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PUBLICATION POLICIES AND INFORMATION FOR CONTRIBUTORS

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

CONTRIBUTIONS: Articles are original contributions in the field of mosquito and related vector control providing information and benefit to the diverse interests in technical development, operations and programs, and management documentation. Papers on controversial points of view are accepted only as constructive expositions and are otherwise generally dissuaded, as is the case with an excessive number of papers on one subject or by one author where imbalance might ensue. Although preference is given to papers of the conference program, acceptability for publication rests on merit determined on review by the editors and the Publications Committee.

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ENCEPHALITIS VIRAL ACTIVITY AND VECTOR POPULATIONS IN CALIFORNIA – PRESENT AND FUTURE CONCERNS[1]

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We appreciated your invitation to present reports on our studies that are supported by special funds for mosquito research from the State Legislature. Originally, I planned to present an overview of the wide variety of studies our research team has developed. However, developments caused me to shift from this objective and to devote the alloted time primarily to the consideration that a resurgence of mosquito populations and vector-borne diseases in California can be expected as a direct result of the approximate 60% cut in funds available for mosquito control.

You will still have an opportunity to acquaint yourselves with the breadth of research being carried out. Our staff is presenting 8 papers: 3 on basic genetic studies on *Culex tarsalis* and *Aedes sierrensis* (Ainsley 1979, Asman 1979; McDonald 1979), 2 on mark-release-recapture studies (Milby 1979; Nelson and Milby 1979), 2 on vector competence (Kramer et al. 1979, Meyer et al. 1979) and 1 on potential use of enzyme markers for studies of field populations (Houk et al. 1979).

BACKGROUND. A primary objective of our research over the past 35 years has been to obtain and analyze records on the levels of mosquito vector populations that are associated with viral activity and clinical cases of western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE). As your programs to control *Cx. tarsalis* and *Aedes melanimon* became increasingly effective encephalitis almost disappeared from our state. Data from the State Department of Health Services dramatically documented this achievement (Figure 1). With this development, we re-oriented our research priorities and focused attention on new research areas such as vector competence to transmit viruses, vector genetics, and statistical modeling of variables that affect the control of vector populations and epidemic occurrences. The evolution of this research effort was reviewed at your 1976 Conference (Reeves 1976).

Now, a new variable has entered into the factors that may lead to a resurgence of encephalitis and malaria in California and that is the passage of Proposition 13. Your Association has projected that this action will decrease the funds for mosquito control from $18,000,000 to $8,000,000 per year once reserve funds and state relief are unavailable. We believe that an inevitable result of this development will be a resurgence of vector populations and that in some years WEE and SLE cases could occur at the levels experienced in epidemics in the 1940's and 1950's. Your President, Mr. Gilbert Challet, and I documented these concerns in testimony before 3 Senate Committees on November 15, 1978.

Today we wish to discuss 4 subjects that should be of interest to this audience:

1. The evidence that favors a resurgence of vector populations and viral activity.
2. Projection of the number of cases of encephalitis to be expected if there is a recurrence of the attack rates experienced in the 1950's.
3. The possible need to utilize a portion of limited funds that are available for a selective control program aimed at control of the older, aged and disease-transmitting adult female mosquitoes.
4. The need to maintain a high level of surveillance on mosquito populations and viral activity if we are to anticipate and prevent epidemics and document the impact of decreased revenues for mosquito control.

RESURGENCE OF VECTOR POPULATIONS AND VIRAL ACTIVITY.–Experience in 1978 illustrated the capacity of *Cx. tarsalis* and the encephalitis viruses to rapidly return to high levels of activity. In the spring, the State Department of Health Services and its consultants recognized that the record levels of rainfall, snowpack and river run-off would pose a challenge to your capacity to control *Cx. tarsalis* and other vectors (California Morbidity, 1978). We did not take into consideration that there would be a drastic decrease in the tax base for vector control, as that decision came later.

