosquito season. Technicians often find that fish are not on hand when they are needed because the district's holding tanks have become temporarily depleted. This generally occurs because the holding facilities at many agencies are too small to hold the numbers of fish required to meet ongoing demands. Some technicians load excessive numbers of fish into their district holding tanks to avert these frequent shortages. This practice usually results in dissolved oxygen deficits which in turn promotes fish stress and often culminates in acute bacterial, fungal or parasitic infections.

The configuration of many holding tanks presents additional disadvantages. Rectangular holding tanks, narrow designs in particular, usually provide easy access to the fish; unfortunately, water circulation patterns within these tanks may be uneven and include areas of negligible circulation. These "dead zones," which accumulate settleable waste material, can support and promote sudden pronounced surges in the growth of undesirable bacterial populations. The proliferating bacteria, in addition to their possible pathogenicity, utilize large quantities of dissolved oxygen. This can result in a critical reduction in the oxygen supply that would normally be available for the fish.

The use of supplemental agitator aeration in such situations will boost circulation and dissolved oxygen levels temporarily, but it is not a complete solution as contributory solid wastes will continue to accumulate. Flushing with fresh water also helps purge suspended waste metabolites. Settleable solids, however, are largely unaffected by this action unless exchange volumes are kept quite high. At most agencies, it is neither practical nor economical to continually flush large volumes of water through holding tanks. As a consequence, tanks are often inadequately operated or poorly maintained, and are not capable of offering fish the best possible short-term environment.

Several ordinary, rectangular tanks had been used for years at the headquarters of the Sutter-Yuba Mosquito Abatement District to hold fish for immediate use. The necessity for an improved and larger-scale fish holding facility was recognized as an urgent priority, and it was apparent that changes would be required in the fish storage system for an expanded biocontrol program. Several factors had to be considered to satisfy the District's requirements. First, any new tanks had to be economical to construct, yet large enough to accommodate substantial numbers of fish for weeks or even months at a time. Second, tank durability was considered to be important due to the District's extremely hard water and projected heavy use. Third, the tanks had to be relatively easy to maintain in an acceptable state of cleanliness. Last, the tanks had to permit ready access to the fish by technicians. Numerous tank designs were considered; but a circular configuration was finally selected because basic materials were on hand that would help minimize construction costs and the completed tanks would very nearly meet all the foregoing criteria.

One unique design feature exhibited by the many circular tanks now used in the aquaculture industry and government hatcheries is their capacity to be essentially self-cleaning (Larmoyeux and Piper 1973). To accomplish this, slow circulating tank water currents are induced through directed perimeter water jets, with the settleable and suspended wastes being slowly transported by vortex action toward a single axial tank drain. This whirlpool effect and facilitation of complete draining are enhanced in the latest tanks with shallow, conical bottoms (Brandt and Morrow 1987, Hawke and Field-Dodgson 1987). Central effluent sumps or enclosed standpipe drains receive the waste-laden water, and it's either discharged or subsequently filtered and recycled at the discretion

of the user.

Fisheries technicians find that sizeable mosquitofish populations survive longer in large, shallow environments than they do in small, deep confines. This may be due to the diluting effect large volumes of water offer, which permits a slower accumulation of toxic waste metabolites. Tanks with large surface to volume ratios promote greater atmospheric gaseous exchange than do those tanks with equal volumes but less surface area. This is advantageous because these tanks can accommodate fish which have constantly varying demands for dissolved oxygen. Injuries induced in captive mosquitofish through density-dependent interaction, stress, or fright responses may also be reduced in larger confines where fish are less crowded. Finally, larger volume holding facilities offer greater temperature stability than do their smaller counterparts. With these and other factors supporting the use of larger holding tanks, and circular tanks in particular, Sutter-Yuba M.A.D.'s personnel designed an enlarged fish holding system.

Materials.

Following the basic tank design of Hauke and Field-Dodgson (1987), which consisted of a thin galvanized steel band embedded in a circular concrete pad, several modifications were incorporated to enhance tank durability and longevity. First, a ring of butt-welded, marine-grade aluminum sheeting would be employed to provide greater rigidity than the thin steel sheeting originally described. A U.V.-resistant plastic liner would be bonded to the inside surface of the embedded aluminum tank wall to protect against corrosion and add needed freeboard.

Panels of Sun-Lite HP plastic sheeting measuring 3 ft x 50 ft x 0.040-in. thickness were purchased from Solar Components Corporation for this application. Actual bonding would be made by running parallel beads of silicone adhesive and a similar waterproof sealant between the two materials around the exposed top and bottom inside edges of the embedded aluminum sheeting. An additional bead of sealant would be used between the extreme bottom edge of the plastic liner and the concrete tank bottom to prevent water from contacting any exposed aluminum.

Construction.

Professional skills were required to construct the shallow, cone-shaped concrete tank bottoms and the 6-in. thick compacted crushed rock bases that were to support the concrete pads for each of the five tanks. A contract for this work was awarded to a local construction firm. Their surveying measurements ensured that the rock bases were uniformly level, and precisely established the locations for the concrete pad forms

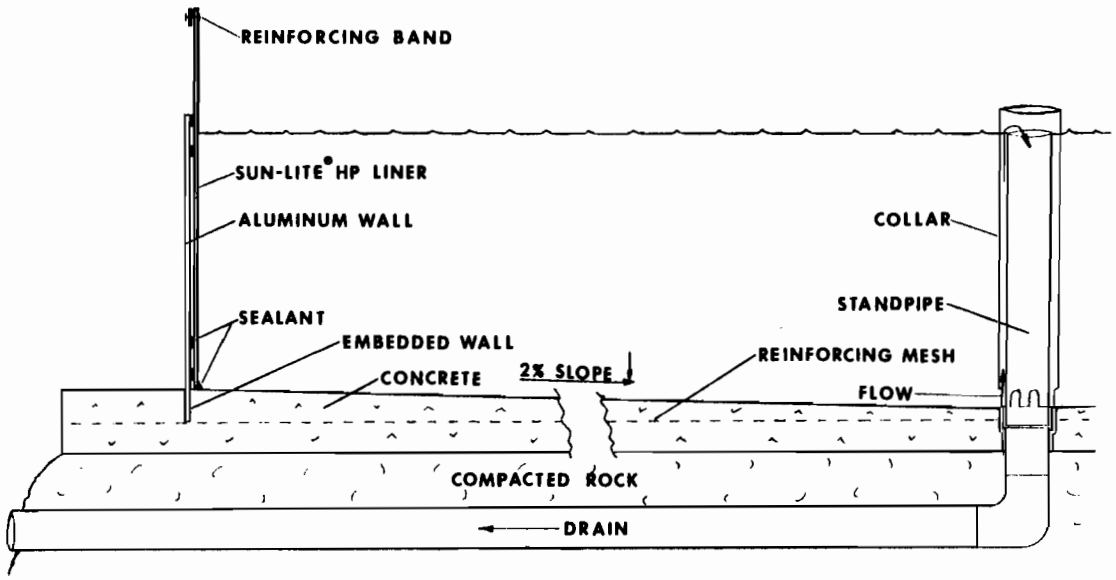

Figure 1.-Tank, partial schematic view.

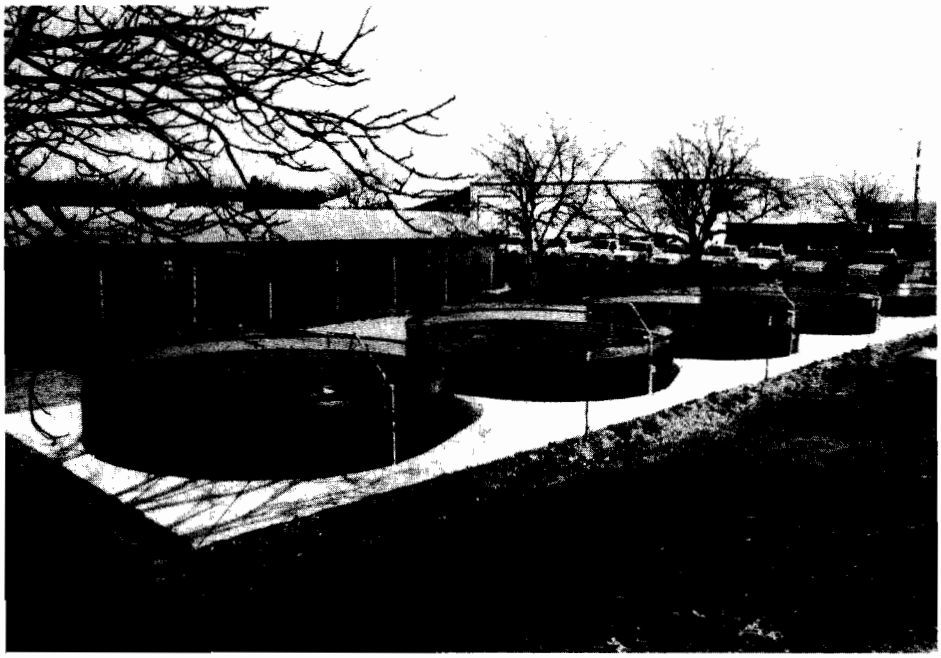

Figure 2.-The completed mosquitofish holding facility consisting of five circular tanks.

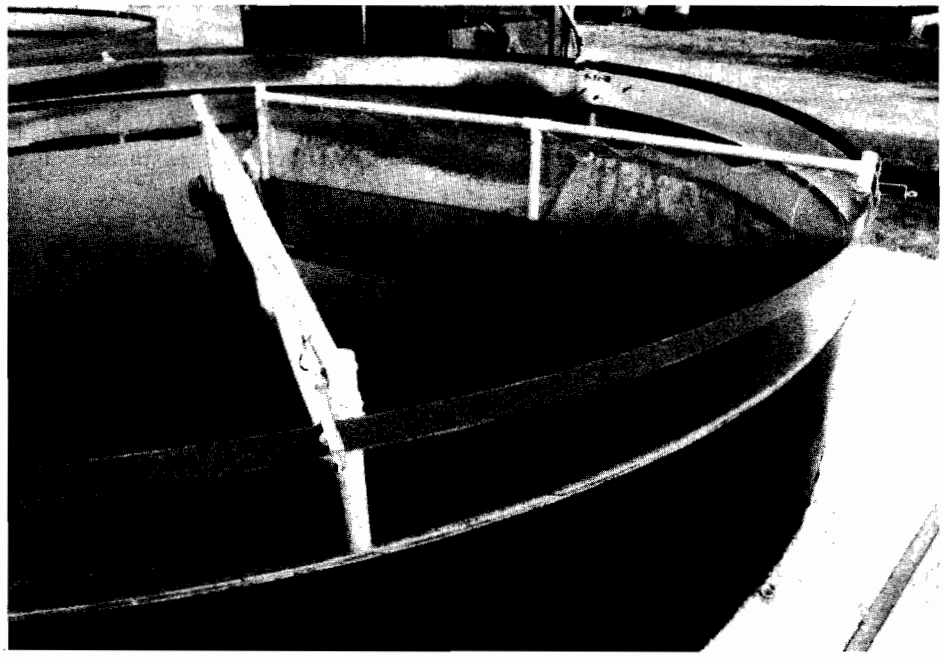

Figure 3.-Crowder net in closed (harvest) position.

as well as the tanks. A rectangular form for a jointed concrete slab 20 ft x 90 ft x 1/2 ft in depth was constructed to accommodate all five tanks. Reinforcement wire grids were suspended upon small concrete pier blocks to strengthen the pads, and the welded aluminum rings destined to be the walls of the tanks were set into place above this wire gridwork. The aluminum tank bands were positioned three in. above the elevation of the compacted rock pad by attaching numerous, short, aluminum legs to the outside bottom edge of each of the metal bands. This was to permit the poured concrete to flow both above and below the bottom edges of the aluminum material serving as the walls of the tanks and create a 3- to 4-in. watertight, concrete seal along the tank bottoms. Lastly, measurements were made to permit fabrication of conical tank bottoms which would slope 2% toward the recessed tank drains (Figure 1).

Once these tank materials and all necessary concrete forms were in place, but prior to the pouring of any concrete, district personnel plumbed in plastic 1-in. water supply and 4-in. drainage lines, as well as plastic (0.5-in.) conduit for electrical service. The concrete was then poured and finished one tank at a time. The interior tank bottoms were wet-cured for 30 days to maximize strength after the initial set-up of the concrete on all five tanks. The tanks were then drained, solvent cleansed, and the plastic liners were bonded to the interior aluminum walls. These bonds were allowed to cure overnight with sacks of sand stacked against the two wall materials to keep them properly positioned. The tanks were leak-tested after they were fully cured by filling them beyond their normal capacity. Final plumbing and electrical work was completed and, after several more fill and flush cycles, bioassay fish were introduced into the new tanks. No indication of any material toxicity was noted at the time so more fish were gradually stocked into the new tanks.

Each completed tank (Figure 2) measured

Table 1.-Basic materials and contract construction cost per tank.

Quantity	Description	Amount
1 ea.	18'x 20' Concrete Pad on Rock Base	$ 1,035.00
48.7 ft.	0.125"x 29" Sheet Aluminum	38.16
50 ft.	0.040"x 36" Sun-Lite HP(R) Plastic Sheet	147.75
5 ea.	Cartridges, Sealer and Adhesive (Dow Corning 8641 and 739)	38.80
Basic tank materials total		= $ 1,259.71

Table 2.-Plumbing costs per tank.

Quantity	Description	Amount
10 ft.	1" Schedule 40 PVC Pipe	$ 5.41
2 ea.	1" SXT Male Adaptor	1.06
1 ea.	1" SXS Elbow 90 Degree	0.66
2 ea.	1" SXT Elbow 90 Degree	1.19
1 ea.	1"x 3/4" SXT Reducer Bushing	0.59
1 ea.	3/4" hose adaptor	0.34
1 ea.	Sweeper Nozzle	2.11
1 ea.	1" Ball Valve	9.87
1 ea.	1" x 2" Schedule 80 Nipple	0.40
25 ft.	4" Class 100 PVC Pipe	36.57
1 ea.	4" SXS Elbow 90 Degree	11.45
1 ea.	4" SXSXS Tee	17.00
1 qt.	711 PVC Cement (on hand)	0.00
Plumbing materials total		= $ 86.65

15.5 ft in diameter with a water depth of 27-28 in. and a freeboard of 10 in., thereby yielding an indi-vidual volume of 3312 gal. and offering a total system volume of 16 560 gal. for all five circular tanks.

Costs.

Actual construction costs for this tank system, as installed at Sutter-Yuba M.A.D., were lessened somewhat because some major building components were purchased at much reduced prices. Commercially-constructed tanks have been gradually decreasing in cost as the aquaculture industry expands; so it may eventually be possible to locate manufacturers that produce circular tanks similar in size and features. The component descriptions, quantities used, and itemized costs are listed in Tables 1-4.

Operation.

Filamentous algae growth often becomes rank in sunlit, shallow ponds during the summer season in central California. Holding tanks are not exempt from this phenomenon, and they must be kept relatively free of nuisance vegetation if they are going to remain in a harvestable condition. Aquatic herbicides generally do not offer a long-term solution to these filamentous algae problems because the vegetation usually rebounds rapidly after chemical treatment, and many of the more common algicides or their indirect effects can be stressful, if not lethal to mosquitofish.

An essentially nontoxic solution to this problem has become available, and while it does not eradicate algae entirely, it does inhibit its development significantly. Aquashade®, a combination of two food-grade organic dyes, imparts a bluish tinge to treated waters with photoinhibition the intended mode of action. Filamentous algae and many vascularized aquatic plants are dependent upon initial germination and growth at sunlit benthic surfaces. This dye compound prevents critical amounts of sunlight from reaching the pond or tank bottom, thus keeping much of the algal development in check. Unfortunately, some algae will continue to grow along shallow pond margins, and must be removed physically or controlled with spot herbicide applications.

Aquashade® is used regularly to inhibit algae growth in the District's new circular tanks, and as wastewater discharges are not being recycled, replacement water additions must be kept to a minimum to avoid diluting the dye-treated tank water. A small electric fountain pump is used to sustain the slow circular currents required to transport suspended wastes toward the center standpipe

Table 3.-Electrical costs per tank.

Quantity	Description	Amount
10 ft.	1/2 " PVC Conduit	$ 5.30
1 ea.	1" PVC Conduit	1.80
1 ea.	1/2" Weatherproof Outlet Box	6.29
2 ea.	1/2" T/A Adaptor	0.48
1 ea.	1/2" PVC Elbow	0.63
5 ft.	12/2 w/Ground, UF Wire	1.11
1 ea.	115 Vac, 15-Amp Receptacle	0.58
1 roll	Rubber Tape	1.42
Electrical material total	=	$ 29.01

Table 4.-Summary costs.

1. Tank Materials (Wall, Liner, Concrete)		$ 1,259.71
2. Plumbing (Supply, Drainage)		86.65
3. Electrical (115VAC Supply)		29.01
Total cost per tank	=	$ 1,375.37
Cost for entire system	=	$ 6,876.85

drain of each tank. Periodic draining of the collected sediments is accomplished by turning on the powerful peripheral jet for a short period of time. This causes the settled wastes to be drawn upward between the standpipe and its collar, and exit the tank through the standpipe's drain. The friction-fitted standpipes can be removed from the recessed bottom fittings of the tanks for complete draining. The self-cleaning efficiency of this circular tank design has not been perfect, but it is an improvement over the manual cleaning system used in the older rectangular tanks. Additional experimentation will be necessary to determine optimal flow rates, circular velocities, and other maintenance details.

Comparatively large numbers of fish have been introduced to several of the tanks with agitators activated to assure adequate dissolved oxygen. This has been necessary because very little dissolved oxygen is introduced through the peripheral jets as the District's wellwater is essentially anaerobic. Experimentation will be conducted on the benefit of special air injection nozzles to alleviate this problem. Maximum fish holding capacities for the tank system have not been determined, so loading tests will be made during summer 1989. Minimal replacement water and fish feed were required during this winter's cold weather, and waste accumulations have as a consequence been insignificant.

Large, circular tanks have a reputation for eing difficult to harvest unless the fish are drained from them. A folding fish crowder has been devised to alleviate this problem for our new tanks by modifying the design of Brandt and Morrow (1987). This one- or two-person crowder was fabricated by stretching and attaching 1/8-in. nylon seine material within two rectangular PVC pipe frames hinged loosely together (Figure 3). One of the two netted frames is anchored to the tank wall, while the other section is walked around the tank to form a narrow, wedge-shaped area from which the fish can be dip-netted. One hundred per cent harvests of the fish have not been achieved because a few fish escape around the edges of the crowder, but refinements should improve the crowder's efficiency.

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FEEDING AND SURVIVAL OF LABORATORY *AEDES SIERRENSIS* LARVAE

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ABSTRACT

First instar larvae of *Aedes sierrensis* were reared in laboratory microcosms containing different sources of nutrition. The food sources consisted of treehole water, a larval food mix of 50% liver powder and 50% yeast powder, leaves of California black oak (*Quercus kelloggii*) and leaves of Pacific madrone (*Arbutus menziesii*). Deionized water served as the control. The results indicated that deionized and treehole water did not sustain larval or pupal development. The food mix treatment had the greatest mean pupal weight but there were no significant differences in mean adult wing lengths among the treatments. The percentages of *Ae. sierrensis* which emerged as adults were: deionized water, 0%; treehole water, 0%; food mix, 29%; oak leaves, 33%; and madrone leaves, 41%.

Introduction.

The western treehole mosquito, *Aedes sierrensis* (Ludlow), is a vector of dog heartworm, *Dirofilaria immitis* Leidy, and deer bodyworm, *Setaria yehi* (Rudolphi), and is considered to be an important biting pest of humans (Weinmann and Garcia 1974, Weinmann et al. 1973). Larvae of *Ae. sierrensis* can be found commonly in treeholes in many areas of western North America (Darsie and Ward 1981).

Leaves, stems and other materials which fall into a water-filled treehole provide a nutrient source which usually supports a detritus-based invertebrate community (Kitching 1971, Woodward et al. 1988). Larval food may be the most important factor limiting the abundance of treehole mosquito populations (Carpenter 1983). Zavortink (1985) studied eight treehole inhabiting *Aedes* species in the southwestern U.S. and reported they are capable of filter-feeding, browsing, and gnawing. Fish and Carpenter (1982) reported that their laboratory study of an eastern species of treehole mosquito, *Aedes triseriatus* (Say), indicated that browsing upon fungi and bacteria on the surface of submerged leaves is of primary importance for larval nutrition. Walker and Merritt (1988) did a field study of *Ae. triseriatus* and reported that leaves are not necessary because treehole water containing bacteria provides sufficient nutrients for larval growth. The present study was undertaken to determine which food source provided the best growth and survival of *Ae. sierrensis* in the laboratory.

Materials and Methods.

The laboratory microcosms (simulated treeholes) consisted of 20 one liter glass containers with lids. Four microcosms contained 500 ml of deionized water only. A mixture of 400 ml of deionized water and 100 ml of filtered treehole water was added to each of the remaining 16 containers. These were used for the treehole water, food mix, oak leaf and madrone leaf treatments.

The larval food mix consisted of 50% Schiff Natural Liverall powder and 50% Yeast 500 plus Vitamins powder. Each of the four larval food mix containers received 0.25 grams of the food mixture.

Leaves of California black oak, *Quercus kelloggii* Newberry, were collected from an experimental site in Kelseyville, California. Three grams of dried oak leaves were added to each of four of the containers.

The last treatment contained leaves of Pacific madrone, *Arbutus menziesii* Pursh, collected near Potter Valley, California. Three grams of dried madrone leaves were added to each of the four remaining containers.

Aedes sierrensis eggs, collected from a laboratory colony, were hatched in filtered treehole water with a dissolved oxygen concentration of <0.1 ppm. The larvae were then rinsed in a petri dish of deionized water in order to remove any organic particles that might have adhered to them during the hatching process. Twenty larvae were added to each of the 20 laboratory microcosms. The larvae were kept at 23±2°C and observed daily. All dead larvae and exuviae were removed daily so that living mosquitoes could not feed on them.

Larvae from two jars of each treatment group were removed from their containers and head capsule widths were measured daily using a dissecting microscope fitted with an ocular micrometer. When the larvae reached the pupal stage they were weighed with an electronic microbalance.

Emerging adults from each food type were collected daily and the sex of each was recorded. Adult wing lengths were measured from the alular notch to the distal wing tip (Bock and Milby 1981) with an ocular micrometer in a dissecting microscope.

The experiment was terminated when all *Ae. sierrensis* had either died or emerged successfully as living adults. Survival, development, wing length, and emergence data were each analyzed by a one-way analysis of variance followed by a Student-Newman-Keuls multiple range test. Sex ratio data were analyzed by Kruskal-Wallis tests (Zar 1974).

Results and Discussion.

The mean percentage of larvae alive in each treatment after ten days was as follows: deionized water, 1%; treehole water, 63%; food mix, 63%; oak leaves, 68%; and madrone leaves, 78%. The percent alive in the deionized water was significantly ($P<0.01$) less than in the other treatments.

Figure 1 presents *Ae. sierrensis* survival rates during the study. On days 16 and 28, the deionized water and treehole water colonies died out, respectively. Head capsule width measurements indicated the larvae in the deionized water colony never matured beyond the second instar, but some of the larvae in treehole water matured to the fourth instar before dying. Another treehole mosquito, *Orthopodomyia signifera* (Coquillett), develops a rose-pink coloration after feeding on the bacterium, *Thiocapsa* sp. (Reeves 1941). No pinkish coloration was observed in the *Ae. sierrensis* in the containers with treehole water.

The percentage of larvae which successfully pupated is indicated in Figure 2. No pupae developed in the deionized water and treehole water treatments. Fish and Carpenter (1982) reported that rapidly decomposing leaf litter supported more mosquito growth than slowly decomposing litter. The madrone leaves decayed relatively slowly in the present study, but there were no significant differences ($P>0.05$) in pupation rates among the food mix, oak leaves, and madrone leaves treatments.

The pupal weight of each individual within two replicates of each treatment group was measured and the results are presented in Figure 2. The larvae reared on the food mix had a significantly ($P<0.05$) higher mean pupal weight (5.5 mg) than did larvae reared on madrone leaves (mean pupal weight of 3.7 mg) or oak leaves (mean pupal weight of 3.6 mg). Gilpin and Langford (1978) reported that *Ae. sierrensis* larvae which were given greater quantities of food formed heavier pupae. The more immediate availability of the powdered food mix to feeding larvae may have also been important in the present study.

Figure 3 plots the cumulative adult emergence against the number of days in the experiment. Pupae from the oak leaf microcosms started producing adults on Day 14. Adult emergence continued for 8 days during which a total of 33% of the first instar larvae in the oak leaf microcosms emerged as adults. Food mix microcosms first produced adults on Day 15. All adults in these microcosms emerged over a 4-day period during which 29% of the *Ae. sierrensis* emerged as adults. Madrone leaf microcosms first produced adults after 16 days. The emergence period lasted until Day 40. During this span, 41% of the *Ae. sierrensis* emerged as adults. There were no statistically significant differences ($P>0.05$) in the numbers of adults which emerged, nor in the sex ratios of the adults from the food mix, oak leaf, and madrone leaf treatments.

The percentage of pupae which emerged as adults (Figure 4) was high (79%) for the madrone leaf treatments. Figure 4 also presents the mean wing lengths of the adult mosquitoes from the three food source colonies. The adults reared on larval food mix had a mean wing length of 2.55 mm followed by madrone leaves with a mean wing length of 2.39 mm and by oak leaves with a mean length of 1.92 mm. A comparison of wing lengths from all treatments indicated females had significantly ($P<0.01$) greater wing lengths than males. However, comparisons of male wing lengths between treatment groups showed no significant differences ($P>0.05$). Similarly there were no marked differences ($P>0.05$) among food mix, oak leaf, and madrone leaf treatments in female wing lengths.

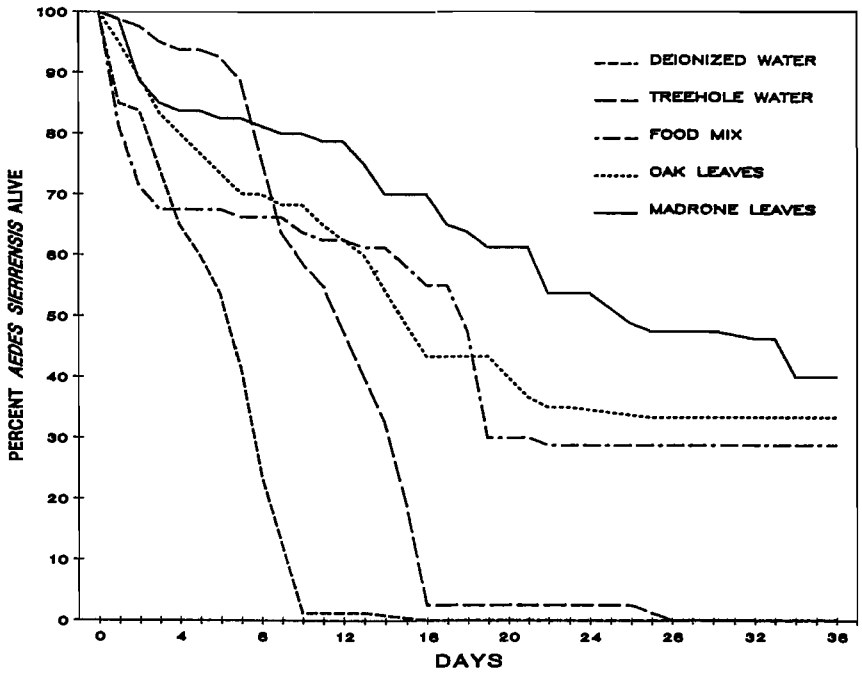

Figure 1.-Survival graph of the percent of *Aedes sierrensis* alive during each date of the study.

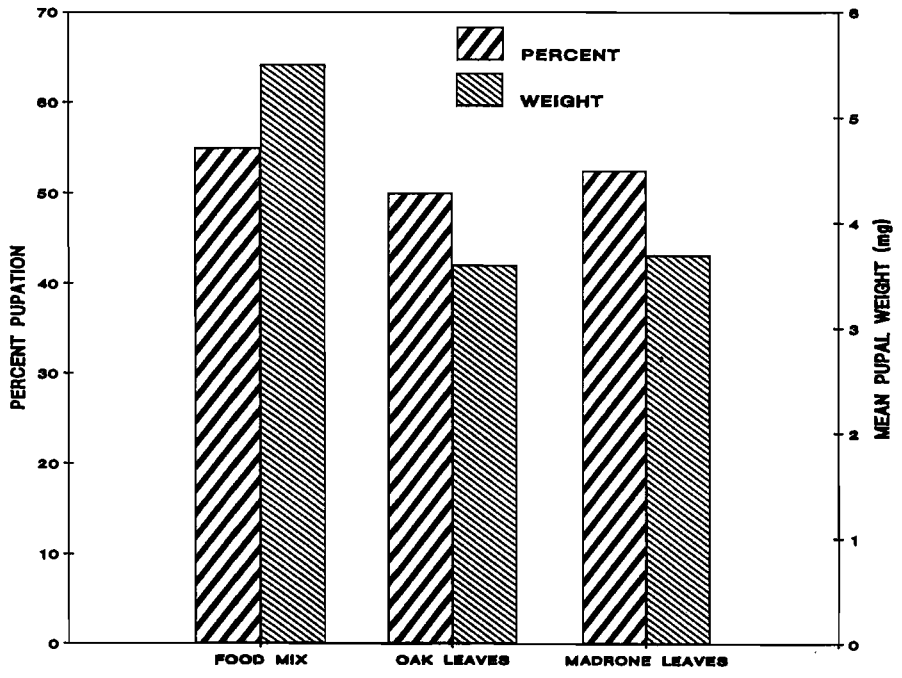

Figure 2.-Percent of *Aedes sierrensis* larvae which pupated, and mean pupal weight. No pupae occurred in the deionized water and treehole water treatments.

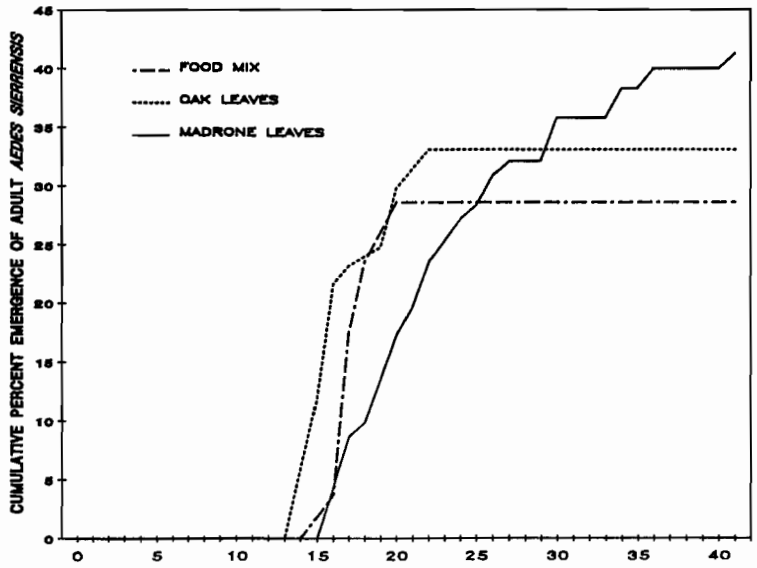

Figure 3.-Cumulative percentage of *Aedes sierrensis* which emerged as adults. No adults developed in the deionized water and treehole water treatments.

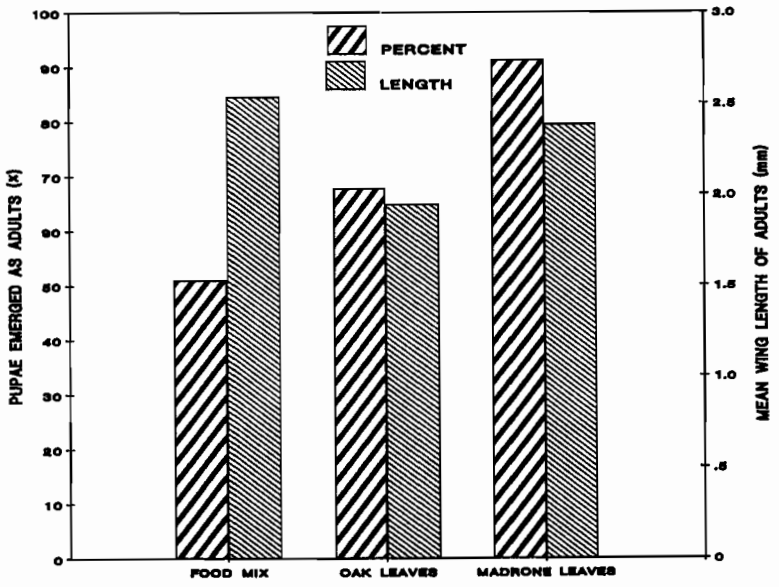

Figure 4.-Percent of *Aedes sierrensis* pupae which emerged as adults, and mean wing length of adults. No adults developed in the deionized water and treehole water treatments.

The considerably greater pupal weight of the food mix mosquitoes did not result in a particularly longer wing length of adults. This may be due to the high pupal mortality in the food mix microcosms.

The idea that filter feeding on bacteria, algae, or other items in treehole water is of primary importance to treehole mosquito larvae was not confirmed in this study. However, the artificial food mix, which may have been ingested by filter feeding, is suitable for rapid laboratory rearing of adults. The relatively slow rate of emergence from the madrone leaf microcosms suggests the larvae may not be able to immediately feed directly on these tough leathery leaves. Even after decomposing organisms had softened the leaves, they did not appear to have pieces removed from them by the larvae. These observations are consistent with the hypothesis that *Ae. sierrensis* larvae browse on fungi and other microbes which have colonized the leaves.

Acknowledgments.

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A MODEL OF POPULATION DYNAMICS OF *CULEX TARSALIS* ON RICE FIELDS IN FRESNO, CALIFORNIA

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ABSTRACT

A life table was constructed for *Culex tarsalis*, the primary vector species of western equine encephalomyelitis. Parameters measured to develop the table included survival in the adult and immature stages, fecundity, egg viability, gonotrophic cycles, and sex ratio. Under the strong predation pressure of natural enemies in Fresno, California rice fields, the rate of increase from generation to generation (R_o) of the species was ca. 1.6X, indicating a relatively stable population density. The intrinsic rate of increase (r) was 0.0246, and mean duration of a generation (T) was 20 days.

Introduction.

Culex tarsalis Coquillett is a primary vector of both western equine encephalomyelitis and St. Louis encephalitis viruses in California (Reeves 1965). Adult mosquitoes are usually collected from natural habitats such as animal burrows, tree cavities, and rock piles, as well as from chicken houses, animal shelters, and under bridges (Mortenson 1953, Loomis and Green 1955, Kliewer et al. 1969). Larvae are found in all categories of ground water, like natural pools or marshy wastelands, but they are usually associated with agricultural usage of water such as rice fields, irrigated pastures, and irrigation seepage water (Bohart and Washino 1978).

Mosquito populations, including *Cx. tarsalis,* are becoming increasingly resistant to chemical pesticides, and the undesirable environmental impact of pesticides has become recognized (Carson 1962, Georghiou et al. 1969, Miura and Takahashi 1974, Schaefer and Wilder 1970, Womeldorf et al. 1971). Both traditional and novel alternative control measures are needed to control mosquito populations for these reasons.

In order to develop new approaches to mosquito control, it is necessary to know the biology of the mosquito, as well as what methods and materials have been used to control mosquitoes in the past. Key data include how the insect survives in its larval and adult stages, where breeding occurs, and the physical and physiological effects of the factors in its living environment.

Moon (1975) developed a statistical model of the basic cycle of western equine encephalomyelitis in Kern County, California, using laboratory and field data available in the literature. White (1980) reported the population dynamics of this species breeding in a marshy canyon surrounded by arid foothills in Kern County.

The objective of this report is to construct life tables to determine the net reproductive rate (R_o), intrinsic rate of population increase (r), and mean generation time (T) of *Cx. tarsalis* breeding on rice fields in the San Joaquin Valley, California.

Materials and Methods.

Rice culture and mosquito control. The study was conducted in the northwest portion of Fresno County, California. Rice fields in this area normally were flooded during the 2-month period between April 15 to June 15. The major mosquito species found was *Cx. tarsalis*, which usually appears 3 to 5 weeks after flooding and is found there throughout the rice season.

Two main strategies, biological and chemical, have been used by the local mosquito abatement district (Fresno Westside MAD) to control mosquitoes. The most important biological agent is the mosquitofish, *Gambusia affinis* (Baird and Girard). Normally, 0.2 lbs. (200 to 400) fish per acre are stocked 7 to 10 days after flooding the fields.

Biology of *Cx. tarsalis* in Fresno area. *Cx. tarsalis* is a multivoltine species that overwinters as diapausing females. Usually, first yearly occurrences of newly blooded or gravid females are collected in the last week of January (Kliewer et al. 1969, Schaefer and Miura 1972). In some years, first occurrences of males (a 1st generation) are collected with light traps during the first week of March, but yields are generally small until the middle to latter part of March. This indicates that most likely the eggs were oviposited in February. The species has bimodal population peaks during the year; the first peak appears at the middle of July, and the second at mid-September (13 years trap collection records, Fresno Westside MAD). The Fresno area has an autogenous strain which is apparently indigenous (Bellamy and Kardos 1958).

Data collection. *Cx. tarsalis* colonies established from wild populations collected in the vicinity of Fresno were used. The colonies were held in 1 ft^3 screened cages in an insectary maintained with a daily light regime of 14L:lOD and a temperature and RH of 27 ± 1° C and 80%, respectively. The caged mosquitoes were provided food in the form of raisins and permitted to take blood from mice twice a week. A paper cup (140 ml) containing 100 ml water for oviposition was placed inside each cage. Oviposited eggs rafts were hatched in the cup and transferred to rearing pans (40 x 25 x 6 cm), each containing 3 liters of deep well tap water (pH 7), powdered rabbit food, and yeast solution. The water in the pans was then gently aerated.

To determine the ratio of autogenous and anautogenous females in the colony, 100 or more pupae were transferred to 16 oz Styrofoam emergence cups with 300 ml of water. Each cup was placed in a 1 ft^3 cage containing a raisin cup for food, and the cages were held in the insectary for a week after adult emergence. The females were then dissected and classified as to Christopher's (1911) stage of egg development. Females in stage III or later were considered as autogenous. This test was repeated five times.

Subcolonies of autogenous and anautogenous strains were established separately to study fecundity. Fifty or more adults of each strain were kept in separate 1 ft^3 cages, provided with a raisin cup, and given access daily to mice from the time of emergence throughout the study period. Both egg raft production and the numbers of eggs per raft were recorded daily. Viability of eggs for both strains was determined by examining and counting eggs from individual rafts hatched in separate cups.

Mathematical model. We used a model essentially identical in principle to that described by Weidhaas (1974). In brief, the net reproductive rate (R_o) is used for computing population growth rates, and it is expressed mathematically as follows:

$$R_o = \Sigma\, \ell_x m_x$$

where l_x is the age specific survivorship, i.e., the probability that an individual which was alive at age 0 will be alive at age x, and m_x is fecundity, which is defined as the mean number of offspring produced to individual age x in a given time period. To determine the R_o of *Cx. tarsalis* calculation, we used the following formula:

$$R_o = [\Sigma(\ell_{x_{aut}} * m_{x_{aut}} + \ell_{x_{anaut}} * m_{x_{anaut}})]\, V\, S_i$$

incorporating two reproductive strains and where V equals viability of eggs, and S is the survival rate of immature stages. The mean generation time (T) and intrinsic rate of increase (r) were estimated from the life table using the following equation:

$$T = \frac{\log_e R_o}{r}$$

The r value was calculated by an interactive substitution method using Lotka's equation (Birch 1948):

$$1 = \Sigma\, \ell_x\, m_x\, e^{-rx}$$

Results and Discussion.

Both the population statistics obtained by the present study and the data available in published references are shown in Table 1. The autogenous rate of the Fresno strain of *Cx. tarsalis* reared by the diet described earlier was ca. 17%, ranging from 0 to 35%.

The first egg rafts deposited by autogenous females appeared on the 3rd day after emergence; most egg rafts were deposited on the 5th day, while anautogenous females produced most egg rafts on the 6th day. The subsequent length of the gonotrophic cycles was four days in both strains (Fig. 1). The mean number of eggs per raft oviposited by anautogenous females was 250 with

Table 1.-Life table statistics, used to calculate the net reproduction rate (R_o), intrinsic rate of increase (r) and mean duration of a generation (T) of *Cx. tarsalis* in Fresno, California rice fields.

Biological events	Statistics	Source
1 Survival Rate (%)		
Eggs	0.930	present study
Larvae		
I	0.478	Miura and Takahashi unpublished data
II	0.323	
III	0.221	
IV	0.039	
P	0.021	
Adult (daily)	0.820	Nelson & Milby, 1982
2 Duration (eggs to emergence)	10 days	present study
3 Fecundity		
Autogenous	80 eggs/raft	present syudy
Anautogenous	250 eggs/raft	
4 Gonotrophic cycle		
1st oviposition		
Autogenous	4 days	present study
Anautogenous	6 days	
2nd and 3rd oviposition		
Autogenous	4 days	present study
Anautogenous	4 days	
5 Sex ratio	1:1	present study

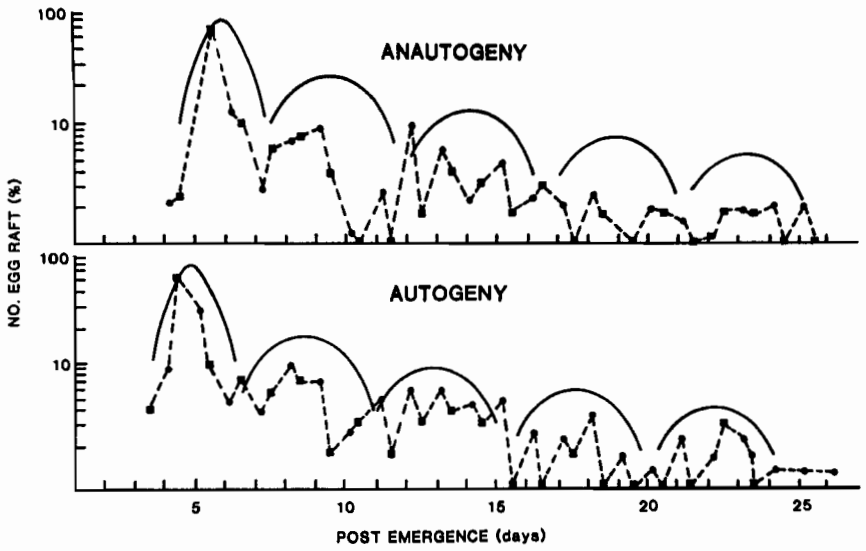

Figure 1.-Gonotrophic cycle of *Cx. tarsalis*. Dotted line, actual daily oviposition rate (in percent); Arch-shaped solid lines, appropriated oviposition peaks.

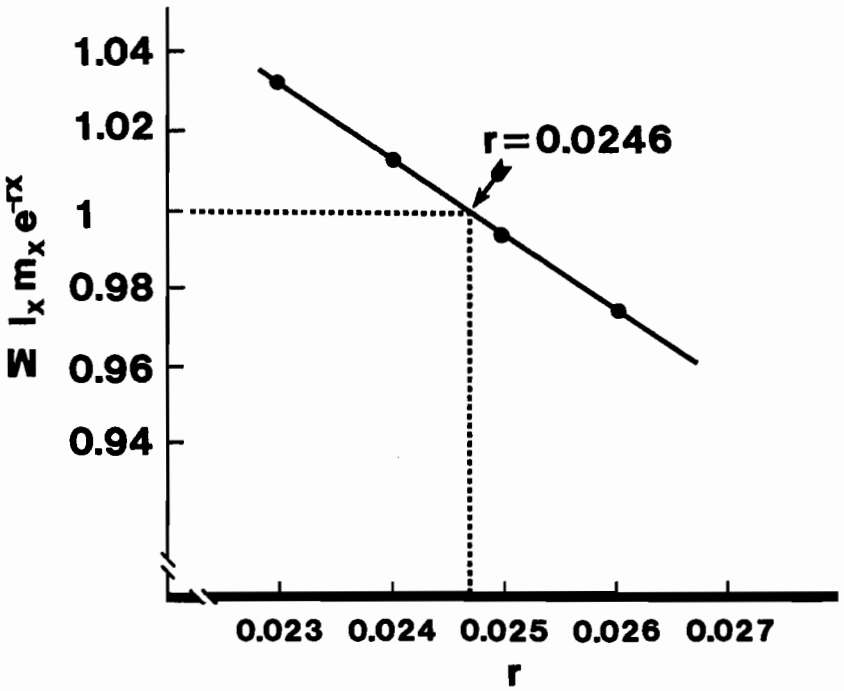

Figure 2.-Graphic approximation of the value of "r" by using Lotka's equation, $1 = \Sigma\, \ell_x m_x e^{-rx}$.

Table 2.-*Culex tarsalis* population model in Fresno, California rice fields.

Day	Life stage	No. individual	No. eggs	Survival rate	ℓ_x	m_x	$\ell_x m_x$
0-2	Eggs	50,000		0.920	0.92000	0	
3	1st instars	46,000		0.519	0.47748	0	
4	II	23,874		0.677	0.32325	0	
5	III	16,163		0.686	0.22175	0	
7	IV	11,088		0.177	0.03925	0	
9	Pupae	1,962		0.525	0.02061	0	
11-12	Adults	1,030		0.820	0.01690	0	
5[a/]	Adults	65	2,600	0.820	0.00627	40	0.2508
6	Adults	260	32,500	0.820	0.00514	125	0.6425
9	Adults	29	1,160	0.820	0.00283	40	0.1132
10	Adults	118	14,750	0.820	0.00232	125	0.2900
13	Adults	13	520	0.820	0.00128	40	0.0512
14	Adults	53	6,625	0.820	0.00105	125	0.1313
17	Adults	6	240	0.820	0.00058	40	0.0232
18	Adults	24	3,000	0.820	0.00047	125	0.0588
21	Adults	3	120	0.820	0.00026	40	0.0104
22	Adults	11	1,375	0.820	0.00021	125	0.0263
25	Adults	1	40	0.820	0.00012	40	0.0048
26	Adults	5	625	0.820	0.00010	125	0.0125
29	Adults	0.5	20	0.820	0.00005	40	0.0020
30	Adults	2	250	0.820	0.00004	125	0.0050
33	Adults	0	0	0.820	0	40	0
34	Adults	1	125	0.820	0.00002	125	0.0025
			63,950			$\Sigma = \ell_x m_x =$	1.6245

[a/] Hereafter = days after adult emergence.

range of 159 to 325, and that by autogenous females was 80 (35-206). The viability of eggs per raft was ca. 93% in both strains.

The summary life table of *Cx. tarsalis* on Fresno rice fields is shown in Table 2. Larval mortality was high during the younger immature stages and this might have been due to predation (Miura in press). Mogi et al. (1984) similarly observed high mortality (99%) of *Culex* sp. in Philippine rice fields. The R_o of the Fresno strain in a rice growing season was 1.6245. This means the population was growing even under heavy pressure from natural predators (Miura et al. 1981). The r value was 0.02465 (Fig. 2) indicating that the population was growing at a rate of 0.02465 per female per gonotrophic cycle. Mean duration of a generation (T) was calculated as 19.7 days.

The data obtained from the life table study indicated that rice fields in the Fresno area produce relatively few mosquitoes on a per acre basis. Nevertheless, rice fields play an important role in mosquito production in the area because rice is usually cultivated in large acreages, and fields remain flooded for five months every year, thereby

providing a vast area for mosquito breeding during a large part of the year. This is contrasted to most other agricultural practices where long term mosquito breeding habitats are not created as a normal part of cultivation.

Acknowledgment.

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MARK-RELEASE-RECAPTURE STUDIES ON CULICINE MOSQUITOES IN SOUTHERN CALIFORNIA DURING 1988[1]

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ABSTRACT

The population ecology of *Culex quinquefasciatus*, *Culex stigmatosoma*, and *Culiseta incidens* was studied using mark-release-recapture methods at a dairy/residential setting in Chino, San Bernardino County, and at the residential community of Rossmoor, Orange County, during the summer of 1988.

Mosquitoes were collected as larvae and pupae from dairy lagoon (Chino) or drainage channel (Rossmoor) breeding sites. They were allowed to emerge, marked with date- and site-specific colored fluorescent dust, and released in late afternoon when less than 24 hours old on three consecutive days at peripheral and/or residential sites. Recapture was attempted for resting, host-seeking, and gravid adults for 9 to 11 consecutive days. Mosquito collections were sorted by sex, species, and mark status, and counted. All recaptured marked, and a sample of females unmarked, were dissected to determine parity.

Few *Cx. stigmatosoma* and *Cx. quinquefasciatus* were recaptured by 32 CO_2 traps, 12 gravid traps and 3 walk-in red boxes operated for nine consecutive days at Chino (Table 1). *Culex stigmatosoma* was more dispersive with 5 of 11 recaptured females taken at traps >1 km distant from the release point (max = 4.3 km), than was *Cx. quinquefasciatus* with 2 of 17 females recaptured at traps >1 km from the release site. Although low recapture rates precluded the determination of cohort-specific dispersal, the abundance of unmarked *Cx. stigmatosoma* and *Cx. quinquefasciatus* females was significantly greater at residential (geometric means = 61.8 and 77.9 females/CO_2 trap night, respectively) than at dairy (7.5 and 37.6) collection sites, indicating that host-seeking females may have infiltrated residential areas from dairy breeding sources.

Only 0.3% of the marked *Cx. stigmatosoma* were recaptured on 13 sampling occasions at Rossmoor, as compared to 11.4% of the marked female *Cx. quinquefasciatus* and 19.3% of the marked female *Cs. incidens* (Table 1). Similar to the Chino experiment, 2 of 4 recaptured *Cx. stigmatosoma* were taken at traps >1 km from the point of release, indicating considerable dispersal in both open dairy and residential habitats. Spatially, *Cx. quinquefasciatus* recaptures remained clumped near both the drainage ditch and backyard release sites until host-seeking commenced 3-4 days after release. The mean daily dispersal rate of marked females ranged from 0.6 to 1.0 km/day during days 3 to 9, and these females readily infiltrated a 0.6 km^2 portion of the Rossmoor community. Marked *Cx. quinquefasciatus* females were recaptured at 25 of 27 trap sites (max. recapture distance = 2.4 km). The duration of the initial gonotrophic cycle (time from modal nulliparous to parous female recapture rates) was six days. The daily loss rate (death + emigration) for females 3 to 9 days of age was estimated horizontally by

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curvilinear regression to be 0.213 for females recaptured within a 0.6 km^2 area of the Rossmoor community. The daily vertical loss rate (death) was estimated from the parity rate of unmarked females to be 0.122, indicating that 0.091 (43%) of daily recapture losses were attributable to emigration from the 0.6 km^2 area. Daily female population size was estimated by the Lincoln Index modified to account for the daily losses in marked females, and averaged (±95% confidence limit) 72 918 ± 22 938 females (population density = ca. 121 000 ± 38 000 females/km^2). The abundance of unmarked females at 16 CO_2 and 12 gravid traps operated daily within the study area averaged 95.6 and 22.5 females/trap night, respectively, which indicated that trapping efficiencies (mean number per trap/population size) were 0.13% and 0.07%.

Marked *Cs. incidens* did not disperse readily, since 88% were recaptured at traps within 50 m of the release site, and 39% of all unmarked females were collected by four traps near a drainage channel breeding site. The daily population losses at this site were estimated horizontally from the recapture rate to be 0.302 and vertically from the parity rate to be 0.205. Losses due to emigration were 0.097, 32% of horizontal losses. Population size at the drainage channel site averaged 484 ± 326 females.

The low recapture rate of *Cx. stigmatosoma* at both Chino and Rossmoor was unexpected and was attributed to dispersal prior to or during the initial host-seeking flight. Planned research will compare the dispersal of teneral females collected as larvae/pupae with mature host-seeking females collected by CO_2 traps in both dairy and residential environments. In contrast, the recapture and survivorship rates of *Cx. quinquefasciatus* and *Cs. incidens* at Rossmoor were higher than expected and will be compared to values estimated in other communities in Los Angeles County.

Table 1. Numbers of mosquitoes released and recaptured in Chino, San Bernardino County, and Rossmoor, Orange County, California, 1988.

Species	Released	Recaptured (%)
	F - M	F - M (F - M)
	Chino	
Cx. stigmatosoma	29,922 - 24,567	11 - 1 (0.039 - 0.004)
*Cx. quinquefasciatus**	2,083 - 889	17 - 8 (0.82 - 0.90)
	Rossmoor	
Cx. stigmatosoma	1,171 - 1,179	4 - 3 (0.3 - 0.3)
Cx. quinquefasciatus	8,961 - 11,249	1,016 - 477 (11.4 - 4.2)
Cs. incidens	425 - 443	82 - 30 (19.3 - 6.8)

*Includes adults collected in CO_2 traps and red boxes.

SIZE-SELECTION BY THE TADPOLE SHRIMP, *TRIOPS LONGICAUDATUS* (NOTOSTRACA: TRIOPSIDAE) ON LARVAE OF THE MOSQUITO, *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE)

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ABSTRACT

The tadpole shrimp (TPS), *Triops longicaudatus*, was found to be a size-dependent predator of *Culex quinquefasciatus* larvae in the laboratory. Additionally, differences in TPS size were accompanied by changes in prey-size preference: larger-sized predators (carinal length = 2.2-5.7 mm) consumed an increasing proportion of larger prey items, while very large TPS (carinal length = 9.9 mm) appeared to be non-selective predators of this mosquito species. Quantified behavioral observations indicated that while second instar mosquito larvae were encountered significantly less frequently than were fourth instar larvae or pupae, they were captured at substantially higher rates and with shorter handling times. It is hypothesized that prey vulnerability has an influence on TPS prey size "preference."

Introduction.

In many predator-prey relationships, the body sizes of both the predator and the prey play an important role in dictating the size of prey items consumed (Krebs 1978). Prey vulnerability may be a more important factor of size-dependent predation than is active selection by invertebrate predators. As prey size increases, so does the efficiency of their escape responses. Invertebrate predators, termed size-dependent predators, have been found to prefer intermediate-sized prey items due to capture and handling tradeoffs (Allan et al. 1987; Pastorok 1981).

Tadpole shrimp (Notostraca: Triopsidae) are inhabitants of transient fresh waters worldwide (Longhurst 1956). According to the classification scheme of Greene (1985), tadpole shrimp (TPS) are cruising raptorial predators. *Triops longicaudatus* LeConte is polyphagous and feeds upon invertebrates and plant material in both daylight and darkness (Scott and Grigarick 1979). These investigators noted that early instar mosquito larvae were consumed in the laboratory. A more detailed study in the laboratory showed this species to be a predator of *Culex quinquefasciatus* Say larvae (Tietze 1987), and it is currently being considered as a biological control agent against mosquitoes. This investigation on the feeding rates of two TPS sizes on mosquito larvae demonstrated that smaller TPS (mean body length = 1.6 cm) consumed first and second instar mosquito larvae at higher rates than for the third and fourth instars, while larger TPS (mean body length = 2.4 cm) consumed all mosquito instars at nearly equal rates (Tietze 1987).

Unlike floodwater mosquitoes (i.e. genera *Aedes* and *Psorophora*), stagnant water mosquitoes in genera *Culex* and *Anopheles* may oviposit during the entire flooding period, thereby producing a heterogeneous assemblage of larval instars and pupae. The ability of growing TPS to feed upon various mosquito instars may establish their effectiveness as biological control agents of stagnant water mosquitoes. The goal of this study was to determine from laboratory experiments whether TPS showed significant instar preference for stagnant water mosquito larvae and pupae, and if so, to determine if these preferences changed as the TPS increased in size. The issue of whether active selection or vulnerability was involved in this predation scenario was addressed through limited behavioral observations.

Materials and Methods.

Tadpole shrimp (*T. longicaudatus*) were reared from eggs in fiberglass microcosms at the UCR Aquatic and Vector Control Research Facility in Riverside. The microcosms were 0.84 m^2 in

area and equipped with float valves to maintain the proper water depth (30 cm). Five microcosms were flooded at different times over a five day period to produce a range of TPS sizes. Larval and pupal stage mosquitoes of the species *Cx. quinquefasciatus* were acquired from a laboratory colony maintained at UCR.

Predator and prey sizes were measured using a dissecting microscope fitted with a calibrated ocular micrometer. TPS size was determined by measuring the length of the carinal suture that divides the carapace along the longitudinal body axis (Linder 1959). Immature mosquito size was measured from the apex of the head capsule to the base of the air siphon for larvae, and from the anterior of the head to the paddles for pupae.

Preference tests were performed in a Gibson environmental chamber. Temperature was maintained at 26 ± 2° C and measured using a continuous hygrothermograph. Illumination within the chamber was provided by one 60 watt cool white fluorescent bulb. Arenas were plastic containers filled with 200 ml of well water at a water depth of 4 cm. Both the TPS and immature mosquitoes were acclimated to the test chambers for one hour prior to treatment. Ten representative mosquitoes of each larval instar, and the pupal stage, were preserved in 70% EtOH. Their body lengths were measured later. Preference tests consisted of exposing individual TPS in the arena to five of each mosquito larval instar and the pupae (25 total). The prey items were added to the arena simultaneously and the time of addition noted. After one hour of predation each arena was inspected for the number of prey consumed. Six replicates were made for each predator size class. Due to the short period of exposure (one hour) replacement of prey consumed was not implemented.

Prey selection was calculated using Chesson's alpha electivity index (Manly et al. 1972; Chesson 1983). Chesson's alpha can be used for calculating prey selection in constant or changing prey densities (Chesson 1978). Alpha was calculated for each prey size class in each arena and each predator size group. An ANOVA (SAS Institute Inc. 1982) was performed to test the null hypothesis: there were no significant differences in selection between predator size groups. Separate ANOVAs and Duncan's Multiple Range Tests (SAS Institute Inc. 1982) were performed to determine whether significant differences exist for prey size preferences within each predator size group.

Results.

Prey selection. Significant differences ($p<0.05$) in prey size preference (a) were found for first, third, and fourth instar larvae, and pupae when comparing six predator (TPS) size classes; preference for second instar larvae was not significantly different ($p>0.05$) between predator size classes.

Prey size preferences of TPS changed dramatically with their growth. The smallest TPS size group (2.2 mm) preferred first instar larvae significantly more than second instar larvae, and did not consume larger instars or pupae. The second predator size group (3.0 mm) fed upon first and second instar larvae without a significant difference ($p>0.05$) in preference. Members of all prey size classes were taken when the TPS had carapace lengths of 4.0 mm or greater. Medium-sized TPS (4.1 mm) showed significant preference for earlier instar larvae, but did feed upon third or fourth instar larvae and pupae. The larger-sized TPS (4.70 mm) showed distinct preferences between all prey size classes other than fourth instar larvae and pupae. TPS with 5.75 mm carapace length exhibited similar preferences for third and fourth instar larvae, and fourth instar larvae and pupae, but registered a significantly greater preference for third instar larvae over pupae. Finally, while the largest TPS (9.9 mm) exhibited no marked difference in preference between first, third, and fourth instar larvae, and pupae, there were notable differences between consumption of these and second instar larvae.

Behavioral observations. Video-recorded predation tests showed that the capture success rate of second instar *Cx. quinquefasciatus* was significantly greater than that displayed for fourth instar larvae or pupae. TPS (7.0 mm) capture success rate on second instar larvae was five times greater in magnitude than on fourth instar larvae, and six times greater than that of the pupae. Similarly, handling time of second instar mosquito larvae was significantly less than that of fourth instar larvae and the pupae. A comparison of the numbers of encounters between the TPS and the three prey items showed fourth instar larvae and pupae had significantly

more encounters than did second instar mosquito larvae.

Discussion.

Tadpole shrimp were found to be size-dependent predators of mosquito larvae, and generally displayed decreasing preferences for increasing larval size. As TPS increased in size, their relative preference for larger prey sizes increased. Distinct prey-size preferences were evident if TPS were separated into arbitrary size groups (i.e. small, medium and large). Small TPS consumed early instar (first and second) mosquito larvae, while medium-sized TPS consumed all available instars but preferred early instar larvae. As the predators grew larger, the relative preference for larger prey items increased. The largest TPS size group appeared to feed upon most prey sizes in a non-selective manner (i.e. the proportion of the prey sizes consumed was equal to that in the environment).

TPS had an overall preference for second instar mosquito larvae. This preference did not change notably with growth of the TPS, and the largest predator group did show greater preference for these than for first, third, and fourth instar larvae, and pupae. Behavioral analysis gave similar results; high capture success and short handling times were registered for second instar larvae. Second instar larvae may be the most vulnerable mosquito larval instar to TPS predation.

Alteration of prey body shape is known to influence predator capture and handling success (Kerfoot 1975 and 1977). Mosquito pupation produces a drastic change in body shape; the linear fourth instar larva evolves to a smaller, round pupa. The latter stage was the least vulnerable to predation and was generally rated the least "preferred" by TPS. Behavioral analysis indicated slightly lower capture success and greater handling times for mosquito pupae than for fourth instar larvae.

While this study was not a definitive test of whether apparent prey size preference was due to active predatory selection or vulnerability of the prey, behavioral observations tended to support the latter hypothesis. The behavioral analysis showed that although the number of encounters between TPS and second instar larvae was fewer in frequency than with fourth instar larvae and pupae, capture success of second instars was far greater. Since second instar larvae were observed (but not quantified) on the video-taped predation sequences to consistently display weaker escape responses (in velocity and distance) when encountering a predator, they might have been more vulnerable to being captured. These observations suggest that vulnerability might have an important influence on size-dependent predation. However, lower encounter rates of second instar larvae may have been in part due to their smaller size, swimming behavior, and position in the water column.

Prey size selection is an important factor to consider when evaluating new biological control agents. The effective level of control may only be realized when there are appropriate relative sizes of predator and prey. For example, in this study we found that smaller TPS (carapace length <4.0 mm) did not consume third or fourth instar *Cx. quinquefasciatus* larvae. In the field, some stagnant water mosquito larvae produced from ovipositions during the first days after flooding may escape predation by becoming third and fourth instars before the TPS have grown to 4.0 mm in carapace length (pers. obs.). Similar situations may occur where prey growth rates are very high, as in the floodwater mosquito, *Psorophora columbiae* (Dyar and Knab), whose eggs hatch immediately upon hydration. If these mosquitoes become third instar larvae before the TPS are large enough to capture and handle them, one would expect them to escape TPS predation.

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COMPARISON OF NEW JERSEY LIGHT TRAPS AND CO_2-BAITED TRAPS IN URBAN AND RURAL AREAS

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Introduction.

New Jersey light traps (NJLT) have been a key element in evaluation of mosquito control programs and in California's Encephalitis Virus Surveillance Program since the 1950s. Studies throughout the Central Valley and in other areas of the United States have shown that, while high NJLT indices for vector species did not necessarily mean that virus activity would occur, western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses were rarely detected when the indices were low. In Kern County, there was a strong positive correlation between virus infection rates in *Culex tarsalis* Coquillett, sero-conversions in sentinel chickens, and NJLT indices for *Cx. tarsalis* (Reeves 1968).

After several years of research on the ecology of WEE and SLE viruses in the Los Angeles metropolitan area, it became apparent that virus activity was occurring despite very low NJLT indices for *Culex* mosquitoes. For example, in 1988, 58% of the sentinel chickens at Sepulveda Basin became SLE antibody positive, yet the peak NJLT index at that site was only 2.1 *Cx. tarsalis* per trap night, and the average for April through October was 0.5 per trap night. We began to suspect that the NJLTs did not sample the vector populations efficiently, perhaps due to interference or competition from indigenous light sources -- both ground level lights and the overall "urban glow."

Sacramento Valley studies.

The results of a study done in cooperation with the Sutter-Yuba Mosquito Abatement District which compared collections of *Cx. tarsalis, Aedes melanimon* Dyar, and *Anopheles freeborni* Aitken in NJLTs with those in CDC traps baited with CO_2 (dry ice) and operated with lights (Milby et al. 1978) were presented 11 years ago. We found that in rural areas the CO_2 traps collected about 3 times as many *Cx. tarsalis* and *Ae. melanimon* as the NJ traps, and that in urban areas the mean ratio for *Cx. tarsalis* was as high as 10-20 times more females in the CO_2 traps. We attributed this difference to distance from known breeding sites. For *An. freeborni* the ratios were inverted, with NJLT collecting 2-3 times as many females as CO_2 traps.

A re-examination of more recent data (1986) from the Sutter-Yuba MAD at rural sites similar to those used in 1977 showed somewhat lower mean ratios of CO_2 trap to NJLT indices than before (Table 1). This difference probably can be explained by the fact that several CO_2 traps were operated weekly at each site, and the distances between the CO_2 traps and the NJ trap were often much greater than in the earlier study. The two sites where NJLTs collected more *Cx. tarsalis* than CO_2 traps (Staas and James) frequently were very windy. However, at each of the eight sites, there was a statistically significant correlation between NJ and CO_2 trap indices over time.

The average numbers per trap night shown in Tables 1-4 are back-transformed Williams' means, which were used in order to dampen the influence of occasional very high or very low weekly indices. The mean ratios are the means of the weekly ratios, where 1 was added to each value to handle cases where the denominator (the NJ trap index except for *An. freeborni*) was zero, which often happens in urban NJLT data.

For *An. freeborni*, the mean ratios of NJLT to CO_2 trap collections also were lower than in the previous study (Table 1). However, the two indices were significantly correlated over time at only four of the eight locations. An example of good correlation between the two measures can be seen in Figure 1 which compares weekly indices for *Cx. tarsalis* and *An. freeborni* in the NJLT and two CO_2 traps at a single location.

Los Angeles Basin.

Comparisons were made for NJLT and CO_2 trap data from four sites in Los Angeles and Orange Counties (Table 2). The weekly indices for

Table 1. Females per trap night, Sutter-Yuba M.A.D., 1986

	Culex tarsalis			*Anopheles freeborni*		
Location	New Jersey traps	CO_2 traps with lights	Mean ratio CO_2+1:NJ+1	New Jersey traps	CO_2 traps with lights	Mean ratio NJ+1:CO_2+1
Barker	16.4	66.1	4.6	3.8	5.5	1.0
Guisti	18.1	52.0	4.0	42.5	6.2	1.0
P. Staas	21.8	9.0	0.6	16.5	1.9	0.9
Gollenbusch	32.4	39.0	1.6	6.5	0.4	0.7
Bennett	49.2	52.9	1.8	141.0	6.1	1.8
James	60.6	21.8	0.9	71.3	4.0	1.1
Sheppard	64.0	59.0	1.5	115.6	16.0	3.2
Dean	104.7	90.2	1.2	224.0	15.8	1.7

Table 2. Females per trap night, Los Angeles and Orange Counties, 1988

	Culex tarsalis			*Culex quinquefasciatus*		
Location	New Jersey traps	CO_2 traps with lights	Mean ratio CO_2+1:NJ+1	New Jersey traps	CO_2 traps with lights	Mean ratio CO_2+1:NJ+1
Harbor Lake	0.1	12.5	15.4	0.2	21.2	28.1
Fullerton	0.2	1.4	2.6	0.3	4.1	5.2
Sepulveda Basin	0.5	3.8	4.2	0.8	14.8	11.1
San Joaquin Marsh	7.7	26.9	5.4	1.0	43.7	38.4

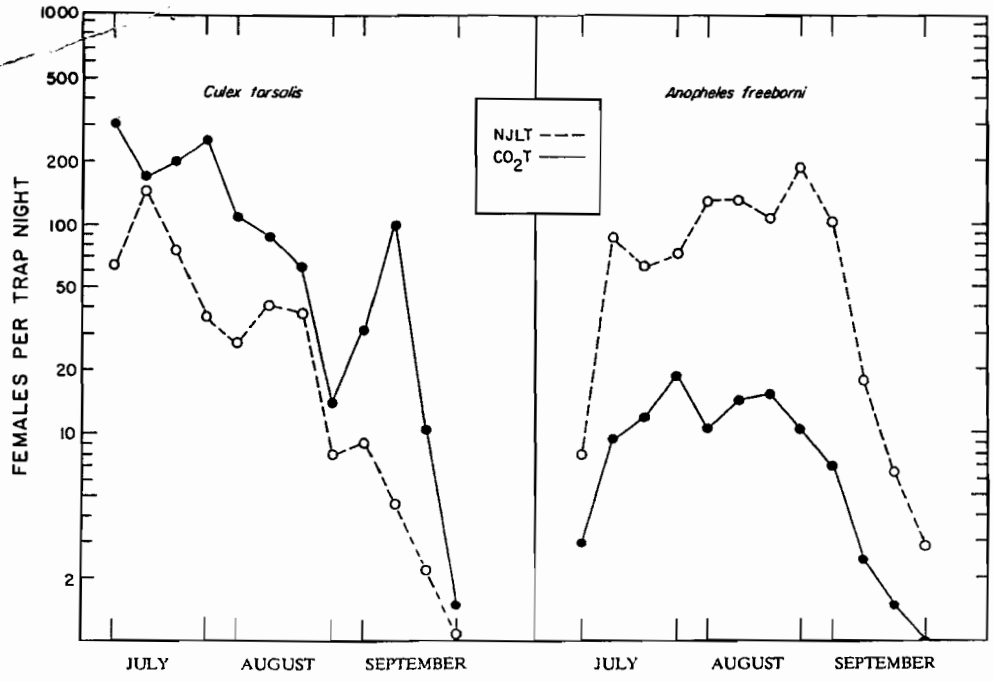

Figure 1. Female mosquitoes per New Jersey and CO_2 trap night, Guisti, Sutter County, 1986.

the two types of traps were significantly correlated over time only at the San Joaquin Marsh site, which is the most rural of the four studied. The number of CO_2 traps operated at each site varied from week to week, and so the geographic area sampled also varied. This could explain not only the large discrepancy for *Cx. tarsalis* at Harbor Lake, but also the lack of correlation between the two measures of abundance.

The mean ratios for *Cx. quinquefasciatus* Say (Table 2) were even higher than those for *Cx. tarsalis*, and again the San Joaquin Marsh was the only location where the two indices were correlated. At all four sites, the CO_2 traps were operated with lights. There were no major competing light sources at the San Joaquin Marsh.

Other urban and rural sites.

Comparisons were also made of NJLT indices with those from CO_2 traps operated without lights at six study areas, three in well-lighted urban areas and three in rural locations (Table 3). These data were more consistent than those from the Sacramento Valley or the Los Angeles Basin, as the CO_2 traps were operated weekly at fixed sites in each area. However, correlations between NJ and CO_2 trap indices were significant at only a few sites. For *Cx. tarsalis* these were Palm Desert, an urban location in Riverside County, John Dale Ranch in Kern County, and Adohr Farms in the Coachella Valley. Both rural sites had little or no indigenous lighting.

Collections of *Cx. quinquefasciatus* were correlated significantly only at Palm Desert and Briano Brothers Dairy near Chino. At Palo Verde, the NJ trap is located near a very bright mercury vapor light and collects very few mosquitoes of either species.

Trap indices for *Psorophora* species at Palo Verde were significantly correlated, with NJ traps collecting approximately the same number of females of this species as CO_2 traps during June through October.

Culex stigmatosoma Dyar (formerly *Culex peus* Speiser) was seldom collected by either type of trap at the locations where both were operated (Table 4). Of the sites where females of this species were collected by both NJ and CO_2 traps, the

numbers per trap night were correlated only at Fullerton and Briano. We continue to seek more effective ways to sample populations of this species.

The influence of moonlight on light trap collections has been documented by several authors (Barr et al. 1960, Bidlingmayer 1967, 1974, Pratt 1948), but we were unable to demonstrate a significant moonlight effect using the data for any of the above species at any sites including rural areas with no competitive domestic lights.

Comparison of CDC traps baited with CO_2 run with or without lights.

In 1978, during a mark-release-recapture study at Poso West near Bakersfield (Milby et al. 1980), we compared the effectiveness of CDC traps baited with CO_2 and operated without lights with that of those run with lights. This is a remote area with no artificial lighting at night. This site was chosen for pilot releases of genetically altered *Cx. tarsalis* because it supported a large natural population that was almost exclusively *Cx. tarsalis*, especially in early summer. Consequently, we have trap comparison data only for *Cx. tarsalis*.

We ran 12 traps (numbers 16 and 1 through 11) each night for 12 days (June 13 through June 24), in a north-south line down a canyon (Nelson et al. 1978). Some portions of the canyon contained more breeding sites and thus supported larger populations of mosquitoes than others, so we used a stratified sampling scheme. The 12 traps were divided into contiguous groups of four; within each

Table 3. Females per trap night, 1988

	Culex tarsalis			*Culex quinquefasciatus*		
Location	New Jersey traps	CO_2 traps without lights	Mean ratio CO_2+1:NJ+1	New Jersey traps	CO_2 traps without lights	Mean ratio CO_2+1:NJ+1
Urban sites						
Smith[1]	0	0.2	-	0.06	3.7	11.5
Palm Desert[2]	0.2	4.9	9.4	0.3	68.4	107.9
Palo Verde[3]	0.2	48.8	154.4	0.05	0.8	3.1
Rural sites						
Briano[1]	0.01	0.4	2.0	0.6	14.2	39.4
John Dale[4]	2.1	22.0	15.2	0.7	8.3	9.8
Adohr Farms[2]	16.3	201.9	22.6	0.3	0.4	1.7

1. San Bernardino Co. 2. Riverside Co. 3. Imperial Co. 4. Kern Co.

group of four each night, two traps were operated with lights and two without. This was determined on the spot each night by tossing a coin. The first two heads in each group of four were run with lights, tails were run without.

The trap counts were analyzed by performing an analysis of variance (ANOVA) on the natural logarithms of the actual numbers of mosquitoes collected. This transformation reduced the variance and normalized the data.

In June, the study area had a very large population of *Cx. tarsalis* (Milby et al. 1980). The collections of females in the traps operated without lights ranged from 126 to 1541 per trap night; in those with lights the mean numbers were 324 to 1772 per trap night. There was significant variation between trap sites within the canyon, but the most important and significant source of variation was the presence or absence of a light. The number of females from traps with lights was generally two times that from traps without lights, regardless of whether the trap site was highly productive or had few females. To put this into statistical language, the two-way interaction between trap site and type of trap was not a significant source of variation in the ANOVA.

Table 4. *Culex stigmatosoma* (formerly *Cx. peus*) females per trap night, 1988

Location	New Jersey traps	CO_2 traps	Mean ratio CO_2+1:NJ+1
Urban sites			
Indio[1]	.005	.006	1.5
Harbor Lake[2]	.013	1.8*	4.3
Palm Desert[1]	.028	.046	1.1
Smith[3]	.16	3.3	7.6
Fullerton[4]	.47	3.0*	4.3
Sepulveda Basin[2]	.89	.42*	0.9
Rural sites			
San Joaquin Marsh[4]	.30	.94*	1.7
Briano[3]	.42	1.4	2.4

* Operated with lights; other locations operated without lights.

1. Riverside Co. 2. Los Angeles Co. 3. San Bernardino Co. 4. Orange Co.

For males, the difference was quite dramatic, since traps operated without lights collected almost no males at all, while those operated with lights averaged 48 males per trap night.

The collections from traps without lights are very "clean," i.e., have almost no moths or midges to slow down processing and tear up the mosquitoes. Thus, to collect female *Cx. tarsalis*, it is probably more efficient to run twice as many traps without lights than to run half as many traps with lights.

Conclusions.

It seems reasonable to suggest that CO_2 traps be considered as a replacement for ineffective NJLTs as a surveillance tool in highly urbanized areas. On the pro side, they collect more *Culex* mosquitoes, especially in areas where there is abundant light from other sources, and do not need to be operated at sites adjacent to an electrical outlet. Collection of a larger sample of a population increases the sensitivity of the monitoring system for measuring population changes. The con side, of course, is that they are more labor intensive since they must be set out just before dusk and picked up just after dawn the following morning, they rarely attract males of any species, which sacrifices information important in locating breeding sources or in some biological studies, and there is an increased cost as CO_2 bait is expensive and unavailable in some areas.

If CO_2 traps are to replace NJLTs for mosquito surveillance in urban areas, careful attention must be given to standardization of both the number of traps operated and their placement in an area. It is probably critical to establish a ratio of NJLT to CO_2 trap indices at a site for several years before the change is made. The Sacramento Valley studies indicate that CO_2 traps are less effective for collecting *An. freeborni*, and this could be important in studies of this species as a pest or in malaria studies.

A controlled evaluation should be made to compare further the effectiveness of CO_2 traps with and without lights in urban areas. The advantage of using traps with only CO_2 as an attractant is that this would at least dampen and might eliminate the effect of competitive lights. The disadvantage would be that it would limit collections to host-seeking females and exclude males which would decrease the sensitivity and value of the sampling.

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THE INFLUENCE OF VEGETATION ON CO_2 TRAP EFFECTIVENESS[1]

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ABSTRACT

The influence of vegetation on CO_2 trap effectiveness for sampling host-seeking female *Culex tarsalis*, *Culex quinquefasciatus*, and *Aedes nigromaculis*, was investigated at a foothill riparian habitat on the eastern side of the San Joaquin Valley in Kern County, California.

Traps were spaced along a linear transect perpendicular to Poso Creek to accommodate sampling mosquitoes in five discrete microhabitats: 1) open hilltop devoid of vegetative cover (2 traps); 2) open pasture with a sparse cover of dried grasses and saltbush (*Atriplex* sp.) (2 traps); 3) peripheral understory of mule fat (*Baccharis viminea*) on the outer north and south margins of Poso Creek (3 traps); 4) shaded understory of mule fat beneath the canopy of cottonwood (*Populis* sp.) and willow (*Salix* sp.) trees (2 traps); and 5) open canopy 5 m above ground level within the cottonwood and willow growth (3 traps). The traps were baited with 2.5 kg of dry ice and operated for three consecutive nights at mid-month from June through October. Collections were sorted to species and sex, and counted. The mean number of females [transformed by LN (y + 1)] collected per trap night per microhabitat was compared by ANOVA and Duncan's multiple range test.

Most host-seeking females of *Cx. tarsalis* and *Cx. quinquefasciatus* were collected by traps posi-

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Table 1. Mean (Mw) number of females collected per CO_2 trap night in each microhabitat at Poso Creek, 1988.

Microhabitat	Mw females collected per trap night *		
	Culex tarsalis	*Culex quinquefasciatus*	*Aedes nigromaculis*
Hill top	27.4 a **	1.4 a	9.2
Open pasture	56.3 a	0.7 a	25.8
Shaded understory	85.6 ab	2.8 a	4.2
Peripheral understory	160.8 c	2.9 ab	23.4
Open canopy	155.2 c	9.6 b	0.8

**Culex tarsalis* and *Culex quinquefasciatus* sampled on 15 trap nights and *Aedes nigromaculis* on 3 trap nights.

***Means followed by the same letter are not significantly different in a Duncan's multiple range test ($P>0.05$).

tioned within the peripheral understory (*Cx. tarsalis* only) and open canopy (Table 1). The mean (Mw) number of female *Cx. tarsalis* collected per trap night was not significantly different between the open canopy and peripheral understory. However, these means were significantly higher than the mean number of females collected by traps operated in the shaded understory, open pasture, and open hill top microhabitats. Although *Cx. quinquefasciatus* was 15-fold less abundant than *Cx. tarsalis*, females were sufficiently numerous to demonstrate that traps operated in the open canopy collected significantly more females than traps operated in the remaining microhabitats (Table 1). The mean numbers of females collected per trap night in the peripheral and shaded understory were nearly identical. Traps positioned on open hill tops and in the open pasture collected relatively few females. Based upon microhabitat affinities exhibited by host-seeking females collected by CO_2 traps, sampling of *Cx. tarsalis* and *Cx. quinquefasciatus* would be most effective if the traps were operated 5 m above ground level within the open canopy of trees, or near ground level along corridors of vegetation.

Intermittent flooding of a pasture in mid-September produced a relatively large hatch of *Aedes nigromaculis*. Preliminary data indicated that females of this species engaged in host-seeking activity in the open pasture and along the peripheral understory of vegetation, but less frequently at the open hill tops, in the shaded understory, or in the open tree canopy (Table 1).

ATTRACTION OF FLORIDA MOSQUITOES (DIPTERA: CULICIDAE) TO ARTIFICIAL LIGHT IN THE FIELD

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ABSTRACT

Six New Jersey light traps were employed in the field to assess attractiveness of adult mosquitoes to six incandescent light sources of different colors and wattages (intensities). White, yellow, orange, blue, green, and red lamps were used. White incandescent and fluorescent lamps were also compared. Seventeen species of mosquitoes were collected in three separate tests using lamps of different intensity and color combinations. *Culex* spp. [*Cx. salinarius*, *Cx. nigripalpus*, and *Cx.* (Mel.) *erraticus*] and *Psorophora* spp. [*Ps. columbiae* and *Ps. ciliata*] were the most abundant mosquitoes. Color ($F=5.14$; 7 and 133 df; $P<0.001$) was much more important than intensity ($F=0.40$; 1 and 133 df; $P>0.999$), with blue light, in general, attracting more adults than the other colors, even though it had the lowest intensity. There were also significant effects of gender ($F=9.86$; 9 and 133 df; $P<0.001$) and genus ($F=2.15$; 38 and 133 df; $P<0.001$) upon the numbers captured by the different colors. Fluorescent light attracted more mosquitoes than incandescent white light. The results suggest that light traps should be equipped with blue incandescent or fluorescent lamps for better collection of the mosquitoes.

Introduction.

Previously, several laboratory and field studies had been conducted on the responsive behavior (primarily host-seeking) of adult mosquitoes to colored visual stimuli; a majority of these studies involved *Aedes* spp. (Brown 1951, 1954, Sipple and Brown 1953, Wood and Wright 1968, and others). Relatively few studies were undertaken on the non-responsive behavior of mosquitoes, as indicated by their catches in traps equipped with various sources (color and intensity) of light (Headlee 1937, Breyev 1963, Jiqun et al. 1984). Ali et al. (1986) reported on the attraction of adult chironomid midges to colored lamps in the field. A considerable number of adult mosquitoes was also trapped in that study. The attraction of adult mosquitoes to the lamps used in various combinations (color and wattage) in the separate tests conducted in central Florida is reported here. Such behavioral information on mosquitoes is useful for their efficient trapping and collection.

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Materials and Methods.

The tests were conducted during the summer of 1984 at the northwest shore of Lake Jessup in Seminole County, Florida. Six New Jersey light traps equipped with suction mechanism (Mulhern 1942) were used and were spaced 20 m apart in a row. Each trap, hung from a pole, was placed ca. 2 m above ground level. The experimental design and the study area were previously described (Ali et al. 1986). A portable 3000-W generator (Dayton, Inc., Dayton, OH) supplied power to lamps fitted in the traps. The lamps used were commonly available incandescent lamps, also known as general purpose A-line lamps (Anonymous 1978). In test 1, all 100-W lamps of white (WL), yellow (YL), orange (OL), blue (BL), green (GL), and red (RL) were used. In test 2, WL, YL, OL, BL, GL, and RL lamps of 25-, 40-, 40-, 100-, 100-, and 100-W, respectively, were employed. In test 3, three 60-W WL and three 60-W warm white fluorescent (FL) lamps (Circlite 60) were compared. All WL-lamps were frosted (inside) while all colored lamps were enameled.

The color lamps used emitted broad bands of wavelengths in the visible spectrum. The wavelengths emitted by BL are between 430 and 490 nm, GL between 490 and 550 nm, YL between 550

and 590 nm, OL between 590 and 620 nm, and RL between 620 and 770 nm (Anonymous 1978, Hollingsworth 1961). The radiant energy emitted by WL and FL was in all visible wavelengths of 390 to 770 nm; the FL light peaks at ca. 180-190 nm, which are shorter than the peak wavelengths of the WL (Anonymous 1978).

The relative brightness of each lamp was measured in a photography dark room using a LI- COR light meter (Li-Cor, Inc., Lincoln, NB) equipped with a photometric sensor (LI-210SB) (Ali et al. 1986). The measurements of light intensity for 100-W WL, YL, OL, BL, GL, and RL were 37.1, 19.7, 11.1, 1.2, 1.4, and 2.1 lux, respectively, while those of 25-W WL, 60-W WL, 40-W YL, and 40-W OL were 4.9, 17.1, 5.8, and 3.1 lux, respectively. The intensity reading for the FL was 15.9 lux.

The traps in each test were activated for a period of 2 h, commencing about half an hour before sunset on each sampling occasion. Tests 1 and 2 were repeated on six different occasions so that each lamp in a test occupied a different pole each time. Test 3 was also conducted on six different occasions, and the FL and WL were positioned alternately on each occasion. The adult mosquitoes collected during each sampling period were identified, sexed, and counted. Due to the small numbers collected of some mosquito species, catches were grouped by genus to increase the sample sizes for the analysis of the effects of light color and intensity on catch.

The significance of the qualitative factors, genus (G), sex (S), lamp type (L), color (C), and the quantitative factor, intensity (I), in attracting the adult mosquitoes was elucidated by contingency table analysis using log-linear models with quantitative factors as previously described (Ali et al. 1984, 1986). The analysis differed from that given in Ali et al. (1984, 1986) in that log-linear model parameters were estimated using weighted least squares regression with an adjustment to reduce bias (problem 8.3 III, Dobson 1983). The weighted regressions were fit using SAS (SAS 1985), and the degrees of freedom were adjusted for observations with zero weights.

Tests 1 and 2 were analyzed together to allow the effects of color to be separated from intensity (Ali et al. 1986). Effects of intensity could not be separated from the other aspects of lamp type in the analysis of test 3, but generic and gender preferences could be examined. The hierarchy of terms added to the log-linear model in each analysis is presented in Table 2. Unprotected least significant difference (LSD) tests ($P = 0.05$) were conducted on arcsine-square root transformed catch proportions across lamp types for each gender in each genus in each test to aid in interpreting the significant effects in the above analyses.

Results and Discussion.

Seventeen species of mosquitoes belonging to *Aedes* spp., *Anopheles* spp., *Culex* spp., *Psorophora* spp., and *Uranotaenia* spp., plus *Coquillettidia perturbans* (Walker), and *Mansonia titillans* (Walker) were collected (Table 1). *Culex* spp. formed 48.2, 36.6, and 76.7% of the total mosquitoes taken in tests 1, 2, and 3, respectively. *Culex nigripalpus* Theobald and *Culex salinarius* Coquillett were predominant in test 1, and *Culex* (Mel.) *erraticus* Dyar and Knab formed 7.2% of the total *Culex* spp. One female *Culex* (Mel.) *pilosus* (Dyar and Knab) occurred in test 1 only. In test 2, among *Culex* spp., *Cx. salinarius* (43.5%), *Cx.* (Mel.) *erraticus* (30.1%), and *Cx. nigripalpus* (26.4%), in that order, were abundant, while in test 3, *Cx.* (Mel.) *erraticus* (68.5%) outnumbered *Cx. nigripalpus* (14.7%) and *Cx. salinarius* (16.8%).

Psorophora ciliata (Fabricius) and *Psorophora columbiae* (Dyar and Knab), occurring in all three tests collectively formed 48.2% (test 1), 58.6% (test 2), and 3.4% (test 3) of the total mosquitoes; the latter species was the single most predominant mosquito collected in both tests 1 and 2 (Table 1). *Aedes sollicitans* (Walker) and *Aedes vexans* (Meigen) were the most abundant of the *Aedes* spp. mosquitoes. The total number of *Aedes* spp. collected in tests 2 and 3 amounted to only one and nine adults, respectively. *Aedes* spp., when most abundant, formed 1.7% of the total mosquitoes in test 1. *Anopheles* spp. were also less common, forming 1.3% of the total mosquitoes in test 1, with *Anopheles crucians* Wiedemann being more abundant than *Anopheles quadrimaculatus* Say. The total number of *Anopheles* spp. trapped in each of the tests 2 and 3 were < 12, adults. *Mansonia titillans*, *Cq. perturbans*, *Uranotaenia lowii* Theobald, and *Uranotaenia sapphirina* (Osten Sacken) also occurred in small numbers (Table 1). Proportions of female mosquitoes in all collections were higher than male mosquitoes; the total females comprised 94.2, 92.0, and 62.7% of the total male and female mosquitoes collected in tests 1, 2, and 3, respec-

tively. The relative increase of total males in test 3 (37.3%) was primarily due to *Cx.* (Mel.) *erraticus* (Table 1).

The number of adult mosquitoes attracted to each lamp (color, intensity, or type) in the combinations of lamps used in the three tests is presented in Figure 1 as a percent of total (male and female) adults of the genera, *Aedes*, *Anopheles*, *Culex*, *Psorophora*, and the total mosquitoes collected in each test. The numbers of *Aedes* spp. and *Anopheles* spp. collected in tests 2 and 3 were too small to be included in Figure 1. The light intensity value of each lamp is also shown in Figure 1.

The highest percent of *Aedes* spp. was attracted to BL (35.3%) and the lowest to YL (6.6%) in test 1. *Anopheles* spp. were collected in significantly higher proportions with BL (54.7%), followed by GL (20.9%), while none occurred at RL during the test. There were no significant differences between *Anopheles* spp. catches taken by WL (9.2%), YL (7.6%), and OL (7.6%). *Culex* spp. (comprising >45% each of *Cx. nigripalpus* and *Cx. salinarius*) were taken the most with YL (28.8%), then BL (22.3%), and the least with OL (4.4%). Most *Psorophora* spp. (comprising of >96% *Ps. columbiae*) were caught using BL (32%) and RL (29%), while no significant differences were found between their catches with WL (9.2%), YL (8.2%), OL (7.0%), and GL (14.6%). The highest numbers of adult mosquitoes were captured with BL (29.3%) and the lowest with OL (5.9%) in the combination of different lamps in test 1.

Culex spp. [*Cx. salinarius* 44%, *Cx.* (Mel.) *eraticus* 30%, and *Cx. nigripalpus* 26%] were at-

Table 1.-Species and numbers[a] of adult mosquitoes collected from New Jersey light traps fitted with various (color and wattage) lamps used in three separate tests conducted near a lake in Seminole County, FL (July-October 1984).

Species	Test 1		Test 2		Test 3	
	♂	♀	♂	♀	♂	♀
Aedes infirmatus	0	0	0	0	1	1
Ae. sollicitans	0	28	0	1	0	5
Ae. taeniorhynchus	0	0	0	0	0	1
Ae. triseriatus	0	1	0	0	0	0
Ae. vexans	1	14	0	0	1	0
Anopheles crucians	4	26	0	13	3	6
An. quadrimaculatus	0	3	0	2	0	14
Coquillettidia perturbans	0	0	0	1	0	2
Culex. (Mel.) *erraticus*	3	85	3	62	153	127
Cx. nigripalpus	18	560	0	57	4	56
Cx. (Mel.) *pilosus*	0	1	0	0	0	0
Cx. salinarius	10	549	0	94	28	41
Mansonia titillans	0	10	0	8	2	54
Psorophora ciliata	12	35	0	2	1	2
Ps. columbiae	98	1080	44	300	3	12
Uranotaenia lowii	1	1	0	3	0	3
U. sapphirina	0	1	0	0	3	10
Totals	147	2394	47	543	199	334

[a] Numbers of a species given under each test are the total number of all replicates of the test.

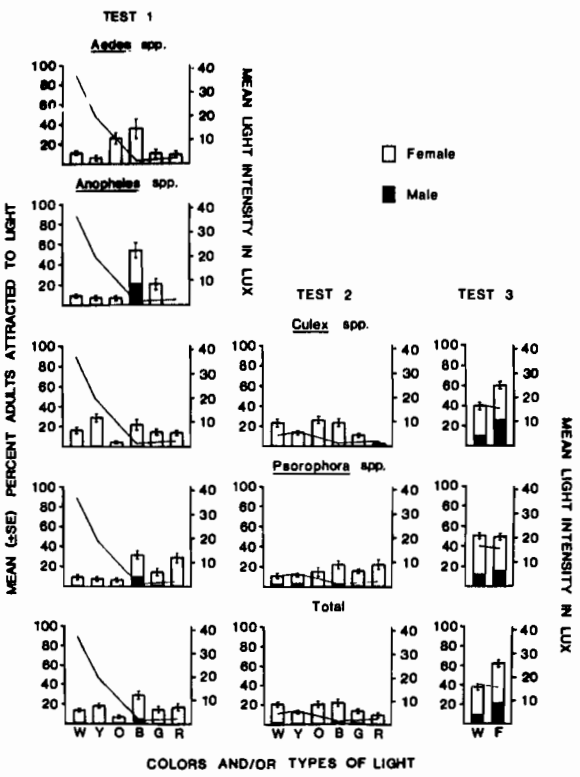

Figure 1.- Attraction of adult mosquitoes in the field to six different color 100-W incandescent lamps (test 1), to six different color incandescent lamps of different wattage (test 2), and to three 60-W white incandescent and three 60-W white fluorescent lamps (test 3). The bars represent the percentage of adults attracted to each lamp, while the line graphs indicate the light intensity of each lamp in a test combination. The W, Y, 0, B, G, and R indicate white, yellow, orange, blue, green, and red incandescent lamps, respectively, while F indicates white fluorescent lamp. In test 2, the lamps were 25-, 40-, 40-, 100-, 100-, and 100-W, respectively.

tracted in almost equal proportions to WL (23%), OL (24%), and BL (23%), but were significantly less numerous at RL (4%) in test 2 (Figure 1). There were no significant differences between *Psorophora* spp. (99% *Ps. columbiae*) attraction to different lamps used in test 2, although BL (21%) caught the most and YL (12%) caught the least. The catches of total mosquitoes taken by different lamps (test 2) were not markedly different.

The statistical comparisons of adult catches by FL and WL in test 3 showed significantly higher catches of *Culex* spp. [*Cx.* (Mel.) *erraticus* 68%, *Cx. salinarius* 17%, and *Cx. nigripalpus* 15%] by FL, while *Psorophora* spp. (83% *Ps. columbiae*) showed no significant preference for either lamp. *Mansonia titillans*, however, was taken in significantly higher numbers at FL, and the trend was the same for total adult mosquitoes taken in test 3.

The results of weighted least-squared error regression analysis for the three tests are presented in Table 2. Intensity ($F=0.40$; 1 and 133 df; $P>0.999$) was not an important factor for lamp preference in the analysis of tests 1 and 2. Terms involving differences in response among the genera ($F=2.15$; 38 and 133 df; $P<0.001$), between the genders ($F=9.86$; 9 and 133 df; $P<0.001$), and among the colors ($F=5.14$; 7 and 133 df; $P<0.001$) were all highly significant. This implies that the pattern of response to the lamp types was different among the genera and genders in at least some of the genera. In general, BL tended to attract the greatest number of adult mosquitoes (particularly *Aedes* and *Anopheles*), while *Psorophora* spp. were as much attracted to RL as to BL, and *Culex* spp. showed a preference to BL over RL.

The analysis of test 3 (Table 2) indicated that FL tended to attract significantly ($F=39.53$; 1 and 46 df; $P<0.001$) more adults than the incandescent WL. The slight preference for FL may have been due to greater emission of light in blue wavelengths by the FL based upon the results of tests 1 and 2.

The response of adult mosquitoes to colored visual stimuli was more towards dark than towards light colors (Howlett 1910, Eckstein 1920); the descending order of preference of colors by *Anopheles maculipennis* Meigen, was red, violet, yellow, and white (Brighenti 1930). Brett (1938) showed that *Aedes aegypti* (L) preferred black, while light-yellowish khaki and yellow were less attractive. However, Magnum and Callahan (1968) showed that *Ae. aegypti* preferred the near-infrared region. Brown (1954) also observed that dark red was the most attractive for *Aedes* spp. mosquitoes, followed by dark blue, light red, and purple. Host-seeking female *Aedes cantator* (Coquillett), *Aedes punctor* (Kirby) and *Cq. perturbans*, generally, were more attracted to low-intensity colors, such as blue, black, and red than to high-intensity colors, such as white and yellow; the strongest response was shown to blue-green (Browne and Bennett 1981). Gilbert and Gouck (1957) found that the darker shades attracted the most *Ae. aegypti*, while the lighter

Table 2.-Weighted least-squared error regression analysis for field studies on adult culicid attraction to artificial light.

Lamp color or type combination	Source of variation[a]	Change in weighted SS	F (df)	% of total SS[b]
		Tests 1 and 2 (3131 adults caught)		
WL, YL, OL, BL, GL, RL	Intensity (I)	5.8	0.4 (1,133)	0.1
	Lamp color (C+CxI)	525.5	5.14** (7,133)	10.6
	Sex (SxI+SxC+SxCxI)	1294.9	9.86** (9,133)	26.1
	Genus (GxI+GxC+GxSxI +GxSxC+GxCxI)	1193.8	2.15** (38,133)	24.1
	Residual	1940.7	---	39.1
		Test 3 (533 adults caught)		
WL,FL	Lamp type (L)	51.9	39.53** (1,46)	31.5
	Sex (SxL)	28.8	10.97** (2,46)	17.5
	Genus (GxL+GxSxL)	23.8	1.51 (12,46)	14.4
	Residual	60.4	---	36.6

** Significant at the 99% confidence level.

[a] Terms added to log-linear model are in parentheses.

[b] % of total weighted sum of squares accounted for by the indicated sources of variation.

shades attracted the most *Aedes taeniorhynchus* (Wiedemann), indicating that different species of the same genus may respond differently to the same visual stimuli. Conversely, Sipple and Brown (1953) showed that certain behavioral responses were shared by a variety of mosquitoes. Thus, comparisons of various studies and generalizations of adult mosquito response to colored visual stimuli should be made with great caution, particularly when the Culicidae contains hundreds of species.

Earlier field observations on the response of flying adult mosquitoes to colored lights had shown that they generally were more attracted to ultraviolet or blue lights (Headlee 1937, Breyev 1963, Jiqun et al. 1984). The results obtained in the present study are compatible with those of Headlee (1937), Breyev (1963), and Jiqun et al. (1984). *Aedes* spp., *Anopheles* spp., *Psorophora* spp., and to some degree *Culex* spp., showed a preference for blue color, although minor preferences to other colors were also shown as already explained.

Results of the present study suggest that in order to collect larger numbers of flying mosquitoes, blue incandescent or, wherever possible, fluorescent lamps should be used in the light traps.

Acknowledgment.

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A LABORATORY EVALUATION OF *LAGENIDIUM GIGANTEUM* IN WATER FROM CONTRA COSTA COUNTY, CALIFORNIA MOSQUITO SOURCES

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ABSTRACT

The oomycetous fungus, *Lagenidium giganteum*, is a promising microbial control agent for mosquito larvae. It has been evaluated in a variety of habitats and has demonstrated the ability to recycle in nature. *L. giganteum* does, however, have some restrictive environmental limitations, such as a low tolerance of organic water pollution and salinity.

Given the environmental constraints of *L. giganteum*, the primary purpose of this study was to determine, through laboratory experiments, in which Contra Costa County habitats the fungus could potentially control mosquito larvae. A second objective was to correlate the efficacy of the fungus with certain water quality parameters.

The efficacy of *L. giganteum* was evaluated in six separate laboratory bioassays over a 3-month period. Water and mosquito larvae were collected from a variety of habitats, such as creeks, marshes, irrigated pastures, artificial containers, and a wild rice field. Water from each source was placed into six plastic cups, and 10 second or third instar larvae from the same source were added to the cups. Three of the cups were inoculated with from 2 to 10 ml of the asexual stage of *L. giganteum*, and three cups without inoculum served as controls. *Lagenidium giganteum* was also evaluated in distilled water during each of the six bioassays. Three days post-inoculation, dead larvae were individually examined under a compound microscope for fungal infection, and the mortality due to infection by *L. giganteum* was calculated. A sample of water from each source was analyzed during the last five bioassays.

The fungus infected 100% of the larvae from the one creek tested and the distilled water during the first bioassay. There was no larval mortality due to the fungus in water collected from the irrigated pasture or marshes.

During the second bioassay, more than 90% of the larvae in water collected from two of the five creeks tested, the wild rice field and the distilled water, were dead and packed with sporangia. There was no larval mortality in water collected from irrigated pastures or marshes. Infection of larvae by *L. giganteum* corresponded to low measurements of turbidity, conductivity, and total dissolved solids. The other water qualities analyzed (pH, hardness, salinity, phosphate concentration, and chemical oxygen demand) did not correspond to *L. giganteum* mortality rates.

No larval mortality due to *L. giganteum* infection was shown in water from any source or in the distilled water during bioassays 3 and 4.

Mortality due to *L. giganteum* was evident in larvae from three of the seven creeks tested, two of the three artificial containers, and the distilled water during the fifth bioassay. The fungus was not effective in water from the one irrigated pasture and marsh tested. Low measurements of water hardness corresponded to high levels of larval mortality due to *L. giganteum*.

During the final bioassay, *L. giganteum* infected larvae from 11 (nine creeks, two artificial containers) of the 14 sources with >90% mortality. There was no larval mortality due to the fungus in water from one artificial container, one creek, or the irrigated pasture. Five water quality parameters (hardness, total dissolved solids, conductivity, chemical oxygen demand, and ammonia nitrogen) were lower from sources with larval mortality due to *L. giganteum* than in water from sources with no fungal infection. There was little correlation between salinity, phosphate concentration, or turbidity and *L. giganteum* mortality rates.