We have analyzed the light trap records from 23 Districts in the Central Valley for 1969, 1977, and 1978 with particular concentration on the urban indices for which there is a continuous record. The last year of a major water surplus in the state was 1969. The seasonal index for *Cx. tarsalis* females in 1978 was higher than in 1969 in 87% of the Central Valley Districts and higher than in 1977 in 80% of the Districts. In some Districts the increases represented a 5 to 10-fold rise over 1977 levels (Table 1).

[1] These studies were supported in part by Research Grant AI-03028 from the National Institute of Allergy and Infectious Diseases, General Research Support Grant I-S01-FR-05441 from the National Institutes of Health, and by U. S. Army Contract/Grants No. DAMD 17-74-C4128 and DAMD 17-77-C-7018 U. S. Army Medical Research and Development Command, Washington, D. C.

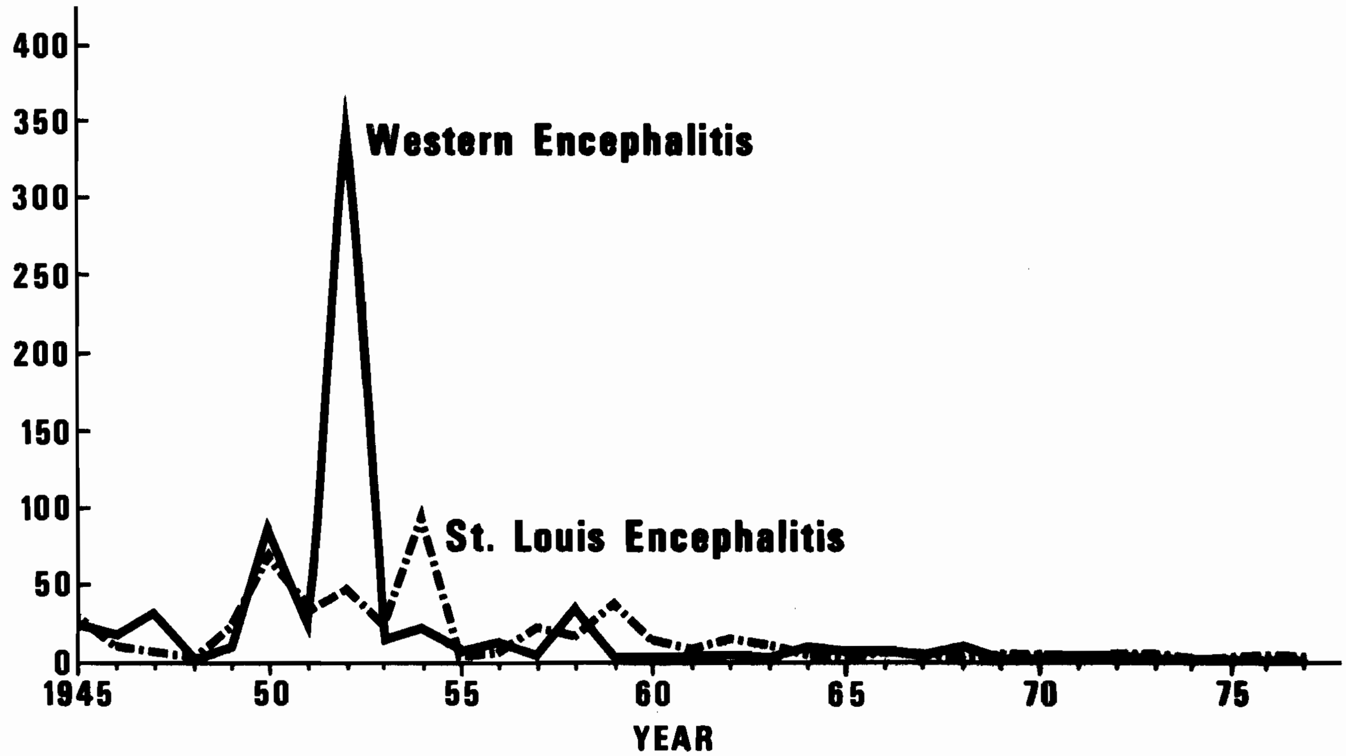

Figure 1. Number of laboratory proven cases of Western and St. Louis encephalitis – California 1945 - 1977. (Source - California State Department of Health.)