Water quality clearly affects the ability of *L. giganteum* to release zoospores and infect mosquito larvae. Thus the fungus has the potential to control a wide range of mosquito species in creeks, artificial containers, and other sources with relatively clean water. The fungus was not effective in either irrigated pastures or marshes in Contra Costa County.

CHAETOTACTIC ANALYSIS OF TWO SPECIES IN THE *ANOPHELES MACULIPENNIS* COMPLEX IN CALIFORNIA

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ABSTRACT

A chaetotactic key was evaluated to distinguish the immatures of *Anopheles hermsi* and *Anopheles freeborni*, both of the *Anopheles maculipennis* complex (Diptera: *Culicidae)*. The key correctly identified 90% and 91% of the individual larvae and pupae tested, respectively. When an average of individuals from each location was used, the key identified 100% of the pupae correctly.

Introduction.

There are three members of the *Anopheles maculipennis* complex in California: *Anopheles freeborni* Aitken, *Anopheles occidentalis* Dyar and Knab, and *Anopheles hermsi* Barr and Guptavanij. *Anopheles freeborni* has long been recognized as an important vector of malaria in California (Moore 1945). *Anopheles hermsi* was implicated recently in malaria transmission in San Diego County (Turley et al. 1986, Cope 1989), and *An. occidentalis* is limited to the coastal regions and only rarely takes human blood meals (Bohart and Washino 1978).

Adults of *An. hermsi* and *An. freeborni* are morphologically identical. The only ways known to distinguish these two sibling species is by differences in the ribosomal RNA (personal communication, Dr. Frank Collins, CDC), or by cross breeding with known reference colonies and looking for sterility in the backcross of the F_1 generation.

J.N. Belkin and W.A. McDonald devised a chaetotaxic key in the 1950s to differentiate the immatures of these two species. It was the purpose of this study to measure the ability of this unpublished key to differentiate a large sample of these two species from a wide geographic area.

Materials and Methods.

Field collections of immature stages were made throughout southern California during the summer months of 1987 and 1988. Larvae were reared in the laboratory at room temperature in pans containing tap water. Pupae were transferred to one gallon adult rearing cages. Mated females were offered a human blood meal. The chromosomes of the ovarian nurse cells were examined by the methods of Menchaca (1986) and Morrison (1985).

After identification to species, the colony was fed again and 100-200 first instar larvae were reared in pans. Early fourth instar larvae were killed in hot water and transferred to alcohol. Newly emerged pupae were placed in wax paper cups. Soon after each adult emerged its exuviae were transferred to alcohol. Permanent slides were prepared using balsam.

The total number of branches of each hair that was examined was counted bilaterally; if one hair was missing the number of branches on the remaining hair was doubled. For larvae, the branches of ventral hair #13 on the third segment, and dorsal hair #2 (the antipalmate hair) on the fourth, fifth, and sixth segments were summed to give a larval index. For pupae, the branches of hairs #2 and #5 on the third segment, and hair #2 on the sixth segment were summed to give a pupal index. Slides were read blindly as each colony was assigned a number before analysis.

Results.

Collections identified as *An. hermsi* were made from 32 locations extending south into northern Baja California in Mexico, north to San Luis Obispo, and east to Lake Hemet in Riverside County. *Anopheles freeborni* were collected from 11 locations in the San Joaquin and Owens Valleys. (Figure l).

A total of 369 *An. hermsi* and 164 *An. freeborni* larvae was analyzed. A comparison of the distribution of the larval index for each species can be seen in Figure 2. The mean larval index for *An. hermsi* was 26.27 with a range of 15-41 and a standard deviation of 3.46. The mean larval index for

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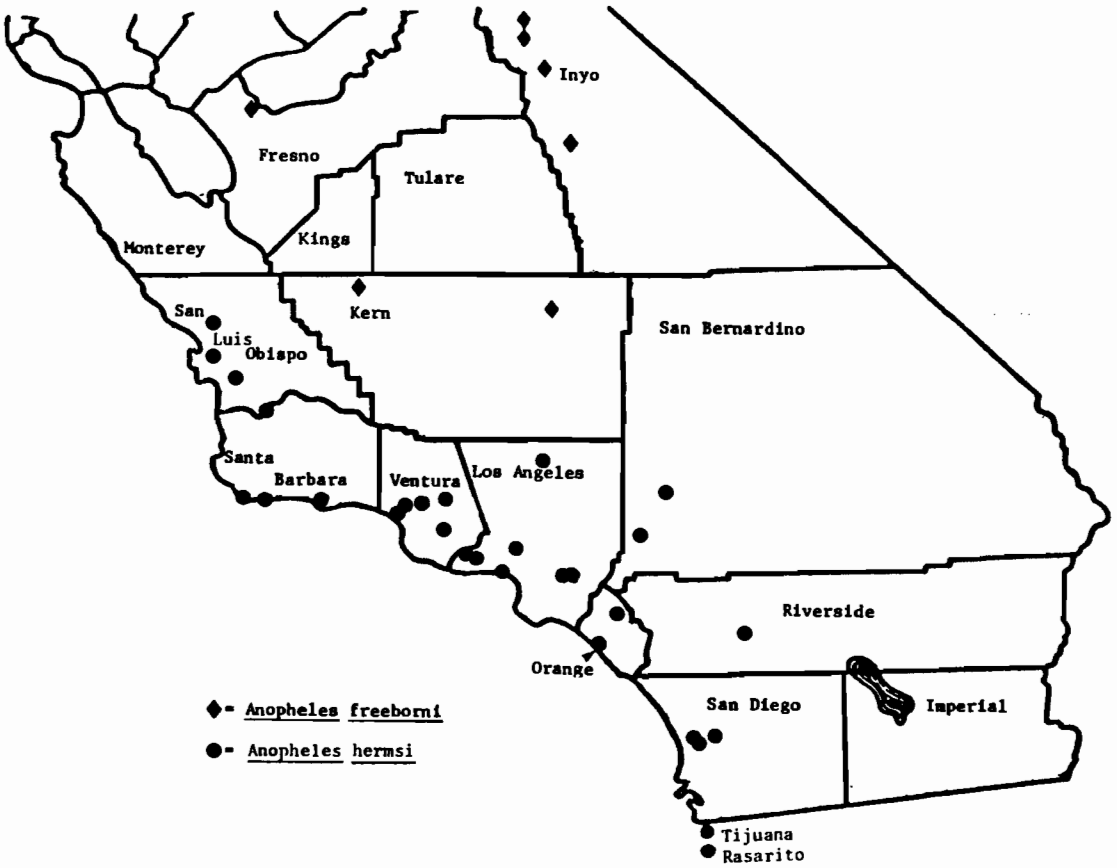

Figure 1.-Collection localities - *Anopheles maculipennis* complex in southern California - UCLA *Anopheles* survey, 1987-88.

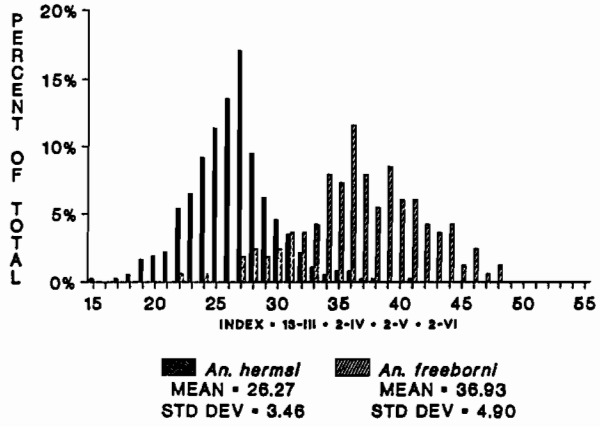

Figure 2.-Distribution of Larval Index.

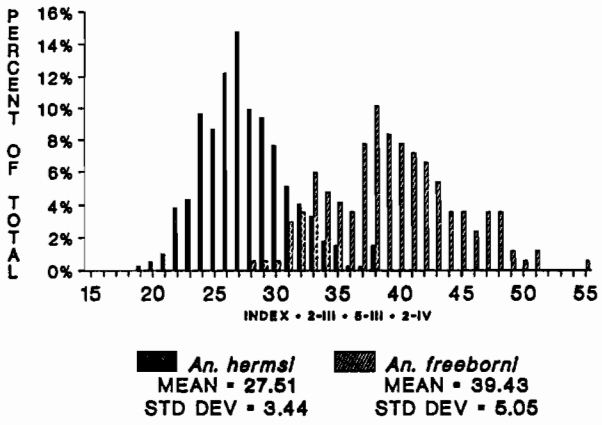

Figure 3.-Distribution of Pupal Index.

An. freeborni was 36.93 with a range of 22-48 and a standard deviation of 4.90. The difference of the means is 10.66 and the p value from a t-test is $p < 0.00001$.

Pupal indices of 393 *An. hermsi* pupae from 31 collections and 168 *An. freeborni* pupae from10 collections are shown in Figure 3. The mean pupal index for *An. hermsi* is 27.51 with a range of 19-38 and a standard deviation of 3.44. The mean pupal index for *An. freeborni* is 39.43 with a range of 28-51 and a standard deviation of 5.05. The difference of the pupal means is 11.92 and the p value from a t-test is $p < 0.00001$.

The key was tested for differentiating both the larvae and pupae. For pupae (Table l) the cut-off number suggested by Belkin and McDonald (less than 33 for *An. hermsi* and 33 or greater for *An. freeborni*) was used. This correctly identified 91.3% of the *An. hermsi* and 91.7% of the *An. freeborni* individuals. The key was used to differentiate the populations by the mean pupal index from each collection location. It correctly identified 100% of both the *An. hermsi* and *An. freeborni* populations.

The cut-off to distinguish larvae using the larval index (Table 2) suggested by Belkin and McDonald was less than 28 for *An. hermsi* and greater than or equal to 28 for *An. freeborni*. This identified 69.9% of the individuals and 87.4% of the populations of *An hermsi*, and 97.0% of the individuals and 100% of the populations of *An. freeborni*. The sensitivity of the key to identify *An. hermsi* can be increased without great detriment to the sensitivity for *An. freeborni* when the cut-off is moved to less than 31 for *An. hermsi*, and 31 or greater for *An. freeborni*. This new cut-off correctly identified 90.2% of both *An. hermsi* and *An. freeborni* individuals and 96.9% and 90.9% of the *An. hermsi* and *An. freeborni* populations, respectively.

Discussion.

All of the *An. hermsi* collections analyzed in this study were from the southern third of the State, from San Luis Obispo County south. There was no north-south or county-by-county trend in the data for either *An. hermsi* or *An. freeborni*.

The large statistical difference in both the lar-

Table 1.-Percent of pupae correctly identified.

	Anopheles hermsi	*Anopheles freeborni*
INDIVIDUAL PUPAE		
	< 33 91.3% (359/393)	≥ 33 91.7% (154/168)
PUPAE BY COLLECTION LOCATION		
	< 33 100.0% (31/31)	≥ 33 100.0% (10/10)

Table 2.-Percent of larvae correctly identified.

	Anopheles hermsi	*Anopheles freeborni*
INDIVIDUAL LARVAE		
	< 28 69.9% (258/369)	≥ 28 97.0% (159/164)
	< 31 90.2% (333/369)	≥ 31 90.2% (148/164)
LARVAE BY COLLECTION LOCATION		
	< 28 87.4% (28/32)	≥ 28 100.0% (11/11)
	< 31 96.9% (31/32)	≥ 31 90.9% (10/11)

val and pupal indices substantiate the work done by Belkin and McDonald. We found that the key correctly identified more individuals and collections when the cut-off point was revised upward from 28 to 31. The larger difference in the means and fewer outliers make the pupal index a better tool for differentiating the two species. The specificity of the key is increased when multiple individuals from a population are sampled. This makes the key useful for identifying unmixed populations.

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ESTIMATION OF ABSOLUTE NUMBERS OF DAMSELFLY POPULATION DENSITIES BY DIPPING SAMPLING DATA

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ABSTRACT

Estimates of relative and absolute densities in ricefield populations of damselfly nymphs (predominantly *Enallagma civile* with few *Ischnura denticollis* and *I. cervula*) were compared using regression method. Equation, X = Y - 0.016, allows estimation of absolute density (X) from relative density index (Y, dipper count). In the rice growing area of Fresno County, nymphal population peaks appeared during July and August, approaching 3 to 5 millions per acre. Presence of submerged vegetation (*Najas* sp. and *Chara* sp.) markedly affected population density of nymphs.

ESTIMATIONS OF GENE NUMBER, HERITABILITY AND DOMINANCE IN QUANTITATIVE INHERITANCE, WITH SPECIAL REFERENCE TO HYMENOPTERA

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ABSTRACT

Insecticide resistance, parasitization rates, and other behavior are often governed by polygenes in insects. A Microscoft BASIC computer program was developed to estimate gene number, dominance, and heritability. Special considerations are presented for haplo/diploid systems, as found in Hymenoptera.

Introduction.

It is increasingly apparent that many behavioral and morphological traits in insects, and other animals, are under the influence of polygenic systems in which inheritance is quantitative (Falconer 1981, Lande 1981, Gardner & Snustad 1984, Legner 1988a,b,c, 1989). Polygenes each exert a slight effect on the phenotype, but they govern together with several or many other genes, a quantitative trait (Falconer 1981, Goodenough 1984). Examples include degrees of resistance to insecticides (Raymond et al. 1986); fecundity and gregarious oviposition (Legner 1987a, 1988a); shape of the head in Drosophila (Val 1977); eye diameter in fish (Wilkens 1971); and human skin color (Harrison & Owen 1964).

When two populations of a particular species demonstrate different degrees of a trait (eg., resistance vs. susceptibility to insecticides), the number of independent and additive genes involved in a polygenic system may be estimated from the means and variances shown in isogenic parental, F_1, F_2, and backcrossed cohorts.

Because dominance and linkage can influence the magnitude of phenotypic expression of a particular trait, the actual number of genes may be correspondingly higher than the estimates derived. Also, because the maximum number of genes cannot exceed the number of chromosomal segments segregating independently in one generation (Lande 1981), it is important to know the recombination index (Darlington 1937), which is derived from the haploid number of chromosomes, plus the mean number of recombination events per gamete. The recombination index is generally only a few times the haploid number of chromosomes (Darlington 1937, White 1973, King 1975, Lande 1981), and thus often may be estimated without cytological investigations.

Varying levels of sophistication are required to obtain the necessary data for estimating minimum gene number, dominance, and heritability, depending on the species and their breeding systems. However, the calculations and considerations are often beyond the scope of many biologists. This paper presents a program to compute the minimum number of active genetic loci for quantitative traits in insects, as well as estimates for dominance and heritability in the broad sense. The reliability of the estimates is qualified with influences of dominance, linkage, and other genetic phenomena. Influences of the experimental environment are estimated by a calculation of the coefficient of heritability.

Procedure.

The minimum number of independent genes with additive effects that contribute to the expression of quantitative traits, such as multiple oviposition and fecundity, can be estimated from the means and the variances of the character in the parental cohorts, their F_1 and F_2 offspring, and backcrossing data, by applying Wright's (Castle 1921) formula:

$$n_E = (\mu p_2 - \mu p_1)^2/(8\sigma^2_s) \leq n$$

[n_E = effective number of genetic factors
μp_1 = mean of parental cohort-1
μp_2 = mean of parental cohort-2
σ^2_s = difference in variances between compared generations]

(see Lande 1981)

Four estimates and their standard errors can be made according to Lande (1981) as follows: n_{E1} considers F_1 and F_2 variances; n_{E2}, the F_1, F_2, P_1 and P_2 variances; n_{E3}, the F_2 and first and second backcross variances; and n_{E4}, the F_1, P_1, P_2, and first and second backcross variances.

Assumptions necessary for the accurate application of Wright's method enumerated by Lande (1981) and Wright (1952) are that the two parental populations have homologous gene sequences so that there is no post-mating reproductive isolation due to chromosomal rearrangements; any number and frequencies of alleles are allowed at each locus within the parental populations; and the loci or segregating factors are not linked and in random combination in each parental population with no significant selection during the experiment. Also, all mating individuals must be chosen at random from the respective populations.

Because the inheritance must be measured statistically, only averages and variances being considered, cytoplasmic influences, gene and environmental interactions, epistasis, etc., are included in the averages and variances. Also, genes may not be equal in their influence on the phenotype. Therefore, even though models that estimate the number of genes based on the assumption of equal effects are oversimplified, it is necessary to make this assumption when the degree of influence of individual genes is unknown (Gardner & Snustad 1984). Such interferences, if present, generally bias gene number estimates downward, however.

Analysis Scales. The scales for analysis should guarantee additivity of the mean phenotypes in F_1, F_2, and backcross populations, and there should be a linearity of P_1, F_1 and P_2 variances when plotted against their means with the extra variance segregating in backcross populations being about half that in the F_2 (Lande 1981). The best scale for analysis is one on which the effects of both genetic and environmental factors are as nearly additive as possible (Wright 1952), although because of a complex of genetic and environmental factors, these effects are in general not wholly additive (Wright 1968). A logarithmic scale is indicated if the coefficients of variability of isogenic strains and their F_1s are all the same (Wright 1952). Whenever interaction effects exist, however, no single transformation can satisfy all available criteria of additivity.

Transformations for data may be selected with the procedure outlined in Wright (1968). Standard deviations are regressed in terms of means among inbred parental cohorts and their F_1s in order to derive a regression formula, $Y = a + bx$. Then, the relationship a/b suggests the transformation function (ie., log (X + a/b). Other kinds of transformations may be appropriate for particular situations, the primary function being to produce additivity among the values.

Special Considerations For Hymenoptera. Because these formulae were derived for diploid-diploid systems, their application to haplo-diploid situations presents some unique considerations about the formation of recombinant males in the F_1 generation. Although normal oogenesis in arrhenotokous Hymenoptera does not deviate from that found in other diploid-diploid organisms, hymenopteran spermatogenesis is highly modified (Crozier 1975). Because hymenopteran males are haploid, marked modifications of spermatogenesis are necessary to ensure that a balanced set of chromosomes is transmitted via the sperm. The principal difference is that the first division is somewhat abortive, with no karyokinesis, so that there is only one true division, an equational one (Crozier 1975). In most Hymenoptera, the sperm of any one haploid male are identical, at least in the genetic components they carry.

Considering a hypothetical hymenopteran example involving two loci in which parental cohorts are homozygous for different alleles at each locus, the F_1 generation of females would be all genetically identical and heterozygous. Assuming that the loci in question are unlinked, each F_1 female would be capable of producing four kinds of

gametes: AB, A'B, AB', and A'B', in equal proportions. Similarly, virgin F_1 hymenopteran females produce four haploid and genetically distinct males from unfertilized eggs: AB, A'B, AB', and A'B'. True F_1 males may then only be derived from F_1 females, and the F_2 generation obtained only by experimentally staggering the generations. However, 50% of these males would be of the parental genotypes (eg., AB & A'B'), as opposed to none of the F_1 females. In this way, the recombinant "F_1" hymenopteran males differ from diploid-diploid systems: there are different kinds of genotypes depending on the number of active loci. Thus, there is no true F_1 generation for haploid males as there is for diploid females.

When crossing F_1 females with males produced by that generation, each free-living, haploid, "F_1" male produces only a single type of gamete, but among the population of males present, all gametes that are produced by the F_1 hybrid female also will be represented. However, at this point each of the different kinds of males (four in the above example) must have equal mating advantage which must be guaranteed, e.g., by manual random selection, in order for the Wright model of random recombination to hold. Also, where large numbers of genetic loci are involved it is essential to have a sufficient number of replicates to ensure that the larger numbers of male genotypes are given equal statistical advantage. Because of the previously mentioned mating influences on female behavior, it is advisable to use only virgin females to measure the quantitative traits.

Haplo-diploid hymenopteran systems also present a situation in which the whole genome is essentially sexlinked. To overcome sex-linkage influences, data from reciprocal F_1 populations and the F_2 populations derived from them may be pooled to achieve the same composition as for autosomal genes in a diploid species, to which Lande's (1981) formulas apply.

Coefficient of Heritability. Estimates of the coefficient of heritability in the broad sense (genetic variance / total variance) can be made using two methods. The first method (H) assumes that inbred parents and the F_1 are genetically homogenous so that all variance observed therein is due to environmental influence. An overall value for environmental variance is derived by averaging the variances for the parents and the F_1. This value subtracted from the total variance, represented by the F_2 variance, gives an estimate of genetic variance (Goodenough 1984). The second method (H_2) simply uses only the F_1 variance as an estimate of environmental influence, and is preferable if the two parental variances are greatly divergent. These estimates are included in the program shown in Appendix I.

Heritability, in the more narrow sense, may be calculated in other ways using regression analyses which may be more appropriate for certain breeding systems, as discussed by Hazel & Terrill (1948), Klein et al (1973), Falconer (1981), Hellmich et al. (1985), and Owen (1989).

Dominance Level. The dominance level (D) in the F_1 progeny may be estimated using the index of Stone (1968), which was derived for monofactorial cases but has been used in polygenic systems (Raymond et al. 1987). The $P \leq 0.05$ confidence limits are derived from formulae in Misra (1968). The parameter "D" may vary linearly from +1, indicating complete dominance, to -1, indicating complete recessivity, and 0 indicating perfect codominance.

Stone's (1968) formula:

$$D = (2 \log F_1 - \log P_1 - \log P_2 / (\log P_1 - \log P_2),$$

may be applied to untransformed data for a value that later can be compared to values computed from transformed data. D is often reduced following a log transformation and may serve in part to judge the acceptability of such transformations.

The following is an annotated listing of <GENEEST.BAS> that summarizes quantitative data and computes estimates of gene number, dominance, and heritability (Appendix I). <GENEEST.BAS> was developed on a Digital Rainbow 100 microcomputer (Z80A 4 MHz cpu, 128 K RAM). It is written in Microsoft BASIC for machines using the MS-DOS and CP/M operating systems. Execution time for calculation varies with the amount of data from ca. 5 to 45 seconds. The escape sequence, CHR$(27)+[4w" sets a dot matrix printer to a horizontal pitch of 16.5 characters per inch. Laser printers will require a different sequence; e.g., the Hewlett Packard Laserjet Series II responds to CHR$(27)+"&k2S" with CHR$(27)+"E" inserted before the program END.

The program requires entries for P_1, P_2, F_1, F_2, B_1, and B_2 data. When there are no data avail-

Appendix I

```
100 REM:  FILE NAME <GENEEST.BAS>
110 LPRINT CHR$(27)+"[4w"
120 PRINT "PROGRAM TO CALCULATE MINIMUM NUMBER OF GENES
     CONTRIBUTING"
130 PRINT "          TO EXPRESSION IN A POLYGENIC SYSTEM"
140 PRINT : PRINT
150 PRINT "DO YOU WANT TO COMPUTE MEANS AND VARIANCES OR ENTER
     THEM"
160 PRINT "  DIRECTLY FROM THE KEYBOARD OR USE EXISTING DATA?"
170 PRINT " ---choose any one <COMPUTE>, <KEYBOARD> or <EXISTING>"
180 INPUT STATUS$
190 IF STATUS$ = "COMPUTE" THEN GOTO 2690
200 IF STATUS$ = "KEYBOARD" THEN GOTO 220
210 IF STATUS$ = "EXISTING" THEN GOTO 3990
220 REM:  GENE ESTIMATES USING KEYBOARD ENTRY OF MEANS AND
     VARIANCES
230 INPUT "IDENTIFY THE DATA";IDENTITY$
240 PRINT "ENTER FOLLOWING VALUES AS INDICATED FOR DATA--
     (";TRANS$ ")"
250 PRINT : PRINT
260 INPUT "Enter mean of Parent-1";MEANP1
270 INPUT "      mean of Parent-2";MEANP2
280 INPUT "      mean of F-1";MEANF1
290 INPUT "      mean of F-2";MEANF2
300 INPUT "      mean of Backcross to Parent-1";MEANB1
310 INPUT "      mean of Backcross to Parent-2";MEANB2
320 PRINT
330 INPUT "Enter the sample size for Parent-1";SIZEP1
340 INPUT "           sample size for Parent-2";SIZEP2
350 INPUT "           sample size for F-1";SIZEF1
360 INPUT "           sample size for F-2";SIZEF2
370 INPUT "           sample size for Backcross to Parent-1";SIZEB1
380 INPUT "           sample size for Backcross to Parent-2";SIZEB2
390 PRINT
400 INPUT "Enter the variance for Parent-1";VARP1
410 INPUT "           variance for Parent-2";VARP2
420 INPUT "           variance for F-1";VARF1
430 INPUT "           variance for F-2";VARF2
440 INPUT "           variance for Backcross to Parent-1";VARB1
450 INPUT "           variance for Backcross to Parent-2";VARB2
460 PRINT
470 PRINT "NOW ENTER UNTRANSFORMED MEAN VALUES FOR THE FOLLOWING:"
480 PRINT
490 INPUT "    untransformed mean of Parent-1"; UMEANP1
500 INPUT "    untransformed mean of Parent-2"; UMEANP2
510 INPUT "    untransformed mean of F-1"; UMEANF1
520 PRINT : PRINT
530 PRINT IDENTITY$
540 NE1 = ((MEANP2-MEANP1)^2)/(8*(VARF2-VARF1))
550 NE2=((MEANP2-MEANP1)^2)/(8*((VARF2)-((1/2*VARF1)+(1/4*VARP1)
     +(1/4*VARP2))))
560 NE3 = ((MEANP2-MEANP1)^2)/(8*(2*VARF2-VARB1-VARB2))
570 NE4=((MEANP2-MEANP1)^2)/(8*((VARB1+VARB2)-((VARF1+(1/2*VARP1)
     +(1/2*VARP2)))))
580 PRINT : PRINT
590 PRINT : PRINT : PRINT
600 REM: STANDARD ERROR OF AN ESTIMATE--Formula 8a (Lande 1981)
610 VARSIGMA1 = (2*(VARF2^2)/SIZEF2) + (2*(VARF1^2)/SIZEF1)
620 VARNE1 = 4*((VARP1/SIZEP1) + (VARP2/SIZEP2))
630 VARNE2 = (MEANP2 - MEANP1)^2
640 VARNE3 = VARSIGMA1
650 VARNE4 = (VARF2 - VARF1)^2
660 LEFT = VARNE1/VARNE2 : RIGHT = VARNE3/VARNE4
670 VARNE = LEFT + RIGHT
680 STANDERROR1 = SQR(NE1^2*VARNE)
690 REM:  STANDARD ERROR OF ESTIMATE--Formula 8b
700 VARSIGMA2 = (2*(VARF2^2)/SIZEF2) + (1/2*(VARF1^2)/SIZEF1) +
     (1/8*(VARP1^2)/SIZEP1) + (1/8*(VARP2^2)/SIZEP2)
710 VARNE1 = 4*((VARP1/SIZEP1) + (VARP2/SIZEP2))
720 VARNE2 = (MEANP2 - MEANP1)^2
730 VARNE3 = VARSIGMA2
740 VARNE4 = ((VARF2 - ((1/2*VARF1)+(1/4*VARP1)+(1/4*VARP2))))^2
750 LEFT = VARNE1/VARNE2 : RIGHT = VARNE3/VARNE4
760 VARNE = LEFT + RIGHT
770 STANDERROR2 = SQR(NE2^2*VARNE)
780 REM:  STANDARD ERROR OF ESTIMATE--Formula 8c
790 VARSIGMA3 =(8*(VARF2^2)/SIZEF2) + (2*(VARB1^2)/SIZEB1)+
     (2*(VARB2^2)/SIZEB2)
800 VARNE1 = 4*((VARP1/SIZEP1) + (VARP2/SIZEP2))
810 VARNE2 = (MEANP2 - MEANP1)^2
820 VARNE3 = VARSIGMA3
830 VARNE4 = ((2*VARF2 - VARB1 - VARB2))^2
840 LEFT = VARNE1/VARNE2 : RIGHT = VARNE3/VARNE4
850 VARNE = LEFT + RIGHT
860 STANDERROR3 = SQR(NE3^2*VARNE)
870 REM;  STANDARD ERROR OF ESTIMATE--Formula 8d
880 VARSIGMA4 = (2*(VARB1^2)/SIZEB1)+(2*(VARB2^2)/SIZEB2)
     +(2*(VARF1^2)/SIZEF1)+(1/2*(VARP1^2)/SIZEP1)
     +(1/2*(VARP2^2)/SIZEP2)
890 VARNE1 = 4*((VARP1/SIZEP1) + (VARP2/SIZEP2))
900 VARNE2 = (MEANP2 - MEANP1)^2
910 VARNE3 = VARSIGMA4
920 VARNE4 = ((VARB1+VARB2)-(VARF1+(1/2*VARP1)+(1/2*VARP2)))^2
930 LEFT = VARNE1/VARNE2 : RIGHT = VARNE3/VARNE4
940 VARNE = LEFT + RIGHT
950 STANDERROR4 = SQR(NE4^2*VARNE)
960 REM:  HERITABILITY COMPUTATION
970 VEP = (VARP1 + VARP2)/2
980 VEF1 = VARF1
990 VE = (VEP + VEF1)/2
1000 VT = VARF2
1010 VG = VT-VE
1020 H = VG/VT
1030 H2 = (VARF2 - VARF1)/VARF2
1040 REM:  DOMINANCE COMPUTATION
1050 REM:  X1 = LOG P1 : X2 = LOG F-1 HYBRID : X3 = LOG P2
1060 REM:  FOR:  D = (2*X2-X1-X3)/(X1-X3)
```

```
1070 X1 = LOG(UMEANP1)/2.3026
1080 X2 = LOG(UMEANF1)/2.3026
1090 X3 = LOG(UMEANP2)/2.3026
1100 NUMERATOR = 2*X2 - X1 - X3
1110 DENOMINATOR = X1 - X3
1120 D = NUMERATOR/DENOMINATOR
1130 X1B = LOG(MEANP1)/2.3026 : X2B = LOG(MEANF1)/2.3026
1140 X3B = LOG(MEANP2)/2.3026 : NUM = 2*X2B - X1B - X3B
1150 DEM = X1B - X3B : DB = NUM/DEM
1160 REM:  PRINT-OUT OF VALUES
1170 PRINT SPC(20);IDENTITY$
1180 LPRINT SPC(20);IDENTITY$
1190 PRINT
1200 PRINT "   VALUES REPRESENT RAW DATA";TRANS$;TR;")"
1210 LPRINT "   VALUES REPRESENT RAW DATA";TRANS$;TR;")"
1220 PRINT : LPRINT
1230 PRINT " Population          N               mean
     variance"
1240 LPRINT " Population          N               mean
     variance"
1250 PRINT "_____________________________________________________
     _____"
1260 LPRINT "_____________________________________________________
     _____"
1270 PRINT : LPRINT
1280 PRINT "Parent-1           ";
1290 LPRINT "Parent-1           ";
1300 PRINT USING "###.##";SIZEP1;
1310 LPRINT USING "###.##";SIZEP1;
1320 PRINT "         ";
1330 LPRINT "         ";
1340 PRINT USING "####.###";MEANP1;
1350 LPRINT USING "####.###";MEANP1;
1360 PRINT "      ";
1370 LPRINT "      ";
1380 PRINT VARP1
1390 LPRINT VARP1
1400 PRINT : LPRINT
1410 PRINT "Backcross to P-1   ";
1420 LPRINT "Backcross to P-1   ";
1430 PRINT USING "###.##";SIZEB1;
1440 LPRINT USING "###.##";SIZEB1;
1450 PRINT "         ";
1460 LPRINT "         ";
1470 PRINT USING "####.###";MEANB1;
1480 LPRINT USING "####.###";MEANB1;
1490 PRINT "      ";
1500 LPRINT "      ";
1510 PRINT VARB1
1520 LPRINT VARB1
1530 PRINT : LPRINT
1540 PRINT "F-1                ";
1550 LPRINT "F-1                ";
1560 PRINT USING "###.##";SIZEF1;
1570 LPRINT USING "###.##";SIZEF1;
1580 PRINT "         ";
1590 LPRINT "         ";
1600 PRINT USING "####.###";MEANF1;
1610 LPRINT USING "####.###";MEANF1;
1620 PRINT "      ";
1630 LPRINT "      ";
1640 PRINT VARF1
1650 LPRINT VARF1
1660 PRINT : LPRINT
1670 PRINT "F-2                ";
1680 LPRINT "F-2                ";
1690 PRINT USING "###.##";SIZEF2;
1700 LPRINT USING "###.##";SIZEF2;
1710 PRINT "         ";
1720 LPRINT "         ";
1730 PRINT USING "####.###";MEANF2;
1740 LPRINT USING "####.###";MEANF2;
1750 PRINT "      ";
1760 LPRINT "      ";
1770 PRINT VARF2
1780 LPRINT VARF2
1790 PRINT : LPRINT
1800 PRINT "Backcross to P-2   ";
1810 LPRINT "Backcross to P-2   ";
1820 PRINT USING "###.##";SIZEB2;
1830 LPRINT USING "###.##";SIZEB2;
1840 PRINT "         ";
1850 LPRINT "         ";
1860 PRINT USING "####.###";MEANB2;
1870 LPRINT USING "####.###";MEANB2;
1880 PRINT "      ";
1890 LPRINT "      ";
1900 PRINT VARB2
1910 LPRINT VARB2
1920 PRINT : LPRINT
1930 PRINT "Parent-2           ";
1940 LPRINT "Parent-2           ";
1950 PRINT USING "###.##";SIZEP2;
1960 LPRINT USING "###.##";SIZEP2;
1970 PRINT "         ";
1980 LPRINT "         ";
1990 PRINT USING "####.###";MEANP2;
2000 LPRINT USING "####.###";MEANP2;
2010 PRINT "      ";
2020 LPRINT "      ";
2030 PRINT VARP2
2040 LPRINT VARP2
2050 PRINT : LPRINT
2060 PRINT "_____________________________________________________
     _____"
2070 LPRINT "_____________________________________________________
     _____"
2080 PRINT : PRINT : LPRINT : LPRINT
```

```
2090 PRINT "                                    Variances Considered"
2100 LPRINT "                                   Variances Considered"
2110 PRINT "                                    ________________________"
2120 LPRINT "                                   ________________________"
2130 PRINT : LPRINT
2140 PRINT "N1 =";NE1 "_+_";STANDERROR1;"----F-1 & F-2 data"
2150 PRINT
2160 LPRINT "N1 =";NE1 "_+_";STANDERROR1;"----F-1 & F-2 data"
2170 LPRINT
2180 PRINT "N2 =";NE2 "_+_";STANDERROR2;"----F-1, F-2, P-1 & P-2
     data"
2190 PRINT
2200 LPRINT "N2 =";NE2 "_+_";STANDERROR2;"----F-1, F-2, P-1 & P-2
     data"
2210 LPRINT
2220 PRINT "N3 =";NE3 "_+_";STANDERROR3;"----F-2, B-1 & B-2 data"
2230 PRINT
2240 LPRINT "N3 =";NE3 "_+_";STANDERROR3;"----F-2, B-1 & B-2 data"
2250 LPRINT
2260 PRINT "N4 =";NE4 "_+_";STANDERROR4;"----F-1, P-1, P-2, B-1 &
     B-2 data"
2270 PRINT
2280 LPRINT "N4 =";NE4 "_+_";STANDERROR4;"----F-1, P-1, P-2, B-1 &
     B-2 data"
2290 PRINT : PRINT : LPRINT : LPRINT
2300 PRINT "DOMINANCE (computed from raw data) = ";D;
2310 LPRINT "DOMINANCE (computed from raw data) = ";D;
2320 IF D = 0 THEN PRINT "--There is perfect codominance"
2330 IF D<.1 AND D>-.1 THEN PRINT "--There is almost perfect
     codominance"
2340 IF D<.1 AND D>-.1 THEN LPRINT "--There is almost perfect
     codominance"
2350 IF D = -1 THEN PRINT "--There is complete recessivity"
2360 IF D = -1 THEN LPRINT "--There is complete recessivity"
2370 IF D>-.9000001 AND D<-.8 THEN PRINT "--There is almost
     complete recessivity"
2380 IF D>-.9000001 AND D<-.8 THEN LPRINT "--There is almost
     complete recessivity"
2390 IF D> -.8 AND D<-.2 THEN PRINT "--There is semi-recessivity"
2400 IF D> -.8 AND D<-.2 THEN LPRINT "--There is semi-recessivity"
2410 IF D = +1 THEN PRINT "--There is complete dominance"
2420 IF D = +1 THEN LPRINT "--There is complete dominance"
2430 IF D<.9000001 AND D>.8 THEN PRINT "--There is almost
     complete dominance"
2440 IF D<.9000001 AND D>.8 THEN LPRINT "--There is almost
     complete dominance"
2450 IF D<.8 AND D>.2 THEN PRINT "--There is semi-dominance"
2460 IF D<.8 AND D>.2 THEN LPRINT "--There is semi-dominance"
2470 PRINT "              (of the trait shown in Parent-2)"
2480 LPRINT "              (of the trait shown in Parent-2)"
2490 PRINT : LPRINT
2500 IF TRAN$ = "Y" THEN GOTO 2550
2510 PRINT "Untransformed Mean Values"
2520 LPRINT "Untransformed Mean Values"
2530 PRINT " P-1 =";UMEANP1;" P-2 =";UMEANP2;" F-1 =";UMEANF1
2540 LPRINT " P-1 =";UMEANP1;" P-2 =";UMEANP2;" F-1 =";UMEANF1
2550 IF TRAN$="Y"THEN PRINT "DOMINANCE (computed from transformed
     data) = ";DB
2560 IF TRAN$="Y"THEN LPRINT "DOMINANCE (computed from transformed
     data) = ";DB
2570 PRINT : PRINT : LPRINT : LPRINT
2580 PRINT "HERITABILITY (H) =";H
2590 LPRINT "HERITABILITY (H) =";H
2600 PRINT "HERITABILITY (H-2)=";H2
2610 LPRINT "HERITABILITY (H-2)=";H2
2620 PRINT : LPRINT
2630 PRINT "      Thus, variability of the trait was influenced
     genotypically by"
2640 LPRINT "      Thus, variability of the trait was influenced
     genotypically by "
2650 PRINT H*100 "--"; H2*100 "%"
2660 LPRINT H*100 "--"; H2*100 "%"
2670 PRINT : PRINT : PRINT : LPRINT : LPRINT : LPRINT
2680 END
2690 REM:  SUBROUTINE TO ENTER RAW DATA AND TO COMPUTE MEAN,
     VARIANCE, ETC.
2700 PRINT
2710 PRINT "IDENTIFY THE KIND OF DATA"
2720 INPUT IDENTITY$
2730 REM: (B=n; G=X; C=total; E=X^2; H=mean; D=summ. X^2;
     M=variance
2740 REM: (P=standard deviation; Q=stand.error of mean; L=coeff.
     of variability
2750 REM:  ROUTINE TO DEFINE MEANS AND VARIANCES FOR GENE
     ESTIMATES
2760 OPEN "O",#1, "GENE.DAT"
2770    PRINT #1, IDENTITY$
2780 GOSUB 2980 :UMEANP1=H:MEANP1=H:SIZEP1=A:VARP1=M : DIMENSION$
     = "DIMEN"
2790 PRINT #1, SIZEP1
2800 FOR I = 1 TO SIZEP1 : PRINT #1,X(I); : NEXT I
2810 GOSUB 2980 :UMEANP2=H:MEANP2=H:SIZEP2=A:VARP2=M
2820 PRINT #1, SIZEP2
2830 FOR I = 1 TO SIZEP2 : PRINT #1,X(I); : NEXT I
2840 GOSUB 2980 :UMEANF1=H:MEANF1=H:SIZEF1=A:VARF1=M
2850 PRINT #1, SIZEF1
2860 FOR I = 1 TO SIZEF1 : PRINT #1,X(I); : NEXT I
2870 GOSUB 2980 :UMEANF2=H:MEANF2=H:SIZEF2=A:VARF2=M
2880 PRINT #1, SIZEF2
2890 FOR I = 1 TO SIZEF2 : PRINT #1,X(I); : NEXT I
2900 GOSUB 2980 :MEANB1=H:SIZEB1=A:VARB1=M
2910 PRINT #1, SIZEB1
2920 FOR I = 1 TO SIZEB1 : PRINT #1,X(I); : NEXT I
2930 GOSUB 2980 :MEANB2=H:SIZEB2=A:VARB2=M
2940 PRINT #1, SIZEB2
2950 FOR I = 1 TO SIZEB2 : PRINT #1,X(I); : NEXT I
2960 CLOSE #1 : GOTO 3850
2970 REM:  SUBROUTINE TO TAKE ENTRIES OF RAW DTA
```

```
2980 LET A = 0 : B = 0 : C = 0 : D = 0
2990 LET E = 0 : G = 0 : ST = 0
3000 PRINT "ENTER OBSERVATIONS IN THE FOLLOWING ORDER:
3010 PRINT "(1) P-1, (2) P-2, (3) F-1, (4) F-2, (5) B-1 and
     (6) B-2"
3020 PRINT "--(Max. No. values = 255)"
3030 PRINT SPC(5) "<when you have entered all your observations,
     begin the "
3040 PRINT SPC(6) "computation by keying-in a -999>"
3050 PRINT
3060 PRINT SPC(6) "                    [to delete a value type
     <12345678>]"
3070 PRINT
3080 IF DIMENSION$ = "DIMEN" THEN GOTO 3100
3090 DIM X(250),Y(250)
3100 REM:  INITIALIZATION OF I TO ONE
3110 IF CYCLE = 0 THEN PRINT "Parent-1 Data for ";IDENTITY$
3120 I = 1
3130 IF CYCLE = 1 THEN PRINT "Enter Parent-2 Data for ";IDENTITY$
3140 IF CYCLE = 2 THEN PRINT "Enter F-1 Data for ";IDENTITY$
3150 IF CYCLE = 3 THEN PRINT "Enter F-2 Data for ";IDENTITY$
3160 IF CYCLE = 4 THEN PRINT "Enter Backcross to Parent-1 Data
     for ";IDENTITY$
3170 IF CYCLE = 5 THEN PRINT "Enter Backcross to Parent-2 Data
     for ";IDENTITY$
3180 COUNT = 1
3190 INPUT X(I)
3200      IF COUNT >1 THEN GOTO 3230
3210       PRINT "                ";COUNT "entry made"
3220      IF COUNT <2 THEN GOTO 3240
3230       PRINT "                ";COUNT "entries made"
3240      LET F = 1
3250      IF X(I) = 12345678# THEN GOTO 3940
3260      IF X(I) = -999 THEN 3360
3270      LET A = A + 1
3280      LET B = B + F
3290      LET G = X(I)*F
3300      LET C = C + G
3310      LET E = (X(I)^2)*F
3320      LET D = D + E
3330      LET I = I + 1
3340    COUNT = COUNT + 1
3350 GOTO 3190
3360 N = I - 1
3370 LET H = C/B
3380 LET M = (D-(C)^2/B)/(B-1)
3390 LET P = SQR(M)
3400 LET Q = SQR(M/B)
3410 LET L = (P*100)/H
3420 PRINT
3430 PRINT : PRINT : LPRINT : LPRINT
3440 PRINT IDENTITY$
3450 LPRINT IDENTITY$
3460 PRINT : PRINT: LPRINT :LPRINT
3470 PRINT "ORIGINAL VALUES":PRINT
3480 LPRINT "ORIGINAL VALUES":LPRINT
3490 IF CYCLE = 0 THEN PRINT "Parent-1 Data"
3500 IF CYCLE = 0 THEN LPRINT "Parent-1 Data"
3510 IF CYCLE = 1 THEN PRINT "Parent-2 Data"
3520 IF CYCLE = 1 THEN LPRINT "Parent-2 Data"
3530 IF CYCLE = 2 THEN PRINT "F-1 Data"
3540 IF CYCLE = 2 THEN LPRINT "F-1 Data"
3550 IF CYCLE = 3 THEN PRINT "F-2 Data"
3560 IF CYCLE = 3 THEN LPRINT "F-2 Data"
3570 IF CYCLE = 4 THEN PRINT "Backcross to Parent-1 Data"
3580 IF CYCLE = 4 THEN LPRINT "Backcross to Parent-1 Data"
3590 IF CYCLE = 5 THEN PRINT "Backcross to Parent-2 Data"
3600 IF CYCLE = 5 THEN LPRINT "Backcross to Parent-2 Data"
3610 PRINT : PRINT : LPRINT : LPRINT
3620 FOR I = 1 TO N : PRINT X(I); : LPRINT X(I); : NEXT I
3630 PRINT : PRINT
3640 LPRINT : LPRINT
3650 PRINT : PRINT : LPRINT :LPRINT
3660 PRINT : LPRINT
3670 PRINT "NUMBER OF OBSERVATIONS = ";A
3680 LPRINT "NUMBER OF OBSERVATIONS = ";A
3690 PRINT "GRAND TOTAL = ";H*A
3700 LPRINT "GRAND TOTAL = ";H*A
3710 PRINT CHR$(27)+"[7m"
3720 PRINT "MEAN = "; H
3730 PRINT CHR$(27)+"[0m"
3740 LPRINT "MEAN = "; H
3750 PRINT "VARIANCE (S^2)= ";M
3760 LPRINT "VARIANCE (S^2) = ";M
3770 PRINT "STANDARD DEVIATION (s) = "; P
3780 LPRINT "STANDARD DEVIATION (s) = "; P
3790 PRINT "STANDARD ERROR OF MEAN (sx) = "; Q
3800 LPRINT "STANDARD ERROR OF MEAN (sx) = "; Q
3810 PRINT "COEFF. OF VARIABILITY (CV) = "; L
3820 LPRINT "COEFF. OF VARIABILITY (CV) = "; L
3830 PRINT : CYCLE = CYCLE + 1
3840 RETURN
3850 PRINT "BASIC DATA IS STORED IN FILE NAMED <GENE.DAT>":PRINT
3860 PRINT "Do you wish to rename the file?--(Y/N)" : PRINT
3870 INPUT R$
3880 ON ERROR GOTO 3920
3890 IF R$ = "Y" THEN PRINT "ENTER NEW FILE NAME" : INPUT N$
3900 IF R$ = "Y" THEN NAME "GENE.DAT" AS N$ :LPRINT:LPRINT N$:
     GOTO 530
3910 IF R$ = "N" THEN PRINT: GOTO 530
3920 PRINT "FILE ALREADY EXISTS...TRY ANOTHER NAME" : PRINT :
     GOTO 3890
3930 END
3940 REM:  SUBROUTINE TO MAKE CORRECTIONS--DELETE AN ENTRY
     FROM ARRAY
3950 LET ST = ST - G
3960 LET D = D - E : C = C - G : B = B - F
3970 LET I = I - 1 : A = A - 1 : F = 0
```

```
3980 GOTO 3190
3990 REM:  SUBROUTINE TO ENTER DATA FROM NAMED DATA FILE
4000 PRINT: PRINT "Enter the name of data file": PRINT
4010 INPUT FILO$
4020 LPRINT "FILE NAME = ";FILO$ : LPRINT
4030 PRINT "FILE NAME = ";FILO$ :PRINT
4040 OPEN "I",#1,FILO$
4050    INPUT #1,IDENTITY$
4060 DIM X(250),Y(250),P1(250),P2(250),F1(250),F2(250),B1(250),
     B2(250)
4070    INPUT #1,SIZEP1
4080    FOR I = 1 TO SIZEP1 : INPUT #1,P1(I)  : NEXT I
4090    INPUT #1,SIZEP2
4100    FOR I = 1 TO SIZEP2 : INPUT #1,P2(I) : NEXT I
4110    INPUT #1, SIZEF1
4120    FOR I = 1 TO SIZEF1 : INPUT #1,F1(I) : NEXT I
4130    INPUT #1, SIZEF2
4140    FOR I = 1 TO SIZEF2 : INPUT #1,F2(I) : NEXT I
4150    INPUT #1, SIZEB1
4160    FOR I = 1 TO SIZEB1 : INPUT #1,B1(I) : NEXT I
4170    INPUT #1, SIZEB2
4180    FOR I = 1 TO SIZEB2 : INPUT #1,B2(I) : NEXT I
4190 CLOSE #1
4200  FOR I = 1 TO SIZEP1 : LET TOT = TOT + P1(I) : NEXT I
4210     UMEANP1 = TOT/SIZEP1 : TOT = 0
4220  FOR I = 1 TO SIZEP2 : LET TOT = TOT + P2(I) : NEXT I
4230     UMEANP2 = TOT/SIZEP2 : TOT = 0
4240  FOR I = 1 TO SIZEF1 : LET TOT = TOT + F1(I) : NEXT I
4250     UMEANF1 = TOT/SIZEF1 : TOT = 0
4260  PRINT "DO YOU WANT DATA TRANSFORMED TO log(X + ??) ?
     -- (Y/N)"
4270  INPUT TRAN$
4280 IF TRAN$ = "Y" THEN GOSUB 4680
4290 FOR I = 1 TO SIZEP1
4300   LET ZX2 = ZX2 + P1(I)^2
4310   LET TOT = TOT + P1(I) : NEXT I
4320   LET C = (TOT)^2/SIZEP1
4330   MEANP1 = TOT/SIZEP1 : VARP1 = (ZX2-C)/(SIZEP1-1)
4340   ZX2 = 0 : TOT = 0
4350 FOR I = 1 TO SIZEP2
4360   LET ZX2 = ZX2 + P2(I)^2
4370   LET TOT = TOT + P2(I) : NEXT I
4380   LET C = (TOT)^2/SIZEP2
4390   MEANP2 = TOT/SIZEP2 : VARP2 = (ZX2-C)/(SIZEP2-1)
4400   ZX2 = 0 : TOT = 0
4410 FOR I = 1 TO SIZEF1
4420   LET ZX2 = ZX2 + F1(I)^2
4430   LET TOT = TOT + F1(I) : NEXT I
4440   LET C = (TOT)^2/SIZEF1
4450   MEANF1 = TOT/SIZEF1 : VARF1 = (ZX2-C)/(SIZEF1-1)
4460   ZX2 = 0 : TOT = 0
4470 FOR I = 1 TO SIZEF2
4480   LET ZX2 = ZX2 + F2(I)^2
4490   LET TOT = TOT + F2(I) : NEXT I
4500   LET C = (TOT)^2/SIZEF2
4510   MEANF2 = TOT/SIZEF2 : VARF2 = (ZX2-C)/(SIZEF2-1)
4520   ZX2 = 0 : TOT = 0
4530 FOR I = 1 TO SIZEB1
4540   LET ZX2 = ZX2 + B1(I)^2
4550   LET TOT = TOT + B1(I) : NEXT I
4560   LET C = (TOT)^2/SIZEB1
4570   MEANB1 = TOT/SIZEB1 : VARB1 = (ZX2-C)/(SIZEB1-1)
4580   ZX2 = 0 : TOT = 0
4590 FOR I = 1 TO SIZEB2
4600   LET ZX2 = ZX2 + B2(I)^2
4610   LET TOT = TOT + B2(I) : NEXT I
4620   LET C = (TOT)^2/SIZEB2
4630   MEANB2 = TOT/SIZEB2 : VARB2 = (ZX2-C)/(SIZEB2-1)
4640 IF TRAN$ = "Y" THEN GOTO 530
4650   UMEANP1=MEANP1 : UMEANP2=MEANP2 : UMEANF1=MEANF1
4660 GOTO 530
4670 REM: SUBROUTINES TO TRANSFORM DATA TO LOG(X + ??)
4680 INPUT "Enter value you wish to be added to X";TR
4690 TRANS$ = " transformed to LOG(X + "
4700 FOR I = 1 TO SIZEP1 : P1(I) = LOG((P1(I) + TR))/2.306 :
     NEXT I
4710 FOR I = 1 TO SIZEP2 : P2(I) = LOG((P2(I) + TR))/2.306 :
     NEXT I
4720 FOR I = 1 TO SIZEF1 : F1(I) = LOG((F1(I) + TR))/2.306 :
     NEXT I
4730 FOR I = 1 TO SIZEF2 : F2(I) = LOG((F2(I) + TR))/2.306 :
     NEXT I
4740 FOR I = 1 TO SIZEB1 : B1(I) = LOG((B1(I) + TR))/2.306 :
     NEXT I
4750 FOR I = 1 TO SIZEB2 : B2(I) = LOG((B2(I) + TR))/2.306 :
     NEXT I
4760 RETURN
4770 LPRINT CHR$(27)+"E"
4780 END
```

able for one of these categories, the user must enter a few dummy values for computation to proceed. Also, all raw data entries must contain some variation.

Included is one example of a transformation to the $\log_{10}(X)$, which may be modified to suit the particular set of data being analyzed by additions or subtractions to the value X. Any negative value entered must leave the raw data >0.

Raw data are stored in a file named <GENE.DAT>, which may be renamed as desired. When all data for a particular cohort have been entered, the computer prints the number of observations, grand total, mean, variance, standard deviation, standard error, and coefficient of variability for those data. When data from all cohorts have been entered, a summarized account is given, together with estimations of gene number, dominance, and heritability.

An example print-out for a cross between solitary and gregarious populations of the South American parasitoid, *Muscidifurax raptorellus* Kogan & Legner, is given in Appendix II.

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Appendix II

FILE NAME = A:89GENE.3

% GREGARIOUS OVIPOSITION -- POOLED CHILE + PERU LINES
VALUES REPRESENT RAW DATA transformed to LOG(X + 3.128)

Population	N	mean	variance
Parent-1	180.00	0.495	1.640959E-06
Backcross to P-1	200.00	0.787	4.508731E-02
F-1	200.00	1.358	7.638136E-03
F-2	234.00	1.312	7.696298E-02
Backcross to P-2	200.00	1.817	1.093634E-02
Parent-2	180.00	1.792	2.912366E-02

Variances Considered

N1 = 3.033752 _+_ .318764 ----F-1 & F-2 data

N2 = 3.19323 _+_ .3530633 ----F-1, F-2, P-1 & P-2 data

N3 = 2.148206 _+_ .331117 ----F-2, B-1 & B-2 data

N4 = 6.218114 _+_ .9174555 ----F-1, P-1, P-2, B-1 & B-2 data

DOMINANCE (computed from raw data) = -.8002845 --There is almost complete recessivity
(of the trait shown in Parent-2)

DOMINANCE (computed from transformed data) = -.569853

HERITABILITY (H) = .8557696
HERITABILITY (H-2)= .9007558

Thus, variability of the trait was influenced genotypically by
85.57696 -- 90.07558 %

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MIGHT WARY GENES ATTENUATE AFRICANIZED HONEY BEES?

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ABSTRACT

The discovery in Hymenoptera of a group of genes capable of partial phenotypic expression in females, following mating with males of different races, suggests a search be made for similar genes in honey bees. If they are found to exist in honeybees, they might be employed to attenuate Africanized bee colonies. However, preintroduction asessments of resulting hybrids are essential to judge long-term effects of hybridization, and to preclude the possible evolution of even more noxious races.

African honeybees, or "killer bees" as they frequently are called, are encroaching on the United States from Mexico. Predictions place their arrival time in south Texas by around 1991, and their appearance in California and Arizona is expected shortly thereafter (probably by 1993) (see Taylor 1985).

A public health problem may be expected within a year of the invasion as people become aware of these bees and succumb to their attacks. However, studies on honeybee behavior at higher latitudes in South America suggest that the public health threat is not as great as these bees' notoriety (Taylor 1985). Nevertheless, mosquito abatement districts in California will undoubtedly be called upon for information about how to deal with the bees and perhaps to exterminate feral colonies.

Most of the characteristics that distinguish African bees from European bees, such as aggressiveness, early-day mating times, degrees of pollen and honey hoarding, etc., are thought to be quantitative and, therefore, under the control of polygenic systems. Unfortunately, because of difficulties inherent in studying quantitative traits in honeybees, knowledge of this phase of their genetics is scant. In fact, Taylor (1985) acknowledged that there is an overall limited understanding of honeybee genetics. Thus, we really cannot predict what will occur following hybridization of African and European races because practically all opinions are being derived from their behavior in South America (Kerr et al. 1982, McDonell 1984, Rinderer et al. 1982, 1984, Taylor 1985). Perhaps some indications can be obtained from other groups of Hymenoptera.

A great deal of information about hymenopteran quantitative inheritance has been gathered recently from parasitic wasps in the genus *Muscidifurax* that attack synanthropic Diptera. For example, in *Muscidifurax raptorellus* Kogan & Legner, a South American species, traits for fecundity and other reproductive behavior are under the control of a polygenic system (Legner 1987b, 1988a,1988b, 1989a). Males in this species are able to change the female's oviposition phenotype upon mating by transferring an unknown substance capable of evoking behavioral changes. It appears as if a proportion of the genes in the female have the phenotypic plasticity, or norm of reaction, to change expression under the influence of the male substance environment. The intensity of this response is different, depending on the respective genetic composition of the mating pair (Legner 1989a). Thus, the genes involved, which regulate phenotypic changes in the mated female, cause partial expression of the traits they govern shortly after insemination and before being inherited by resulting progeny.

The behavioral changes after mating seem permanent, as was revealed by increases or decreases in the response maintained for 16 days following a particular mating (Legner 1987a, and unpub. data). Furthermore, subsequent experimentation showed there is no switchback in behavior following a second mating with a male of the opposite genotype (Legner 1989a, and unpub. data).

Such genes, that are capable of phenotypic expression in the mated female before being inherited by her progeny, have been called "wary genes" because of their partial expression in the environment before chromosomal inheritance in offspring. Also, because inheritance of such genes seems to occur in a stepwise manner the entire process has been referred to as "accretive inheritance" (Legner 1988b, 1989a).

Indeed, the ability of the male substance to switch a polygenic locus on or off in the mother suggests that a locus may exist in two states: active and inactive. Polygenic loci have been thought generally to be occupied by distinct genes coded for a fixed kind of expression (Wright 1968). However, Falconer (1981) has suggested that variants of regulatory genes may be responsible for the expression of polygenic characters. The switching on or off hypothesis fits in with this idea.

In the process of hybridization, wary genes are thought to quicken the pace of evolution by allowing natural selection to begin to act in the parental generation (Legner 1988a, 1989a). Wary genes, which are detrimental to the hybrid population, might thus be more prone to elimination, and beneficial ones may be expressed in the mother before the appearance of her active progeny.

If such a system prevails in honeybees, greater importance could be placed on drones because it may be possible for African or European drones to convey directly to unmated queens of either race some of their own racial characteristics. The rapid Africanization of European bee colonies in South and Central America could be explained partly by this process, although early-day mating of African drones has been considered primarily responsible (Taylor 1985).

It is admittedly presumptuous at this time to infer similarities in the genetics of genera *Apis* and *Muscidifurax*, and the presence of wary genes in both. Some speculation seems justified where similarities might exist, however, especially as there is general agreement that permanent control of the Africanized bees will probably involve genetic manipulation and mating biology (The Calif. Bee Times 1988). If present, wary genes could offer a means to the abatement of this potentially severe public health pest. However, the possible occurrence of similar hybridization events in honeybees, as has been observed in *Muscidifurax*, would dictate extreme caution in setting into motion any processes that might lead to the formation of new races. Available means for identifying hybridized colonies and extirpating Africanized queens (Page & Erickson 1985, Taylor 1985) are tedious and imperfect. With the understanding that hybridization events and wary genes of the kind found in *Muscidifurax* have yet to be substantiated in *Apis*, the following suggestions for African bee abatement are tentative.

Considerations For Deploying Wary Genes in Abatement.

Wary genes could be used to induce in queen bees immediate behavioral changes such as a reduced dispersal tendency, greater susceptibility to winter cold, lower fecundity, or even a preference for subsequent matings to occur in the afternoon when European drones are most active.

Africanized queens that mate with different races of European drones might exhibit immediate postmating depression in some cases, as was reported recently in some species of *Muscidifurax* (Legner 1988c). However, the offspring of crosses between African queens and certain races of European drones might be expected to show heterosis, expressed as increased fecundity and stamina, while other crosses involving different races of European bees might produce a negative effect. Crosses between hybrid queens and hybrid males could result in superactive queens after mating, followed by even more highly active progeny, as was observed recently in *M. raptorellus* (Legner, unpub. data).

Selection favoring the superactive hybrids would tend to guarantee the survival of both parental races and a continuous formation of hybrid bees, as has been suggested for *Muscidifurax* (Legner 1988c). Such a process could direct events leading to the relatively rapid evolution of a new race. A superiorly adapted race might displace Africanized bees and prevail in the area. Of course this race also would have to display desireable characteristics of honey production, pollination, and nonaggression to be acceptable.

Mating European queens with races of drones from feral northern European populations might cause such queens to acquire increased winter tolerance and give rise to hybrids that have even greater tolerances. On the other hand, having drones available that possess a reduced winter

tolerance could increase winter kill.

The selection of appropriate populations for intraspecific crosses is critical to avoid detrimental outcomes from negative heterosis, or hybrid dysgenesis, as well as undesirable positive heterotic behavior, such as an increased aggressiveness. Preintroduction assessments are essential to reveal such tendencies (see Legner 1988c).

The introduction of alien alleles into a population by hybridization utilizing naturally evolved parental populations would probably be less risky than introducing genetically engineered ones where no natural selection has acted priorly. Researchers, working to inject laboratory-engineered products into natural populations, should consider what kind of behavior will be demonstrated once heterosis has had a chance to act. Unless the engineered populations can be completely isolated reproductively from resident wild populations, there is considerable risk involved.

We could imagine a lot of other possibilities. However, the first step should involve a more thorough understanding of honeybee genetics, and whether or not enough similarity exists with known hymenopteran systems to derive safe and viable strategies. Certain aspects of genetics are as yet unclear in Hymenoptera, which was demonstrated recently with the discovery of paternal influences in males (Legner 1989b). However, there is a clear rationale for pre-introduction assessments as presently advocated for parasitic Hymenoptera (Coppel & Mertins 1977, Legner 1986, 1988c).

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DEVELOPMENT AND SURVIVAL OF MOSQUITOES IN AN IPM RICE MANAGEMENT PROGRAM

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Introduction.

Rice fields in northern California are major breeding sites of *Culex tarsalis* (Coquillett) and *Anopheles freeborni* (Aitken). It has been observed in past studies that the larval abundance of these mosquito species varies considerably from one field to another (Case and Washino 1979). Because of this, a project was initiated to investigate factors which are potentially responsible for this trend. Some of those factors include predation, edaphic factors, and crop management practices.

Initial studies uncovered a lack of continuity in population trends related to presence or absence of predaceous flatworms, field age (whether or not the field had been previously planted in rice), water source (well vs. ditch water), and field preparation (i.e., contour or laser planing) which decreased the utility of these factors for prediction of larval abundance (Palchick and Washino, 1985). However, the same study produced preliminary data on an association between water temperature, herbicide use, water depth, and numbers of mosquito larvae. This provided impetus for further investigations which were conducted in experimental rice fields in cooperation with the University of California Rice Integrated Pest Management program. Work in experimental plots eliminated uncontrolled variation in cultural practices found in commercial fields and allowed us to concentrate our efforts on the factors of water depth and weed management strategies. This paper summarizes the results of a preliminary analysis for the years 1984 through 1987.

Materials and Methods.

Study sites were located in Colusa County in 1984 and 1985, and in Sutter County from 1985 through 1987. Both were designed similarly with a 36 plot field divided by a central irrigation canal forming 18 straight-levee checks on either side. In Colusa, the checks measured 19.5 m x 128.0 m, while Sutter checks were 30.5 m x 93.0 m on the untreated side and 30.5 m x 125.0 m on the treated side. Four water management regimes (e.g., depth) were used: shallow (2.5-7.6 cm in Colusa, 2.5-5.1 cm in Sutter), medium (7.6-12.7 cm in both), deep (12.7-17.8 cm in Colusa, 15.2-20.3 cm in Sutter), and discontinuous flood which was drained and refilled (12.7-17.8 cm in Colusa, 15.2-20.3 cm in Sutter). These were arranged randomly in each of three replicates. The major difference between the two sites is that Colusa had uniform herbicide treatment in all 36 plots while Sutter had one side (18 plots) which remained untreated.

The survivorship and development of *Cx. tarsalis* and *An. freeborni* were investigated by placing laboratory strains of first instar larvae of both species into 1-gallon plastic, screened cages to minimize any effect from predation. The 1984 and 1985 studies were based on the extent of larval development at the end of either a 7- or 12-day observation period in each water treatment. In 1986 and 1987, larvae were observed continuously (i.e., daily) until pupal formation was observed. Pupae were then collected and held for emergence in the laboratory. The mean number of days taken for development from first larval instar to pupae was determined, and results evaluated for significant differences using Chi-square or Duncan's multiple-range test. Dipping was used to assess the native larval abundance.

Results and Discussion.

The larval survival pattern (Table 1) for *Cx. tarsalis* showed inconsistency from year to year and by study sites. Survival was highest in deep water plots and lowest in shallow water plots in the 1984 Colusa studies. This trend was not observed in 1985 as survival in all plots did not differ. The 1986 Sutter data showed the reverse of the 1984 results with lowest survival occurring in deep plots, while all other water management regimes produced

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similar survival. In 1987 there was high larval mortality in 22 of the 48 screened cages, presumably due to lowering of water for herbicide treatment, but observations of remaining larvae showed the highest survival pattern was associated with deep water plots.

Comparisons between the herbicide-treated and untreated plots for 1986 through 1987 in Sutter showed no relationship for *Cx. tarsalis*.

For *An. freeborni*, no significant differences for water or weed management regimes were found in 1986. In 1987, larval survivorship was generally low in shallow water plots and highest in deep water plots; this pattern was similar to *Cx. tarsalis* in 1984.

Larval development for *Cx. tarsalis* at both field sites in 1984, 1985, and 1987, was consistently more retarded in shallow water plots. No other plots showed termination of larval development at the third or earlier larval stages in either the 7-day (1984) or 12-day (1985) trials. In 1986, the larval development pattern did not differ significantly among the populations in plots with varying depths, but in 1987, the results again showed slowest development in the populations confined to the shallow plots.

Table 1.-Larval survival for *Cx. tarsalis* and *An. freeborni* in experimental rice fields with varying water management practices in Colusa and Sutter Counties, CA.

Year[1]	Percentage Larval Survival							
	Cx. tarsalis				*An. freeborni*			
	shal.	med.	deep	disc.	shal.	med.	deep	disc.
1984	55	75	87	64		----------		
1985	65	72	56	74	71	79	63	67
1986	38	35	18	41	14	18	12	14
1987	12	03	24	09	07	20	22	15

[1]1984(x=180), 1985(x=720), 1986(x=270), 1987(x=180)

Table 2.-Mean[1] larval development time for *Cx. tarsalis* and *An. freeborni* from first instar to pupae in experimental rice fields with varying water management practices in Sutter County, CA.

H20 Mgt. Practice	*Cx. tarsalis*		*An. freeborni*	
	1986	1987	1986	1987
shallow	10.11a	10.99a	21.79a	22.47a
medium	9.89a	9.83cb	19.56ab	20.37a
deep	9.85a	9.30c	16.41b	19.89a
disc.	9.47a	10.37ab	18.83ab	19.54a

[1]means with different letters are significant ($p<.05$)

No differences in larval development time were observed for *Cx. tarsalis* in plots treated with herbicide compared to untreated plots. However, for *An. freeborni*, development time was much shorter in treated than in untreated plots (15.1 and 30.2 days, respectively) during the 1987 tests. Unlike the results observed in previous years, it appears that the heavy stand of weeds, especially barnyard grass, *Echinochloa crusgalli* (L), may have contributed substantially to the extended larval development time in the untreated plots. Similarly, in 1986, during the second of two *An. freeborni* trials, prolonged development to a lesser extent was also observed when weeds became slightly taller than rice (13.22 days with herbicide vs 14.04 days without herbicide).

Water depth was another important factor for *An. freeborni*. Results for 1985 showed a greater number of larvae still in second instar in shallow water (i.e., 15 in shallow vs. 3 in medium, 7 in deep and 0 in discontinuous), and fewer attaining fourth stage (i.e., 3 in shallow vs. 4 in medium, 20 in deep and 26 in discontinuous). Results of 1986 data (Table 2) similarly yielded a trend of slow development in shallow water, but trials in 1987 showed no differences between water treatments.

Water depth and weed management practices emerged as most influential on larval survival and development in the course of investigating a variety of factors which may affect *An. freeborni* and *Cx. tarsalis* production in rice fields under controlled experimental conditions. It was hypothesized that shading from the unusually tall, dense plant canopy resulted in lower water temperatures, which in turn prolonged the larval development time. Mean temperature readings in the weedy plots were consistently lower than those in the weed-controlled plots (Figure 1).

While temperature data from the experimental rice fields with varying depths have not been analyzed for the four-year duration of the present study, results from earlier *in situ* studies indicated that temperatures in rice fields with shallow water management were subject to greater fluctuations and extremes than were fields with deep water management (Washino unpubl., Milby and Meyer

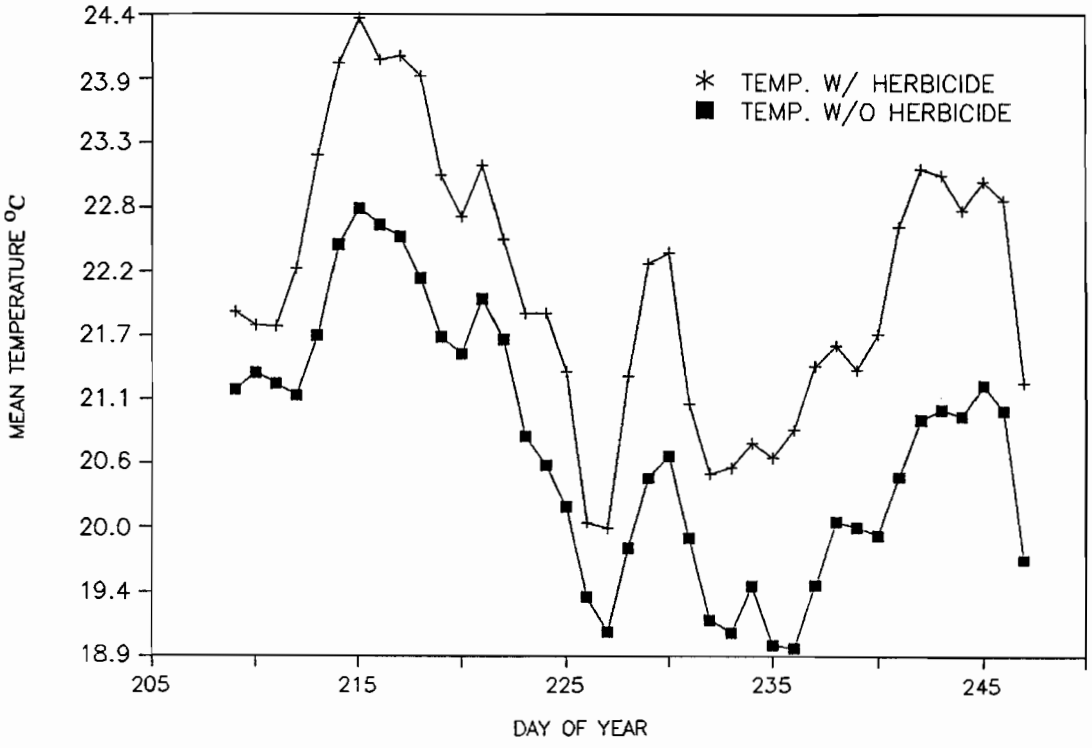

Figure 1.-Mean daily temperatures from plots without herbicide treatment in 1987 Sutter County experimental rice fields.

1986). Bailey and Gieke (1968) had previously reported on the major effects of water temperature on development and survival of *An. freeborni* and *Cx. tarsalis* larvae under both laboratory and simulated field conditions.

Results of the present study suggest that temperature was a most influential factor, but no definitive conclusion can be reached until the 4-year temperature data are analyzed and related to the factors of water depth and weed management. It is unfortunate that the naturally occurring mosquito larval population was too low to yield useful data to augment observations made on caged mosquito populations. In particular, data on the ovipositional attractiveness, and the survival and development of the field population in response to the variables studied, would have been a most useful adjunct to the study.

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THE INFLUENCE OF VEGETATION AND MOSQUITOFISH ON *CULEX TARSALIS* ABUNDANCE IN DUCK CLUB PONDS IN SOUTHERN CALIFORNIA

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ABSTRACT

The effectiveness of current mosquito abatement measures using mosquitofish was evaluated in two 1.8 ha duck club ponds in the Coachella Valley of southern California. The effects of *Gambusia* stocked at current operational densities (1.4 kg/ha), vegetation, and their interaction, were studied in 16 m^2 enclosures. As compared to non-vegetated plots, *Culex tarsalis* larval populations were considerably larger in plots that contained dense vegetative growth only along the pond perimeter, or both perimeter vegetation and dense stands of emergent vegetation in the pond interior. Mosquitofish stocked during the late summer and early autumn at 1.4 kg/ha did not significantly reduce mosquito larval populations in duck club plots. Concurrent studies in 36 m^2 ponds yielded equivalent results. *Culex tarsalis* larval abundance in ponds without fish did not differ significantly from that in ponds where *Gambusia* was stocked at 1 kg/ha. However, larval abundance in ponds containing mosquitofish stocked at the extremely high density of 4 kg/ha differed significantly from those in the control (without fish) and 1 kg/ha *Gambusia* treatments.

The interactions among the 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 developmental sites, complicate mosquito control efforts in duck club ponds. Mosquitofish populations in typical duck club ponds are subject to factors (predacious birds, thermally-stressful conditions, and, probably, low food abundance) that reduce survivorship and recruitment.

Introduction.

Duck club ponds in southern California have been identified as important developmental sites for *Culex tarsalis* Coquillett (Durso and Burguin 1988) and several other mosquito species. In the Coachella Valley, several thousand acres bordering on the northern end of the Salton Sea are flooded annually for recreational duck hunting. U.S. Fish and Wildlife regulations currently permit California duck clubs to provide supplemental forage to migrating waterfowl. Under these regulations, duck club managers stock small, peripheral ponds with additional forage and maintain large expanses of relatively open water on which ducks alight after foraging.

The duck ponds are disked in mid-summer and flooded during late August. Water is pumped into the ponds from wells, enters each pond via dropboxes, and flooding is completed after four to six weeks. If supplemental forage is provided, regulations stipulate that ponds must be kept full of water through approximately mid-January. In most ponds, water persists through mid-March.

Vegetation management practices differ among the duck clubs. Whereas the pond interiors are typically disked, the vegetation along the perimeter dikes is either removed or cut. When cut, the cuttings are left in place and inundated as the ponds are flooded. In those duck clubs where the perimeter vegetation is not removed, or where thick emergent vegetation develops (usually in the first pond flooded) ponds support large populations of mosquito larvae .

The prevailing method of mosquito abatement is to stock the ponds with the mosquitofish, *Gambusia affinis* (Baird and Girard), at initial densities approximating 1.4 kg/ha. Additional control measures utilizing insecticides are sometimes necessary, but are often difficult to carry out because access to the ponds is restricted during duck hunting season.

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Despite the stocking of mosquitofish, large numbers of adult mosquitoes are trapped in the vicinity of duck clubs (Durso and Burguin 1988). The effectiveness of current mosquito abatement measures using mosquitofish was studied during 1988 by examining the effects of *Gambusia* stocked at current operational densities (1.4 kg/ha), vegetation control, and the interaction between these two factors on mosquito larval populations inhabiting duck club ponds.

Materials and Methods.

The effects of vegetation and *Gambusia* on *Cx. tarsalis* larval abundance were studied in 16 m^2 enclosures. As flooding began during mid-August, three enclosures were positioned in each of two 1.8 ha ponds at the Adohr Valley Farms Duck Club in Mecca, California. Each enclosure was divided into four plots with partitions constructed of fiberglass window screening (approximately 7 openings/cm), buried in the bottom sediment, and supported by wooden stakes. Four treatments were randomized within three blocks (enclosures) in each pond: vegetation alone (V), non-vegetated alone (N), vegetation and *Gambusia* (FV), and non-vegetated and *Gambusia* (FN).

Interior vegetation in the N and FN plots was removed by hand and shoreline vegetation was removed with a shovel. A small amount of vegetation (approximately 0.5 m^2) was left along the partitions in the FN plots as a refuge for the fish, and in the N plots as a control for the FN vegetation. *Gambusia* adults were captured from a local pond after one week, weighed, and added to the appropriate plots.

The vegetation cover differed in the two ponds. One-quarter of the surface of Pond 1 was not disked and it supported a dense cover of grasses that was dominated by bearded sprangletop (*Leptochloa fascicularis* (Lam.)). The perimeter vegetation was primarily spikerush (*Eleocharis macrostachya* Britt.), with a thick growth of Bermuda (*Cynodon dactylon* (L.)), and saltgrass (*Distichlis spicata* (L.)). The enclosures in Pond 1 were situated within the dense vegetation. Pond 2 was typical of the remaining ponds in the duck club; the pond interior lacked emergent vegetation and the perimeter was surrounded by a dense growth of Bermuda and saltgrasses.

Mosquitoes, macroinvertebrates, and zooplankton were sampled with a 350 ml dipper. A stratified sampling procedure was followed in which three dips were taken (1) along the shore and (2) 3 m from the shore in each plot and in the pond adjacent to each block. The thick vegetation in Pond 1 precluded sampling by net. The enclosures were sampled weekly from late August through the start of duck hunting season in mid-October. The ponds were sampled once in late October after the enclosures had been removed. The contents of each set of 3 dips was combined, preserved in alcohol, identified and enumerated in the laboratory under a dissecting microscope. Mosquitofish in each plot were sampled every other week using a baited minnow trap that had been lined with window-screening. Water temperature was monitored with minimum-maximum thermometers.

The effects of fish, vegetation, and experimental manipulation (plots vs. pond) were compared for the Pond 1 data by a repeated measures ANOVA and linear contrasts. Because samples from plots through time were presumably autocorrelated, the effect of time (sample date) was removed from the main effects. Tests of this factor violate the assumptions of the ANOVA and are not reported here. To satisfy the assumptions of the ANOVA, counts from 1 m and 3 m strata were combined, log-transformed (count + 1), and analyzed for the entire larval population (1st though 4th instars). Because larvae were not captured in non-vegetated plots on 19 September, analyses were repeated after deleting this date from the data set (29 August through 10 October). The results reported did not change.

The mean and the variance of larval counts in the non-vegetated plots of Pond 2 were zero for most sampling dates, and parametric statistical analysis of these data was inappropriate. We compared the ranks of larval abundance in Pond 2 plots using Wilcoxon's Signed-Ranks tests. Larval counts in vegetated and nonvegetated plots were analyzed separately for each block and combined across blocks. Nominal values are provided in the discussion.

Results and Discussion.

Vegetation. The presence of emergent vegetation in the pond interiors and perimeter vegetation along the pond dikes significantly increased *Cx. tarsalis* larval populations as compared to non-vegetated plots. In Pond 1, mosquito larvae in vegetated plots were initially very abundant and de-

clined between four and six weeks after the pond was flooded (Fig. 1a). This trend was similar to that observed for *Cx. tarsalis* larvae in ponds at the Aquatic and Vector Control Research Facility (Oasis, California) located approximately 3 km northwest of Adohr Valley Farms (Mulla 1986, 1989). The abundance of mosquito larvae in non-vegetated plots in Pond 1 was significantly lower than that in vegetated plots ($F_{1,8}$ = 24.85, p = 0.001).

The phenology of the larval population differed in Pond 2. As compared to vegetated plots in Pond 1, *Cx. tarsalis* larvae were less abundant from 29 August through 19 September (Fig. 1b). Larval abundance increased markedly between 19 and 26 September. After the enclosures were placed into the ponds, approximately two-thirds of the water entering the ponds during mid-August was diverted elsewhere. Whereas water levels in Pond 1 were reduced very little, water levels in Pond 2 declined to a point where the shoreline vegetation was barely inundated. On about 19 September, the majority of water was diverted again into Ponds 1 and 2 and the shoreline vegetation in Pond 2 was reinundated to pre-diversion levels. Rising water levels, which reinundated the shoreline vegetation, and the vegetation that developed during the 5-6 weeks since manipulations were made, increased the available larval habitat and, perhaps, preferred mosquito oviposition sites.

Culex tarsalis larvae were considerably more abundant in vegetated plots than in non-vegetated plots of Pond 2 (Blocks combined: T_S = 0, $p < 0.01$). Unlike the blocks in Pond 1, larval populations in the blocks of Pond 2 were markedly heterogeneous. Very few larvae were captured in the plots of one block (Block A). Larval counts in vegetated plots did not differ significantly from those in nonvegetated plots (Block A: T_S = 5.5, $p > 0.05$). However, in the other two blocks (Block B and C), *Cx. tarsalis* larvae were significantly more abundant in vegetated plots than in the non-vegetated plots (Block B: T_S = 0, $p < 0.01$; Block C: T_S = 0, $p < 0.01$).

The differences of vegetation cover among the blocks were congruous with the effects reported above. By 26 September, vegetation extended approximately 1.5 m from the shoreline into the vegetated plots in blocks B and C . As compared to the vegetated plots in blocks B and C, the vegetation in the V and FV plots of block A was less extensive and did not differ noticeably from adjacent non-vegetated plots.

<u>*Gambusia affinis.*</u> Mosquitofish stocked at 1.4 kg/ha did not significantly reduce *Cx. tarsalis* larval populations as compared to plots that lacked fish. For reasons given below, the effect of mosquitofish was tested only for plots in Pond 1. The effect of *Gambusia* was not significant ($F_{1,8}$ = 2.63, $p > 0.14$). Also, the fish by vegetation interaction ($F_{1,8}$ = 0.05, $p > 0.8$) and pond versus plot comparison ($F_{1,8}$ = 0.42, $p > 0.53$) were not significant.

Concurrent studies conducted at our Aquatic and Vector Control Research Facility, in which we examined the effects of mosquitofish stocking density (0, 1, and 4 kg/ha) on *Cx. tarsalis* yielded equivalent results (Walton and Mulla, unpublished data). During late August and September, mosquito larval abundance in mesocosms stocked with 1 kg/ha *G. affinis* did not differ significantly from that in mesocosms without fish. However, mosquito larval populations were reduced significantly when *Gambusia* was stocked at the extremely high density of 4 kg/ha. Interestingly, a similar study during the spring provided different results. As compared to non-fish controls, mosquito larval populations were reduced markedly and equally by mosquitofish stocked at 1 and 4 kg/ha (Walton et al. unpublished data).

We were unable to assess the effect of *Gambusia* in Pond 2 because the fish were eliminated from all plots. Mosquitofish probably were eliminated by predation and thermal stress. Ardeids (herons and egrets) were observed in large flocks in and around duck club ponds undergoing inundation. Prior to moving to newly flooded habitats, these predators were observed foraging in the enclosures and throughout both ponds. In addition to predation, the shallow water depths, reduced interior vegetation, and hot desert temperatures created thermally-stressful conditions for *Gambusia* (Fig. 2a). Maximum water temperatures during the period from 29 August through 12 September exceeded *Gambusia*'s thermal maximum (Castleberry and Cech, unpublished data). The denser vegetation cover and lower water temperatures in Pond 1 permitted some fish to survive and reproduce in all plots containing fish (Fig. 2b).

<u>General Discussion:</u> Current mosquito abatement methods using mosquitofish in duck clubs of the Coachella Valley are, at times, ineffec-

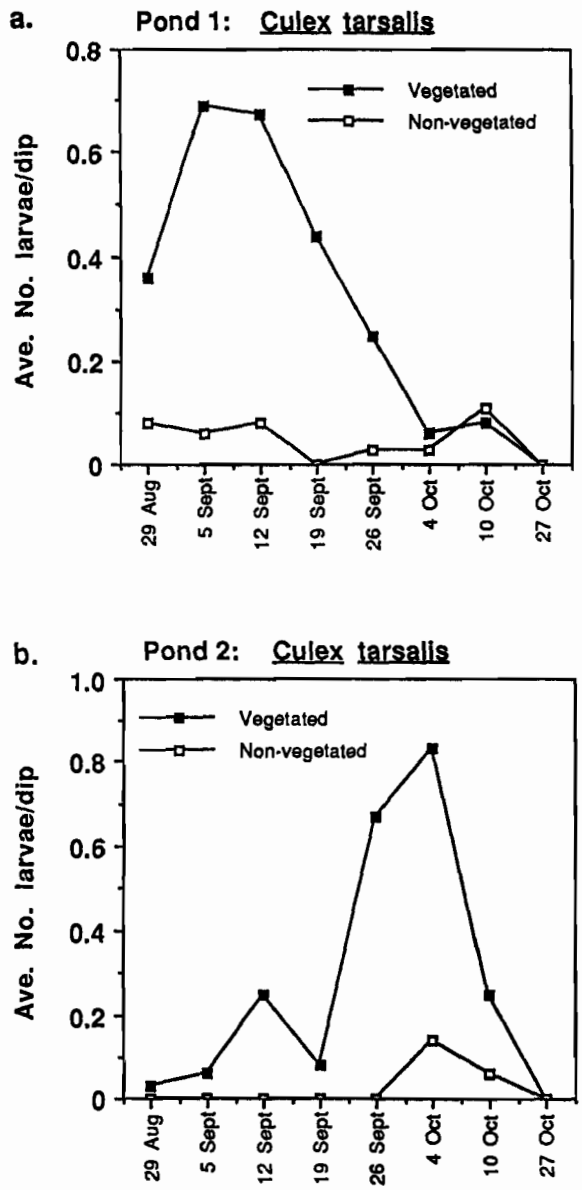

Figure 1.-Average abundances of *Cx. tarsalis* larvae in vegetated and non-vegetated plots in (a) Pond 1 and (b) Pond 2. Averages were computed after combining data from V and FV plots (vegetated) or N and F plots (non-vegetated).

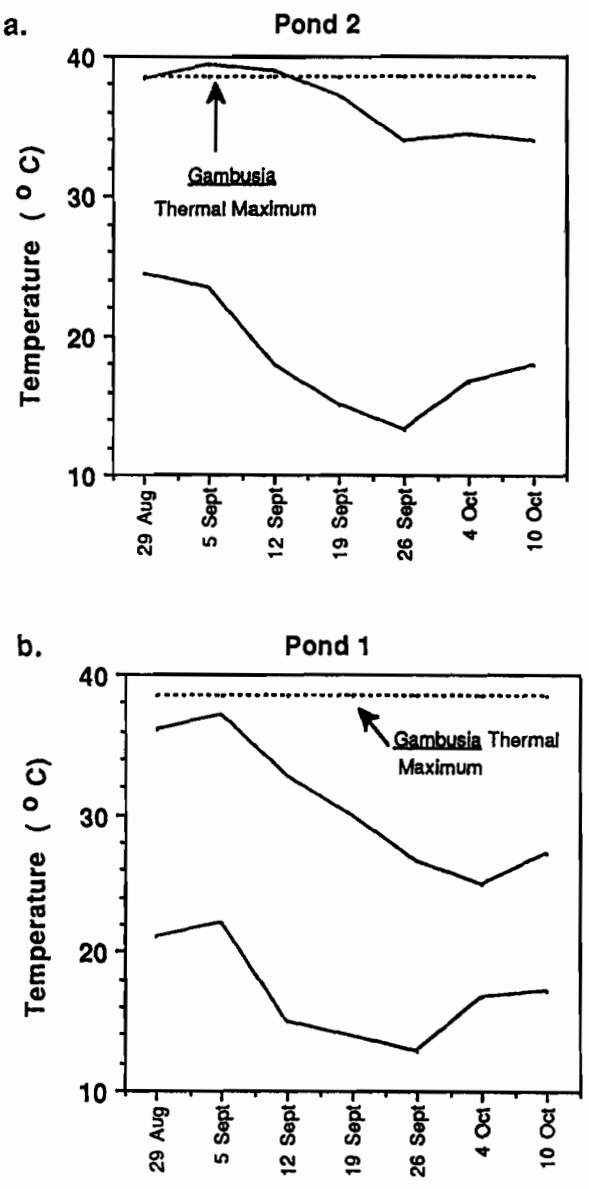

Figure 2.-The maximum and minimum water temperatures recorded in (a) Pond 2 and (b) Pond 1 during 1988. The broken line is the thermal maximum for *G. affinis* (Castleberry and Cech, unpublished data).

Table 1.-Percent of *Gambusia affinis* digestive tracts containing terrestrial or molluscan prey in three studies during 1988. n = sample size.

Study	Terrestrial	Snails	
U.C.R. Ponds (Spring):	3	0	(n=35)
U.C.R. Ponds (Fall):	36	0	(n=22)
Adohr Valley Farms:	62	35	(n=52)

tive or, at the least, complex. The interactions among the 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 developmental sites, complicate mosquito control efforts in duck club ponds.

Several factors reduce recruitment and survival of *Gambusia*. First, mosquitofish undergo a photoperiodically-induced reproductive decline during the autumn (Sawara 1974, Milton and Arthington 1983). This decline coincides with or precedes the time at which *Gambusia* are stocked into duck club ponds. Studies conducted at our Aquatic and Vector Control Research Facility in the Coachella Valley confirm that, during the fall, *Gambusia* stocking densities must be greater than would be required to achieve a comparable level of mosquito control during the spring or early summer.

Second, mosquitofish mortality is high in typical duck club ponds because of reduced vegetative cover and shallow water depth (approximately 22 to 30 cm). *Gambusia* succumb to predation by birds and to thermal stress where water temperatures approach 40°C. High water temperatures additionally must reduce mosquitofish reproduction (Coykendall 1980), and restrict fish to cooler microhabitats such as small stands of emergent vegetation or dense shoreline vegetation. In Pond 2, predation, thermal stress, and water diversions restricted the small, resident mosquitofish population to the dense emergent vegetation in one corner of the pond.

Third, aquatic macroinvertebrate abundance in duck club ponds, as compared to the 36 m^2 ponds at our Coachella Valley facility, is relatively low. Given the proximity of the duck club ponds to the Salton Sea and the salinity of the soil in the Coachella Valley, the macroinvertebrate fauna was dominated by halophilic and, presumably, more euryhaline species. Aquatic macroinvertebrate abundance in Pond 1 was 2 to 5 times lower than that observed in our Oasis ponds (Fig. 3a). In more typical duck club ponds, such as Pond 2, aquatic macroinvertebrates were even less abundant (Fig. 3b). Total aquatic macroinvertebrate abundance in Pond 2 was 10 to 18 times lower than that observed in the Oasis ponds.

Although aquatic macroinvertebrate abundance in duck club ponds was lower than that recorded in the Oasis ponds, zooplankton populations (cladocerans and ostracods) in the duck club ponds were larger than those in the Oasis ponds (Figs. 4a and 4b). During the four to five weeks after flooding, duck club zooplankton could differ from that in the Oasis ponds by more than an order of magnitude.

Whereas small-sized *Gambusia* were observed to feed primarily on zooplankton and chironomid midges, larger-sized *Gambusia* (> 1.5 cm standard length) in duck club ponds were incorporating terrestrial and molluscan (snails) prey into their diets. The proportion of *Gambusia* digestive tracts containing terrestrial or molluscan prey was

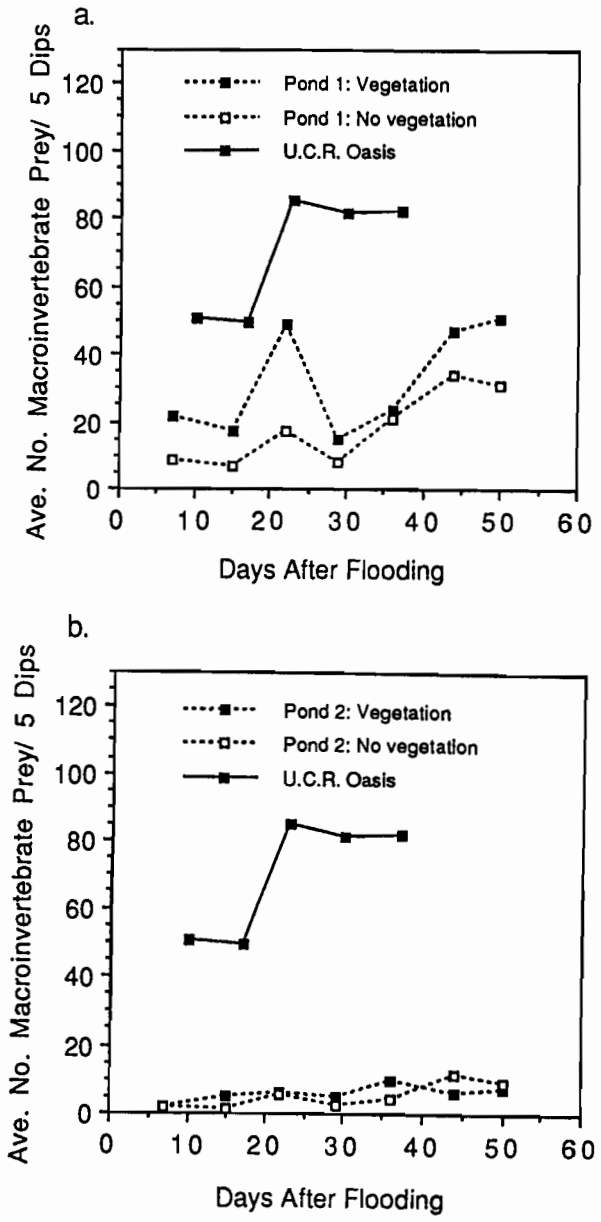

Figure 3.-The average number of aquatic macroinvertebrate in prey five 350 ml dipper samples from duck club plots and 36 m^2 ponds at the U. C. R. Aquatic and Vector Control Research Facility (Oasis, California) during the late summer and early autumn 1988. a. Comparison of macroinvertebrate abundance in plots of Pond 1 and the Oasis ponds; b. Comparison of macroinvertebrate abundance in plots of Pond 2 and the Oasis ponds.

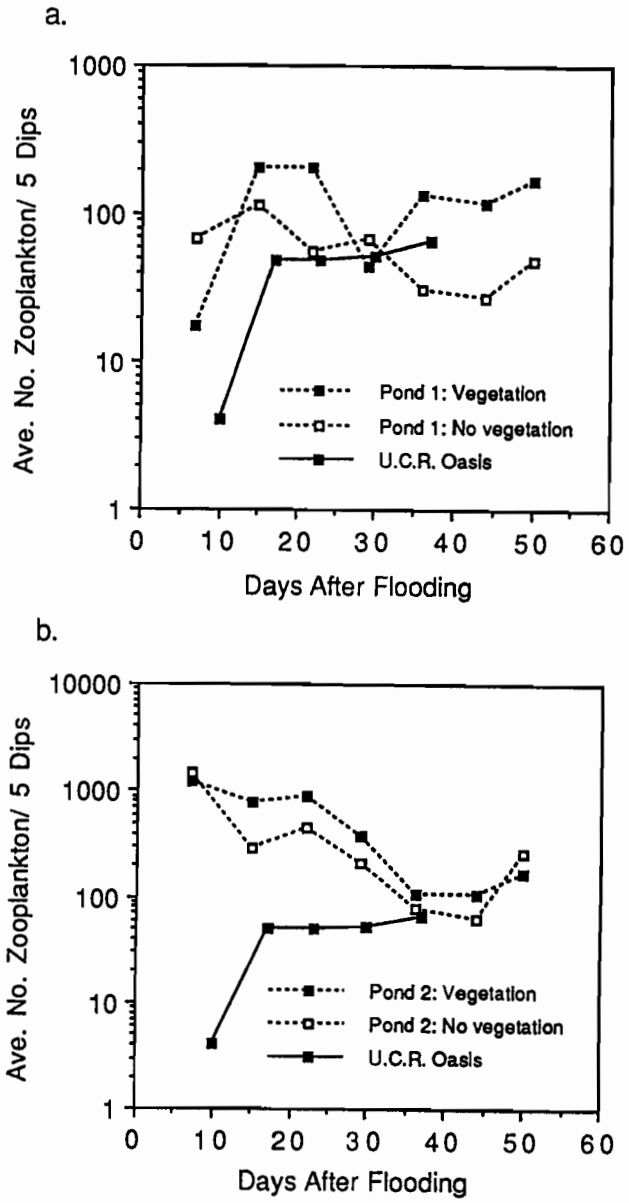

Figure 4.-The average number of zooplankton (cladocerans and ostracods) in five 350 ml dipper samples from duck club plots and 36 m^2 ponds at the U. C. R. Aquatic and Vector Control Research Facility (Oasis, California) during the late summer and early autumn 1988. a. Comparison of zooplankton abundance in plots of Pond 1 and the Oasis ponds; b. Comparison of zooplankton abundance in plots of Pond 2 and the Oasis ponds.

greater for mosquitofish in duck club ponds than for mosquitofish in the Oasis ponds (Table 1). Although snails were present in the Oasis ponds, *Gambusia* did not feed on them when alternate prey were abundant. Norland and Bowman (1976) suggested that food supply was an important determinant of mosquitofish population size. It is not known at the present time whether adult *Gambusia* in duck club ponds, by preying on more sclerous terrestrial prey or more heavily armored molluscan prey, meet their metabolic demands or suffer further reproductive declines. Unlike molluscivorous fishes, *Gambusia* never cracked the shells of *Physa* sp. or *Gyraulus* sp..

The late stocking date, reduced food abundance, and reduced interior vegetation decreased mosquitofish reproduction and survival in Coachella Valley duck club habitats. Mosquitofish populations in typical duck club ponds, which lack interior vegetation, are reduced by predation and thermal stress. Although increased vegetative cover might reduce the natural mortality factors operating in duck club ponds, studies in a variety of habitats have shown that emergent vegetation decreased mosquitofish effectiveness as a biological control agent (Craven and Steelman 1968, Meisch 1985, Orr and Resh 1987, Morton et al. 1988).

Vegetation and water management practices in California duck clubs will change if the proposed amendments to U.S. Fish and Wildlife Service regulations are enacted in 1991. If the supplemental forage practice is discontinued, the flooded acreage will likely increase to accommodate the need to provide waterfowl with attractive natural forage. Duck clubs will maintain a mosaic of densely vegetated "foraging" habitats and relatively open, non-vegetated "resting" or "loafing" habitats. The latter are also preferred for duck hunting.

In addition, flooding will be prolonged to enhance the forage that attracts migrating waterfowl. Flooding is currently completed in 4 to 6 weeks. Flooding will occur for longer periods in order to provide the waterfowl with natural forage at various growth stages, and it will also provide *Cx. tarsalis* with preferred ovipositional and larval developmental sites (newly-flooded habitats: Fanara and Mulla 1974). Such changes in vegetation and water management will probably exacerbate current mosquito problems.

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POPULATION GROWTH OF *GAMBUSIA AFFINIS* AT THREE STOCKING DATES IN WILD RICE

Arthur E. Colwell[1] and Richard Garcia[2]

ABSTRACT

Mosquitofish (*Gambusia affinis*) were stocked in experimental wild rice (*Zizania palustris*) plots at either 4, 15, or 28 days post-flooding. Some food (e.g., flying aquatic insects, terrestrial insects, isopods, arachnids) was available even to the early stocked fish. Outflow monitoring indicated fish emigration from the plots was not extensive early in the season, and was not markedly different among stocking dates throughout the study. Some zooplankton (e.g., cladoceran) densities became significantly lower in the plots with fish than in the control (without fish) plots, but the mosquitofish reproduced well throughout the season. At the termination of the study (110 days post-flooding) there were no notable differences among stocking dates in the stomach fullness indices, condition factors, weights, lengths, pregnancy rates, numbers of embryos per female, or densities of mosquitofish.

Introduction.

The mosquitofish, *Gambusia affinis* (Baird and Girard), is the most widely used agent for the biological control of mosquitoes (Meisch 1985). It has been introduced into drainage ditches, ponds, and many other small aquatic habitats. It can also be effective in controlling rice field mosquitoes (e.g., Hoy and Reed 1971). Stocking mosquitofish is usually more economical than chemical larviciding (which may require repeated applications by aircraft) for rice field mosquito control (Lichtenberg and Getz 1985). As mosquito populations develop pesticide resistance, mosquitofish may be relied upon more heavily to control rice field mosquitoes.

The stocking rate (amount of fish per area) for mosquitofish in rice fields has been studied extensively (reviewed by Meisch 1985). However, the less-studied factor of the stocking date may also be important. If fish were planted in rice fields too early in the season, they might (1) starve to death before there are prey in the field, (2) eat enough food to survive but reproduce poorly, (3) eat newborn mosquitofish when food is scarce and refuges for fry are unavailable, (4) be eaten by predators (Britton and Moser 1982) before refuges develop, or (5) leave the fields to escape predation or search for food. If mosquitofish were planted too late they might not have time to reproduce and develop a population density sufficiently high to control immature mosquitoes.

Farley and Younce (1977) found that stocking fields of white rice (*Oryza sativa* Linn.) 15 to 25 days after seeding gave the best mosquitofish population growth. However, the optimum fish stocking date in fields of wild rice (*Zizania palustris* Linn.) has not been studied. Wild rice can attain heights of 3 meters and has a much fuller canopy than the 1 meter tall white rice (Kramer and Garcia 1988). Densities of mosquitoes (e.g., *Culex tarsalis* Coquillett, *Anopheles freeborni* Aitken) can be very high in wild rice, and the stocking rate of mosquitofish necessary to control mosquitoes is higher for wild than white rice (Kramer et al. 1988). The present study was undertaken to determine the effect of stocking date on the development of mosquitofish populations in wild rice.

Materials and Methods.

The study was conducted in the 0.1 ha experimental wild rice plots described by Kramer et al. (1988). Each plot had an independent water supply which was screened to prevent the entry of fish into the plot.