We found that the seasonal average levels of *Cx. tarsalis* were high enough in over half of the Central Valley Districts to expect WEE virus to be active and in at least 2 Districts there were sufficient vectors that WEE and SLE cases could occur (Table 1). We did not experience the peak levels of *Cx. tarsalis* that were associated with high viral activity and epidemics in the 1950's. A major credit must be given in the prevention of such increases in vector populations to your control efforts including the use of a major proportion or all of reserve funds and whatever emergency relief funds became available in some Districts.

As a second type of study, we maintain sentinel flocks of chickens in Kern, Glenn, Butte, Sutter, Yuba and Placer Counties as a part of the general surveillance program on encephalitis viruses in California. We have records of viral activity and vector populations in these areas that extend over many years. Blood tests on over 400 sentinel chickens from these areas in 1977 gave no evidence that WEE or SLE viruses were active. In fact, since 1973, antibodies to WEE or SLE had developed only occasionally in a single bird or a few birds at isolated sites in the Central Valley indicating that the viruses were just persisting or were reintroduced briefly. In 1978, the situation changed dramatically as WEE infection rates soared. Thirty-seven % of chickens became infected in the Sacramento Valley flocks and 15% in the Kern flocks (Table 2). Infection occurred at 20 of 21 flock locations in the Central Valley. There was no evidence that SLE viral infection occurred in these birds.

The above development gave us another opportunity to look at the relationship between light trap indices for *Cx. tarsalis* females and viral activity. Seasonal light trap indices had ranged from 0.2 to 200 per trap night at the 21 sites. An analysis of the relationships of vector populations to the percent of sentinel chickens that had become infected with WEE virus showed a very significant correlation (p = .004) (Table 2).

Dr. Emmons in his presentation on this program will report the diagnosis of 12 clinical cases of WEE in horses. It is of particular interest that 8 of the 12 cases came from the Sacramento Valley where the highest levels of vector population and sentinel chicken conversions occurred. The only diagnosed case of SLE was from Southern California and this fit with the recoveries of SLE virus from mosquitoes in that region.

To summarize, WEE virus resurged in the Central Valley in 1978 and the levels of vector population rose significantly in many Districts. The sequence of events confirmed earlier observations (Reeves 1968, 1970; Olson et al. 1979) that light trap indices are a valuable predictor for viral activity and that WEE virus will be active when vector populations are below those required for transmission of SLE virus. If the *Cx. tarsalis* populations in future years remain as high or become higher than in 1978, we expect that WEE virus will be active and that human and equine cases will occur. With any increase in *Cx. tarsalis* populations, SLE virus should reappear in the Central Valley. The isolations of SLE virus from the Imperial Valley indicate it is still highly endemic in California (Emmons et al. 1978; Work et al. 1977). We are now analyzing weekly *Cx. tarsalis* light trap indices to determine how they correlate with viral activity. This could lead to development of an early prediction model and increase your ability to utilize the records for early program decisions.

EXPECTED NUMBER OF HUMAN CASES IN FUTURE YEARS.–We did a rather involved analysis to determine what number of WEE and SLE cases would occur if attack rates that prevailed for WEE in 1952 or for SLE in 1954 occurred again

Table 1.--Seasonal light trap indices of *Culex tarsalis* females from 23 mosquito control agencies in the Central Valley, 1969, 1977 and 1978.