All the rice plots were flooded on 23 May 1988, and seeded with wild rice on 24 May 1988. Three (randomly selected) control plots received no fish. Three early (4 days post-flooding), three medium (15 days post), and three late (28 days post) plots were each stocked with mosquitofish at a rate of 1.0 kg/ha.

Mosquitofish populations in the plots were monitored biweekly for 3.5 months with small mesh (0.3 cm apertures) minnow traps. Four traps (one

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per each side of the plot) were placed (at randomly selected sites) in each plot at 1100 hrs and removed at 0900 hrs on the following day. All fish were counted and returned to their respective plots. However, at the termination of the study, a sample of 10 randomly selected fish was taken from each plot and frozen. The mosquitofish were dissected later and the stomach fullness index (SFI) for each fish was calculated according to the following formula (Windell 1971):

$$SFI(pptt) = \frac{\text{weight of stomach contents (g)} \times 10^4}{\text{weight of fish (g)}}$$

The condition factor (K), indicating the fitness of each fish, was calculated from the formula (Tesch 1971):

$$K = \frac{\text{weight of fish without viscera (g)} \times 10^7}{\text{standard length of fish (mm)}^3}$$

Female fish were dissected for reproductive data. Embryos were classified by the methods of Colwell and Schaefer (1983).

The fish exiting each rice plot were collected by a metal screen bag (1.0 mm mesh apertures) attached to each outflow box. The exiting fish were counted once per week but were not returned to the study site.

To determine whether any flying insects appeared in the rice plots soon after flooding, twenty 350 ml dip samples were taken in each of two early plots immediately before the fish were stocked. Samples were preserved in 80% ethanol and all animals present were identified and counted.

Plankton densities were monitored by collecting 63 dip samples (350 ml each) at randomly selected perimeter stations from each plot at 9 and 107 days after flooding. The samples were concentrated in the field with a net having 0.3 mm apertures. Samples were preserved and analyzed by the methods of Colwell and Schaefer (1980).

Water temperatures in each of six plots were recorded every hour by a data logger. The study was terminated at 110 days post-flooding. Temperature and outflow trap data were analyzed by single regression analysis. Other data were analyzed by one-way analysis of variance followed by Student-Newman-Keuls multiple range tests (Zar 1974).

Results and Discussion.

There was some concern that early in the season there would be inadequate cover (algae or macrophytes) in the plots for fish to hide from predators, so early planted fish might leave the fields to avoid predation (or search for food). Farley and Younce (1977) reported that two weeks after stocking, three times as many fish were leaving early (0-7 days post-seeding) stocked white rice fields as compared with medium (15-25 days post-seeding) stocked fields. However, in the present wild rice study, no fish from the early stocked plots appeared in the outflow collections during the first 30 days post-flooding (Figure 1). There were no

Table 1.-Numbers of organisms per dip in wild rice plots at 4 days postflooding.

Organism	Number per dip sample
Insecta	
Formicidae (A)	0.03
Apocrita (A)	0.03
Culex tarsalis (L)	0.08
Aedes melanimon (L)	0.13
Syrphidae (L)	0.28
other Diptera (A)	0.03
Heteroceridae (A)	0.15
Staphylinidae (A)	0.03
Curculionidae (A)	0.03
Dytiscidae (A)	0.03
other Coleoptera (A)	0.05
Belostoma flumineum	0.03
Aphididae	0.08
Collembola	0.05
Arachnida	0.05
Isopoda	0.05
Copepoda	1.75
Cladocera	0.20
Gastropoda (*Physa*)	0.13
Oligochaeta	0.03

L = larva, A = adult.

Table 2.-Numbers of organisms per liter in wild rice plots at 9 days and at 107 days post-flooding.

Organism	No fish		Fish early		Fish medium		Fish late	
	9 days	107 days	9 days	107 days	9 days	107 days	9 days	107 days
Chrysophyta (diatoms)								
Melosira sp.	5426.5	0.0	7752.1	0.0	1550.4	0.0	775.2	15504.2
Other Pennate Diatoms	1783.0	17364.7	4341.2	16667.0	4496.2	18992.6	1317.9	17917.3
Chlorophyta (green algae)								
Pediastrum sp.	0.0	0.0	0.0	155.0	0.0	232.6	0.0	155.0
Scenedesmus sp.	0.0	77.5	0.0	465.1	0.0	310.1	0.0	77.5
Rotifera (rotifers)								
Asplanchna sp.	0.0	0.0	0.0	310.1	0.0	155.0	0.0	387.6
Polyarthra sp.	0.0	0.0	0.0	77.5	0.0	0.0	0.0	155.0
Keratella sp.	0.0	155.0	0.0	0.0	0.0	0.0	0.0	310.1
Philodina sp.	0.0	0.0	0.0	0.0	0.0	155.0	0.0	0.0
Conochilus sp.	0.0	0.0	0.0	232.6	0.0	77.5	0.0	0.0
Brachionus sp.	0.0	155.0	0.0	0.0	0.0	0.0	0.0	387.6
Cladocera (water fleas)								
Ceriodaphnia sp.	3.6	102.2	0.0	6.1	6.0	3.6	3.6	1.8
Simocephalus sp.	0.0	0.6	0.0	0.0	0.0	0.0	0.0	1.2
Chydorus sp.	0.0	38.1	0.0	3.6	0.0	13.3	0.6	7.3
Copepoda (copepods)								
nauplii	0.0	1.2	0.0	5.4	10.3	6.1	1.2	24.8
Acanthocyclops sp.	19.4	21.8	11.5	29.0	16.3	38.7	20.6	81.0
Ostracoda (seed shrimp)	0.0	182.0	1.8	7.9	3.0	29.0	0.0	65.3

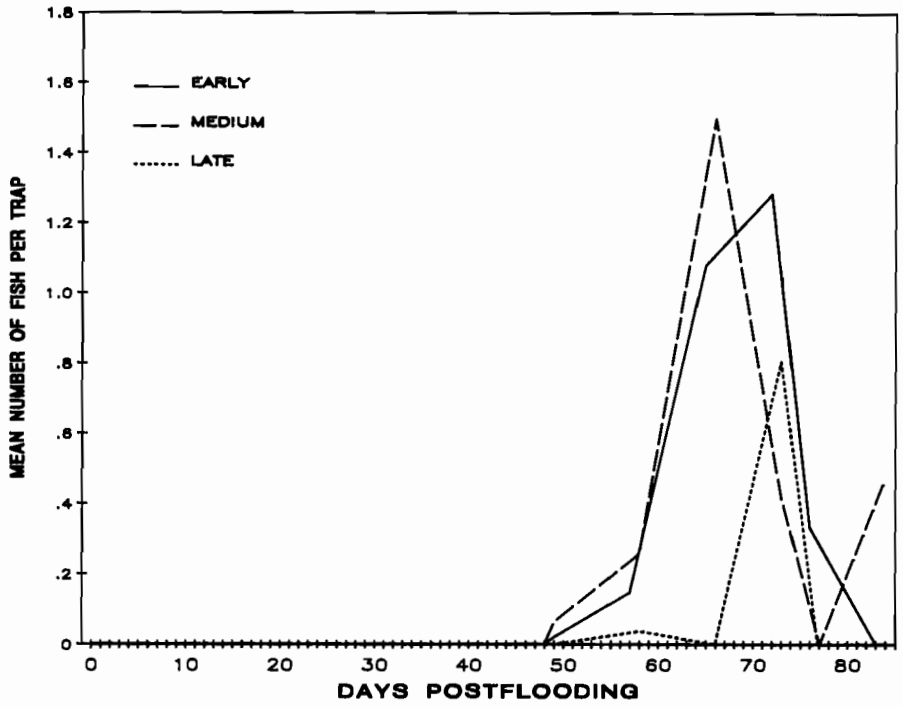

Figure 1.-Mean number of mosquitofish per plot per day collected in outflow traps at plots stocked with fish early (4 days post-flooding), medium (15 days post), or late (28 days post) in the wild rice growing season.

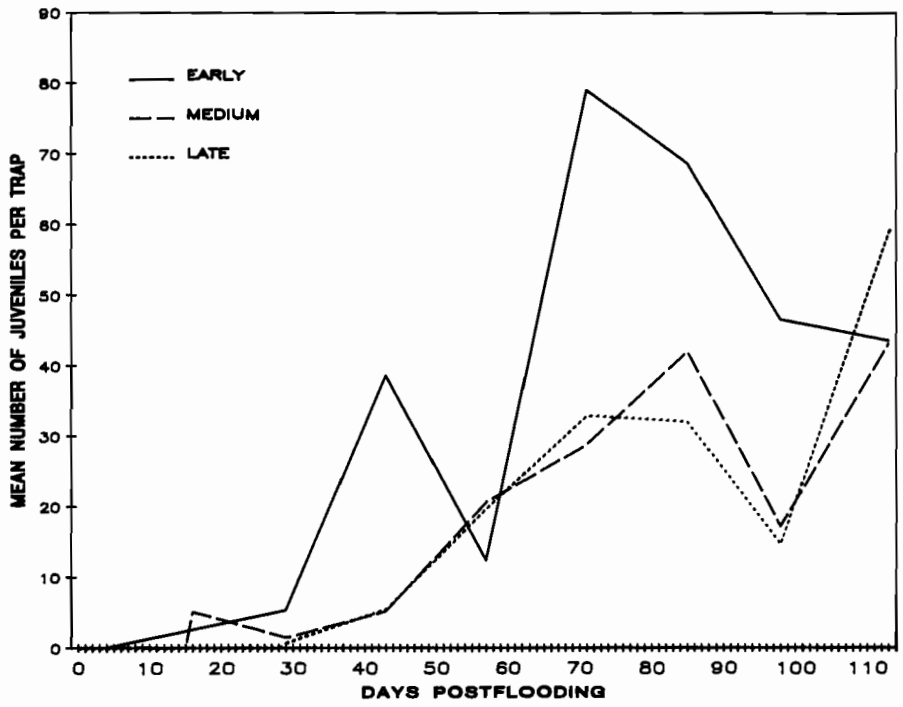

Figure 2.-Mean number of juvenile mosquitofish collected per trap per day by small mesh minnow traps in plots stocked with fish early, medium, or late in the season.

significant differences ($P>0.05$) among the three stocking dates in the total fish leaving the fields. Maximum exodus rates of ca. one fish per plot per day occurred during a period (60-75 days post-flooding) when there were thousands of fish in each plot. Therefore, the exodus may not be important in reducing total numbers of wild rice field fish. Coykendall (1981) found that mosquitofish emigration from white rice fields was positively correlated with water temperature. The number of fish leaving the wild rice plots during the present study was not correlated ($P>0.05$) with water temperature. This exodus may have been due to a dispersal phase which normally occurs at that time of the season or to overcrowding (Robbins et al. 1987).

In some white rice studies, borrow pit ditches adjacent to levees might have provided open-water channels which facilitated movements of mosquitofish. In the present wild rice study there were no borrow pits so the water depth and plant density was more uniform throughout the laser-leveled fields.

Table 1 indicates that there was some food available in the plots prior to the stocking of the early fish. Some adult aquatic insects had apparently flown into the plots. Terrestrial insects, isopods, and arachnids might have been in the plots when they were flooded or may have fallen from the levees into the water.

Later in the season the truly aquatic organisms (Table 2) reproducing in the rice plots may become the main food supply for the mosquitofish. Some of these organisms (e.g., phytoplankton) are not normally eaten, but cladocerans are usually the predominate food organisms for mosquitofish (Washino and Hokama 1967). Cladocera were significantly ($P<0.05$) more abundant in the control (without fish) plots than in the plots with fish.

If fish stocked early in the rice growing season found enough food to survive but were undernourished, they might not reproduce well. If they did reproduce but there was no cover (rice plants or algae) for the juveniles, the hungry adults in early stocked fields might eat their young (Dionne 1985). However, neither of the above possibilities was confirmed in the present study (Figure 2). The early stocked fish reproduced well, and there were no significant differences ($P>0.05$) in the numbers of juveniles collected in the early, medium, and late stocked plots at the termination

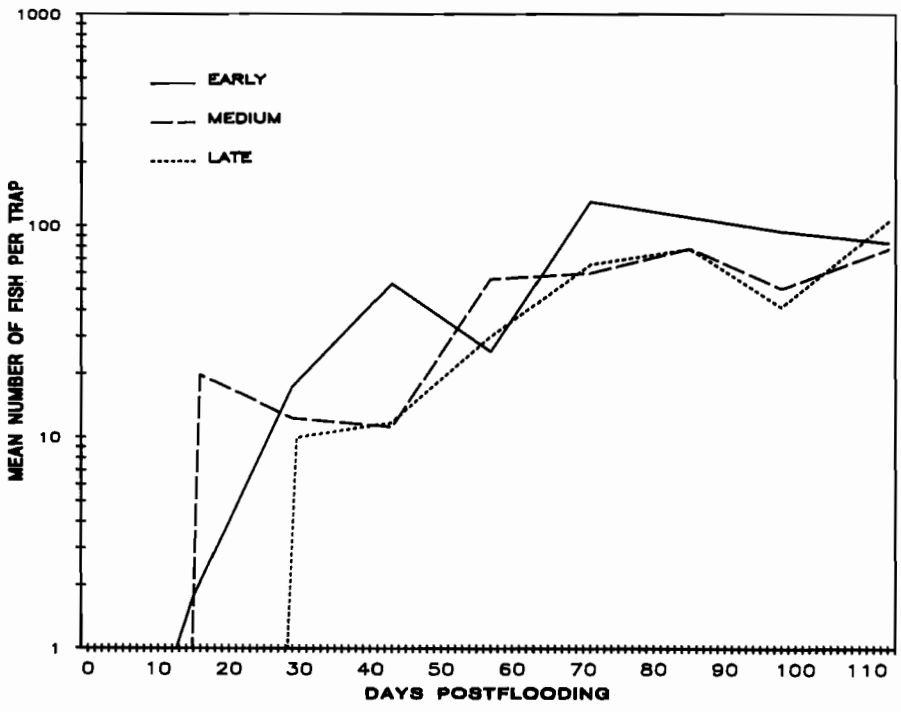

Figure 3.-Mean number of total mosquitofish collected per trap per day by minnow traps in wild rice plots stocked with fish early, medium, or late in the season.

Table 3.-Data for early, medium and late stocked wild rice plots from female mosquitofish collected at 110 days post-flooding.

Plot (No.)	Mean condition factor (K)	Mean weight of fish (g)	Mean weight of stomach contents (g)	Mean stomach fullness index (pptt)	Mean standard length (mm)
7	208.0	0.625	0.017	272.0	31.1
14	159.8	1.023	0.039	381.2	40.0
16	182.8	1.122	0.060	534.8	39.5
Mean early	183.5	0.923	0.039	396.0	36.9
1	180.5	1.228	0.030	244.3	40.8
13	196.1	0.925	0.050	540.5	36.1
15	171.1	1.007	0.057	566.0	38.9
Mean medium	182.5	1.053	0.046	450.3	38.6
3	161.7	1.004	0.025	249.0	39.6
4	171.8	1.011	0.030	296.7	38.9
18	181.5	1.144	0.068	594.4	39.8
Mean late	171.6	1.053	0.041	380.1	39.4

Table 4.-Data for early, medium, and late stocked rice from female mosquitofish collected at 110 days post-flooding.

Plot (No.)	Females pregnant (%)	Mean number of embryos per pregnant female				
		Mature	Tailed	Eyed	Early	Total
7	36.4	5.3	21.3	7.3	0.0	33.8
14	62.5	0.0	4.6	7.6	8.8	21.0
16	45.5	0.8	26.2	16.6	0.0	43.6
Mean early	48.1	2.0	17.4	10.5	2.9	32.8
1	45.5	3.6	19.0	0.0	15.4	38.0
13	62.5	0.4	16.4	11.8	0.0	28.6
15	80.0	3.9	5.5	10.6	4.8	24.8
Mean medium	62.7	2.6	13.6	7.5	6.7	30.5
3	60.0	3.2	0.3	21.2	11.5	36.2
4	50.0	21.8	8.4	0.0	3.6	33.8
18	60.0	1.3	28.7	0.0	2.2	32.2
Mean late	56.7	8.8	12.5	7.1	5.8	34.1

of the study (110 days post-flooding).

The mean total numbers of mosquitofish were not markedly different ($P>0.05$) among the early, medium, and late stocking dates on the final sampling date. Mosquitofish densities reached ca. 100 fish per trap (Figure 3). These data support the suggestion by Kramer et al. (1988) that the mosquitofish carrying capacity may be higher in wild rice than in white rice fields.

Miura and Takahashi (1987) reported that fish collected at the end of a study in white rice plots were "extremely light and slimmish" due to a deficiency of food resources, but the possible effect of stocking date on this problem is unknown. Fish collected at the end of the present study in wild rice plots appeared robust. There were no significant differences ($P>0.05$) between early, medium, and late stocked plots in the weights of mosquitofish, weights of stomach contents, lengths of fish, stomach fullness indices, or condition factors (Table 3). Miura and Takahashi (1987) suggested that late season food shortages in white rice plots could result in reduced fecundity of the mosquitofish. However, late season pregnant mosquitofish (Table 4) in the wild rice plots averaged 32.8 (early), 30.5 (medium), and 34.0 (late) total embryos per female (no significant differences). These values compare favorably with the 13.0 embryos per pregnant female reported for the same month in nearby small ponds (Colwell and Schaefer 1983).

Overall, the results of this study indicated that early stocking of mosquitofish did not result in a population decrease due to starvation, or to fish exiting the plots or due to excessive predation. The early stocked fish reproduced well throughout the season. Late stocked fish also reproduced well and developed high population densities. These small-plot findings suggest that it may be useful for agencies concerned with the biological control of mosquitoes to stock mosquitofish into large commercial wild rice fields at any time during the period (4 to 28 days post-flooding) studied.

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THE EFFECTS ON YIELD OF *BTI* TREATMENTS AT THE FLOWERING STAGE OF RICE

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Introduction.

Rice growers in the Sutter-Yuba Mosquito Abatement District expressed concern about possible crop damage during the 1986, 1987, and 1988 applications of *Bacillus thuringiensis*, serotype H14 (*Bti*). They felt applications of *Bti* during the critical flowering stage might decrease grain yields, and for this reason the District avoided treatments with *Bti* during the flowering stage for the three seasons. This impacted approximately three weeks and up to fifteen thousand acres each year of potential control efforts. Historically, peak larval density of *Anopheles freeborni* Aitken occurs about the time of rice flowering.

No data were available on *Bti* crop injury specific to rice. The manufacturers' labels for *Bti* make no mention of avoiding applications to rice during the flowering stage. Trials were established to examine the effects of *Bti* treatments during various stages of rice flowering on grain yield and other related parameters.

Materials and Methods.

The University of California Cooperative Extension allowed the District to use one of its rice research sites in South Sutter County, near Nicolaus, California for the small plot work done in 1987. Maintenance and harvest of the crop were performed by the University of California Cooperative Extension.

The randomized complete block design was used for this trial. There were six treatments, including controls, with five replications each in a randomized, complete block design. Treatments were assigned to five blocks from a table of random numbers. The 30 experimental plots were 10 x 20 ft each. The six treatments were as follows: 1) *Bti* applied when 20% of the rice stand had flowered, 2) *Bti* applied when 50% of the rice stand had flowered, 3) *Bti* applied when 100% of the rice stand had flowered, 4) *Bti* applied at all three stages, 5) no *Bti* applied, plot walked through as when making an application and 6) no *Bti* applied, plot not walked through.

All *Bti* applications were made with Zoecon's Teknar™ (600 International Toxic Units). The dosage rate was 32 ounces per acre, the maximum labeled rate. Applications were made using a Herbi® hand-held atomizer. Calibration was performed with *Bti* finished spray mix (diluted three parts *Bti* to one part water) to determine the swath width (10 feet), and flow rate (2.0 oz./min.). Application speed was adjusted in the field to 2.31 miles per hour. The droplet size for material delivered by the Herbi very closely resembled the droplet size of the Micronair® rotary atomizer used in the District's normal rice field applications, as indicated by both the manufacturers' specifications. The dates the actual applications took place were as follows: Treatment 1 on August 19, Treatment 2 on August 24, Treatment 3 on September 1, Treatment 4 on August 19, 24, and September 1, and Treatment 5 on September 1.

The rice, cultivar M-201, was harvested on October 21, 1987. The University of California Cooperative Extension used a specially designed harvester, which cut a 7 1/2 foot swath down the center of each plot. The grain from each plot was weighed and the moisture content measured with a Motomco® moisture meter. The average height of the rice stand was also recorded. The values for the weight of the grain and the moisture content were used in calculating the yield in pounds per acre. Final dry grain yield was standardized to 14%.

A 40 acre rice field near Yuba City, California was utilized for the field trail performed in 1988. The field was farmed by one of the District's Board of Trustee members. A simple strip design was used with two treatments, sprayed and non-sprayed, with five replications. Ten experimental plots were arranged in the field, side by side, alternating treatments and controls. Each plot was approximately 200 x 520 ft.

The treatment was made when 50% of the rice stand had flowered, which occurred on August 7. The application was made with Zoecon's Teknar (600 International Toxic Units) at a rate of five ounces per acre, neat. Applications were made using a Cessna Ag Wagon 250 airplane equipped with

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Table 1.-The effects of *Bti* applications on rice height, moisture at harvest, and yield, 1987 study.

Treatment Time	Plant Height (cm)	Harvest Moisture Content (%)	Grain Yield (LB/A) @14% H20
1. 20% headed	78	21.2	10 519
2. 50% headed	77	21.4	10 477
3. 100% headed	76	21.3	10 757
4. 20+50+100%	77	21.0	10 587
5. Walk through	77	21.1	10 550
6. Untreated	77	21.1	10 613
CV (%)	4.3	2.3	2.3
LSD (.05)	NS	NS	NS

Table 2.-The effects of *Bti* application on rice moisture at harvest and yield, 1988 trial.

Treatment	Harvest Moisture Content (%)	Grain Yield (LB/A) @14%H20
1. Sprayed when 50% headed	25.4	9013
2. Untreated	26.2	8964
CV (%)	8.7	6.6
F- Value	0.70	0.05

two Mini Micronair® rotary atomizers. This was the same application rate and equipment used in the District's operational rice field control program.

The rice, cultivar M-201, was harvested on September 29. The grower harvested each plot using a John Deere 7700 rice harvester with a 15.5 foot swath. Two subsamples of 200-250 ft. in length were taken from each plot. Each sample was weighed on a portable scale and a small sub-sample was taken to determine moisture content. These figures were used to calculate grain yield in pounds per acre at 14% moisture content.

Results.

None of the treatments in the 1987 small plot trial had a significant effect on plant height, moisture content of harvested grain, or grain yield (Table 1). Variation was very small in all parameters measured, lending credence to the results. In the 1988 field trial, the spray treatment had no significant effect on moisture content of harvested grain or grain yield (Table 2). Again, variance was very small.

Conclusion.

We conclude that the application of *Bti* to rice in various stages of flowering had no significant effect on grain yield, height, or crop maturity (as measured by harvest moisture content). The District will recommend to its board that applications of *Bti* continue throughout the entire rice growing season for 1989.

ASSESSING *LAMBORNELLA CLARKI* AS A POTENTIAL BIOLOGICAL CONTROL AGENT FOR *AEDES ALBOPICTUS*

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The Asian Tiger Mosquito, *Aedes albopictus* (Skuse), was first discovered in the United States in Houston, Texas in 1985, and it now occurs throughout much of the eastern and southern regions of the country (Hawley et al. 1987, Moore et al. 1988). While this important vector species does not presently occur in California, there are few available data for evaluating the potential for its successful establishment in the state. Ecological features demonstrated in newly colonized regions of the United States such as cold tolerance, variable photoperiodic diapause responses, and desiccation-resistant eggs (Hawley 1988), suggest that *Ae. albopictus* has an adaptable life history that may allow invasion of the woodland ecosystems of California. On the other hand, the prolonged yearly dry season and high summer temperatures in many areas of the state pose significant, and perhaps insurmountable, barriers to permanent establishment by this species.

Research examining several aspects of the potential for establishment by *Ae. albopictus* in California treeholes was initiated during 1988. One of the major objectives of this program is to assess the suitability of *Ae. albopictus* as a host for the parasitic ciliate, *Lambornella clarki* Corliss and Coats, an indigenous natural enemy that infects larvae of the western treehole mosquito, *Aedes sierrensis* Ludlow (Clark and Brandl 1976, Egerter and Anderson 1985). *Lambornella clarki* is widely distributed in treeholes throughout California (Washburn and Anderson 1986) and may pose a biological barrier against the colonization and persistence of *Ae. albopictus*. Further, it may be possible to develop this parasite into a manipulated biological control agent for *Ae. albopictus* and other container-breeding mosquitoes.

We have elucidated the major life history features of this treehole-inhabiting protozoan during the past five years with funding provided by the National Institutes of Health and the University of California Mosquito Research Program. The life cycles of *L. clarki* and *Ae. sierrensis* are tightly synchronized with the seasonal pattern of rainfall and photoperiod. *Aedes sierrensis* populations survive the dry season as desiccation-resistant eggs, while *L. clarki* is maintained in dry treeholes in desiccation-resistant cysts. First instar larvae of *Ae. sierrensis* hatch within several hours of treehole flooding during the first winter rains, and motile forms of *L. clarki* appear about 10 to 20 h later. These free-living ciliates are called trophonts, and they feed on bacteria and other microorganisms living in treehole water. Shortly after escaping from desiccation-resistant cysts, some of the trophonts undergo a morphological change and transform into spherical, parasitic cells called theronts. The theront is the host-seeking stage that forms invasive cysts on the cuticles of host mosquito larvae. Trophonts that differentiate into theronts are irreversibly committed to parasitism. Theronts lack the buccal cavity of trophonts; they are apparently nonfeeding, and in the absence of appropriate mosquito hosts, these forms die within 24 to 48 h (Washburn et al. 1988). Trophont populations can persist apparently indefinitely in the absence of larval mosquitoes.

Theronts that successfully encyst on first instar larvae penetrate the cuticle and enter the hemocoel where they establish an endoparasitic existence within a few days of treehole flooding. These endoparasitic forms feed on hemolymph contents and multiply until they completely fill the body of the host. Parasite amplification is temperature dependent, and hosts typically succumb to ciliate infections 20 to 28 days after theront encystment. Endoparasitic *L. clarki* transform back into trophonts which escape through cuticular lesions at the time of host death. Approximately 24 h after escaping from the cadavers, some of the released trophonts undergo a synchronized division phase, and the daughter cells differentiate into theronts which attack surviving larvae. This transformation is apparently under endogenous control and provides the mechanism for horizontal transmission within mosquito populations during the

prolonged period of larval development during the winter. Late instar larvae of *Ae. sierrensis* that are attacked by theronts may eclose successfully as infected adults in the spring. Adult females are parasitically castrated by endoparasitic *L. clarki* that enter the ovaries, and such females disperse trophonts in much the same way that uninfected females disperse eggs among treeholes. Trophonts form the desiccation-resistant cysts as treeholes dry in early summer. These cysts and the desiccation-resistant eggs of *Ae. sierrensis* carry parasite and host populations, respectively, over the summer months until the winter rains begin the cycle again.

Theronts are also produced by ciliate populations following exposure to mosquito larvae. Larvae of *Ae. sierrensis* and certain other mosquito species release a waterborne substance that induces synchronous cell division and theront formation by free-living, trophont populations of *L. clarki* (Washburn et al. 1988). Induction provides an ecologically flexible response to the presence of mosquito larvae which consume trophonts while filter feeding. Thus, mosquito larvae are both predators and hosts of *L. clarki*.

It is clear that several criteria are necessary for successful parasitization of *Ae. albopictus* and other mosquito hosts. First, larvae need to produce the factor that induces transformation of trophonts into theronts. Second, theronts must recognize and encyst on the larval cuticle. Third, encysted theronts must produce enzymes for penetrating the host cuticle; finally, ciliates must circumvent the immune response of the host and persist and amplify in the hemocoel after penetrating the cuticle. In this report, we present preliminary data from laboratory experiments examining the production of the induction cue by larval populations of *Ae. albopictus*, and we compare the host preference of theronts offered *Ae. albopictus* and *Ae. sierrensis* larvae. Both assays are quantified by comparing the rates of theront formation and encystment between *Ae. albopictus* and our laboratory strain of *Ae. sierrensis*.

Induction is assessed by exposing aliquots of trophont populations to filtered treehole water previously containing larvae of *Ae. albopictus* or *Ae. sierrensis*. To evaluate parasite responses to comparable host populations, the larval populations are standardized for developmental stage, rearing temperature, and nutrient regime during the 48 h conditioning period. We mass-produce trophonts in vitro in flasks containing a dilute mixture of cerophyl and vitamins. These trophonts are harvested and aliquoted into a series of petri plate replicates containing water previously conditioned by larvae of one of the two species. The control group is similar to the treatments in all respects except it lacks any mosquito larvae during the conditioning period. This control treatment assesses the endogenous rate of theront formation in the absence of mosquitoes. Ciliate populations in the control and conditioned waters are maintained for 48 h to allow for induction and theront formation. Then first instar *Ae. sierrensis* are added to each replicate and maintained for 24 h; at the end of 24 h, the larvae are removed, stained with amide black, and the number of cuticular cysts per larva are counted. First instar larvae provide a standard substrate for theront encystment, but are too small to ingest the ciliates. The bioassay is quantified by comparing the parasite attack rates (the number of cuticular cysts formed) on *Ae. sierrensis* larvae by *L. clarki* populations exposed to water conditioned by larval populations of the two test species.

Representative data from two laboratory induction experiments are shown in Figure 1. The induction response of one of our *L. clarki* strains, designated SF, exposed to water conditioned by a North American strain of *Ae. albopictus* or *Ae. sierrensis* maintained at either 11 or 16°C during the conditioning period was examined in this test. No significant differences in the number of encysting theronts from cell populations exposed to larvae of the two species were found at either temperature. Thus, under these laboratory conditions, the numbers of theronts formed by *L. clarki* trophonts exposed to larvae of *Ae. albopictus* or *Ae. sierrensis* were the same.

Our protocol for evaluating the host preference of *L. clarki* theronts is quantified much like the induction response protocol. Theronts are produced from trophont cultures induced by water conditioned by *Ae. sierrensis* larval populations and are subsequently aliquoted into dishes containing larvae of *Ae. sierrensis* or *Ae. albopictus*, or both species in the host preference bioassay. Host recognition and preference are quantified by comparing ciliate encystment rates on *Ae. albopictus* with encystment rates on *Ae. sierrensis*. The encystment ratio between single species treatments can be compared to the ratio from within mixed populations to determine if theronts actively dis-

criminate between hosts or simply encyst on whatever larvae are present.

Data in Figure 2 illustrate results from laboratory experiments comparing attack rates of *L. clarki* theronts (strain SF) on larvae from two North American strains of *Ae. albopictus* with attack rates on our laboratory strain of *Ae. sierrensis*. Theronts encysted at higher rates on *Ae. sierrensis* than on either strain of *Ae. albopictus* in both experiments. The ratio of encystment reflects the host preference of theront populations and is calculated as the number of cysts on *Ae. sierrensis* divided by the number of cysts on *Ae. albopictus*. For example, in the top graph, the encystment ratio between the single species treatments is 5.67, and within the multiple species treatment the ratio is 5.94. Thus, in this test, theronts of this strain of *L. clarki* at-

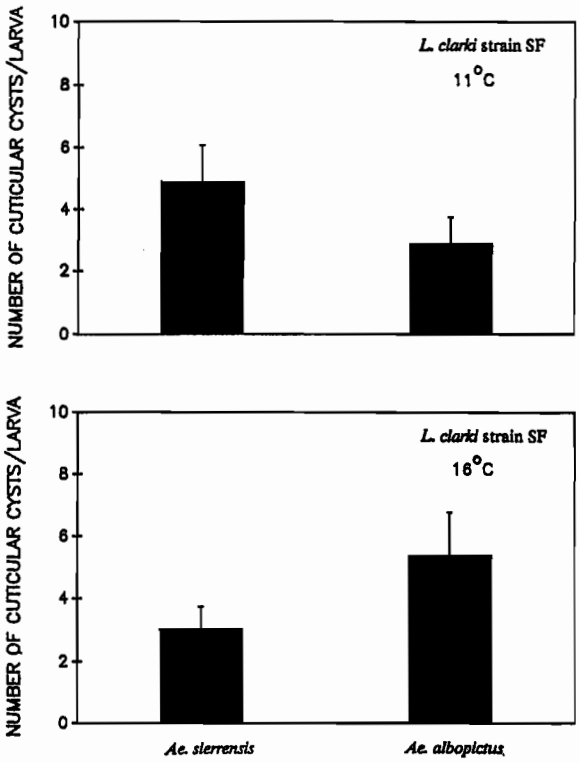

Figure 1.-Laboratory assessment of the induction response of *L. clarki* trophonts exposed to water conditioned by *Ae. albopictus* and *Ae. sierrensis* at 11 and 16°C. Each bar represents the mean number of cuticular cysts on first instar *Ae. sierrensis* from 10 replicates of each treatment; each replicate contained 10 larvae, and bars represent one standard error of the mean. No significant differences in induction were found in ciliate populations exposed to water conditioned by larvae of the two species at 11°C (ANOVA, F = 1.898, p = 0.1852) or at 16°C (ANOVA, F = 2.257, p = 0.1504).

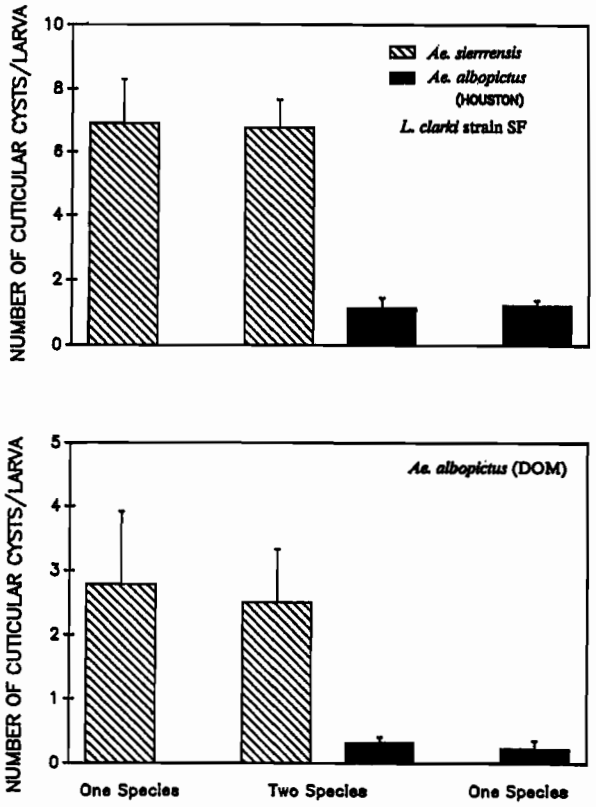

Figure 2.-Laboratory assessment of the host preference of *L. clarki* theronts exposed to larval populations consisting of first instar *Ae. sierrensis* or *Ae. albopictus* or both species. For both experiments, 10 replicates were used for each treatment, and bars represent means with one standard error. Single species populations consisted of 10 larvae of the designated species, and mixed species populations consisted of 5 larvae of each species. In both experiments, significantly fewer theronts encysted on *Ae. albopictus* than on *Ae. sierrensis* larvae in comparisons between single species treatments, and within multiple species treatments (ANOVA, $p < .01$ for all cases).

tacked *Ae. sierrensis* larvae at rates nearly 6 times that of *Ae. albopictus* larvae. The similarity in encystment rates for single and two species treatments in these and additional tests we have conducted suggests that theronts actively discriminate between hosts and do not randomly attack any larvae that are present.

In conclusion, these preliminary results demonstrate that larvae of *Ae. albopictus* meet two of the criteria necessary for being a suitable host for *L. clarki*. Specifically, *Ae. albopictus* larvae induce trophont populations at rates that are equivalent to the natural host, and they are recognized as hosts by theronts. While the encystment response of the SF strain of *L. clarki* is strongly biased towards *Ae. sierrensis*, we need to conduct replicated tests with different strains of *L. clarki* to quantify potential differences in host preference. We are currently maintaining seven strains of *L. clarki* in vitro in our laboratory. It may also be possible to employ laboratory selection for developing strains that exhibit an enhanced preference for *Ae. albopictus*. Finally, in addition to these aspects of host suitability, other responses, such as parasite amplification and dispersal by infected adults, need to be examined before the potential of *L. clarki* as a biological control agent for *Ae. albopictus* can be evaluated.

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EPIZOOTICS OF *LAMBORNELLA CLARKI*: LOCAL EXTINCTIONS OF NATURAL TREEHOLE POPULATIONS OF *AEDES SIERRENSIS*

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Host-parasite interactions between the western treehole mosquito, *Aedes sierrensis* (Ludlow), and the parasitic ciliate, *Lambornella clarki* Corliss and Coats, have been examined in field studies during the past several years in breeding sites at the University of California Hopland Field Station in Mendocino County. One objective of this effort has been to quantify the impact of *L. clarki* on larval mosquito population dynamics and adult production. For determining ciliate infection levels in natural treehole populations, we periodically removed a sample of 50 larvae from each treehole and returned these to the laboratory where they were examined under the microscope at 100 - 400X. Because the invasive cysts and endoparasitic ciliates of *L. clarki* are large and visible over this range of magnifications, we were generally able to determine by external inspection if an individual was parasitized.

In concurrent studies, we have simulated the onset of the winter rains by artificially flooding *L. clarki*-positive treeholes during the fall months in order to monitor the sequence of events that occur when treeholes first fill with rainwater. By collecting water and larval samples at 12 h intervals for the first 4 or 5 days after flooding, we have been able to determine the phenology of *Ae. sierrensis* egg hatch and the appearance of free-living trophonts of *L. clarki* released from resting desiccation-resistant cysts. We have also been able to estimate initial larval and trophont densities, record the timing and incidence of the first parasite cycle, and measure the relative abundance of the major protozoan species that provide part of the resource base for developing larvae.

Using this data base to extrapolate the impact of *L. clarki* on mosquito populations in breeding sites has proven to be a formidable task. One difficulty in interpreting seasonal data on infection levels is that *L. clarki* has several asynchronous parasite cycles during the prolonged period of host development which may last for 5 to 7 months. In nature, larvae die 14 to 28 days after initial infection, and ciliates released from cadavers attack and infect surviving larvae. The total parasite-induced mortality in a treehole mosquito population is the sum of hosts eliminated during all parasite cycles. Estimating mortality is complicated by processes such as larval recruitment from newly-hatched eggs, habitat drying and reflooding, and a variety of other factors. Quantifying actual parasite-induced host mortality for each treehole population requires accurate census data on temporal changes in the age structure and size of populations, and acquiring these data from many breeding sites without destructive sampling has been prohibitive. Nonetheless, by monitoring sufficient numbers of treehole populations for several years we have found several predictable features of host-parasite interactions that appear to be ultimately linked to the production of adult mosquitoes.

The seasonal extinction of *Ae. sierrensis* in some treeholes resulting from natural epizootics of *L. clarki* has been documented; in other breeding sites, we have found consistently low enzootic levels throughout the prolonged period of larval development (Egerter and Anderson 1985, Washburn and Anderson, 1986). The highest number of hosts affected by *L. clarki* in most treehole populations was found shortly after flooding of the breeding site, either by natural accumulation of rainfall or from artificial filling with deionized water. A subpopulation of trophonts of *L. clarki* that was released from desiccation-resistant cysts transformed into parasitic theronts and attacked the newly-hatched larvae within 48 - 84 h. This synchronous attack phase marks the beginning of the first parasite cycle and was quantified by measuring the proportion of larva with cuticular cysts from each treehole population.

Following theront encystment, *L. clarki* ciliates form small holes in the integument and invade the host's hemocoel where they begin an endoparasitic existence (Clark and Brandl, 1976). In examining larval samples from most treeholes, we found a lower proportion of hosts with endoparasitic ciliates compared to the proportion of hosts with cuticular cysts in previous samples. We attributed the

failure of some ciliates to successfully establish infections to three biological processes. First, cuticular penetration provides a portal of entry for other facultatively parasitic microorganisms, including fungi and bacteria, that are common treehole inhabitants (Washburn et al. 1988). These microorganisms use the invasion hole created by *L. clarki* to enter the host, and the secondary infections they create are usually lethal to both the host larva and ciliate parasite. Second, some first instar larvae mount a successful immune response and melanize and kill the invading ciliate shortly after it penetrates the cuticle. Third, full cuticular cysts containing *L. clarki* ciliates are shed by some larvae that molt to the second instar during the first few days after hatching.

While the relative importance of these three processes varied greatly among treehole populations, we did find a general relationship between infection rates in the first parasite cycle and mosquito success. If the percentage of infected larvae was below approximately 25%, we usually observed low enzootic levels of *L. clarki* infections in subsequent samples collected throughout the period of larval development. In contrast, in most holes where rates were initially greater than about 50%, most larval mosquitoes were killed by ciliate infections during the winter and virtually no adults emerged. Overall, we observed the elimination of resident larval populations by ciliate epizootics in approximately 10% of the treehole breeding sites occupied by *L. clarki* during this study. It is important to note that *L. clarki* is a facultative mosquito parasite that is well suited for surviving throughout the winter in treeholes without mosquitoes. Unlike certain other natural enemies, *L. clarki* does not bring about its own demise when it eliminates its host; rather, free-living trophonts of *L. clarki* persist by feeding on bacteria and other microorganisms.

In summary, our findings indicate that the parasitic ciliate, *L. clarki*, causes the natural extinction of *Ae. sierrensis* populations in certain treeholes. Ciliate epizootics can often be predicted from data on events occurring shortly after treeholes first flood with rainwater, particularly the success of the first wave of theront attack. The intensity and success of the first parasite cycle which begins with theront encystment on first instar larvae of *Ae. sierrensis* is modulated by a variety of density dependent and independent factors. For example both the nutrient availability and water temperature influence the molt schedule of larvae and may calibrate the level of successful ciliate infections in the first cohort of larvae. These and other factors, interacting in complex ways, may set the tempo for subsequent parasite cycles during the prolonged period of larval development by modulating the densities of both host and parasite populations.

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EFFICACY AND PERSISTENCE OF *BACILLUS SPHAERICUS, BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS,* AND METHOPRENE AGAINST *CULISETA INCIDENS* IN TIRES

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ABSTRACT

Used tires are an important larval habitat for many species of mosquitoes, some of which are important disease vectors. Tires pose a special problem for mosquito control because it is difficult for pesticides to penetrate the larval habitat, and many tire-breeding mosquitoes are resistant to some of the commonly used pesticides. The bacterial pathogens, *Bacillus sphaericus* and *Bacillus thuringiensis* var. *israelensis* (*Bti*), and the insect growth regulator, methoprene, may provide other options for mosquito control agencies.

Bacillus sphaericus has demonstrated prolonged larvicidal action in some treated habitats, and dead mosquito larvae have been shown to serve as growth medium for the bacteria. This study compared the persistence, growth, and control potential of *B. sphaericus* at three dosage rates in tires containing cadavers of *Culiseta incidens*, and in tire water with all dead larvae removed.

Liquid formulations of *Bti* and methoprene are generally effective for only a short time, but their efficacy may be prolonged in the shade. This study evaluated the efficacy and persistence of *B. sphaericus*, *Bti*, and methoprene against *Cs. incidens* larvae in tires exposed to full sunlight as opposed to tires shaded by a dense canopy of trees.

Field studies were conducted at two sites. At site #1, 2 liters of water and 50 second instar *Cs. incidens* were added to each of 21 tires, which were then inoculated with either 3.75 ppm, 7.5 ppm, or 15 ppm of *B. sphaericus* (2362), or were not inoculated (controls). At each dosage rate, dead larvae were either removed from or left in the tire water. Percent mortality was determined three days post-treatment. Seven days after the *B. sphaericus* inoculation, larvae were again added to each tire and the percent mortality determined. This procedure was repeated weekly over a ten week period.

The efficacy of *B. sphaericus*, *Bti*, and methoprene against *Cs. incidens* larvae in tires was evaluated in open sun versus shaded habitats at site #2. Tires containing 2 liters of water and 50 second instar *Cs. incidens* larvae were inoculated at dosage rates of 15 ppm *B. sphaericus*, 15 ppm *Bti* (Vectobac™ AS, 600 ITU), 2.5 ppm methoprene (Altosid™ SR10), or were not inoculated (controls). There were three replicates of each of the four treatments in both the sun and the shade. The percent mortality was evaluated three days post-inoculation in the *B. sphaericus* and control tires, and two days post-inoculation in the *Bti*-treated tires. Since methoprene prevents adult emergence, its efficacy was assessed by checking the tires every two days for pupae, which were removed and placed in individual emergence containers for observation. Every week, for up to 13 weeks, larvae were added to the tires and the mortality or emergence rate determined as above.

At site #1, there was greater than 90% mortality for ca. two weeks post-treatment in tires treated with 7.5 and 15 ppm *B. sphaericus* and containing cadavers, and for ca. one week in the other treated tires. Mortality exceeded 50% for nine weeks in all tires treated with 15 ppm *B. sphaericus*, for nine and six weeks in the 7.5 ppm treated tires with and without cadavers, respectively, and for four weeks in all 3.75 ppm tires. In all tires, there was an initial decline in the mortality rate and then an increase approximately four to six weeks post-inoculation, suggesting an amplification of the pathogen. At the lower treatment rates, the continual presence of dead larvae in the tire water apparently increased the effectiveness of *B. sphaericus*, since mortality rates were generally higher in tires with cadavers. At 15 ppm, mortality rates were similar in tires with or without cadavers.

At site #2, both *B. sphaericus* and *Bti* provided prolonged control of *Cs. incidens* larvae in shaded tires (>90% mortality for five and two weeks, >50% mortality for ten and four weeks, with the two bacteria, respectively), and controlled mosquitoes in sun-exposed tires adequately for approximately one week. Methoprene inhibited the emergence of ca. 90% of the larvae present at the time of treatment, but not of larvae subsequently introduced into either the sun-exposed or shaded tires.

EVALUATION OF SELECTED LARVIVOROUS FISHES FOR MOSQUITO CONTROL IN WASTEWATER: EFFECTS OF WATER HYACINTH DENSITY

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ABSTRACT

The effect of water hyacinth (*Eichhornia crassipes*) density on the ability of mosquitofish (*Gambusia affinis*) to control mosquitoes in wastewater marshes was investigated. Four replicates of eight treatments were arrayed in a randomized block design using 32 207 L tanks. Two tanks in each block contained 0, 8, 16, and 32 hyacinth each. Fifty mosquitofish were placed in one tank of each density. Eight *Culex pipiens* egg rafts were introduced into each tank every other day throughout the 43-day experiment and mosquito emergence was monitored daily. More mosquitoes emerged from tanks with low hyacinth density; high densities of hyacinth reduced mosquito emergence to very low levels. The mosquitofish eliminated emergence at all hyacinth densities. No effect of hyacinth density could be discerned on the ability of the fish to control mosquitoes.

RATIONALE FOR THE DESERT PUPFISH, *CYPRINODON MACULARIUS*, AS A COMPLEMENT TO *GAMBUSIA* IN MOSQUITO CONTROL

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ABSTRACT

The desert pupfish, *Cyprinodon macularius*, possesses attributes that would make it an attractive substitute and/or complement to *Gambusia affinis* for mosquito control. Potential benefits include improved mosquito control in a wider variety of habitats, reduction of annual mosquitofish restocking activity, a minimum of piscivorous habits, and improbability of indiscriminate translocation to endangered species' habitats. All of these, plus the aesthetic beauty of this native fish, should stimulate its consideration for wider use in the integrated control of mosquitoes in California.

Introduction.

Research in the past two decades with the desert pupfish, *Cyprinodon macularius* Baird and Girard, as a biological mosquito control agent, has revealed several attributes which would make this native species a logical complement to *Gambusia affinis* (Baird and Girard) (Legner and Medved 1974, Legner et al. 1975, Walters 1976, Walters and Legner 1980). The pupfish, stocked in late spring (June) at 3625 5-weeks old fish per acre (8957/ha.) in shallow natural ponds, caused all mosquito breeding to cease four weeks after introduction, whereas in ponds stocked with 290 000 *G. affinis* per acre (716 560/ha.), mosquito breeding continued at a significant level for >8 weeks (Legner et al. 1975). This pattern was observed repeatedly during the 1975-1988 period (Legner and Warkentin, unpub. data).

The desert pupfish displays other characteristics which would make it a worthy supplemental biological control agent. It consumes all instars of mosquito larvae and pupae. In the absence of mosquito prey, *C. macularius* forages mostly in the benthos, consuming benthic chironomid midge larvae, detritus, aquatic vegetation, and snails. *Gambusia affinis*, foraging mostly at the surface, consumes large numbers of floating terrestrial insects, immature chironomids, and snails which are attached to vegetation (Walters 1976, Walters and Legner 1980).

Although both species forage similarly on mosquito predators and zooplankton, the pupfish appears to forage more effectively in habitats with cover, such as rice (Walters 1976), and mosquito upsets or phytoplanktonic blooms (Hurlbert et al. 1972) are not produced at spring stocking rates of 3625 total pupfish per acre (8957/ha.) (Walters and Legner 1980).

Gambusia affinis reproduces more rapidly in shallow ponds and a greater impact on mosquitoes might be expected, but lower numbers of *C. macularius* are equally effective.

Cyprinodon macularius exhibits little piscivorous behavior compared to *G. affinis*. It is also considerably more euryhaline in its natural habitat and adapts easily to both fresh and saline water (4.6-6.8%) conditions (Barlow 1958a,b; Kinne 1960, Kinne and Kinne 1962). In fact, Kinne (1960) regarded *C. macularius* as one of the few truly holeuryhaline animals. By comparison, *G. affinis* exhibits high mortality at the same salinities (Ahuja 1964), and is normally found only in field salinities below 2.5%. Pupfish will also tolerate water temperatures in desert pools in the range of 8° to 44° C (Brown 1971, Brown and Feldmeth 1971, Lowe and Heath 1969, Taylor and Minkley 1966).

The desert pupfish cohabits with a great variety of other fishes and aquatic organisms in natural habitats and irrigation drains (Fig. 1) throughout the Lower Sonoran Desert of California, Arizona,

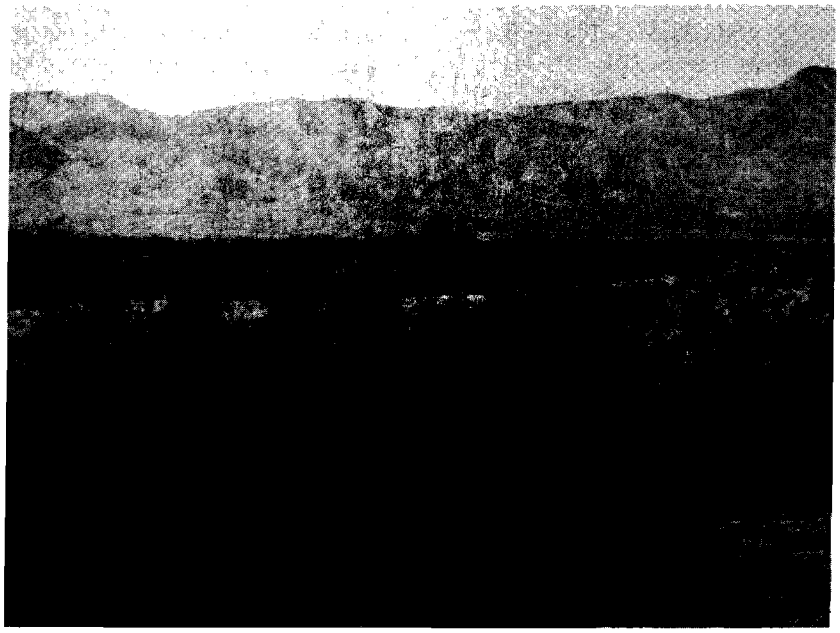

Figure 1. Irrigation drain near the northwest shore of the Salton Sea, California, supporting high populations of *Cyprinodon macularius* Baird and Girard.

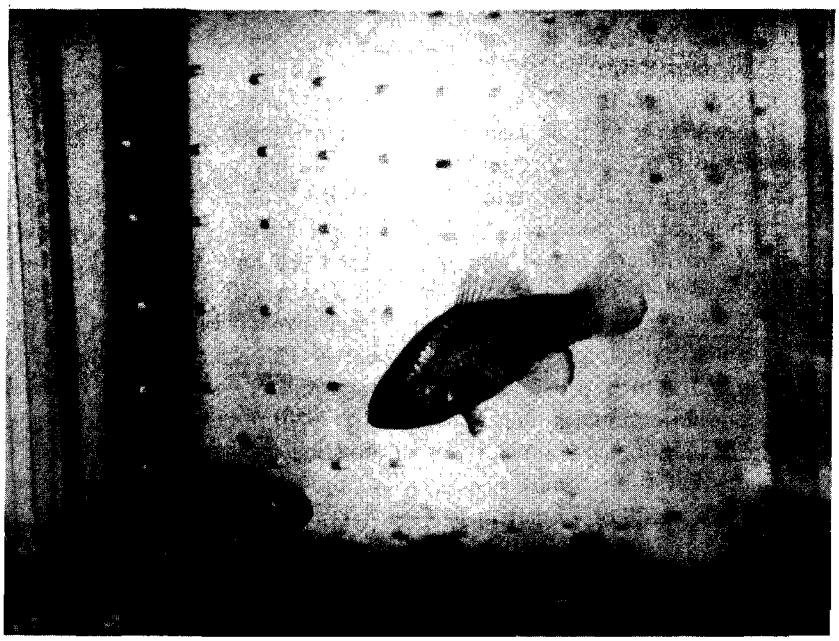

Figure 2. *Cyprinodon macularius* Baird and Girard males in aquarium at the University of California, Riverside. (photo by Max Badgley).

and northwestern Mexico. This life habit is accomplished with apparent minimal ecosystem disruption (Coleman 1929, Cowles 1934, Cox 1972, Legner and Medved 1974, Legner and Walters 1980, Walker, Whitney and Barlow 1961). The pupfish is also an attractive species (Fig. 2), where males take on a deep blue or purple-blue nuptial coloration (Walters 1976).

Different sizes of *C. macularius* school together in small groups of 20-25 fish. Smaller individuals tend to remain near the shoreline or around emergent vegetation. The small schools cover an entire pond habitat at all depths, as opposed to *Gambusia* that school in large communities near the surface and shore. *Cyprinodon macularius* remains near the shallow, warmer water of the shore at night time, while scattered activity is found during daylight.

Reasons For Diminished Attention.

Given the comparable or superior biological mosquito control capabilities of desert pupfish, one asks why haven't they been given increased attention in the biological-integrated control of mosquitoes in the southwestern United States?

Early in this decade, when most of the above mentioned attributes of pupfish as biological mosquito controls were known, a strong argument was given against their use by the California Department of Fish and Game. It was decided that introducing *C. macularius* in the Central Valley of California might result in the extinction of native fish species, and that their widespread use might cause loss of related species in the genus through hybridization. This negative evaluation did not consider the naturally lower population densities of pupfish compared to *Gambusia*, the practical absence of pisciferous habits, and their obvious harmonious coexistence with a great variety of indigenous and exotic fishes and aquatic organisms in the Sonoran Desert. Paradoxically, the proven negative impact of *G. affinis* on endemic aquatic fauna (Deacon and Bunnell 1970, Mallars and Fowler 1970); cyprinid fishes (Miller and Hubbs 1960); juvenile black bass (Myers 1965); and other native fishes (Deacon and Bunnell 1970), was condoned.

Anyone who has worked with both species of fish would minimize any threat of indiscriminate movement of *C. macularius*. The desert pupfish is extremely difficult to catch because of its very fast swimming activity when trapped or endangered. It is not possible to mass produce this species in captivity without a thorough knowledge of its behavior (see Crear and Haydock 1971, and Walters 1976). For that reason, the translocation of pupfish could not be accomplished without guidance by trained personnel. It is also expected that reproduction of *C. macularius* in most Central Valley sites would not succeed because sexual maturity in this fish is favored only at water salinities of 35 p.p.t. (near seawater), yet optimum development of immature fish occurs only in fresh water (Kinne 1960, Kinne and Kinne 1962). Such essential habitat combinations, intermingled with certain critical temperatures for development (Kinne 1960, Kinne and Kinne 1962), are not likely to exist outside of the lower Sonoran Desert area, and successful breeding would require even greater human control of the fish.

The gains that could be realized with *C. macularius* through greater mosquito control in a wider variety of habitats and reduced annual restocking activity, plus the aesthetic beauty of this North American native, are desirable considerations.

Annual restocking of *Gambusia* is usually required on a broad scale in California at the present time. A fish such as *C. macularius*, that might be capable of persisting from year to year in a wider range of habitats and which gave comparable mosquito control, could also be expected to attain a balance with its prey at lower average densities. This would reduce the biomass of fish in any given habitat, eliminate large scale stocking activities, and generally reduce the threat against native fishes.

In her study comparing *C. macularius* with *G. affinis*, Walters (1976) concluded that *Cyprinodon*'s higher adaptability to salinity and temperature extremes makes it more practical for introduction into more diverse environments. Its insignificant piscivorous habits would make any impact on other native fish species negligible or at least reduced over that of *Gambusia*. However, Walters felt that the environmental impact of either fish species must be correlated with specific ecosystems and the abundance of beneficial insect predators in relation to critical mosquito densities.

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INOCULATION AND SPREAD OF PARASITIC WASPS TO CONTROL FILTH FLIES IN POULTRY HOUSES

E. F. Legner[1], W. D. McKeen[2], and R. W. Warkentin[3]

ABSTRACT

Liberations of two species of laboratory-reared parasitic wasps, *Muscidifurax zaraptor* and *Muscidifurax raptorellus*, on a 280 000 bird poultry ranch during spring of 1987 resulted in a significant parasitism build up and spread. The retention of a 15 cm pad of manure following cleaning operations in summer (August and September) did not preserve parasitoid populations, and this suggested a need for additional parasitoid introductions after cleaning.

Introduction.

Filth-breeding flies are a major problem for poultry and dairy producers and feed-lot operators in many areas of the United States.The problem is particularly acute in southern California where rapid suburban expansion has encroached on agricultural areas. Because of problems associated with unilateral chemical fly control, integrated management programs for these flies have been under development in several regions (Axtell 1970, Legner and Dietrick 1974, Petersen and Meyer 1983). Natural enemies are important control components, particularly in the more stable manure communities found in many caged-layer, dairy, and feed-lot operations.

Among the natural enemies, parasitic wasps have received the greatest research emphasis, and most of the published work to date has dealt with observations on seasonal occurrence (Ables and Shepard 1976a,b; Legner and Brydon 1966, Legner and Greathead 1969, Legner and Olton 1971, Mullens et al. 1986, Petersen and Meyer 1983, Rutz and Axtell 1980), and experimental parasitoid releases (Legner and Dietrick 1974, Morgan et al. 1975, Olton and Legner 1975, Rutz and Axtell 1979). However, there is no clear description of the numbers of parasitoids to release, how long the benefits from liberations persist, nor which species or strains are best suited for different climates and seasons.

We follow the dispersal of two parasitic wasp species on a poultry ranch in the present study, and measure their impact on experimentally controlled numbers of hosts in the habitat.

Methods and Materials.

Two species of hymenopterous parasitoids were introduced on a 280 000 bird poultry ranch near Highland, California during spring of 1987, and their rate of spread and impact on house fly hosts were studied. Weekly releases of cohorts of a Denver, Colorado population of *Muscidifurax zaraptor* Kogan and Legner and a (Peru X Chile) hybrid of *Muscidifurax raptorellus* Kogan and Legner, were made from April 21 to June 9, 1987. The latter cohort bore a behavioral genetic marker of gregarious oviposition (>one egg laid per host individual). This enabled positive identification of female parasitoids in a group where only male characters are positive (Kogan and Legner 1970, Legner et al. 1976).

Two separate blocks of poultry houses were chosen for each parasitoid species. Each block consisted of 42 rows (185 m long) of opposed cages, 2-3 birds per cage. Manure accumulated under the cages in a typical cone configuration and was ca. 0.5 m high at the start of the experiment. Manure rows were separated by concrete walkways.

Rows were alternated for each treatment consisting of a control, a single release rate (1X) and a double release rate (2X). Releases and samples were confined to the mid 15.2 m section of each 185 m-long row. Weekly parasitoid releases were made from April 21 to June 9th of *Muscidifurax zaraptor* (1X - 560 parasitoids; 2X - 1120 parasitoids) and *Muscidifurax raptorellus* (1X = 3500; 2X = 7000). There were four replicates of each treatment.

All manure was removed to a 15 cm pad during the 26 August to 8 September interval. Sites were monitored for parasitism at weekly intervals

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from 21 April to 9 June, and 22 September to 10 October.

Sampling was accomplished by employing the sentinel bag technique (Rutz and Axtell 1979, Mullens et al 1986). The bags were constructed of 6.3 mesh/cm fiberglass window screen, and each one contained 25 *Musca domestica* puparia that were 12-18 hr old at the start of an exposure. The weekly distribution of 16 bags was evenly spaced along the 15 m midsection of a row. The bags were placed in the dry friable manure (natural larval fly pupation sites) along the edges of the walkways, and each one was covered with 1-2 cm of dry manure. All puparia were distributed randomly within the bag by shaking it during bag placement. Upon collection one week later, the bags were opened in the laboratory and the puparia were transferred to 46 cm^3 plastic screened vials for parasitoid and host emergence. Puparia which failed to yield either adult flies or parasitoids were dissected to assess aborted parasitism.

Two thermographs were placed at two sites on the ranch to record the air temperature along the edges of the walkways.

Analyses of variance were performed on percentage data after transformation to the arcsin sqrt(% + 1/2). Duncan's multiple range tests (Steel and Torrie 1980) were used to detect significant differences at $P \leq 0.05$. Correlation analyses on untransformed values tested the effects of temperature on parasitization and host kill intensity.

Results and Discussion.

Tables 1 and 2 show the percentage of pupae that were parasitized in the *M. zaraptor* and *M. raptorellus* release poultry houses, respectively, and the accompanying total pupal mortality. Table 3 gives the weekly mean maximum, minimum, and average temperatures taken near the sentinel bag placement sites for the entire parasitoid release period.

Parasitism and Pupal Mortality.--Parasitism of pupae in the sentinel bags in the *M. zaraptor* release houses increased steadily from zero on April 21st to 29.6-44% on June 9th (Table 1). There was no significant tendency for a higher parasitism by *M. zaraptor* in any of the three treatments (control, 1X, & 2X), and this indicated a high parasitoid dispersal rate. Parasitism by the resident *Spalangia cameroni* Perkins followed a similar, apparently random distribution, and increased steadily as the experiment progressed. Pupal mortality increased similarly.

The same trend was observed in the *M. raptorellus* release houses (Table 2). However, this parasitoid either dispersed farther from the release sites, or it demonstrated a lesser parasitization rate, because percent parasitism was not as high as with the previous species (Tables 1 & 2).

Temperature Influences.--Table 3 shows that the average temperatures during the entire parasitoid release period remained quite uniform, with no significant warming trend. Therefore, the observed increases in parasitism were probably due to inherent population characteristics of *S. cameroni* and a combination of population trends and cumulative effects of parasitoid releases in the *Muscidifurax* parasitoids. Random samples of pupae from manure showed that native *Fannia* spp. accounted for 95.6% of the natural fly breeding. These pupae were also parasitized by all three parasitoids, and served as wild hosts for parasitoid population increases.

Correlation analyses performed to test the relationship between the degree of parasitism and temperature during the first three days of pupal exposure, when more than 90% of parasitism occurred, indicated no special relationships for *M. zaraptor*. However, *M. raptorellus* showed a significant correlation with maximum and average temperatures (r = 0.544, 0.516, 15 df, respectively). It appears that *M. raptorellus*, of South American origin, may have a slight preference to parasitize at warmer temperatures.

Effects of *Muscidifurax* Releases on *Spalangia*.--There was no marked relationship between *S. cameroni* activity and the number of *Muscidifurax* individuals liberated. This conclusion was based on the derived correlation coefficients, all of which were less than 0.1.

Parasitoid Dispersal.--The gregarious oviposition behavior of *M. raptorellus* distinguished this species from all other parasitoids active during the study interval. Therefore, it was possible to examine the dispersal pattern of this species over both experimental sections of the poultry ranch.

Data that were accumulated at all sentinel bag exposure sites and averaged over the two-month study interval, are shown in Table 4. *Muscidifurax raptorellus* distributed to all parts of both experimental areas, with 16.4% of total recoveries being made in the *M. zaraptor* release sections

Table 1. Percent of 25 *Musca domestica* sentinel pupae parasitized by *Muscidifurax zaraptor* and *Spalangia cameroni*, and total mortality of pupae in poultry manure at Highland, California during April-June, 1987. ------sentinel bag assessment in *M. zaraptor* release houses.[a]

	PERCENT PARASITIZED BY						PUPAL MORTALITY (%)		
Week	*M. zaraptor*			*S. cameroni*					
beginning	Contr.	1X	2X	Contr.	1X	2X	Contr.	1X	2X
4/21/87	0	0	0	1.6	4.4	8.5	21.2	28.8	38.2
4/28/87	0	0	1.3	7.8	4.5	0	22.7	20.2	12.5
5/05/87	0	0	0	5.6	14.4	2.3	13.2	25.0	6.7
5/12/87	1.0	7.2	1.8	4.6	2.3	1.5	33.0	38.2	39.0
5/26/87	0	18.2	0	17.9	25.0	31.0	33.5	63.8	53.2
6/02/87	6.8	5.5	7.0	37.0	46.0	39.5	58.5	63.0	64.2
6/09/87	29.6	31.2	44.0	21.1	27.7	12.8	71.9	70.5	82.2

[a] Control = no parasitoids released
1X = 560 *M. zaraptor* released per week.
2X = 1120 *M. zaraptor* released per week.

Table 2. Percent of 25 *Musca domestica* sentinenl pupae parasitized by *Muscidifurax raptorellus* and *Spalangia cameroni* and total mortality of pupae in poultry manure at Highland, California, during April-June, 1987 --------sentinel bag assessment in *M. raptorellus* release houses.[a]

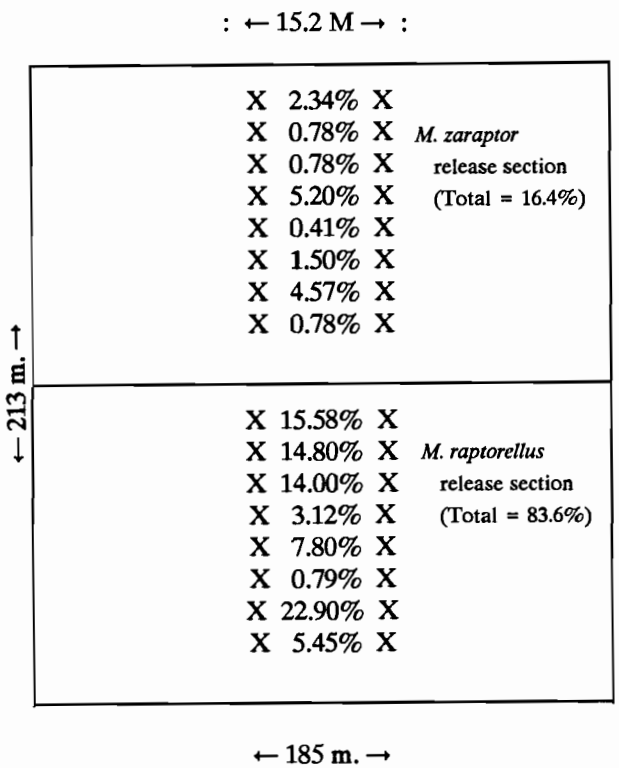
	PERCENT PARASITIZED BY						PUPAL MORTALITY (%)		
Week	*M. raptorellus*			*S. cameroni*					
beginning	Contr.	1X	2X	Contr.	1X	2X	Contr.	1X	2X
4/21/87	0.3	0	1.5	6.1	9.8	2.0	56.4	52.2	62.8
4/28/87	0	0.5	0.8	5.2	5.2	1.0	18.2	18.0	19.0
5/05/87	7.3	4.0	12.5	2.0	0.3	0.5	21.2	15.3	28.5
5/12/87	0.2	0.3	0	3.4	0.7	4.2	30.4	30.2	27.8
5/26/87	1.7	5.0	1.8	32.1	28.0	32.5	46.5	48.1	50.7
6/02/87	1.3	9.8	0	44.8	42.2	49.0	68.4	69.5	72.5
6/09/87	6.1	5.3	2.3	31.6	31.8	25.2	73.6	83.3	74.5

[a] Control = no parasitoids released
1X = 3500 *M. raptorellus* released per week.
2X = 7000 *M. raptorellus* released per week.

Table 3. Average temperatures recorded near the site of sentinel bag placement in poultry manure at Highland, CA, during the April 21 to June 9, 1987 period.

Week	MEAN TEMPERATURE (°C)		
Beginning	Maximum	Minimum	Average
4/21/87	27.8	11.4	19.5
4/28/87	24.4	12.2	17.8
5/05/88	33.1	14.7	23.1
5/12/87	28.5	15.7	20.7
5/19/87	22.0	12.1	16.5
5/26/87	24.3	11.7	17.4
6/02/87	33.3	15.6	23.7
6/09/87	28.5	14.6	19.9

Table 4.-Distribution of gregarious *Muscidifurax raptorellus* over both experimental sections of the poultry ranch at Highland, California during the April to June, 1987 interval.



(Table 4). As only the center of each row was examined critically for parasitism, the actual distribution of parasitoids was undoubtedly much greater along the various rows. This was verified by observations of *M. raptorellus* parasitism in random samples of *Fannia* species taken from other parts of the ranch.

Effects of Manure Removal.--Removal of manure to a 15 cm pad by September 8th was devastating to parasitoid activity. There was no carryover of released species and only the resident *S. cameroni* persisted, and it caused less than 1% parasitism of the sentinel pupae. Total pupal mortality also averaged under 8% (data not shown). It is apparent that without reintroduction of parasitoids following manure removal in late summer, fly pupal survival is very high, and immigration of native parasitoids is too slow to be of significance in population control.

Acknowledgments.

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EVALUATION OF AEROSOL APPLICATIONS USING EXPERIMENTAL AND OPERATIONAL MATERIALS DURING 1988

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Introduction.

A total of 27 coldfogger test line applications was made in Solano, Madera, Glenn, and Colusa Mosquito Abatement Districts during the 1988 mosquito season. Various applications of malathion, malathion/piperonyl butoxide (PBO), malathion/pyrethrin, malathion/resmethrin, pyrethrin/PBO, resmethrin/PBO, permethrin/PBO, phenothrin, chlorpyrifos, and chlorpyrifos/pyrethroid were made against caged, laboratory-reared, susceptible *Culex pipiens* Linnaeus, and wild-caught *Culex tarsalis* Coquillett, *Cx. pipiens*, *Aedes melanimon* Dyar, and *Anopheles freeborni* Aitken. Summaries of applications are shown in Table 1. Results, as an indication of expected operational success, ranged from poor to excellent. The most successful of the duplicated runs are described with emphasis placed on the wild *Cx. tarsalis* populations.

Methods and Materials.

Two types of vehicle-mounted coldfoggers were used in these tests: district built units as described by Whitesell (1973), and the commercially built Leco HD9. All foggers produced similar droplet sizes as measured by University of California, Davis (LASER Droplet Measurement equipment) according to William Steinke (pers. comm.). Various formulations and chemical concentrations were used and will be described for selected applications. Applications were made during temperature inversion or laminar flow conditions, and when wind speeds were lower than 10 mph. Disposable paper and nylon net cages, as described by Townzen and Natvig (1973), were used to evaluate mosquito mortalities. All mortality counts were made 12 h following the applications. Cages were affixed to the top of a 42 in surveyor's lath and placed at 0 ft, 200 ft, 400 ft, 600 ft, 1320 ft, and as noted to 2640 ft, 3960 ft and 4800 ft downwind.

Susceptible *Cx. pipiens* from the Environmental Management Branch laboratory colony were used as indicators of successful application. Wild caught mosquitoes were used as indicators of operational insecticide efficacy. Wild caught mosquitoes were captured by mechanical aspiration from resting stations or biting collections, and from CO_2-baited EVS traps.

Description and Results.

Malathion/PBO-Malathion-resistant *Cx. tarsalis* from the Colusa MAD (Townzen et al., 1987) were challenged with malathion synergized with PBO on four occasions in 1988. Figure 1 shows the mortalities of laboratory-reared *Cx. pipiens* and wild-caught *Cx. tarsalis* for the July 12 application. The July 12 application was considered the most successful of the four, having the highest mortality counts for *Cx. tarsalis*. PBO was mixed 2:1 with 91% malathion and applied at 11.1 fl oz/min on this run. Vehicle speed was 10 mph. South by southeast winds (cooling winds) prevailed throughout the day of the test. Laminar flow conditions were measured during the application which began at 2010 h. Both the 32 ft and the 8 ft thermister measured 81°F. All applications indicated that PBO did not synergize malathion under operational conditions.

Chlorpyrifos-Dursban®, 6 lb/gal chlorpyrifos, formulated to 2 lb/gal was evaluated twice at Glenn County MAD and once at Solano County MAD. The higher mortalities of the two Glenn tests and the Solano application are discussed below. Omitted from the discussion are the three experimental chlorpyrifos/pyrethroid formulations evaluated against organophosphate (OP)-resistant *Cx. tarsalis* at the Colusa MAD. The formulator requested that the specifics be kept confidential.

Solano-Meteorological measurements showed a 1.5°F temperature inversion with wind speed of 8-9 mph. Two lb/gal Dursban was applied at 11.8 fl oz/min with a vehicle speed of 10 mph. Starting time was 0630 hrs. Figure 2 shows the mosquito mortalities 12 hours after the application. Mosquito mortalities of the fogger application were compared to filter paper susceptibility tests as reported by Mac Thompson (in this Proceedings).

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Table 1.-Summary of 27 coldfogger applications of operational and experimental insecticides against caged mosquitoes during the 1988 season.

Date	Location	Chemical	Mosquito Species	Results
6/21	Colusa	Malathion/PBO	*Cx. pipiens, Cx. tarsalis, Ae. melanimon, Ae. vexans*	Poor
6/21	Colusa	Pyrethrum/Malathion	*Cx. pipiens, Cx. tarsalis*	Fair
6/28	Colusa	Malathion/PBO	*Cx. pipiens, Cx. tarsalis*	Poor
6/28	Colusa	Pyrethrum/Malathion	*Cx. pipiens, Cx. tarsalis*	Great
6/29	Colusa	Malathion/PBO	*Cx. pipiens, Cx. tarsalis*	Poor
6/29	Colusa	Pyrethrum/Malathion	*Cx. pipiens, Cx. tarsalis*	Great
7/12	Colusa	Malathion/PBO	*Cx. pipiens, Cx. tarsalis*	Poor
7/12	Colusa	Pyrethrum	*Cx. pipiens, Cx. tarsalis*	Great
7/13	Colusa	Malathion/Resmethrin	*Cx. pipiens, Cx. tarsalis*	Poor
7/13	Colusa	Phenothrin	*Cx. pipiens, Cx. tarsalis*	Poor
7/27	Glenn	Dursban	*Cx. pipiens, Cx. tarsalis*	Poor
7/28	Glenn	Dursban	*Cx. pipiens, Cx. tarsalis*	Poor
8/30	Colusa	Permethrin/PBO	*Cx. tarsalis, Ae. melanimon, An. freeborni*	Good
8/30	Colusa	Permethrin/PBO	*Cx. tarsalis, Ae. melanimon, An. freeborni*	Fair
8/30	Colusa	Permethrin/PBO	*Cx. tarsalis, Ae. melanimon, An. freeborni*	Poor
8/31	Colusa	Dursban/Pyrethroid	*Cx. tarsalis, Ae. melanimon, An. freeborni*	Mixed
8/31	Colusa	Dursban/Pyrethroid	*Cx. tarsalis, Ae. melanimon, An. freeborni*	Mixed
8/31	Colusa	Dursban/Pyrethroid	*Cx. tarsalis, Ae. melanimon, An. freeborni*	Poor
9/1	Colusa	Permethrin/PBO	*Cx. tarsalis, Ae. melanimon, An. freeborni*	Good
9/1	Colusa	Permethrin/PBO	*Cx. tarsalis, Ae. melanimon, An. freeborni*	Good
9/1	Colusa	Permethrin/PBO	*Cx. tarsalis, Ae. melanimon, An. freeborni*	Poor
9/3	Madera	Resmethrin	*Cx. pipiens, Cx. pipiens*	Good
9/14	Madera	Pyrethrum	*Cx. pipiens, Cx. pipiens*	Fair
9/21	Solano	Resmethrin	*Cx. pipiens, Ae. melanimon*	Great
9/21	Solano	Pyrethrum	*Cx. pipiens, Ae. melanimon*	Great
9/22	Solano	Dursban	*Cx. pipiens, Ae. melanimon, Cx. tarsalis*	Great
9/22	Solano	Malathion	*Cx. pipiens, Ae. melanimon, Cx. tarsalis*	Mixed

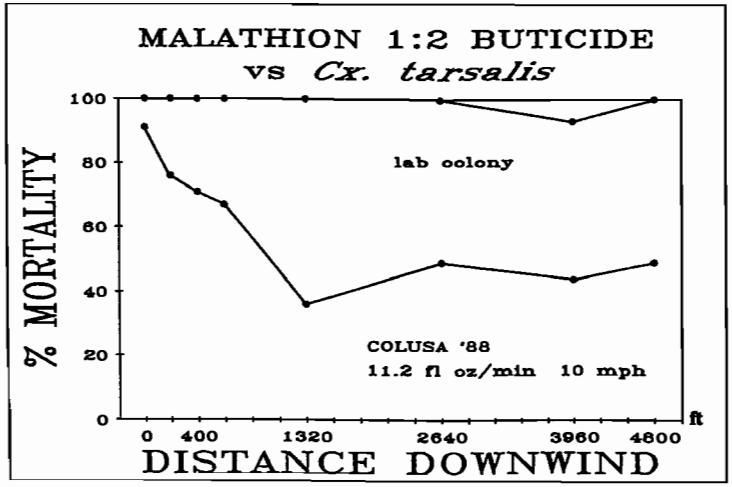

Figure 1.-Mortality of caged wild *Culex tarsalis* and laboratory-reared *Culex pipiens* following coldfogger applications of PBO synergized malathion, Colusa MAD, July 12, 1988.

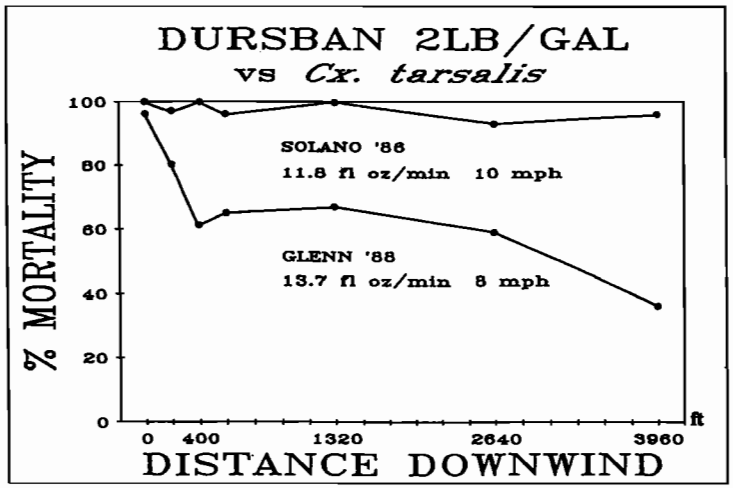

Figure 2.-Mortality of caged *Culex tarsalis* following coldfogger applications of Dursban, Solano County MAD, September 21, 1988, and Glenn County MAD, July 27, 1988.

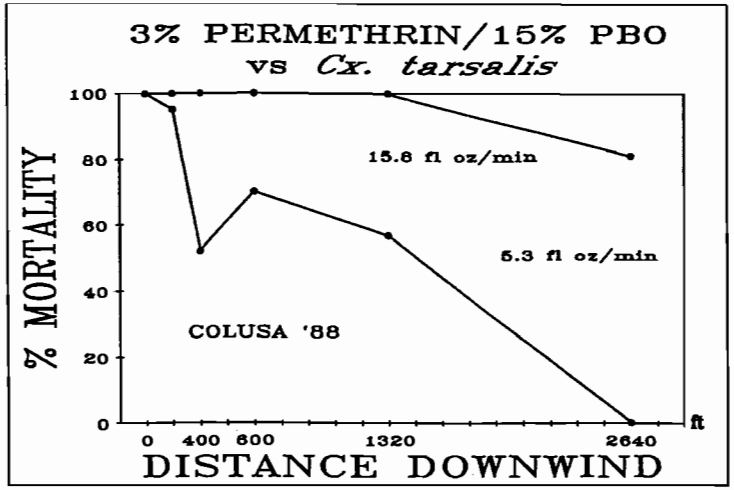

Figure 3.-Mortality of caged *Culex tarsalis* following coldfogger applications of PBO synergized permethrin at 5.3 and 15.8 fl oz/min, Colusa MAD, August 30, 1988.

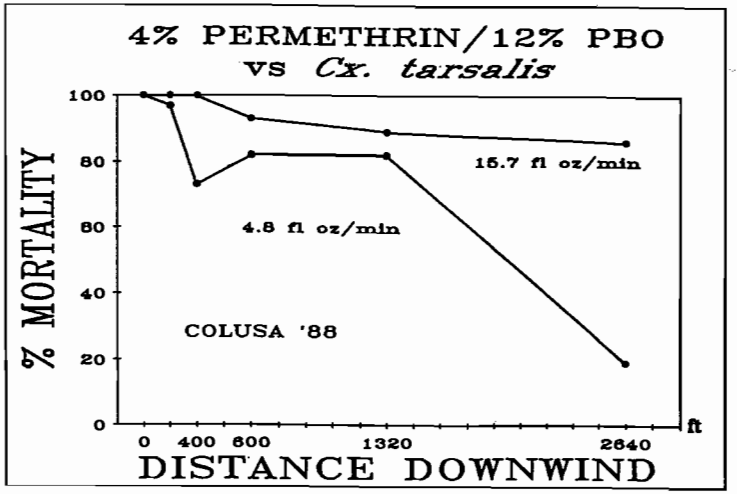

Figure 4.-Mortality of caged *Culex tarsalis* following coldfogger applications of PBO synergized permethrin at 4.8 and 15.7 fl oz/min, Colusa MAD, September 1, 1988.

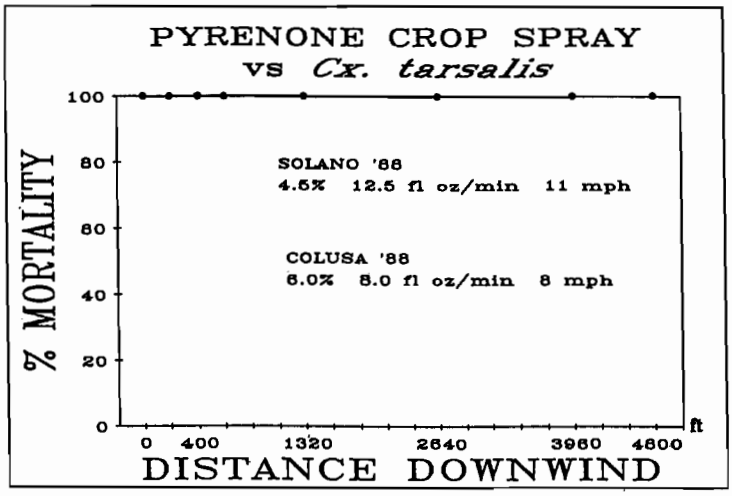

Figure 5.-Mortality of caged *Culex tarsalis* following cold-fogger applications of Pyrenone Crop Spray at Solano County MAD, September 22, 1988, and Colusa MAD, July 7, 1988.

Both fogger and paper tests utilized mosquitoes from the same EVS light trap collections and were completed the same day. Similar results indicated that in a wild population of "borderline" resistant *Cx. tarsalis*, 5% would be expected to survive an operational application.

Glenn-Two lb/gal Dursban was applied at 13.7 fl oz/min at a vehicle speed of 8 mph. The material was applied at 2110 h with a temperature inversion of 2.0°F and wind velocities of 2-3 mph. Evaluation of the Glenn run indicated a resistant population (Figure 2). The first cage, at ground zero, did not have 100% mortality, and mortality beyond 400 ft was approximately 50%. Again, filter paper tests indicated a resistant population with field mortalities of 50% to be expected.

Permethrin-Six experimental applications of permethrin synergized with PBO were made at the Colusa MAD test area. Three percent permethrin/15% PBO and 4% permethrin/12% PBO were applied at the flow rates of 5, 10, and 16 fl oz/min. Caged *Cx. tarsalis*, *Ae. melanimon*, and *An. freeborni* were used to evaluate chemical efficacy. On-site weather monitoring indicated temperature inversions of 0.5°F to 2.5°F with wind speeds of 5-7 mph. Vehicle speed was 10 mph.

No significant differences in mortality were observed in the three mosquito species exposed; each was equally susceptible to the formulations used. Mortality counts for the 10 and 16 fl oz/min rates were comparable and not significantly different. Ten fl oz/min was equally effective as the higher rate. All 5 fl oz/min applications were operationally unsatisfactory.

The mortalities of caged *Cx. tarsalis* at output rates of 5 and 16 fl oz/min for both formulations are shown in Figures 3 and 4. Significant numbers of mosquitoes termed "one-leggers" and "spinners" were observed in all cages. Although biologically dead mosquitoes (BDM), they were intentionally not included in the mortalities shown. When including BDMs in the graphs, mortalities in all cages would approach 100%, even to a distance of 2640 ft downwind.

Both 10 and 16 fl oz/min showed the BDM phenomenon. The 5 fl oz/min flow rate had few BDMs and mostly survivors. The significance of the one-legged/spinner event is related to the pesticide. An insecticide concentration approaching a sub-lethal dose is indicated when mosquitoes are found in this condition. Our evaluation shows permethrin/PEO to be a promising material for control of OP-resistant *Cx. tarsalis*. Further testing at higher concentrations is planned for the 1989 mosquito season.

Pyrethrin-Pyrethrin (Pyrenone Crop Spray 6/60) was evaluated against laboratory-reared *Cx. pipiens* and wild-caught *Cx. tarsalis* at Solano County and Colusa MADs.

Colusa-The application was made during laminar flow conditions with wind velocities of 5-6 mph. Cages were placed to a maximum distance of

4800 ft downwind. Vehicle speed was 8 mph and output was 8 fl oz/min. Figure 5 shows 100% mortality of *Cx. tarsalis* in all cages.

Solano-Pyrenone Crop Spray (6/60) was diluted with water (3:1) to make a 4.5% formulation. A 3°F temperature inversion and wind velocities of 6-7 mph were recorded at the site during the test. Cages were placed to 3960 ft downwind. Mortalities of 100% were observed in all cages (Figure 5).

Discussion and Conclusion.

Applications against wild-caught adult *Cx. tarsalis* showed considerable organophosphate resistance. However, successful control of *Anopheles* and *Aedes* mosquitoes would be achieved with the chemicals used in this study. Tests indicated that Pyrenone Crop Spray is the material of choice for the successful control of OP-resistant adult *Cx. tarsalis* in the crop areas tested. Continued evaluation of materials for future consideration will be necessary for efficacy determination.

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AERIAL APPLICATION OF SCOURGE® IN ORCHEX 796 OIL AGAINST *CULEX TARSALIS* DURING NON-INVERSION CONDITIONS

C. H. Schaefer[1], F. S. Mulligan, III[1], and H. L. Clement[2]

ABSTRACT

Scourge® is a possible replacement for Baygon® (propoxur) against OP-susceptible and OP-resistant strains of adult mosquitoes. Orchex 796 oil is a suitable diluent at 1:2.33 v/v. Application of this formulation at 1 fl. oz/A (0.004 lb (AI)/A) controls adult *Culex tarsalis* using the spray system described. The effective swath width was ca. 120 ft under daylight, temperature lapse conditions, with a 2-3 mph crosswind. If applications could be made under temperature inversion conditions which are limited to 30-45 minutes before darkness and the same period after daybreak, the effective swath could be increased by ca. 2-fold. Frequently, however, work schedules require aerial applications outside of the brief periods of temperature inversion, and swath width will have to be adjusted accordingly.

Introduction.

During the past year, Mobay Corporation announced it would discontinue marketing the carbamate Baygon® (also known as propoxur) as an outdoor mosquito control agent. Mosquito abatement agencies in California, especially in the southern San Joaquin Valley, have depended heavily on the use of this compound for controlling organophosphorus-resistant (OP-R) adults since 1968. Baygon is effective against OP-R strains of *Culex tarsalis* Coquillet, and could have been used to prevent population build-up of this species during a threat of an encephalitis epidemic (Schaefer et al. 1985, Reisen et al. 1985). With the withdrawal of Baygon from the mosquito adulticide marketplace, it is imperative that a replacement(s) be found as soon as possible.

The Mosquito Control Research Laboratory has evaluated potential adulticides for over 20 years. All new organophosphorus compounds that were available in recent years have shown cross-resistance against OP-R strains of *Cx. tarsalis* and *Culex quinquefasciatus* Say. No new, promising carbamates have become available. Thus, it has become imperative to evaluate all possible replacements, of which there are few.

SBP-1382 (5-benzyl-3-furyl) methyl 2, 2-dimethyl-3-(2 methyl-l-propenyl) cyclopropanecarboxylate is also known as resmethrin and as Scourge when formulated with 1 part active ingredient to 3 parts piperonyl butoxide synergist. This synthetic pyrethroid has been evaluated as a mosquito control agent beginning almost 20 years ago. The purpose of this paper is to describe recent (1988) evaluations, which include careful consideration of the best diluent available and of the meteorological conditions under which aerial adulticiding would have to be conducted.

Previous Studies.

SBP-1382 was first evaluated by the Mosquito Control Research Laboratory in 1970. Initial tests were conducted against OP-S and OP-R strains of mosquito larvae. A summary of these results is given in Table 1. While susceptibility was apparent for *Aedes nigromaculis* (Ludlow), the approximate 3X difference in the LC_{90}s for the OP-S and OP-R strains of *Cx. tarsalis* was cause for concern; although a less than 5X difference cannot be considered as strong evidence of cross-resistance.

Three field tests against adult *Ae. nigromaculis* were made in 1976 using a 2.0 lb (AI)/gal EC formulation. A relatively high dose of 0.01 lb (AI)/gal water/acre was used since an earlier test with lower doses (ground applications using a cold-fogger) yielded poor results. A 40 A alfalfa field in Kings County, having a heavy adult population of *Ae. nigromaculis*, was treated at 0930 hrs during clear, calm conditions using a 60 ft swath in the first trial. No reduction in the adult population was apparent. A second test on another 40 A field in Kings County under similar conditions yielded the same result. Due to the possibility that the active ingredient decomposed due to photoinstability, a third test was applied at daybreak (as soon as enough light was present for the pilot to be able to see obstacles) on a 20 A pasture. The sky

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was clear, the wind calm and the temperature 65°F, but no knock-down or reduction in the numbers of *Ae. nigromaculis* adults was apparent. Thus, even at this high dose (0.01 lb (AI)/A), no efficacy was obtained.

The toxicity of resmethrin (SBP-1382 designation no longer used by this date) to an OP-S laboratory strain of *Cx. quinquefasciatus* was compared to that of an OP-R strain colonized from the storm drains in Fresno, CA in 1981. Also, the OP-R strain was treated with 1 part resmethrin plus 5 parts piperonyl butoxide synergist. These results are summarized in Table 2. The OP-R strain showed an LC_{90} ca. 14-fold greater than the OP-S strain, indicating cross-resistance. However, through synergism with PBO, the LC_{90} of the OP-R strain was reduced by five-fold.

Aerial adulticide trials were made in 1983 using 1 part resmethrin to 3 parts PBO, and diluted with Klearol oil (Whitco Chemical Co.). The spraying system (24-80015 nozzles at 60 psi), was calibrated to deliver 3 fluid oz/A (0.007 lb (AI)/A). Three applications to lines of caged *Cx. tarsalis* adults (each cage 20 feet apart for 600 feet) gave highly variable results. The spray particle sizes were relatively large (volume mass diameters, VMDs, averaged 122 microns) and the results were not considered to be operationally effective.

1988 Studies.

In reconsidering the potential of Scourge for aerial adulticiding, several questions had to be addressed prior to new field evaluations: 1) under what meteorological conditions, i.e. inversion or lapse temperature profile, should the material be tested?; 2) what atomization system should be used?; and 3) what diluent would most likely be effective in getting the active ingredient plus synergist to the target?

Consideration of meteorological conditions. Mosquito abatement districts have been advised in the past to conduct adulticiding operations under periods of temperature inversions in order to maximize lateral movement (effective swath width). However, our experience was that such temperature inversions in the San Joaquin Valley did not occur when aerial applications were attempted. A recording weather station (R. M. Young Model 41402J Temperature Delta-T Indicator), which measures and records temperatures at 8 and 32 feet above ground, and wind velocity and direction at 15 feet above ground, was set up at the Kern MAD and operated during July, August and September in order to clarify this matter. A consistent pattern of temperature inversions occurred. Temperature inversions generally began 30-45 minutes before darkness, remained throughout the night and reverted to lapse conditions 30-45 minutes after daylight began. The strongest inversions (4-6° C) generally occurred between 2200 and 2400 hrs. Frequently, inversion conditions did not start until dusk, and they sometimes terminated before daylight. Such brief periods just before darkness in the evening and just after sunrise in the morning do not provide adequate time for operational aerial applications unless the work can be accomplished in 30-45 minutes. Therefore, it is important to define spraying parameters that can be used under daylight conditions, where temperatures decline with increasing altitude at the adiabatic lapse rate.

What atomization system should be used? Due to relatively poor results in most previous trials, it was considered desirable to attempt to obtain a much smaller droplet spectrum (ca. 50 microns VMD). Therefore, the spray boom was equipped with 4 Beecomist electric nozzles having 25 micron sintered, stainless steel sleeves; these were spaced at 8 feet and 20 feet laterally from the fuselage on each wing of an Ayres Thrush aircraft.

What diluent would likely assist in getting the insecticide and synergist to the target? Comparison of commercially-available oil diluents led to the choice of Orchex 796. This oil has been used in crop protection for many years and it functions as a spreader, sticker, penetrant and low-volatility carrier.

1988 Field Trial. Spraying Systems Co. number 4961-35 flow regulators were used, and the boom was pressured at 20 psi to emit 0.11 gal/min per nozzle to achieve the desired application rate of 1 oz formulation per acre. The Ayres Thrush was flown at 140 mph at an altitude of 15-20 feet. Scourge was diluted with Orchex 796 oil at 1:2.33 v/v. A calibration trial was made and the above system delivered 1 fluid ounce per acre, assuming a 200-foot swath. Measurement of the spray droplets from teflon-coated slides held in a battery operated rotator, showed a VMD of 47 microns.

A remote site in western Kern County was used to determine efficacy of Scourge diluted with Orchex 796 using the above application parameters. Adult mosquitoes (*Cx. tarsalis*) were placed at 20-

Table 1.-1970 tests on the toxicity of SBP-1382 to susceptible and resistant strains of mosquito larvae (in ppm).

Species	Strain	LC_{50}	LC_{90}
Culex quinquefasciatus	OP-S	0.0061	0.010
Culex tarsalis	OP-S	0.0081	0.013
Culex tarsalis	OP-R	0.016	0.038
Aedes nigromaculis	OP-S	0.0055	0.010
Aedes nigromaculis	OP-R	0.0080	0.014

Table 2.-Susceptibility of adult *Culex quinquefasciatus* to resmethrin and resmethrin synergized with piperonyl butoxide (in ppm).

Treatment	Strain					
	OP-susceptible[a]			OP-resistant[b]		
	test no.	LC_{50}	LC_{90}	test no.	LC_{50}	LC_{90}
Resmethrin only	1	0.011	0.025	1	0.040	0.22
	2	0.011	0.017	2	0.054	0.24
	3	0.013	0.036	3	0.043	0.60
	Avg.	0.012	0.026	Avg.	0.046	0.35
Resmethrin plus 5X PBO				1	0.0095	0.082
				2	0.0045	0.082
				3	0.0083	0.045
				Avg.	0.0074	0.070

[a] Laboratory strain
[b] Strain isolated from underground storm drain lines in Fresno.

foot intervals in cages held on 4 ft stakes. The application was made 45 minutes after daylight (7/26/88). The temperature was 27.0°C at 8 ft and 26.9°C at 32 ft (Δ T = - 0.1°C). A temperature inversion was present earlier (before 0640 hrs) and this changed to the lapse condition prior to 0659 hrs, when the application was made. The wind was 2-3 mph from the southeast and the relative humidity was 50%.

Complete adult mortality occurred in cages 120 ft downwind of the flightline, and 35-90% mortality in those 120-260 feet downwind. Had the application been made earlier, while the temperature inversion was present, an effective swath of 200 ft (+) would almost certainly have been obtained. Thus, application under the lapse condition still allowed for control but the effective swath width was reduced by ca. 50%. Also, the effective swath width (120 ft) was partly determined by the 2-3 mph crosswind. The effective swath would have been reduced to the aircraft wingspan plus some lateral movement due to wing tip vortices (probably 60-65 ft overall) in a no-wind condition.

Spray droplet spectra at 60 and 160 ft downwind collection points showed VMDs of 57 and 51 microns, respectively.

Therefore, using the aerial application system described, Scourge diluted with Orchex 796 (1:2.33 v/v) will provide control of adult mosquitoes, but the swath width must be adjusted (reduced by about 50%) during temperature lapse conditions. Such conditions occur in the San Joaquin Valley during daylight hours except 30-45 minutes before darkness, and the same interval after daybreak.

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THE USE OF PROPOXUR TO CONTROL ADULT *AEDES* POPULATIONS

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Introduction.

Solano County is one of the northern San Francisco Bay area counties. It extends from San Pablo Bay and Suisun Bay north to the cities of Winters and Davis, and covers a total of 827 square miles. All of it is served by the Solano County MAD. The city of Dixon is located in the northern portion of the county, approximately 18 miles southwest of Sacramento. This area is known for its agricultural productivity, with cattle and sheep being among the most important commodities raised.

The practice of contracting with an aerial applicator to spray *Aedes* mosquitoes in irrigated pastures in the Dixon area originated during the early 1960s. Objectives of the aerial spray program at that time were to: (1) protect livestock and ranch workers from stress caused by hordes of *Aedes nigromaculis* (Ludlow) and, to a lesser degree, *Aedes melanimon* Dyar and (2) prevent these populations from entering adjacent rowcrop properties and disrupting field workers there. Aerial treatment delays occur often in the pasture areas situated 7 to 10 miles southeast of Dixon, this is due to prevailing winds from the south and southwest in excess of 10 mph which persist during daylight hours.

By 1984, the District had contracted with the same flying service for more than 10 years, and used organophosphorus (OP) insecticides throughout the entire period. Fenthion and malathion were used to treat larvae, adults, and occasionally both stages. Applications had achieved satisfactory results consistently. During the summer of 1984, however, control failures occurred on 20% of the acreage treated with fenthion (used at the maximum allowable rate), and on 5% of the acres treated with malathion (used at half the allowable rate) against *Ae. nigromaculis* (Table 1). All Baygon® applications were successful.

Based upon the increasing occurrence of adult failures observed in the field, and confirmation of incipient level larval resistance to OP insecticides through laboratory testing, the decision was made to implement the following changes in the aerial spray program: (1) treat the adult stage only; (2) increase the malathion rate to the maximum allowed; (3) discontinue the use of fenthion over irrigated pasture, and (4) increase the use of Baygon®, the carbamate propoxur. This chemical had been shown to be effective against OP-resistant *Aedes* populations of the Central Valley (Gutierrez et al. 1974) after several seasons of use, and it has been used successfully in the San Joaquin Valley since 1968 (Schaefer et al. 1985).

Applications of Baygon® and malathion (used at the high rate) achieved satisfactory results during the remainder of the 1984 mosquito season. Failures began to reappear early in the summer of 1985, however, after a brief period of successful control using both Baygon® (13% of the acreage treated) and malathion (14% even at the maximum rate), as shown in Table 2. Consequently, a plan

Table 1.-Aerial applications over irrigated pasture during 1984 to control adult *Aedes*.

	Acres Treated	Failures	
Baytex	2594	512	19.7%
Malathion	2946	154	5.2%
Baygon	371	0	-------
Total	5911	666	11.3%

Table 2.-Aerial applications over irrigated pasture during 1985 to control adult *Aedes*.

	Acres Treated	Failures	
Baygon	1251	161	12.9%
Malathion	1144	159	13.9%
Baytex	130	0	-------
Total	2525	320	12.7%

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was developed to determine the causes of these failures, since the *Aedes* should have been particularly susceptible to propoxur. The plan included the following items: (1) check the quality of the Baygon® 70% WP; (2) observe mixing and loading procedures for errors and review inventory records; (3) monitor application techniques and weather conditions; (4) check the activity of the adult mosquitoes; (5) conduct adult cage tests to evaluate the performance of the insecticide in the field; and (6) conduct bioassays in the laboratory and compare these data to those of other populations known to be operationally susceptible.

The purpose of this paper is to discuss the measures undertaken by the District at two study sites to resolve the control problems we were experiencing with aerial applications.

Field Test Sites.