Agency and trap record	1969	1977	1978
Sacramento Valley			
Butte (Rural)	3.4	30.3	44.6^C,S,W^
Colusa (Rural)	N.A.	2.4	15.9^W^
Glenn (Rural)	N.A.	11.9	45.6^C,S,W^
Los Molinas (Rural)	N.A.	0.6	5.9^W^
Sacramento/Yolo (Urban)	1.3	0.3	4.1^W^
Shasta (Urban)	0.8	N.A.	2.3^W^
Sutter-Yuba (Urban)	1.4	0.8	3.8^W^
Tehama (Urban)	10.7	N.A.	9.5^W^
Northern San Joaquin Valley			
Eastside (Urban)	0.1	0.1	0.1
Merced (Urban)	1.2	0.3	0.7
No. San Joaquin (Urban)	1.1	0.4	1.1^W^
San Joaquin (Urban)	0.2	0.2	0.3
Turlock (Urban)	0.3	0.1	0.1
Southern San Joaquin Valley			
Consolidated (Urban)	0.4	0.0	0.1
Delano (Rural)	N.A.	0.5	5.2^W^
Delta (Urban)	0.6	0.1	0.2
Fresno (Urban)	0.1	0.0	0.1
Fresno Westside (Urban)	1.2	0.6	2.8^W^
Kern (Urban)	4.1	0.6	0.2
Kings (Urban)	1.5	0.4	1.2^W^
Madera (Urban)	1.2	N.A.	0.3
Tulare (Urban)	0.5	0.8	1.7^W^
West Side (Urban)	4.6	1.6	1.6^W^

N.A. = not available
W = Index high enough for WEE viral activity
S = Index high enough for SLE viral activity
C = Index high enough for human encephalitis to occur

in the human population that presently resides in the San Joaquin and Sacramento Valleys. We took into consideration that:

1. The population of the Central Valley has almost doubled - from 1,831,800 in 1952 to 3,239,700 in 1978.
2. In the 1950's, inapparent infections produced high immunity rates in older residents. Currently inapparent infections are uncommon in all age groups. As examples, earlier the immunity rate for WEE in samples from the 20-49 age group was around 19%, now it is less than 4%. SLE immunity rates in the same age group have gone from 24% in earlier samples to less than half that level. In children less than 10 years of age there are few or no immunes. This means that a majority of the population has been protected from infections by mosquito bite for the past 10 to 20 years and the result is a decrease in both clinical disease and immunity levels.
3. The ratio of clinical to inapparent infections in the different age groups in future epidemics will be the same as in the past. Infants and the elderly will be particularly high risk groups for WEE and SLE respectively.

If vector populations and viral activity returned to the levels of past epidemic years, we could now expect to have 686 rather than 368 confirmed WEE cases as in 1952 and 207 rather than the 95 confirmed SLE cases that occurred in 1954 (Table 3). If both diseases occurred, in the same summer, there would be 893 cases and that means that 25 of each 100,000 residents of the Central Valley would have encephalitis. Many of the hospitalized cases in infants and elderly persons will have a permanent debilitating brain damage that requires their continuous care for many years. Many more persons - perhaps 1,000 of every 100,000 residents would have a less severe unrecognized infection that did not require intensive medical care and hospitalization.

Table 2.-Correlations[1] of seasonal female *Culex tarsalis* light trap indices and proportion of chickens infected with WEE virus, Central Valley, 1978.

Seasonal light trap index		Chicken infections		County
Rank	♀*Cx. tarsalis*/ trap night	Rank	% positive	
1	200.0	3	61	Butte
2	114.5	12	16	Kern
3	111.6	17	10	Sutter
4	44.6	1	80	Glenn
5	28.9	5	48	Glenn
6	23.2	7.5	32	Yuba
7	21.1	5	48	Yuba
8	18.3	5	48	Placer
9	15.5	10.5	18	Butte
10	14.8	14.5	13	Kern
11	9.4	10.5	18	Kern
12	6.8	7.5	32	Kern
13.5	6.2	13	14	Kern
13.5	6.2	14.5	13	Kern
15	4.0	18	9	Kern
16	3.9	20.5	5	Yuba
17	3.5	2	70	Yuba
18	3.1	9	25	Kern
19	2.6	16	11	Butte
20	1.0	20.5	5	Butte
21	0.8	19	8	Kern
22	0.2	22	0	Kern

[1] Rank correlation coefficient = .594 ($p < .01$).