Pocket Ranch.-This 1250 acre ranch, comprised entirely of irrigated pasture, is located approximately 10 miles southeast of Dixon. A 50 acre area of the ranch (an area commonly sprayed by aircraft) was used for this test. No field failures, using either the OPs or Baygon®, had occurred on this property. The Pocket Ranch was judged a suitable test site because it produces high numbers of adult mosquitoes in several accessible fields.

Borden Ranch.-The 143 acre Borden Ranch (also comprised solely of irrigated pasture) is located about seven miles southeast of Dixon. An 80 acre area of the ranch served as the test site. This property had been heavily pressured with OPs for over 10 years. A number of field failures occurred on this ranch prior to the test during the summer of 1985.

Materials and Methods.

Private laboratory analysis.-A sample of the WP product, and a sample collected from the airplane tank (the mix used for the Borden Ranch test), were sent to an independent laboratory for analysis of percent active ingredient.

Field tests.-Baygon® 70% WP was used in the two field tests at the maximum allowable rate (0.07 lb. AI/acre). Each tank mixture was made at the rate of 0.1 lb of Baygon® 70% WP per 1.0 gal of water and applied at the rate of 1.0 gal per acre as indicated by the applicator. Both applications were made using a Cessna A188B Ag Truck equipped with a 280 gal capacity tank. Twenty-two nozzles equipped with D-8 orifices and No. 45 disks operated at 20 psi were used in both tests. The nozzles were directed straight back during the Pocket Ranch test and back and down at a 45° angle for the Borden Ranch test. Flight speed was 110 mph at elevations of 10-15 ft during the Pocket Ranch test, and 15-20 ft during the Borden Ranch test.

A line of caged, wild mosquitoes was used to evaluate the effectiveness of the Baygon® under field conditions. Laboratory-reared adults from on-site collected *Ae. nigromaculis* pupae were transferred into disposable cages (Townzen & Natvig 1973) at the rate of 20 adults per cage. The cages were attached to 4 ft metal stakes in the field. Protocol requires a second set of cages containing susceptible laboratory colony adults to be attached to each stake next to the wild cage. These mosquitoes are used to demonstrate the effectiveness of the application itself (Townzen et al. 1987). No colony adults were available for our tests. Measurements of wind speed and direction, as well as temperature, were taken at a distance of 4-5 ft from ground level.

The cages were collected immediately after each test and placed separately into plastic bags (to prevent cross contamination) after the cotton rolls (dental type, 1/4" x 1 1/2") were remoistened with water. Mortality was recorded 12 hr posttreatment for the caged adults. Pre- and 24 hr post-test adult activity were recorded by taking leg counts of 30 seconds (per person) at three locations along the cage line in each test.

Pocket Ranch Test.-The Pocket Ranch Test was conducted at 1200 hrs on August 20, 1985. The temperature was 73° F with a southwest wind of approximately 6 mph under clear skies. The cage line (spaced at 10 ft intervals) extended 170 ft into the pasture from the west fence line, perpendicular to the Ag Truck's flight path. The pilot began downwind and proceeded in a north to south pattern from east to west across the prevailing wind and the cage line.

Borden Ranch Test.-The Borden Ranch Test was conducted at 1303 hrs on August 28, 1985 under clear skies with a temperature of 80° F and a 5 mph south to southwest wind. The cage line (spaced at 50 ft intervals) extended 950 ft into the pasture from the south fence line, and perpendicu-

lar to the flight path. The pilot began in the southwest and flew west to east across the prevailing wind.

Adult bioassay.-Monitoring the susceptibility of adult *Ae. nigromaculis* and *Ae. melanimon* populations to propoxur and the OP insecticides from heavily OP-pressured pastures began at our District in 1986. Adults reared from field collected pupae were tested 2-4 days postemergence. Various aspects of the bioassay method used to determine susceptibility levels of adult mosquitoes have been described by Georghiou and Metcalf (1961), Georghiou and Gidden (1965), and Case and Kauffman (1984). In this contact toxicity bioassay, 2 ml doses of insecticide solution of known strength are applied to glass filter papers (Whatman® glass microfibre, GF/A, 9.0 cm diam). The adults are transferred from rearing buckets using a mechanical aspirator. The exposure period is 1 hr, after which the adults are knocked down with CO_2 gas (metered @ 3 liters/min) and transferred to holding containers. Mortality is counted 24 hr posttreatment. For further information refer to Thompson (1989).

Results and Discussion.

Private laboratory analysis.-Laboratory analysis determined the Baygon® WP sample contained the full amount of active ingredient (71.7%). Communication with the manufacturer regarding this lot number did not reveal any complaints from other MADs using Baygon® of this lot in California.

Mixing and loading procedures were observed by District staff and judged to be executed properly. The tank mix sample submitted from the Borden Ranch Test was determined to be of proper concentration (0.79% AI allowing for hydrolysis at the rate of 1.5% per day).

Field tests.-The Pocket Ranch Test cage line mortalities (Table 3) averaged 94.7% which indicates that good coverage was achieved overall. At least one gap occurred within the cage line at the 20 ft station. Had this skip not occurred, the overall cage line mortality would have been 99.6%. Pretreatment leg counts were made the morning of August 20 and posttreatment counts (Table 4) revealed an overall 97.0% reduction of adults on site.

Borden Ranch Test line mortalities (Table 5) averaged 97.3% which also indicates good coverage. The application, however, appears to be inade-

Table 3.-Cage station mortalities of the Pocket Ranch propoxur aerial application against *Aedes nigromaculis*, August 20, 1985.

Station (ft)[1]	12 hr cage mortality (%)[2]
0	100
10	94
20	12
30	100
40	100
50	100
60	100
70	100
80	100
90	100
100	100
110	100
120	100
130	100
140	100
150	100
160	100
170	100

[1] From west fence line.

[2] Total test average = 94.7%.

Table 4.-Pocket Test leg counts.

	Leg Counts [1]		
Station	Pre	Post	% Reduction
1	20	1	95
2	24	1	96
3	20	0	100
Average	21.3	0.66	97.0%

Table 5.-Cage station mortalities of the Borden Ranch propoxur aerial application against *Aedes nigromaculis*, August 28, 1985.

Station (ft)[1]	12 hr cage mortality (%)[2]
0	100
50	97
100	100
150	97
200	100
250	100
300	98
350	100
400	100
450	88
500	100
550	100
600	100
650	90
700	100
750	85
800	100
850	95
900	96
950	100

[1] From south fence line.

[2] Total test average = 97.3%.

Table 6.-Borden Test leg counts.

	Leg Counts [1]		
Station	Pre	Post	% Reduction
1	17	0	100
2	33	2	94
3	34	5	85
Average	28	2.3	93%

quate because only 60% of these cages achieved 100% mortality, while in the Pocket Ranch Test, 89% of the cages showed a complete kill. Pretreatment leg counts were made the morning of August 28, and posttreatment counts revealed an overall 93% reduction (Table 6).

The District contracted with another aerial applicator prior to the 1986 season. The spray system employed by the new contractor utilized 46 #3 WhirlJet Nozzles operated at 10-12 psi with an output rate of 1.0 gal/A. All Baygon® applications using this system and conducted during the 1986 through 1988 mosquito seasons were successful.

Adult bioassays.-The results of propoxur laboratory tests conducted against *Ae. melanimon* are shown in Table 7. Solano County MAD data are similar to those of Sacramento County - Yolo County MAD, whose populations are reportedly operationally susceptible to Baygon®.

Overall *Ae. nigromaculis* test results (Table 8) are similar to those reported for *Ae. melanimon* in that all tests have LC_{90}s less than 3.00 $\mu g/cm^2$. Unfortunately, the predominant species shifted to *Ae. melanimon* on the Pocket Ranch during 1986, and remained so through 1988. Consequently, the only data on *Ae. nigromaculis* against propoxur was obtained from the Borden Ranch. An aircraft test conducted by Scott E. Monsen, Butte County MAD, on the the Sutter-Yuba MAD population revealed operational susceptibility to Baygon®. Laboratory and field test results (and operational notes) appear to indicate the propoxur data presented in Tables 7 and 8 describe susceptible populations. Additionally, Dixon area *Ae. nigromaculis* malathion bioassays also have susceptible results (Thompson 1989).

Conclusion.

The problems experienced by the Solano County MAD with aerial Baygon® applications in the Dixon area were not due to resistance, but rather to operational procedure. They can be attributed to the use of an inadequately equipped nozzle system and, possibly, inconsistent application technique.

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Table 7.-Results of bioassays conducted in the laboratory for propoxur against adults of specified *Aedes melanimon* populations. LC_{50}s and LC_{90}s ($\mu g/cm^2$) obtained by probit analysis (heterogeneity = 1.00).

POPULATION	LC_{50}	95% INTERVAL	LC_{90}	95% INTERVAL	RATIO[1]
Solano Co. MAD					
-The Borden Ranch	0.920	0.775-1.10	2.23	1.74-3.31	2.42
-The Pocket Ranch	0.907	0.757-1.12	1.54	1.22-2.43	1.70
Sacto. Co.-Yolo Co. MAD	2.14	2.01-2.27	2.89	2.69-3.20	1.35

[1]LC_{90}/LC_{50}.

Table 8.-Results of bioassays conducted in the laboratory for propoxur against adults of specified *Aedes nigromaculis* populations. LC_{50}s and LC_{90}s ($\mu g/cm^2$) obtained by probit analysis (heterogeneity = 1.00).

POPULATION	LC_{50}	95% INTERVAL	LC_{90}	95% INTERVAL	RATIO[1]
Solano Co. MAD					
-The Borden Ranch	0.856	0.724-1.01	2.28	1.86-2.97	2.66
Colusa MAD	1.72	1.58-1.87	2.98	2.67-3.45	1.73
Sutter-Yuba MAD	0.857	0.737-0.999	2.36	1.91-3.11	2.75
East Side MAD	1.42	1.15-1.77	2.57	2.04-3.53	1.81
Turlock MAD	1.06	0.878-1.26	2.68	2.16-3.64	2.53

[1]LC_{90}/LC_{50}.

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SUSCEPTIBILITY LEVELS OF ADULT MOSQUITOES TO THE ORGANOPHOSPHORUS INSECTICIDES IN CALIFORNIA

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Introduction.

The resistance surveillance program of the Environmental Management Branch (EMB) has been based since its inception in 1963 on testing field-collected mosquito larvae in the laboratory to measure the susceptibility of important species to organophosphorus (OP) insecticides. Laboratory test results were compared with the observed performance of these larvicides in the field, and from this evidence thresholds were established for each OP (summarized in Thompson 1985, 1986). Thresholds interpret the response of a test population in a laboratory bioassay, and are used to predict the probable response of the field population to an operational application of the insecticide (Womeldorf et al. 1966, Gillies et al. 1968).

Field trials, especially coldfogger tests (Womeldorf et al. 1973), were the primary method used by EMB until recently to measure the effectiveness of insecticides on local populations of adult mosquitoes (Townzen et al. 1987 and 1989). These tests have the distinct advantage of measuring the efficacy of an adulticide under normal operational conditions. Missing from our surveillance program, however, have been laboratory-generated susceptibility data on adult mosquitoes to complement this operational information. Laboratory bioassays measure the response of a population to the chemical over a range of known doses under controllable environmental conditions. Thus, laboratory test data can be conveniently and accurately reproduced under similar conditions so that the response of mosquitoes in one geographical area can be compared to that of another.

The purpose of this report is to present the findings of an adult mosquito susceptibility survey which has been actively in progress since 1987. Objectives of the survey are: 1) to obtain a statewide, skeletal overview of the susceptibility of vector mosquito species, especially *Culex tarsalis* Coquillett, in the laboratory to the OP-active ingredients malathion and chlorpyrifos (Dursban®); and 2) to describe the characteristics of susceptible and resistant bioassay data using interpretive thresholds developed from information on the response of adult populations to the operational use of the OPs in companion fogger test evaluations.

Methods and Materials.

Culex adults were collected by dry-ice baited EVS light-trapping, the preferred method (females only), or as first instar larvae through pupae and reared to the adult stage in the laboratory. *Aedes* species were most often collected at either the late fourth instar larval or pupal (preferably) stage. Occasionally these adults were collected by sweepnetting.

Culex larvae were fed finely ground laboratory rat chow once or twice a day. *Aedes* larvae usually did not require feeding, but when they were fed, several alfalfa rabbit pellets were put into each enamel rearing pan. Pupae were harvested from their pans daily, and placed into an eight-ounce plastic food container containing about 100 ml water. One container with approximately 500 pupae (covering about 3/4 of the water's surface area) was placed in a 3.5 quart plastic food container rearing bucket for emergence. A one foot length of surgical stockinette sleeve was affixed over a 4 inch square hole on the side of the bucket for access. A nine inch diameter circle of nylon net material covered the bucket and was secured by the lid, which had a 5 inch diameter hole cut into it. Emerged adults were provided moistened raisins for an energy/food source, and for water a saturated, thick piece of absorbent cotton (2 in square, covered by a glass petri dish) was available to the adults on the netting at all times.

Stock solutions were prepared from purified insecticide standards purchased from Chem Service, Inc. (West Chester, PA) by wt/vol dilution in reagent grade acetone (A.S.C.). Serial dilutions were made from these stock solutions (1.0%) to prepare the range of doses ordinarily encountered

in the adult bioassay for each active ingredient. The serial test solutions were poured into pre-labeled, round amber bottles, and stored in a refrigerator after being mixed.

The fundamentals of the contact toxicity filter paper test used in this study were described by Georghiou and Metcalf (1961), and Georghiou and Gidden (1965). Bioassay methodology is outlined as follows: 2 ml doses of insecticide solution of known concentration were applied to 9.0 cm glass microfibre filter circles (Whatman® GF/A). Usually two or three replicate filters were used for each dose of a bioassay. An attempt was made to use a dose range starting low enough not to produce any mortality, and then increasing sequentially in concentration to a high dose that would be expected to achieve 100% mortality. The number of doses per bioassay varied from 6-12, depending upon the availability of test specimens. Normally eight doses were used with two control filters. The filter circles were placed on the heads of small finish nails (#4) of a "nailboard" made of 3/4 inch plywood (20 in x 28 in). The nails had been driven into the plywood, leaving 3/4 of an inch of nail above the surface, in a 1-in square grid pattern. With the filters suspended on the nailboard, acetone was applied to the control filters, and then the other filters were treated with test solutions beginning with the lowest dose. The treated filters were allowed to air dry for 5-10 minutes before they were individually rolled up and placed firmly against the inner surface of a glass shell vial (25 mm x 95 mm) previously labeled according to the dosage. After loading all of the filters, each vial was covered with a small piece of fiberglass window screen material (about 3.5 in square), and tightly secured with one or two rubberbands. A slit was cut across the taut screen with a scalpel to accommodate the transfer of adult mosquitoes into the vial.

Unlike the technique described by Case and Kauffman (1984), adults were not anesthetized with CO_2 gas and chilled before transfer. A hand-held mechanical aspirator (size D battery operated, Hausherr's Machine Works, Toms River, N.J.) was used to gently collect flying adults directly from the rearing bucket. The mosquitoes were then "puffed" by mouth back through the removable adapter into the shell vial via the screen slit. Once the adults were loaded, the slit was interlocked and the time (within the current minute) was marked on the vial. Approximately 20-25 adults (2-4 days postemergence), preferably females, were introduced into each vial unless precluded by low numbers. In situations such as those, males (mixed proportionally with mostly females) were included because the response of both sexes is frequently the same (WHO/VBC/81.805). Females should be used exclusively when possible.

The vials, after they had been loaded with adults, were placed in a rack to keep them organized and resting in a horizontal position. The rack was stored in a cool dark area of the laboratory after the last vial was loaded and the time marked on it. The mosquitoes were exposed to the treated filters for one hour. As the one hour period lapsed on the first control vial (and subsequently for the others), the vial was quickly put into a "knockdown chamber" (a plastic, 2.5 cup capacity food container) and covered with the lid inverted. The chamber was supplied with CO_2 gas, metered by a medical regulator (e.g. Victor VMG5LN) at the rate of three liters/minute. The adults were exposed to the CO_2 for 30 seconds, the screen was removed, and the anesthetized mosquitoes were transferred into pre-labeled eight-ounce plastic food containers and covered with clear lids that had a cotton dental roll (slightly moistened) stapled to the inside. Mortality was recorded 24 hours post-treatment. Bioassay data were evaluated by probit analysis (Finney 1971) using a computer program developed by Raymond (1985) where appropriate.

Results and Discussion.

Results of the 56 bioassays presented in the following tables were selected from 49 malathion (54%) and 42 chlorpyrifos (46%) bioassays (91 total) conducted during 1984 (6%), 1987 (37%), 1988 (44%), and early 1989 (13%) against 62 populations of four species of mosquitoes. Often an OP was tested on a single population more than once. In those cases, and when more than one population of a single species was tested at an agency, one bioassay was selected to represent the response of that species to the OP for the agency where the mosquitoes were collected.

Table 1 contains bioassay results of the *Cx. pipiens* Linnaeus complex colony maintained by EMB in Sacramento. These mosquitoes are used (in disposable paper cages clipped to lath stakes) during fogger tests as "air samplers" to monitor the effectiveness of the operation, since they have been proven to be operationally susceptible to both

Table 1.-Results of bioassays conducted in the laboratory against adults of the *Culex pipiens* complex colony maintained by EMB in Sacramento. LC_{50}s and LC_{90}s ($\mu g/cm^2$) obtained by probit analysis (heterogeneity = 1.00).

CHEMICAL	LC_{50}	95% INTERVAL	LC_{90}	95% INTERVAL	RATIO[1]	STATUS[2]
Malathion	5.70	5.30-6.11	8.79	8.09-9.75	1.54	S
Chlorpyrifos	0.119	0.101-0.142	0.417	0.308-0.674	3.50	S

[1]LC_{90}/LC_{50}. [2]Resistance status: S, susceptible.

Table 2.-Results of bioassays conducted in the laboratory for malathion against adult *Aedes melanimon* populations of specified mosquito control agencies. LC_{50}s and LC_{90}s ($\mu g/cm^2$) obtained by probit analysis (heterogeneity = 1.00).

AGENCY	LC_{50}	95% INTERVAL	LC_{90}	95% INTERVAL	RATIO[1]	STATUS[2]
Colusa	5.09	4.67-5.47	6.78	6.27-7.56	1.33	S
Sacramento-Yolo	4.45	4.10-4.83	5.65	5.18-6.34	1.27	S
Solano Co.	2.96	2.68-3.32	4.51	3.91-5.58	1.52	S
Madera Co.	4.41	3.90-4.97	5.61	4.98-6.66	1.27	S

[1]LC_{90}/LC_{50}. [2]Resistance status: S, susceptible.

Table 3.-Results of bioassays conducted in the laboratory for malathion against adult *Anopheles freeborni* populations of specified mosquito control agencies. LC_{50}s and LC_{90}s ($\mu g/cm^2$) obtained by probit analysis (heterogeneity = 1.00).

AGENCY	LC_{50}	95% INTERVAL	LC_{90}	95% INTERVAL	RATIO[1]	STATUS[2]
Colusa	8.09	7.18-9.22	11.6	10.0-14.8	1.43	S
Sutter-Yuba	5.90	4.84-6.58	8.46	7.32-15.3	1.43	S

[1]LC_{90}/LC_{50}. [2]Resistance status: A, susceptible.

Table 4.-Results of bioassays conducted in the laboratory for malathion against adult *Aedes nigromaculis* populations of specified mosquito control agencies. LC_{50}s and LC_{90}s ($\mu g/cm^2$) obtained by either graphic or probit analysis as indicated.

AGENCY	LC_{50}	95% INTERVAL[1]	LC_{90}	95% INTERVAL	RATIO[2]	STATUS[3]
Sutter-Yuba	15.4	11.9-19.9	64.9	46.1-104	4.21	R
Solano Co.	4.40	4.01-4.86	7.70	6.73-9.29	1.75	S
Contra Costa	4.39	3.96-4.87	7.68	6.67-9.41	1.75	S
East Side[4]	12.2	---	--	---	--	R
Turlock[5]	8.79	---	15.4	---	1.75	R
Madera Co.[6]	8.79	---	--	---	--	R
Consolidated	7.13	3.50-11.2	55.1	31.8-160	7.73	R

[1]95% Intervals determined by probit analysis (heterogeneity = 1.00); plateaus by graphic analysis. [2]LC_{90}/LC_{50}. [3]Resistance status: R, resistant; S, susceptible. [4]Plateau at 63% mortality. [5]Plateau at 91% mortality. [6]Plateau at 88% mortality.

Table 5.-Results of bioassays conducted in the laboratory for malathion against adult *Culex tarsalis* populations of specified mosquito control agencies. LC_{50}s and LC_{90}s ($\mu g/cm^2$) obtained by either graphic or probit analysis as indicated.

AGENCY	LC_{50}	95% INTERVAL[1]	LC_{90}	95% INTERVAL	RATIO[2]	PLATEAU[3]	STATUS[4]
Pine Grove	4.71	---	--	---	--	88	R
Shasta	8.19	6.12-11.2	41.1*	25.6-91.6	5.02	--	R
Butte Co.	11.6	---	--	---	--	64	R
Colusa	15.4	---	--	---	--	75	R
Sutter-Yuba	--	---	--	---	--	51	R
Sacramento-Yolo	6.59	---	--	---	--	55	R
Solano Co.	7.85	---	--	---	--	69	R
Alameda Co.	17.0	---	--	---	--	49	R
Fresno Co. UNC[5]	8.58	8.14-9.04	11.9	11.1-13.1	1.39	--	S
Madera Co.	--	---	--	---	--	28	R
Southfork	7.06	6.72-7.47	8.45	7.90-9.48	1.20	--	S
Northwest	5.97	---	--	---	--	87	R
Coachella Valley	6.59	---	11.6	---	1.76	97	R
Imperial Co.	11.9	---	21.4	---	1.80	93	R

[1]95% Intervals determined by probit analysis (heterogeneity = 1.00). [2]LC_{90}/LC_{50}. [3]% mortality, determined by graphic analysis. [4]Resistance status: R, resistant; S, susceptible. [5]Uncontrolled area, not within the boundaries of an organized mosquito control program. *Michael A. Seth tester, extrapolated LC_{90}.

Table 6.-Results of bioassays conducted in the laboratory for chlorpyrifos against adult *Aedes melanimon* populations of specified mosquito control agencies. LC_{50}s and LC_{90}s ($\mu g/cm^2$) obtained by probit analysis (heterogeneity = 1.00).

AGENCY	LC_{50}	95% INTERVAL	LC_{90}	95% INTERVAL	RATIO[1]	STATUS[2]
Colusa	0.0541	0.0500-0.0579	0.0746	0.0686-0.0852	1.38	S
Sacramento-Yolo	0.0878	0.0783-0.0977	0.158	0.139-0.186	1.80	S
Solano Co.	0.0134	0.0114-0.0153	0.0357	0.0301-0.0449	2.66	S
Madera Co.	0.0504	0.0435-0.0578	0.0771	0.0657-0.104	1.53	S

[1]LC_{90}/LC_{50}. [2]Resistance status: S, susceptible.

Table 7.-Results of bioassays conducted in the laboratory for chlorpyrifos against adult *Anopheles freeborni* populations of specified mosquito control agencies. LC_{50}s and LC_{90}s ($\mu g/cm^2$) obtained by probit analysis (heterogeneity = 1.00).

AGENCY	LC_{50}	95% INTERVAL	LC_{90}	95% INTERVAL	RATIO[1]	STATUS[2]
Colusa	0.101	0.0868-0.116	0.188	0.158-0.247	1.86	S
Sutter-Yuba	0.0811	0.0680-0.0946	0.159	0.132-0.214	1.96	S

[1]LC_{90}/LC_{50}. [2]Resistance status: S, susceptible.

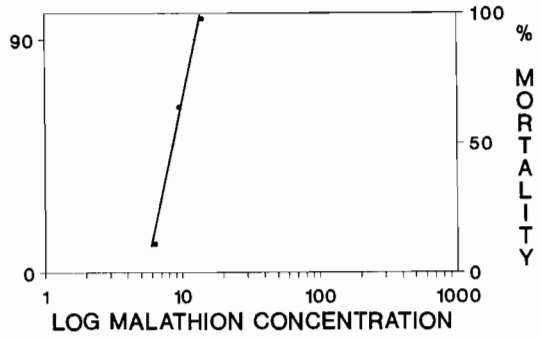

Figure 1.-Graphic analysis of the Wonder Valley *Culex tarsalis* data (Fresno County) showing a steep slop; LC_{90}/LC_{50} ratio = 1.40.

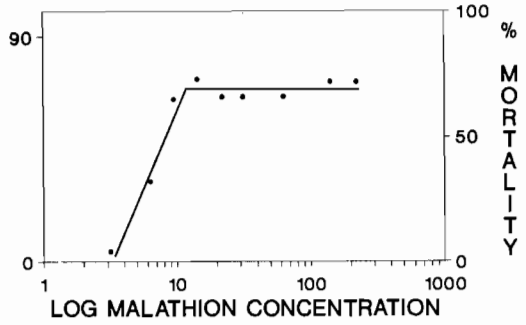

Figure 2.-Graphic analysis of the Grizzly Island *Culex tarsalis* data (Solano County) showing a plateau at 69% mortality.

Dursban® and malathion. Fogger tests conducted under adequate meteorological conditions with recommended dosages usually kill these mosquitoes, and measure the effectiveness of the insecticide to caged wild populations (clipped to each stake next to the colony cage). Experience with the fogger test, though, has demonstrated that our laboratory colony is not as susceptible to chlorpyrifos as it is to malathion (described in detail later).

The colony, *Aedes melanimon* Dyar (Table 2), and *Anopheles freeborni* Aitken (Table 3) test results are models of malathion susceptible data. LC_{90}/LC_{50} ratios are less than 2.00, indicating steep-sloped regression lines (Figure 1) of populations responding homogeneously to the insecticide over a narrow range of concentrations. This range, for these bioassays, covers a series of relatively low concentrations below 31.4 $\mu g/cm^2$ suggesting susceptibility. Fogger test results (Townzen et al. 1987, 1989, and unpublished data) have shown *Ae. melanimon* and *An. freeborni* populations from Colusa MAD to be operationally susceptible to malathion. *Aedes melanimon* from Solano County MAD, too, can be controlled effectively with malathion applied by aircraft. In another fogger test, Contra Costa MAD's *Ae. nigromaculis* Ludlow was also found to be susceptibile, substantiating the laboratory test result on this population (Table 4), which is strikingly similar to those of *Ae. melanimon*, *An. freeborni*, and the laboratory colony. Control difficulties, however, were reported with *Ae. nigromaculis* populations from Sutter-Yuba, East Side, Turlock, and Madera County MADs. Mortality plateaus (Figure 2) were evident in the test data of the latter three agencies.

Preliminary findings appear to demonstrate that LC_{90}s greater than a threshold concentration tentatively established at 31.4 $\mu g/cm^2$, or plateaus that extend into concentrations higher than this threshold, seem to depict resistant populations. At this time, however, field and companion laboratory studies to locate definitively this concentration for malathion (and another for chlorpyrifos) are not complete. Survivors of the threshold in a laboratory bioassay identify the resistant individuals of a test population that survive an application of the insecticide in the fogger test, and consequently, operational failures of some degree should be anticipated when treating the wild counterpart of that population in the field.

Plateaus are typical of *Cx. tarsalis* malathion bioassays (Table 5). The Shasta MAD data may not be an exception. This population was not tested to concentrations greater than the threshold. A plateau may have been located had this been done. Ordinarily, if a plateau is situated below 90% mortality, an LC_{90} can not be determined by graphic analysis (Figure 2). This is why there are so many missing LC_{90}s in Table 5. Analysis of the Sutter-Yuba and Madera County data show plateaus only, no LC_{90}s or LC_{50}s. The Coachella Valley and Imperial County bioassays have susceptible LC_{90}s, after which a plateau extends into concentrations higher than the threshold, indicating resistance. The other populations, with the exception

Table 8.-Results of bioassays conducted in the laboratory for chlorpyrifos against adult *Aedes nigromaculis* populations of specified mosquito control agencies. LC_{50}s and LC_{90}s ($\mu g/cm^2$) obtained by probit analysis (heterogeneity = 1.00).

AGENCY	LC_{50}	95% INTERVAL	LC_{90}	95% INTERVAL	RATIO[1]	STATUS[2]
Shasta	0.746	0.616-0.913	2.84	2.12-4.23	3.81	R
Solano Co.[3]	0.314	0.258-0.376	1.02	0.806-1.42	3.25	R
Contra Costa	0.0773*	0.0440-0.105	0.309	0.260-0.394	4.00	S
East Side	0.856	0.696-1.04	2.91	2.25-4.14	3.40	R
Turlock	0.489	0.393-0.584	1.30	1.05-1.78	2.66	R
Madera Co.	0.0882	0.0736-0.103	0.198	0.163-0.268	2.24	S
Consolidated	0.0298	0.0247-0.0369	0.0762	0.0565-0.129	2.56	S

[1]LC_{90}/LC_{50}. [2]Resistance status: R, resistant; S, susceptible. [3]Glenn Yoshimura tester. *Extrapolated LC_{50}.

Table 9.-Results of bioassays conducted in the laboratory for chlorpyrifos against adult *Culex tarsalis* populations of specified mosquito control agencies. LC_{50}s and LC_{90}s ($\mu g/cm^2$) obtained by either graphic or probit analysis as indicated.

AGENCY	LC_{50}	95% INTERVAL[1]	LC_{90}	95% INTERVAL	RATIO[2]	STATUS[3]
Pine Grove[4]	0.0565	---	0.722	---	12.8	R
Shasta	0.0564	0.0390-0.0745	0.229	0.162-0.404	4.06	S
Butte Co.	0.104	0.0688-0.144	1.11	0.674-2.46	10.7	R
Glenn Co.	0.211	0.142-0.288	1.27	0.870-2.22	6.02	R
Colusa	0.172	0.135-0.214	1.35	1.02-1.94	7.85	R
Sutter-Yuba	0.136	0.0626-0.198	0.924	0.585-2.63	6.79	R
Sacramento-Yolo[5]	0.0879	---	0.879	---	10.0	R
Solano Co.	0.106	0.0897-0.124	0.349	0.279-0.466	3.29	S
Alameda Co.	0.324	0.252-0.417	1.63	1.10-2.99	5.03	R
Fresno Co. UNC[6]	0.0780	0.0704-0.0862	0.162	0.142-0.190	2.08	S
Southfork	0.0325	0.0306-0.0354	0.0425	0.0381-0.0525	1.31	S
Northwest	0.0751	0.0607-0.0907	0.487	0.363-0.728	6.48	S
Coachella Valley	0.635	0.569-0.708	1.99	1.71-2.40	3.13	R
Imperial Co.	0.818	0.726-0.914	2.96	2.55-3.54	3.62	R

[1]95% Intervals determined by probit analysis (heterogeneity = 1.00); plateaus by graphic analysis. [2]LC_{90}/LC_{50}. [3]Resistance status: R, resistant; S, susceptible. [4]Plateau at 89% mortality. [5]Plateaus at 81% mortality. [6]Uncontrolled area, not within the boundaries of an organized mosquito control program.

of two, have plateaus of low enough % mortality to prevent determination of an LC_{90}. These populations are responding to the chemical heterogeneously over a wide range of concentrations, surviving those which denote resistance. The Grizzly Island population of Solano County MAD is not as tolerant to malathion as most of the others listed in the table. Populations with plateaus of lower % mortality are more tolerant of the chemical than those having plateaus of higher % mortality. This is presumably because of a greater frequency of resistant individuals.

Fogger tests were conducted against the Colusa, Solano County and Imperial County *Cx. tarsalis* populations, and they confirmed operational resistance. Generally, the % mortality of the plateau was the average level of control demonstrated in the fogger operation, whereas adults of the laboratory colony were killed concurrently. Overall, malathion does not appear to be the appropriate choice of insecticide to use against *Cx. tarsalis* to interrupt the spread of a disease outbreak by eliminating these vector mosquitoes. Test populations of the Sacramento Valley, San Joaquin Valley, and Southern California CMVCA Regions are resistant to this insecticide. Fortunately, models of susceptibility were discovered at Wonder Valley (Fresno County) and Southfork MAD in Kern County, east of Lake Isabella. These data are dramatically similar to those describing the laboratory colony, *Ae. melanimon*, *An. freeborni* and susceptible *Ae. nigromaculis*.

Test results for *Ae. melanimon* (Table 6), except for Solano County MAD, and *An. freeborni* (Table 7) are models of chlorpyrifos susceptibility, having LC_{90}s less than the threshold tentatively established at 0.628 $\mu g/cm^2$, and ratios less than 2.00. The colony (Table 1) has a ratio greater than 2.00 and is not a "model" by definition. The colony has been shown to be susceptible to Dursban® in fogger tests (Townzen et al. 1987), but since the LC_{90} is nearly equal to the threshold, the limit of susceptibility, some adults of the colony have survived these tests when they were conducted under less than optimal meteorological conditions, or when low rate dosages were used (unpublished data). Fogger trials on Colusa and Solano County *Ae. melanimon* and Contra Costa *Ae. nigromaculis* (Table 8) corroborate the susceptible laboratory bioassay findings of these populations. Control problems were reported with *Ae. nigromaculis* at Shasta and East Side MADs.

Mortality plateaus were not common with *Cx. tarsalis* chlorpyrifos bioassays (Table 9). They were evident only in the Pine Grove and Sacramento-Yolo data, occurring before the LC_{90} in either case. Wonder Valley and Southfork MAD *Cx. tarsalis* also fit the definition of a chlorpyrifos susceptible model. Fogger tests conducted on the Solano County population revealed operational susceptibility and confirmed resistance on the Glenn County collection. Resistance is indicated in other tests of the Sacramento Valley, Coastal, and Southern California Regions, and therefore, operational failures should be anticipated when treating the wild mosquitoes of these test populations. Chlorpyrifos may also be an inappropriate choice of insecticide to use on adult *Cx. tarsalis*.

Conclusion.

Fogger operations were employed in this study to evaluate the performance of two OP insecticides on selected adult collections of four mosquito species. They provided the empirical evidence needed to tentatively establish interpretative thresholds for the laboratory bioassay: LC_{90}s greater than a threshold concentration of 31.4 $\mu g/cm^2$ for malathion, and 0.628 $\mu g/cm^2$ for chlorpyrifos, respectively, denote resistance.

Findings of this report contrast the susceptibility of *Ae. melanimon* and *An. freeborni* to the prevalence of *Ae. nigromaculis* and *Cx. tarsalis* resistance. This survey will be complete when more *Cx. tarsalis* data are gathered from mosquito control agencies of the San Joaquin Valley, the Los Angeles Basin, coastal areas of Southern California, desert locations along the Colorado River, and the Owens Valley.

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NOVEL TESTS FOR ORGANOPHOSPHATE INSECTICIDE RESISTANCE IN SINGLE MOSQUITOES: AN OVERVIEW OF RECENT PROGRESS AND OUTLINE OF FILTER PAPER TEST[1]

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Four test procedures have resulted from collaborative research at Riverside, California, and Montpellier, France, on methods for the detection of high esterases that are responsible for organophosphate resistance in *Culex* mosquitoes. Three of these procedures analyze the efficiency of these enzymes by the use of naphthyl acetate as a substrate, and determination of the amount of naphthol generated in the reaction. They are a "Filter paper" esterase test (FP/Est test), a Nitrocellulose membrane esterase test (NC/Est test) and a Microtitration esterase test (MT/Est test). The fourth test uses an immunological reaction (dot blot) to detect esterases B. The procedure for the most practical of these tests, the "filter paper test," is outlined at the end of this overview.

In designing these tests, we have investigated the physical parameters of each test to the extent necessary in order to minimize extraneous variations, and to provide maximum diagnostic sensitivity. The temperature requirements for each test, methods for homogenization of insects, types of buffers and reagents, dilution factors, appropriate volumes of aliquots, incubation and staining periods, and other aspects were thoroughly examined. Likewise, biological factors associated with enzyme availability were studied in order to optimize detection efficiency. Sufficient enzyme activity was present in both adults and larvae, and both life stages can be used satisfactorily. However, larval body fat occasionally presented interferences, especially in the NC/est test which uses nitrocelloluse membrane as homogenate support. Thus, when choice exists, young adult insects are preferred.

Since gene amplification is probably a common resistance mechanism, we have considered how its presence may affect the interpretation of test results. In the highly resistant strain of *Culex quinquefasciatus* Say, Tem-R, the level of amplification is ca. 250-fold. The history of evolution of this strain under selection pressure (L.E. Ranasinghe, 1976, Ph. D. dissertation; Ranasinghe and Georghiou, 1981, Pestic. Sci. 10: 502-08; Pasteur et al. 1980, Proc. Calif. Mosq. Vector Control Assoc. 48: 69-73), indicates that this high level of resistance was not reached in a single step, but in several. There is no doubt that in natural populations, a variety of amplification levels of the esterase B1 gene co-exist along with non-amplified (susceptible) genomes. The consequence of this phenomenon is that the diagnostic tests may determine whether an insect contains more copies of the esterase B1 gene than a susceptible insect, but will not reveal whether the insect is homozygous- or heterozygous-resistant. This is because the same number of copies of the gene, say 100, may conceivably be present in a heterozygous-resistant individual as well as in a homozygote in which the number of gene copies on the two sister chromosomes totals 100. This situation does not present any unusual problems with quantification of resistance. It only masks the distinction between the two kinds of resistant genotypes (RS and RR).

The tests developed have both qualitative and quantitative features. The qualitative detection of resistance is the minimum requirement for each test: the insect is classified either as susceptible or resistant. This is accomplished readily with any one of the three biochemical tests and the phenotype can be recognized visually. With the dot-blot test, this is at present possible only for esterases B. The biochemical tests, however, differ in quantitative

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efficiency. Higher accuracy is inherent in the microtitration test, although the sensitivity of the FP/Est test is sufficiently high as to make it adequately reliable. None of the tests is presently able to reveal the spectrum of organophosphate cross-resistance. This must rely on conventional bioassay. Similarly, the biochemical tests, as they now stand, cannot distinguish between type A and type B esterases, or between isozymes of each esterase type. These determinations can be made early by starch gel electrophoresis in a modestly equipped central laboratory. As antibodies of esterases become available, immunoassays that discriminate between esterase types A and B will be feasible.

In certain populations, more than one esterase may be present. For example, esterases B1, A2 and B2 may be found together in certain populations in the U.S. Similarly, esterases A2 and B2 are frequently found together in several countries. Since each esterase may contribute a different degree of resistance, quantification of resistance from the total esterase activity against naphthyl acetate is presently difficult, if not impossible. A further complication arises where additional mechanisms of resistance, besides esterases, i.e. insensitive AChE, glutathione-S-transferase, or MFO may contribute to resistance. However, such cases are rare, and must not detract from the usefulness of the present tests.

An important consideration is the cost of each test. The most economical is the FP/est test. In its simplest form, its main cost is the cellulose filter paper, i.e. approximately $0.021 per 100 insects. An automatic pipette dispensing 2 ul and 100 ul drops is desirable and costs $90. It may be replaced by capillary tubes. In its more advanced version, which includes a quantification feature, the test utilizes a densitometer (Tobias RCX) costing $1,245. However, this instrument is not mandatory, since the spots may be read visually against a reference chart, or the papers may be sent to a central laboratory for densitometer reading.

Biochemical tests must be viewed as supplements of traditional bioassays. They have a number of advantages that are not present in bioassays:

a. The results of tests are unequivocal: an insect possessing the resistance mechanism can only be resistant. In contrast, in diagnostic dose tests, some insects may have survived due to inaccurate dosing, thus confirmation is required either through repetition of the test or by testing the offspring of the survivors.

b. Results are obtained within minutes. Thus the tests can be conducted by a field man in a hotel room, or during brief visits to various sites or laboratories, without the need for returning after 24 hours to read the results.

c. Insect specimens can be preserved for several days in liquid nitrogen or dry ice and processed together at a later time or at some distant laboratory.

Limitations of the biochemical tests are:

a. Relatively high cost of tests involving quantification of resistance.

b. Procedures that involve quantification require some technical skills.

The inability of the present biochemical test methods to provide clear information on the degree of resistance and the spectrum of cross resistance, mandates that conventional bioassays (determination of complete dose-response lines; diagnostic dose tests) be preserved.

It is expected that biochemical tests (FP/Est test, MT/Est test) will be employed in conjunction with electrophoresis to identify the mechanism of resistance present, and subsequently to monitor the frequency of resistant individuals in the population. Periodic bioassay tests will provide information on the level of resistance present and on the spectrum of cross resistance.

It is also expected that eventually a battery of diagnostic tests using biochemical approaches will be developed so that the homogenate of single insects can be tested for each of the known mechanisms of resistance.

Dot blot immunoassays appear to be, at present, the most difficult ones to introduce due to the problem of obtaining large quantities of antibodies of good quality for both esterases A and B. Because of the very low likelihood that immunoassay will be applied as a diagnostic tool

for resistance in the immediate future, emphasis at the present time should be placed on biochemical tests.

APPENDIX

MODIFIED FILTER PAPER ESTERASE TEST*
(FP/Est test)

Equipment and Supplies

Strips of Whatman filter paper No. 2 (or No. 42), 10.5 x 3.5 cm.
One 10 ml test tube
Porcelain plate with 12 cavities (Fisher, Lexington, Mass., Ref. 13-754)
Micropipette, for 2 ul and 100 ul drops
Insect forceps
Glass petri dishes or similar containers
Tissue paper (such as Kleenex)
*Densitometer, RCX model, Tobias Associates, Ivyland, Pa.

CHEMICALS

Mono-sodium phosphate
Di-sodium phosphate
Alpha-naphthyl acetate (Sigma, St Louis, Mo., Ref N8505)
Fast Garnet GBC salt (Sigma, Ref. F0875)
Ethanol, absolute
Triton X-100
*Alpha-naphthol

STOCK SOLUTIONS (keep refrigerated)

Di-sodium phosphate (0.1 M)
Mono-sodium phosphate (0.1 M)
Phosphate buffer (0.1 M, pH 6.5), made from the two above solutions
Homogenization buffer: phosphate buffer (0.1 M, pH 6.5) containing 0.5% of Triton X-100
Substrate solution (2% *alpha*-naphthyl acetate in ethanol)

WORKING SOLUTIONS

Solution A (to be prepared just before use)
5 ml of substrate solution in 95 ml of phosphate buffer
Solution B (to be prepared daily)
150 mg Fast Garnet GBC in 100 ml of water
Solution C, 500 ml of water

PROCEDURE

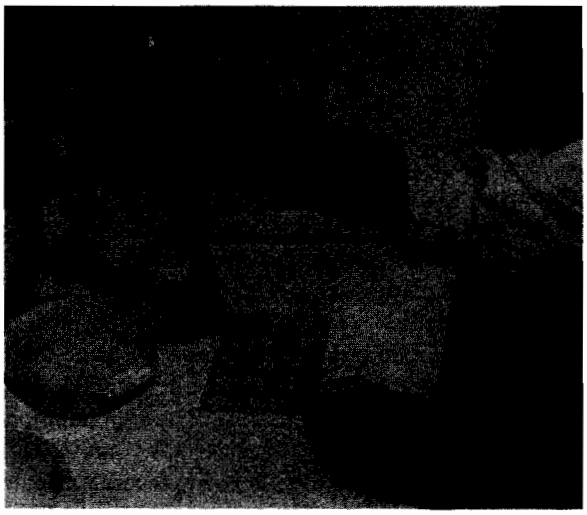

Figure 1.
- Using forceps, place a single insect (adult or larva) in each cavity of the porcelain plate.
- Deposit 100 ul of homogenization buffer in each cavity.

Figure 2.
- Grind mosquito using bottom of test tube. Carefully wipe the bottom of test tube with tissue paper after processing each mosquito to avoid contamination.

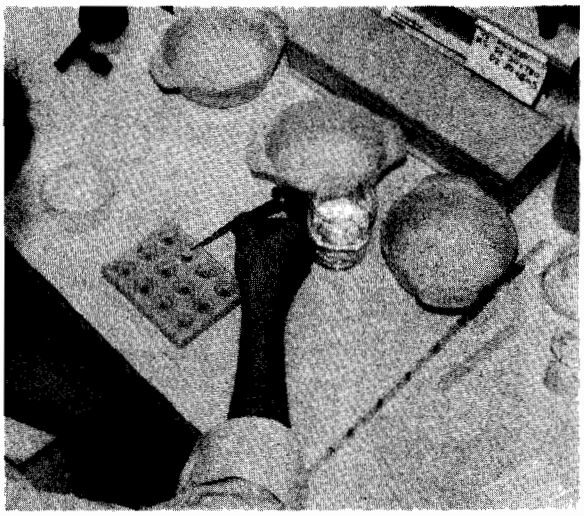

Figure 3.
- Deposit a small (1 cm x 1 cm) piece of a single layer of tissue paper on top of the homogenate in each well.

Figure 5.
- Deposit this homogenate on the Whatman filter paper as follows: hold the filter paper from one corner with index and thumb while the automatic pipette is held with other hand. The homogenate is deposited by bringing the tip of the pipette lightly in contact with the filter.

Figure 4.
- Aspirate 2 ul of homogenate through the layer of tissue paper.

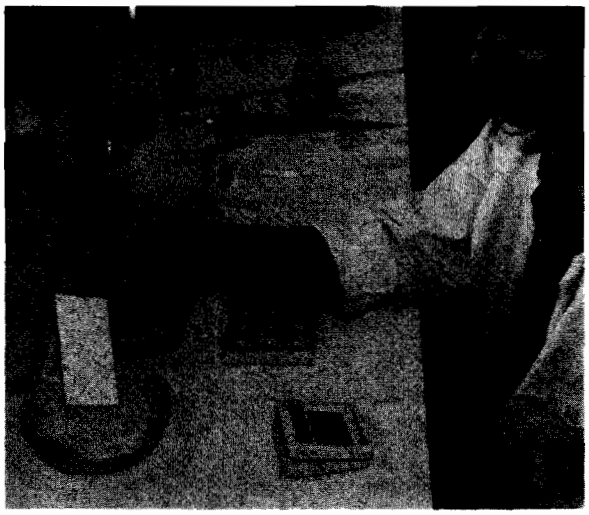

Figure 6.
- After processing a maximum of 10-20 homogenates (approximately 2 min.), immerse the filter paper in solution A for 60 seconds.
- Blot the filter paper between two layers of tissue paper.

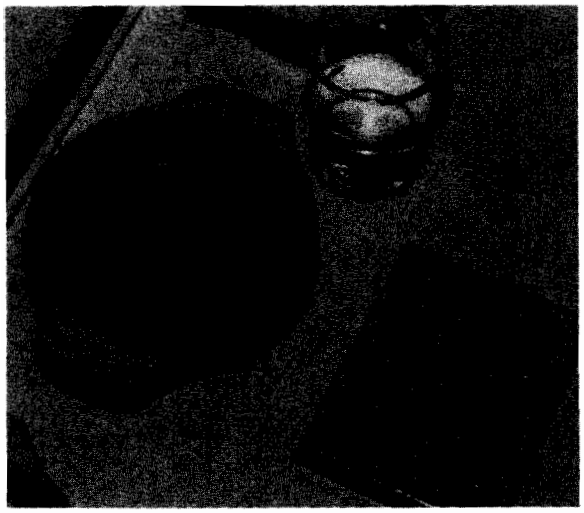

Figure 7.
- Transfer the filter to solution B for 60 seconds. The presence of esterase and hence of resistance is revealed by the development of a purple color at the site of each deposit. The absence of color, or very slight evidence of it, indicates susceptibility.

Figure 8.
- Dip the filter briefly in solution C.

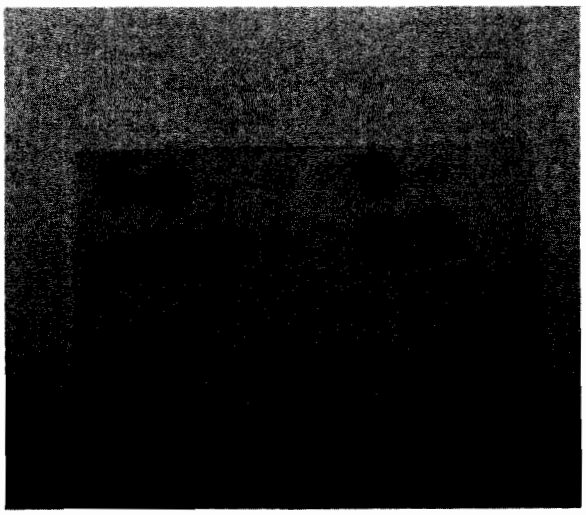

Figure 9.
- Allow to dry on tissue paper. Dried filters can be stored indefinitely in a note book or in plastic bags for reference. The absence of light improves shelf life.

DENSITOMETRIC ANALYSIS OF THE SPOTS WITH RCX DENSITOMETER

- Use dry filter papers
- Blank the Tobias RCX densitometer on an area of the filter paper where there is no deposit.
- For each filter, measure the optical density (OD) of each spot that was developed from each homogenate.

NOTE. If RCX densitometer is not available, a filter paper on which known concentrations of naphthol have been processed may be used for visual reference.

*Items marked by asterisk are essential only for densitometric analysis.

ul = microliter.

This test is described in Pasteur, N. and G. P. Georghiou, 1989. Improved filter paper test for detecting and quantifying increased esterase activity in organophosphate-resistant mosquitoes (Diptera: Culicidae). J. Econ. Entomol. 82(2): 347-353.

SURVIVORSHIP AND GONOTROPHIC CYCLE LENGTH IN *AEDES MELANIMON* IN THE SACRAMENTO VALLEY OF CALIFORNIA

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ABSTRACT

Survivorship and gonotrophic cycle length were estimated in Sacramento Valley populations of *Aedes melanimon* during 1987 and 1988 using mark-release-recapture (MRR), and age-grading methods. Survivorship was estimated at 0.90 per day in a MRR study conducted in September 1987, 0.84 per day in a MRR study in August 1988, and 0.89 per day in an age-grading study conducted in August 1988. These data indicate that *Ae. melanimon* females are long-lived compared to other Central Valley mosquito species. Gonotrophic cycle length was estimated at five days in both MRR studies, based on a 5-day interval between peaks in the number of recoveries of marked females per day. The association of peaks in recoveries with shifts in the parity state of recovered females was confirmed by determining the parity state of recovered females in the 1988 study. Results from the age-grading study confirmed the 5-day gonotrophic cycle estimate obtained in the MRR studies. The implications of high survivorship and a short duration of the gonotrophic cycle to vectoring capability are discussed.

Introduction.

Aedes melanimon Dyar is an important pest mosquito in the Central Valley and elsewhere in California. More important, from a public health standpoint, is the association of this species with arboviruses. *Aedes melanimon* is the mosquito most frequently found infected with California encephalitis virus (CEV), and transovarial transmission (TOT) of the virus in Central Valley populations of *Ae. melanimon* has been demonstrated (Turell et al. 1982). Because of its close association with CEV, *Ae. melanimon* is considered the principle reservoir and maintenance host for CEV in California (Reeves et al. 1983). The importance of horizontal transmission to the maintenance of the virus is not known.

Aedes melanimon is also associated with western equine encephalomyelitis virus (WEE). Though *Culex tarsalis* Coquillett is the principle vector of this disease in California (Reeves and Hammon 1962), the history of virus isolations from field collected *Ae. melanimon* (Emmons et al. 1972, 1973, 1980, 1981, 1986, Reisen 1984, Hardy 1987) indicates that it plays a role in the epidemiology of this virus as well. Laboratory studies have shown that *Ae. melanimon* is highly susceptible to oral infection with WEE and transmits the virus readily by bite (Hardy 1987).

In spite of its importance, little is known about the biology of *Ae. melanimon*, including whether populations have the capacity for sustained horizontal transmission of CEV, WEE, and other arboviruses. To investigate the biological capacity of *Ae. melanimon* populations for virus transmission, we studied the population biology of this mosquito beginning in 1987. The goal of this project was to obtain estimates for some of the population-based factors which could contribute to its vectoral capacity (Garret-Jones and Grab 1964, Birley 1980) for CEV and WEE. Among the factors studied were adult female survivorship, abundance, duration of the gonotrophic cycle, and the host feeding pattern. This paper summarizes the findings from three studies which provide evidence that *Ae. melanimon* females may have the potential to serve as efficient horizontal vectors of arboviruses. The studies were conducted on the Colusa National Wildlife Refuge in Colusa County, California. Colusa County has been the site of several isolations of CEV and WEE virus isolations from *Ae. melanimon* and the study area has abundant populations of this mosquito.

1987 Mark-Release-Recapture Study.

A mark-release-recapture (MRR) study was conducted in mid September 1987. Approximately

5500 female *Ae. melanimon* were marked and released. Recoveries of marked females were made for 13 consecutive days using CDC traps baited with CO_2. Approximately 1% of the released females were recovered, and daily survivorship was estimated based on the rate of decrease in the number of recoveries per day. The results indicated that *Ae. melanimon* females at this time of the year were long-lived with a daily survivorship probability (DSP) of 0.90 per day. This exceeds estimates published for *Cx. tarsalis* (McHugh and Washino 1986, Reisen et al. 1983). The natural abundance of *Ae. melanimon* in the study area was estimated at an average of approximately 20 million females in the 20 hectare study area using the modified Lincoln Index of Abundance. In addition to having a high DSP, the pattern of recoveries of marked females per day provided evidence suggesting that the gonotrophic cycle of the marked females was approximately five days in duration. This was based on a 5-day interval between peaks in the number of recoveries per day.

1988 Mark-Release-Recapture Study.

A second MRR study was conducted in mid-August 1988 at the same site as that of the 1987 study. Approximately 14 000 marked females were released, and recoveries were made for 14 consecutive days. The proportion of marked females recovered was similar to the 1987 MRR study, with 0.94% of the released females being recovered over the 14-day period. Survivorship was estimated at 0.844 per day.

The parity state of recovered females was determined by ovarian dissection beginning four days post-release. The results of these dissections showed that the parity state of recovered females shifted progressively from nulliparous to 1 parous to 2 parous in the course of the study. Three peaks in the number of recoveries per day were observed and associated with the recovery of nulliparous, 1 parous and 2 parous females respectively. It was concluded that interval between peaks in recoveries per day was indicative of the duration of the gonotrophic cycle of the marked females. Based on a 5-day interval between peaks in 1- and 2 parous females, the duration of the gonotrophic cycle was estimated at five days. Abundance was calculated to be approximately 900 000 females in the study area each day.

Age Grading Study.

An age-grading study, to confirm the survivorship and gonotrophic cycle length estimates obtained from the MRR studies, was conducted at the same site as the 1987 and 1988 MRR studies. Changes in the daily abundance of nulliparous and parous females in the native population were monitored from 1 August to 1 September, 1988 using the number of females caught daily in five CDC traps. The population of parous females was estimated by determining the parity state through ovarian dissection (Detinova 1962) of a subsample of females caught in the traps each day. The parity rate estimate was applied to the total number of females captured in the traps to estimate the number of nulliparous and parous females each day.

These data were analyzed using the method of Birley and Rajagopalan (1981) to determine the best estimate of the duration of the gonotrophic cycle, and the survivorship per gonotrophic cycle. Since the approximate duration of the gonotrophic cycle was known, the survivorship estimates from the age-grading study and MRR study could be compared.

Using data from 21 days of sampling, the gonotrophic cycle was determined to be five days. The survivorship per gonotrophic cycle was estimated at 0.55, which converts to a daily survivorship probability of 0.89 per day.

Discussion.

The survivorship estimates of 0.844-0.90 per day obtained from these studies indicated that *Ae. melanimon* is a long-lived mosquito. Survivorship estimates obtained for other Central Valley mosquito populations included 0.69 - 0.85 per day for *Cx. tarsalis* (Milby 1969, Reisen et al. 1983, McHugh and Washino 1986), and 0.74 - 0.76 per day for *Anopheles freeborni* Aitken (McHugh and Washino 1986).

Though all three studies indicated that *Ae. melanimon* females are long-lived, differences exist between the estimates. The difference between the 0.84 estimate from the 1988 MRR study, and the 0.89 figure obtained in the age-grading study conducted on the native mosquito population at the same time as the MRR study, may have resulted from a limitation of the MRR technique in estimating survivorship. In a MRR study, dispersal of

marked mosquitoes outside of the study area can not be distinguished from mortality within the study area. Therefore, high rates of dispersal of marked mosquitoes out of the study area will decrease the survivorship estimate.

A comparison of the mean distances of recoveries of marked females from the release points in the 1987 and the 1988 MRR studies suggest that females dispersed farther from the release point in the 1988 study than they did in 1987.

The results of the ovarian dissections in the 1988 MRR, which indicated that peaks in the number of recoveries per day were associated with the recovery of females of shifting parity states, suggest that the interval between peaks can be used to estimate the duration of the gonotrophic cycle in MRR studies. Therefore, the 5-day interval between peaks observed in the 1987 and 1988 studies can be considered the duration of the gonotrophic cycle in both studies. Additional evidence for this was provided by the findings from the age-grading study, in which the 5-day gonotrophic cycle length estimate was identical to that obtained in the MRR studies.

These studies suggest that *Ae. melanimon* has the potential to be an efficient horizontal vector of arboviruses based on high daily survivorship and a relatively short duration of the gonotrophic cycle.

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INDUCTION OF *LAMBORNELLA CLARKI* (PROTOZOA: CILIOPHORA: TETRAHYMENIDAE) BY *AEDES SIERRENSIS* (DIPTERA: CULICIDAE) LARVAE: FINE-TUNED BIOLOGICAL CONTROL OF MOSQUITOES BY A PROTOZOAN

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ABSTRACT

Lambornella clarki is a facultative parasite of the western treehole mosquito, *Aedes sierrensis*. The parasitic form of this ciliate is induced by mosquito larvae via a water-borne cue. We report the influence of larval density, larval feeding, and temperature upon the formation of this induction cue. We also report on the thermal and temporal stability of the induction cue. These characteristics are discussed in terms of the specificity of interactions between the parasite and its host, and in terms of the biological control of container-breeding mosquitoes.

Introduction.

Aedes sierrensis (Ludlow) is the dominant treehole mosquito of the Pacific coastal range and the foothills of the Sierra Nevada (Bohart and Washino 1978, Washburn and Anderson 1986). Also occupying treeholes within this region is the protozoan *Lambornella clarki* Corliss and Coats. In the absence of mosquitoes, *L. clarki* populations persist in water-filled treeholes as teardrop-shaped, bactivorous, free-living trophonts. Trophonts reproduce by binary fission, and when treeholes begin to dry they form dessication-resistant cysts, the form in which ciliates survive treehole drying during the hot, dry summers that are typical of California.

When *Ae. sierrensis* shares treeholes with *L. clarki,* larvae effectively eliminate free-living protozoa, including *L. clarki*, from treehole water (Washburn et al. 1988a). An additional form of *L. clarki,* the theront, develops in the presence of these larval predators. This spherical, non-feeding form of the ciliate searches for *Ae. sierrensis* larvae, recognizes larvae of this (and certain other mosquito) species as suitable hosts, and forms cysts on their cuticles. The ciliate exits its cyst and invades the host through the larval cuticle; once inside the hemocoel of the host, *L. clarki* cells utilize host metabolites to replicate, often completely filling the hemocoel. Heavily infested mosquito hosts are killed. Free-living trophonts are formed within the dead or moribund host; these ciliates escape into the surrounding treehole water through ruptures in the larval cuticle.

In an earlier publication (Washburn et al. 1988a), we reported that larvae need not be present to induce theront formation. When a population of free-living *L. clarki* is exposed to water from which *Ae. sierrensis* larvae have been recently removed, a proportion of the trophonts is induced to form theronts. If larvae are not subsequently made available to these cells, the theronts invariably die.

We argued that predation by *Ae. sierrensis* larvae was responsible for an extreme trophic level shift in one of their prey species, *L. clarki.* The parasitic response of *L. clarki* to larval predation pressures makes it the most important natural enemy of *Ae. sierrensis* throughout its range in California (Washburn and Anderson 1986). The formation of a host-seeking theront from a free-living trophont is a commitment by that individual cell to parasitism. Furthermore, *L. clarki* demonstrates host specificity for certain mosquito larvae. These features of *L. clarki* may be exploited for the effective biological control of other container-breeding mosquito species both in their native ranges and following their introduction into the continental United States. In order to apply *L. clarki* to the biological control of container-breeding mosquitoes, it is first necessary to understand its interactions with its native host.

Theront formation by *L. clarki* is mediated by a water-borne chemical cue. Free-living trophonts

thrive in the absence of larvae, and the commitment to parasitism is irreversible; therefore, the induction cue should indicate the presence of larval predators specifically and reliably. On the other hand, the induction factor should neither be a persistent substance nor one formed by treehole processes unrelated to mosquitoes; parasitic forms would then be induced independently of mosquito predation and in the absence of suitable hosts. In this report, we present preliminary laboratory results characterizing the chemical cue which induces theront formation. These results include the effect of temperature on induction cue formation, thermal and temporal stability of the induction cue, the effect of larval density on theront formation, and the role of larval feeding on formation of the induction cue.

Materials and Methods.

All *Ae. sierrensis* were from a laboratory colony originally collected from Fresno, California, and maintained since 1974. Eggs were produced by adult females blood-fed on anesthetized mice. Larvae were raised in autoclaved treehole water (diluted to 15% of its original concentration with deionized water), and fed ground laboratory rat chow. *Lambornella clarki* were maintained in cerophyl cultures (Washburn et al. 1988a).

Efficacious production of an induction cue by mosquito larvae was tested using an assay outlined in Washburn et al. (1988a). In this assay, fourth instar larvae were washed and counted into dilute solutions of autoclaved treehole water. Ground rat chow or trout pellets were added to the arenas, and larvae were permitted to feed for 48 h or more in an unlit incubator set to 11° C. Experimental controls contained dilute autoclaved treehole water and ground rat chow but no larvae.

At the end of the incubation period, the contents of the larval arenas were poured through Whatman #1 filter paper, and portions of the filtrate (i.e. conditioned water) and replicates of equal volumes of *L. clarki* cerophyl cultures were aliquoted into clean dishes. Cell cultures were selected from several geographical strains available; robust and rapidly dividing cells (as determined by daily counts of one milliliter samples) were used for all experiments. Cells were maintained with the conditioned larval and control waters for 48 h at 11° C after which 10 newly-hatched (less than 24 h) *Ae. sierrensis* larvae were added to each replicate. Dishes containing cells, conditioned water, and first instar larvae were returned to the incubator. After 24 h, the larvae were removed and stained with amide black dye; the number of cuticular cysts per larva was determined under a compound microscope (100X to 400X). Newly-hatched larvae provide a suitable substrate for encystment but do not themselves induce theront formation within the time of exposure. The average number of cuticular cysts per larva was determined for each replicate; significantly higher numbers of cuticular cysts per larva in the conditioned water than in the controls was evidence for the presence of an induction cue.

Elaborations of the above procedure were used to characterize the production and nature of an induction cue. Portions of conditioned water were treated as outlined below, and the change in induction response following treatment was determined by comparison with that of unmanipulated conditioned water (e.g. autoclaved compared with non-autoclaved water). Alternatively, variations from the standard conditioning procedure resulted in differences in theront formation and numbers of cuticular cysts providing clues concerning the properties of induction cue formation.

Temperature effect on induction cue formation.-Sixty *Ae. sierrensis* larvae were counted into each of four containers holding 50 ml dilute treehole water (density = 1.2 larvae/ml) and an excess of food. One container each was placed into an unlit incubator set to 6°, 11°, 16°, or 21° C. The containers were removed after 48 h and the contents filtered; the filtrate from each temperature setting was tested for theront induction with two strains of *L. clarki.*

Heat stability of induction cue.-Three hundred washed larvae were placed in 150 ml dilute treehole water and provided with an excess of food (density = 2.0 larvae/ml) at 11° C. Following conditioning, the water was filtered and divided into two equal volumes. Half of the conditioned water was autoclaved for 15 minutes, and the remainder was held at 11° C until it was used. After the treated solution had cooled, both were exposed to cells according to the procedure outlined.

Effect of larval density and dilution on induction cue.-In a preliminary experiment, equal portions of dilute treehole water with equal masses of food received larval densities of 3.16, 0.32, 0.03, or 0 larvae/ml. These larval densities encompassed most of the range of larval densities reported by

Hawley (1985) for natural *Ae. sierrensis* populations in treeholes in Oregon. The conditioning period for all these density treatments was eight days, after which induction potentials were assessed.

Subsequently, in a separate experiment, a larval density series was coupled with a dilution series and induction responses were compared. A larval density series of 0.32, 0.19, 0.13, 0.08, 0.05, 0.03, and 0 larvae/ml was prepared for conditioning. A dilution series was prepared by diluting treehole water conditioned by larvae at a density of 0.32 larvae/ml; dilutions were made to mimic solutions conditioned by larvae at the same densities listed. It was assumed that the induction potentials of these two series would have been similar if larvae produced the induction cue at a constant and characteristic rate.

Persistence of induction cue.-Larvae and an excess of food were placed into one of seven identical containers holding dilute treehole water (density = 3 larvae/ml). All seven containers were placed in an unlit incubator at 11° C. After 24 h, the larvae and food were filtered from the treehole water of the first container (Day 6 in Figure 4), washed and transferred to the second container (Day 5). Pupae were replaced with fresh, washed larvae. The same procedure was followed each day for a week, until the water of each container had received 24 h conditioning by the same larvae. All containers were handled similarly whether or not they held larvae on a given day. Only the time elapsed since larval presence differed among containers. The induction potential of each solution was assessed at the same time with the same *L. clarki* cell culture.

Larval feeding and induction cue formation.-Equal numbers of washed larvae were added to equal volumes of dilute treehole water (density = 1 larva/ml) in a preliminary experiment. One treatment received powdered rat chow, while the other received no food; both were incubated for six days at 11° C. Water samples conditioned by fed larvae and larvae deprived of food were compared for induction potential.

In a subsequent experiment, equal numbers of larvae were exposed to three substrate treatments to investigate the role of larval feeding. These treatments included equal masses of ground trout pellet, kaolin, and penicillin (see Dadd 1973, Dadd and Kleinjan 1976) in double-distilled water (density = 0.8 larva/ml). Larvae were allowed to replace their gut contents with the substrates for 24 h. Then they were again washed to minimize transfer of residual material, placed in distilled water with fresh supplies of the appropriate substrate, and allowed to feed at 11° for 24 h. The mixtures were then filtered and added to two strains of *L. clarki* to test for induction potential.

Results.

Temperature effect on induction cue formation.-The effect of temperature upon the formation of induction cue was significant among the treatments for one of the two strains tested ($p = 0.005$, one-way ANOVA); the results for that strain are shown in Figure 1. There were no significant differences among the means for the other strain ($p = 0.07$, one-way ANOVA). The number of cuticular cysts due to the 16° C treatment was highest for both strains of *L. clarki* used. During the 48 h conditioning period, 5, 8, 23, and 27% of the 60 larvae pupated in the 6°, 11°, 16°, and 21° C treatments respectively.

Heat stability of induction cue.-Larval conditioning activity which resulted in an average of 2.03 ± 1.19 cysts per larva, was virtually nullified by autoclaving. No cysts were formed from populations of cells exposed to autoclaved water which had been conditioned by larvae.

Effect of larval density and dilution on induction cue.-Significantly more cysts ($p << 0.001$, one-way ANOVA) were formed at the highest larval density (i.e. 3.16 larvae/ml) (mean = 9.3 ± 2.9 cysts per larva) in the preliminary larval density experiment, than at the other densities tested. Larvae at a density of 0.32 larvae/ml induced a mean of 2.1 ± 1.0 cysts per larva, and the lower densities tested resulted in no cuticular cysts.

The second larval density experiment, which was paired with a dilution series, similarly suggested that the production of an induction cue increased with larval density. In the density series, a few cysts, attributable to background activity, were formed in the control. All treatments with larvae present induced the formation of significantly more cysts in the density series (Figure 2). However, in this density series, induction activity of a conditioned solution was not correlated with larval density; only 11% of the variance in the numbers of cuticular cysts was explained by larval density. On the other hand, 90% of the variance in cyst production for the dilution series was explained by the larval density equivalent generated by dilution

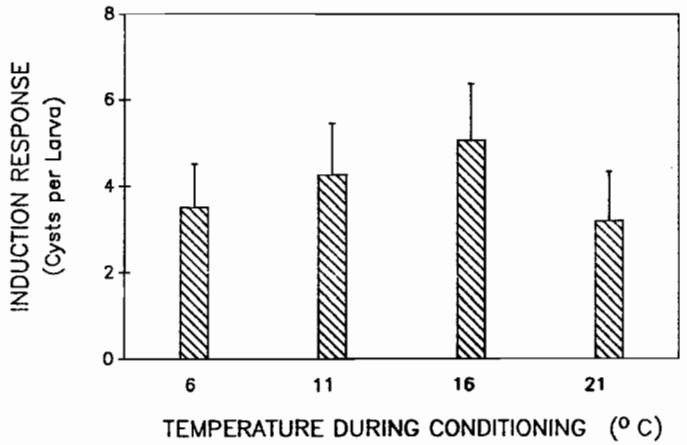

Figure 1. Effect of temperature on induction cue production by *Aedes sierrensis* larvae. Bars represent mean ± one standard deviation for ten replicates of *Lambornella clarki* populations.

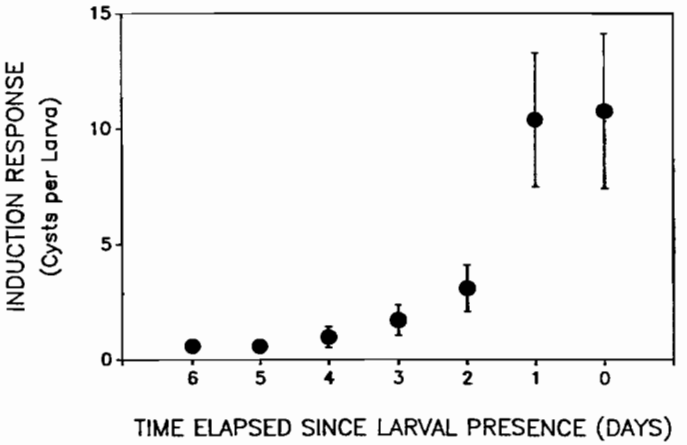

Figure 3. Persistence of induction cue in conditioned treehole water from which *Aedes sierrensis* larvae have been removed. Each treatment was conditioned for 24 h by larvae which were transferred daily to the next treatment. All treatments were used to induce theronts at time = 0. Points represent the means of ten replicates; bars represent one standard deviation.

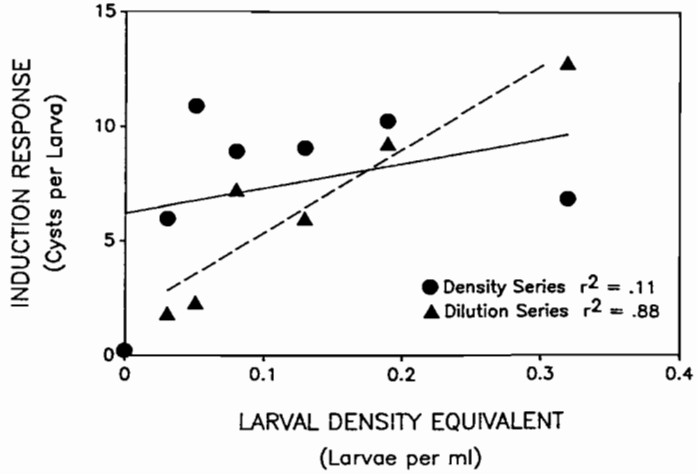

Figure 2. Effect of larval density and dilution on induction of *Lambornella clarki* populations. The density series was prepared by adding different numbers of fourth instar *Aedes sierrensis* larvae to treehole water during conditioning. The dilution series was prepared by diluting conditioned treehole water.

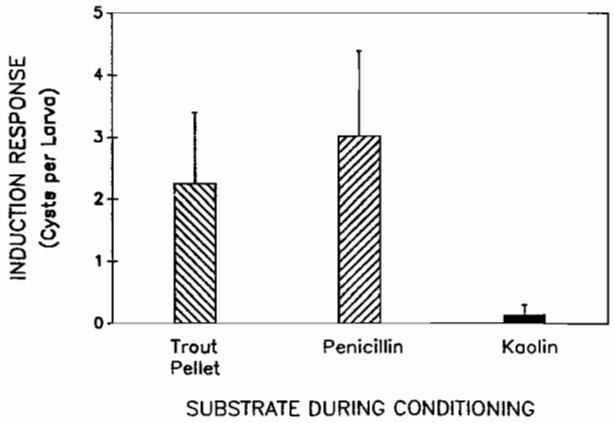

Figure 4. Production of induction cue by *Aedes sierrensis* feeding on different substrates. Similar larval populations were fed ground trout pellets, the antibiotic penicillin, or the clay kaolin during a 24 h conditioning period. Bars represent induction response of ten replicated populations of *Lambornella clarki* to conditioned water treatments (mean ± one standard deviation).

(Figure 2). Since induction potentials of the paired density and dilution series were dissimilar (Figure 2), larval production of the induction cue was not constant.

Persistence of induction cue.-Larvae at a density of three larvae per milliliter treehole water produced enough induction cue to generate more than 10 cysts per larva (Day 0 and Day 1, Figure 3) within a 24 h conditioning period. This induction activity decreased following removal of larvae from conditioned water to a significantly lower level of induction activity ($p << 0.01$, one-way ANOVA). The loss of induction activity was greatest between 24 and 48 h after larvae had been removed from conditioned water.

Larval feeding and induction cue formation.-Significantly more ($p < 0.01$, Student's t test) cuticular cysts were formed by cells exposed to water conditioned by fed larvae (1.9 ± 1.2 cysts per larva) than by those exposed to water conditioned by starved larvae (0.9 ± 0.3 cysts per larva) in the preliminary larval feeding experiment. These results were consistent with previous experiments (J. O. Washburn and D. R. Mercer, unpublished data; also see Table 1 in Washburn et al. 1988a). Apparently larval feeding was necessary for production of the induction cue.

Larvae were fed inert or non-nutritive substrates to further clarify the role of feeding in the production of the induction cue. Results for one of the two *L. clarki* strains tested are shown in Figure 4. Considerably more cysts per larva were formed by populations of *L. clarki* exposed to water conditioned by larvae feeding on trout pellet powder or penicillin than on kaolin, a non-nutritive clay. More cuticular cysts were formed in the penicillin treatment than in the trout pellet treatment, but the difference was not significant. Larval survival and pupation rates were similar among the treatments during the conditioning period.

Discussion.

The water-borne chemical cue which induces transformation of *L. clarki* trophonts to host-seeking theronts is released by actively feeding *Ae. sierrensis* larvae. The induction cue is formed within 24 h in sufficient quantities to induce high numbers of theronts. The cue is not a heat-stable compound since it is destroyed or dissipated by autoclaving, admittedly a severe and unnatural treatment. Neither does it persist longer than two days after larval feeding stops. Free-living trophonts are less likely to form theronts at a time when the hosts required by this parasitic form are disappearing. *Lambornella clarki* only rarely encysts on mosquito pupae, although it recognizes and attacks all larval instars (J. O. Washburn and D. R. Mercer, unpublished observations). Larvae which halt feeding in preparation for pupation should release little or no induction cue into their treehole habitat. *Lambornella clarki* populations are able to respond quickly to larval predation pressure. This response is fine-tuned temporally to a substance associated with larval feeding which disappears when induction to parasitic forms becomes a liability.

Hawley (1985) reported larval densities of *Ae. sierrensis* ranging from fewer than one to more than six larvae per milliliter (number of first instar larvae to appear divided by the maximum observed treehole volume for the season) in natural treeholes in Oregon. We used larval densities as high as 3.16 fourth instar larvae per milliliter for our experiments. Within the ranges of larval densities tested, higher densities of larvae caused greater proportions of induced cells from free-living populations. For conditioned water (produced by holding 316 larvae in 100 ml treehole water) diluted to mimic decreasing larval densities, 90% of the variance in induction response was explained by concentration of induction cue (i.e. larval density equivalents).

Individual *L. clarki* are variable in their response to the induction cue. Similar populations of ciliates responded differently to increasing concentrations of induction cue, instead of exhibiting an all-or-nothing response. Apparently there is no uniform threshold for theront production. This means that relatively low predation by a few *Ae. sierrensis* larvae would induce few ciliates to become parasitic in natural treehole populations. Successful infection of a larva by a single theront is sufficient to kill that larva; ciliates divide within their hosts, protected from larval predation. On the other hand, greater numbers of cuticular cysts serve as portals for infection by opportunistic pathogens which kill both larva and ciliate parasites (Washburn et al. 1988b). Thus, a variable response by individual cells to the concentration of induction cue is adaptive at both the individual and the population levels.

The lack of correlation between induction response and larval density in the density series may

be explained in part by this variable response to induction cue, but also by variation in production of induction cue among individual larvae. We are currently conducting experiments meant to measure this variability, and to correlate variability of induction cue production with larval age (as indicated by time to pupation).

The induction response to larvae feeding on different substrates is more intriguing than informative. Larvae feeding on the non-nutritive substrate kaolin induced few theronts. This result was consistent with other experiments in which larvae feeding on non-nutritive substrates did not cause high levels of induction. The relatively high numbers of cuticular cysts recovered in the penicillin treatment suggest that the induction cue results from some larval digestive process, and not from the bacteria (at least none susceptible to the antibiotic) associated with larval guts. It is not clear, however, what, if any, nutritive benefit was gained by larvae feeding on penicillin during the conditioning period. Perhaps higher cuticular cyst numbers in the penicillin treatment reflected lower mortality of susceptible theronts in those treatments since bacterial growth was limited.

Lambornella clarki responds to a water-borne induction cue intimately associated with *Ae. sierrensis* larvae feeding on nutritive substrates present in treehole water. Free-living trophonts respond in a variable manner to the induction cue, proportionally more theronts formed at higher concentrations of the cue. The cue accumulates rapidly in the water while larvae are feeding, but disappears quickly when feeding stops. *Lambornella clarki* cannot complete an infection cycle in all mosquito species nor can it survive in all mosquito habitats. However, further characterization and identification of this induction cue will aid in identifying situations in which this treehole inhabitant might be used for the biological control of container-breeding mosquitoes.

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EVALUATION OF MOSQUITOFISH (*GAMBUSIA AFFINIS*) GROWTH AND FECUNDITY IN RELATIONSHIP TO FRESHWATER INVERTEBRATE POPULATIONS

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ABSTRACT

Eight of the District's mosquitofish culture ponds were filled and fertilized on different dates, and three separate zooplankton density analyses were made for each pond on the day the ponds were stocked. Zooplankton samples were taken 17 times during the 12-week study period. The organisms in these samples were identified to the nearest taxon, and included in the cumulative list of orders collected per sample for each pond throughout the survey. The relative abundance of zooplankton was calculated for each pond by averaging all zooplankton samples and determining the mean number of zooplankton per liter throughout the study period. Ponds were ranked according to their relative zooplankton abundance. All ponds were stocked with fish taken from the District's overwintering ponds at the same location in Elk Grove, CA. Mosquitofish samples (10 females per week per pond) were taken each week, and their length, weight, fecundity, gut contents, and Fulton's Condition Factor were determined. Gut contents and relative zooplankton abundance were compared. Prey abundance and prey preference were closely correlated for most orders of zooplankton. Chironomid larvae were the most commonly selected prey item. *Gambusia affinis* fecundity was recorded numerically by removing the gonads and counting the number of hydrated eggs. Fecundity data were recorded for each fish and averaged over the study period. The study ponds were ranked according to mean number of mature eggs per fish per pond. The condition of each fish sampled was determined by using Fulton's Condition Factor ($K = \frac{W}{L^3} \times 100\,000$). This factor is used to compare the condition (amplitude) of a fish, and is based on the hypothesis that the heavier fish of a given length are in the best condition. Condition factors for each pond for the last eight weeks of the study were recorded, and the mean condition of the fish from each pond was used to rank ponds by condition. Trends in the data did indicate a correlation between zooplankton densities, fecundity, and condition. The use of cottonseed meal as a fertilizer in four of the study ponds may have affected the gonads of the adult fish. Cottonseeds contain a pigment known as gossypol, which has antioxidant properties and is a polymerization inhibitor. Additional work is needed to determine its specific effects on mosquitofish.

Introduction.

The importance of abundant zooplankton populations in aquaculture has been cited frequently in the literature. Krumholz (1948) suggests that differences in fecundity among different mosquitofish stocks can be attributed to corresponding differences in the pond fertility, with a high fertility level resulting in an abundant crop of plankton. Culturing zooplankton at the District's mosquitofish hatchery pond prior to fish stocking has been a standing operational procedure since 1984. The use of zooplankton inoculum in District ponds has increased our over-winter survival rate for mosquitofish by nearly 30 percent.

The ramifications of differences in pond production were not totally understood during the spring 1987 rearing season. Most ponds had met physical and chemical parameters set over the years, and abundant zooplankton populations were established in all ponds before stocking. Plankton

populations were assessed at number per liter, but they were not identified to the nearest taxon or monitored during the rearing cycle.

District fisheries personnel developed a protocol in 1988 to study the effects that zooplankton abundance had on mosquitofish growth and fecundity. By accessing relative abundance of different orders and species of zooplankton in each pond, and comparing fish gut contents, prey preference was established. It was postulated that by identifying select species of zooplankton and recording which zooplankton communities were most beneficial to both mosquitofish growth and fecundity, it would be possible to set parameters to increase pond production in the future.

Materials and Methods.

Mosquitofish culture ponds were fertilized on different dates to culture different zooplankton densities. Ponds 3 and 12 were used as zooplankton inoculum ponds to maintain a constant and fresh supply of zooplankton for the over-wintering mosquitofish. These two ponds had the highest density zooplankton populations at stocking time. Pond 3 had 1046.52 planktors per liter, and pond 12 had 362.44 planktors pre liter. Study ponds 4, 5, 10, and 11 were filled on 18 February 1988, and had moderate zooplankton populations (25-85 planktors per liter) at stocking time. Study ponds 6 and 9 were filled and fertilized on 9 March 1988, and had no zooplankton populations at stocking time. All eight study ponds were stocked on 12 March 1988 from the District's over-wintering ponds in Elk Grove, California.

Zooplankton samples were taken 17 times during the 12-week study period, and all ponds were sampled at least once a week on the same day, beginning at 0730 hours. A 1-1/2 in. diameter Honda pump with a flexible impeller was used to draw a 10 liter sample from the southeast corner of each pond. The end of the suction hose was started at the pond bottom and pulled up slowly through the water column during sampling. The pump was set to pump 20 liters per minute so as to supply an adequate volume of water to collect some of the more mobile aquatic insects.

The 10 liters sampled were filtered through a 240-micron mesh net and reduced to a 10 ml sample. Using a random sample chart (Cox 1976), 10 squares in the bottom of a culture dish were chosen by assigning numbers on the "X" and "Y" axes of the sample grid. The total number of each order for the 10 squares sampled was determined and then divided by 10 to get the average number per square. That number was then multiplied by 68 (total number of squares) and divided by 10 to get the average number of each order per liter. The sum of all orders was totalled and expressed as the total number of organisms per liter. Pond zooplankton were identified to the lowest practical taxon (Pennak 1978) and expressed in the number of orders per liter, and again as the number of organisms per liter.

Mosquitofish were collected each week from our study ponds by seining the east quarter of the ponds. The fish were consolidated in the seine and 10 females were removed with dip nets. These fish were killed immediately, put on ice, and returned to the laboratory for analysis. Fish length (total) to the nearest millimeter, and weight (wet) to the nearest 0.01 gram were recorded. Stomachs were removed to the pyloric caeca, opened, and the contents put onto a slide for microscopic identification. Prey organisms were counted and identified to the nearest taxon.

Mosquitofish fecundity was determined by removing the gonads and counting the number of hydrated eggs. Eighty fish from each of the eight experimental ponds were used for this study. Fecundity data were recorded for each fish per pond and averaged over the final eight weeks of the study.

The eight experimental ponds were of the river rock rip-rap design and had the following physical description: 68 ft x 300 ft, with 0.46 acre surface area, 1.84 acre ft capacity, 5 ft maximum pond depth, 4:1 slope (run over fall), and the sides lined with 50 tons of 3-6 in. river rock cobble. This rip-rap provided reproductive sites for gravid females as well as harborage for newly-released fry from the sight-feeding adults. All eight ponds were stocked at the rate of 50 lbs. fish per pond.

Ponds 3 through 6 were fertilized initially with 100 gallons of chicken manure and two gallons of liquid inorganic fertilizer (L.I.F.), ammonium polyphosphate (10-34-0). The L.I.F. was applied with a hand can and spread over the entire pond surface. Each Monday, after water chemistry tests were completed, any pond that had a Secchi disk reading greater than six inches was refertilized with 50 gallons of chicken manure and one gallon of the L.I.F. The use of chicken manure as a pond fertil-

izer has been standard procedure at the hatchery for the last five years. We experimented with other organic and inorganic fertilizers during that time but never attained the level of production achieved with chicken manure.

In 1988 we experimented with cottonseed meal as an organic fertilizer in four of our study ponds. After filling ponds 9 through 12, each one was fertilized with 200 pounds of cottonseed meal and two gallons of L.I.F. (10-34-0). Each Monday, any pond that had a Secchi disk reading greater than six inches was refertilized with 50 pounds of cottonseed meal and one gallon of the L.I.F.

In addition to the zooplankton populations in our study ponds, the fish were fed the Sutter-Yuba M.A.D. mosquitofish feed each day, according to estimated biomass present and water temperature. The custom feed was not intended to be a complete diet when it was formulated, but rather as a supplement to forage organisms (zooplankton) that were found in the pond culture program.

Results and Discussion.

The weekly zooplankton populations often fluctuated from one sample date to the next. By averaging the 17 zooplankton samples per pond over the entire study period, we were able to rank the ponds according to average number of zooplankton per liter. Fecundity and condition of sample mosquitofish also changed from week to week, thereby making the 95% confidence interval of our sample means attainable. In order to display the trends in our data, condition and fecundity were also ranked (Table 1).

Ponds 6 and 9 had the lowest mean number of zooplankton per liter during the study period, and they ranked eighth and seventh in condition, respectively. Ponds 6 and 9 ranked sixth and eighth in mean fecundity category, suggesting that low zooplankton population levels were not adequate to attain maximum condition and fecundity. Pond 12 had the highest mean number of zooplankton per liter, and it ranked first in condition and seventh in fecundity. This anomaly may be explained by the high Secchi disk readings (25 in. plus) through the first 10 weeks of the 12-week study. The high disk readings indicated that this pond had less primary production and the zooplankton populations (844.16 per liter) were foraging directly on fish food, detritus material, cottonseed meal, or bacterial blooms. Pond 12 was the only pond that had high Secchi disk readings throughout the study period. For most species of fish, good condition and high fecundity go together. The anomaly of pond 12 will require further study.

Pond 4 ranked first in fecundity, second in relative zooplankton abundance, and fourth in condition. The first four ranks for condition varied by only four-one hundredths (0.04), so they should be considered as one, or equal in rank.

The trends most evident with this ranking procedure occurred in ponds 6 and 9, with their low mean number of planktors per liter in relationship to the poor condition and low fecundity of the fish. These data suggest that only moderate zooplankton populations (275-375 per liter) may be needed in the rearing ponds before mosquitofish are stocked in the spring. A supplemental feed ration must also be given to the fish daily to maintain these zooplankton populations.

With zooplankton populations at moderate levels, phytoplankton blooms can also be maintained. This would enable the fertilizer nutrients to go directly to the primary producer (phytoplankton) and make for a more stable food web by reducing changes in pH and dissolved oxygen levels.

The species list (Table 2) for each study pond was compiled from the zooplankton samples. *Daphnia similis* was present in all of the study ponds other than ponds 6 and 9, but this cladoceran did not appear in any of the pond samples until the eighth week of the study, and was considered to have had little impact on fish condition and fecundity that late in the study period. Another cladoceran, *Simocephalus vetulus*, was not taken from ponds 4, 6, 9, and 10. The absence of this organism alone was not thought to have had a negative impact on condition or fecundity because ponds 4 and 10 were in the top four ranks of both categories. It is possible that the absence of both species of cladocerans from ponds 6 and 9 did impact the fecundity and condition of the fish taken from them. Missing zooplankton species will be pumped from pond to pond in 1989 to increase zooplankton diversity throughout all of the culture ponds.

Gut contents and relative zooplankton abundance were compared as percent number available to percent number consumed (Figures 1 and 2) to determine prey selectivity. Frequency of occurrence was included in Figures 1 and 2, and expressed as a percentage of guts in which each food

Table 1.-Ranking of each study pond by the relative abundance of zooplankton, mean fecundity, and the mean of Fulton's Condition Factor for 80 adult female mosquitofish per pond.

Rank	1	2	3	4	5	6	7	8
Mean Zooplankton/Liter	844.16	362.43	310.68	291.68	280.82	277.48	140.93	123.44
Pond No.	12	4	10	3	5	11	6	9
Mean Fecundity	80.6	60.59	57.83	55.83	51.20	46.88	46.44	42.44
Pond No.	4	3	10	11	5	6	12	9
Mean Fulton's Condition Factor	1.44	1.43	1.42	1.40	1.39	1.35	1.27	1.24
Pond No.	12	10	11	4	3	5	9	6

Table 2.-Species list for study pond zooplankton

Organism	Pond Number							
	3	4	5	6	9	10	11	12
Ceriodaphina reticulata	X	X	X	X	X	X	X	X
Simocephalus vetulus	X		X				X	X
Moina sp.	X	X	X	X	X	X	X	X
Daphnia similis	X	X	X			X	X	X
Cyclops vernalis	X	X	X	X	X	X	X	X
Diaptomus sp.	X	X	X	X	X	X	X	X
Filinia sp.	X	X	X	X	X	X	X	X
Hexarthra sp.	X	X	X	X	X	X	X	X
Polyarthra sp.	X	X	X	X	X	X	X	X
Euchlanis sp.		X	X	X	X	X	X	
Platyias quadricornis	X	X	X	X	X	X	X	X
Brachionus sp.		X	X	X	X	X	X	X
Chironomid larvae	X	X	X	X	X	X	X	X
Corisella sp.	X	X	X	X	X	X	X	X
Notonectidae	X	X	X	X	X	X	X	X
Ostracoda	X	X	X	X	X	X	X	X

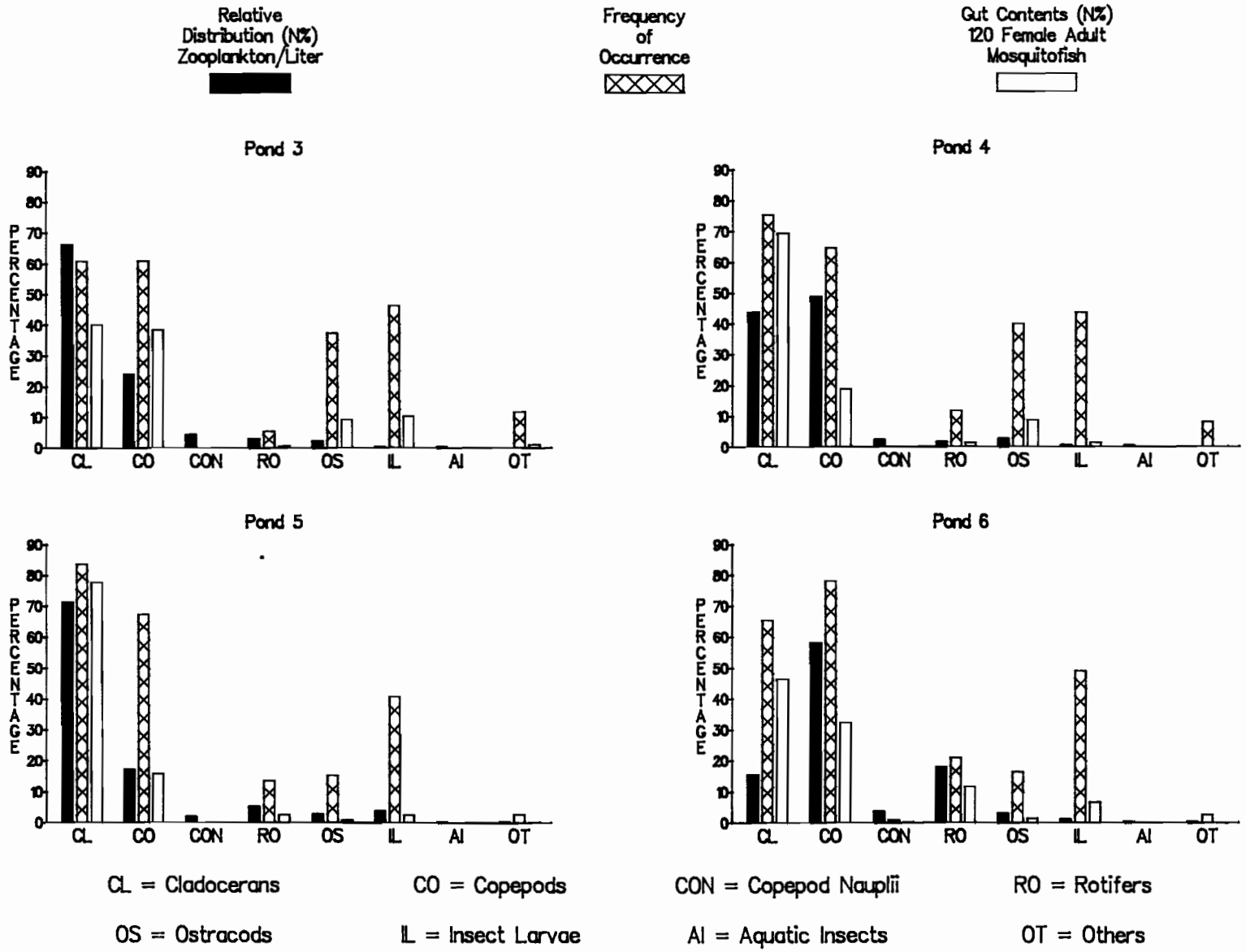
Relative Distribution (N%) Zooplankton/Liter
Frequency of Occurrence
Gut Contents (N%) 120 Female Adult Mosquitofish
Pond 3
Pond 4
Pond 5
Pond 6
PERCENTAGE
0 10 20 30 40 50 60 70 80 90
CL CO CON RO OS IL AI OT
CL = Cladocerans
CO = Copepods
CON = Copepod Nauplii
RO = Rotifers
OS = Ostracods
IL = Insect Larvae
AI = Aquatic Insects
OT = Others

Figure 1.

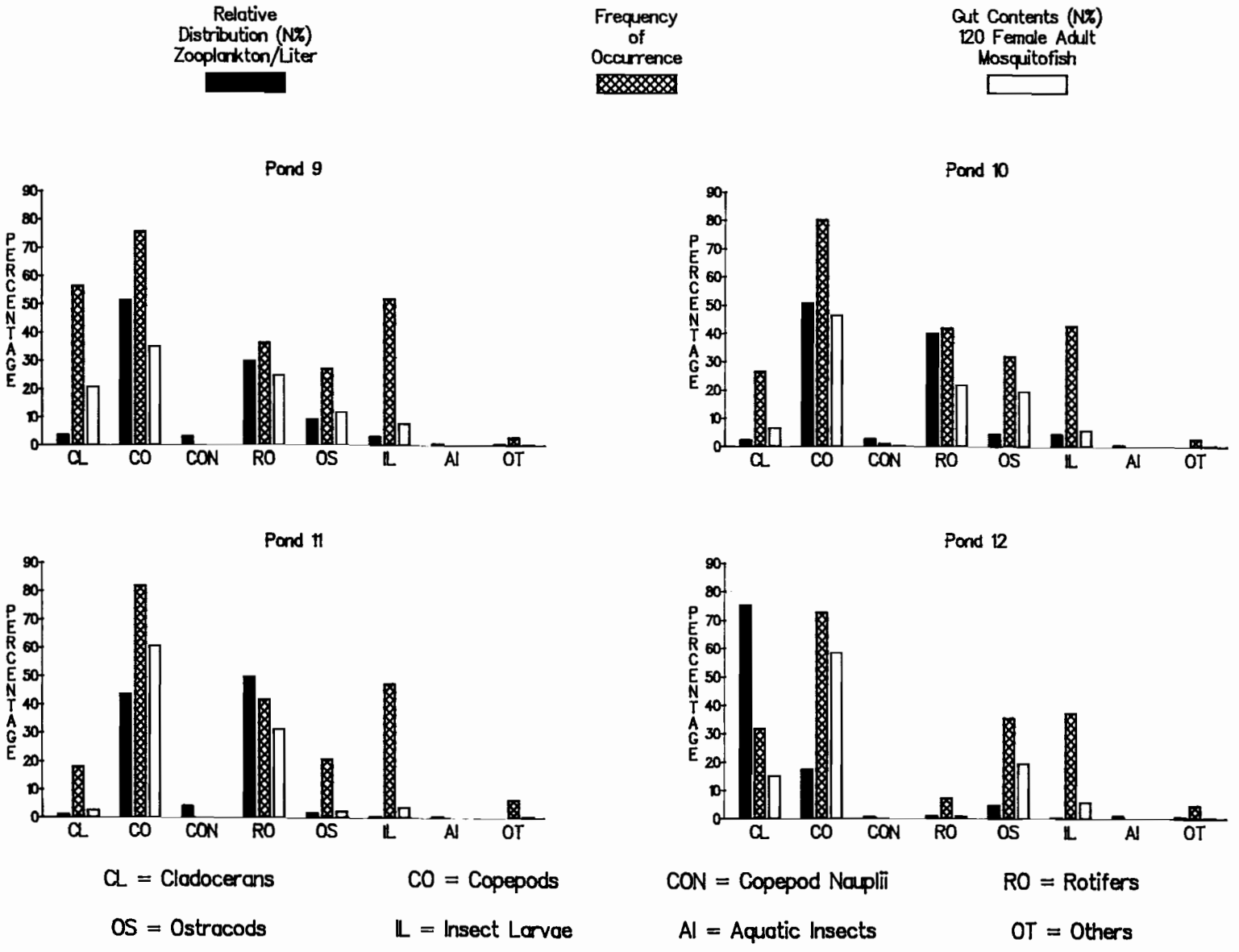
Relative Distribution (N%) Zooplankton/Liter
Frequency of Occurrence
Gut Contents (N%) 120 Female Adult Mosquitofish
Pond 9
Pond 10
Pond 11
Pond 12
PERCENTAGE
CL CO CON RO OS IL AI OT
CL = Cladocerans
CO = Copepods
CON = Copepod Nauplii
RO = Rotifers
OS = Ostracods
IL = Insect Larvae
AI = Aquatic Insects
OT = Others

Figure 2.

item was found. According to the gut analysis data, chironomid larvae were the prey item most often selected by the fishes. However, a higher average of midge larvae was sampled in the guts than the median number picked up by the zooplankton sampling gear. This discrepancy might have been due to the ability of the fish to feed from between the rocks or on the pond bottoms, while the design of the plankton sampler permitted it to touch the bottom but not necessarily remove organisms from the sediment. The relatively large size of the chironomid larvae also made them more vulnerable to the sight-feeding mosquitofish.

In pond 12, 75.24 percent of the food items available were cladocerans (Figure 2). However, only 15 percent of the prey selected were of this order, and they were only recovered in 31.82 percent of the guts examined. Conversely, in pond 12, copepods comprised only 17.37 percent of the prey items available, but made up 58.4 percent of the diet, and were found in 72.73 percent of the stomachs examined. This pattern of prey selectivity and avoidance occurred to a lesser extent in pond 3 (Figure 1). The optimal diet theory (Gardner 1981) predicts that a predator should drop less profitable prey items from the diet at high prey densities. This suggests that the cladocerans available were less profitable than the copepods that were selected because of the high prey densities (Table 1) in ponds 3 and 12.

The use of cottonseed meal as an organic fertilizer in four of the study ponds may have had a negative effect on the gonads and development of the fish in ponds 9 through 12, although, the fish in ponds 10, 11, and 12 had the highest condition rating. Cottonseeds contain 0.03 to 0.2 percent of a yellow pigment known as gossypol. This aldehyde has antioxidant properties and is a polymerization inhibitor.

Friedman (1972) found that the most common symptom associated with gossypol toxicity is a depressed growth rate and a decreased utilization of feed for body weight gain. Ponds 9 through 12 were each fertilized with a total of 600 pounds over the study period. Fish in all four of the cottonseed meal-fertilized ponds cut down on their feeding four weeks after the study was completed. The condition of these fish was very poor and few adult females sampled had hydrated eggs. The ovaries of most female fish sampled were cloudy white. Perhaps the gossypol inhibited development of the gonads of the mosquitofish before affecting their condition. This might explain the good condition of the fish in pond 12 in comparison to their low fecundity. The young of the first spawn were alive but were in the same poor condition as the adult fish. Very few second round fry were seen in any of the four ponds. Of the 15 ponds used for spring culture in 1988, the four fertilized with cottonseed meal had the lowest yields.

Schaible (1934) found that gossypol would cause a decrease in egg production in chickens. Growth inhibition was also observed in fingerling channel catfish fed levels of dietary cottonseed meal greater than 17.4 percent or free gossypol of 0.09 percent and higher (Dorsa 1982). No reference to gossypol toxicity in mosquitofish was found in the literature, but the findings of this study indicate that investigation is needed to evaluate the effects of gossypol on mosquitofish growth and fecundity.

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