You will note that the above projections do not include estimates of cases in the large population that lives in Southern California. We have never experienced an epidemic in that region and the number of sporadic cases and available background information do not allow accurate predictions. However, we know that WEE and SLE viruses have been active in parts of the southern area in most recent years (Emmons et al. 1978; Work et al. 1977). It is to be expected that a state-wide epidemic would include some Southern California populations and this probably would add sufficient cases to increase the total number to over 1,000 cases.

In a separate analysis Olson (1977) predicted that there would be at least 100 WEE cases in horses (81 per 100,000) in a single year if the attack rates in past epizootics recurred in the present equine population. We would remind you that the State Virus Laboratory reported 12 confirmed WEE cases in horses this past summer.

The cost of responding to an epidemic of the above magnitude would be much higher than the annual cost of preventing an epidemic which has been our objective for over 20 years. Recent experience is relevant to our concern. In 1978, there was a water surplus and we were concerned there would be an epidemic. A number of areas of California were declared disaster areas because of physical damage from flooding. These conditions led mosquito control agencies to request $2,700,000 in disaster relief funds, $392,000 was approved and the latest figures indicate that $125,000 finally was

Table 3.--The expected number of WEE and SLE cases if the infection rates for previous epidemic years recurred in the 1978 Central Valley population.

Age	1952	Percent with antibody in 1952[1]		Cases		Attack rate per 100,000 susceptibles		1978	Percent with antibody in 1973-76		Predicted cases	
	Population	WEE	SLE	1952 WEE[2]	1954 SLE[3]	WEE	SLE	Population	WEE	SLE	WEE	SLE
<1	41,300	0	3.5	103	0	294.9	0	54,846	0	0	137	0
1-4	170,200	4.4	6.9	44	10	27.0	6.3	214,639	0	0	58	14
5-9	174,400	7.0	10.9	29	16	17.9	10.3	332,771	0	1.3	60	34
10-19	267,600	14.1	24.8	35	24	15.2	11.9	660,610	1.5	3.0	100	77
20-29	282,900			19	10	8.3	4.7	473,980	2.2	8.1	38	20
30-39	285,400	19.0	24.2	21	11	9.1	5.1	364,234	3.0	14.9	32	16
40-49	231,400			27	9	14.4	5.1	386,584	7.7	12.8	51	17
50+	378,600			90	15	29.3	5.2	743,036	3.4	24.1	210	29
TOTAL	1,831,800			368	95			3,239,700			686	207

[1] From Reeves and Hammon, 1962.
[2] From Longshore et al., 1956.
[3] From Olson, 1977.

awarded. This amount of money, in actuality, provided little or no relief - it was "peanuts". The paper work to obtain the funds amounted to what looked like a late spring and early summer blizzard. The use of reserve funds and an unusual level of dedicated effort by the mosquito control staffs at least partially minimized the problem in 1978. However, as was indicated earlier, there still was a resurgence of vector populations, viral activity, and cases.

Present decreases in funding will lead to a rapid erosion of our capacity to maintain effective control programs. Losses that are already occurring or can be anticipated are in:

1. Professionally trained staff,
2. Equipment becoming antiquated or non-functional,
3. Source reduction programs such as drainage and diking systems and waste water sumps cannot be maintained,
4. Decreases will occur in continuing education of water users and the population regarding mosquito production and disease risk,
5. Presently effective information gathering and warning systems regarding vector populations, viral activity and clinical cases can become less effective.

California has been known for some time as the state with a mosquito control and mosquito-borne disease prevention program second to none in the world. The population has been protected from disease and pest annoyance. We have heard it said that if an epidemic does threaten, we will now have to turn to State and Federal Agencies for emergency funds, manpower, equipment and materials, and that they can replace the resources we have lost on short notice. That is not true. In the event of an impending epidemic of encephalitis, with cases beginning to occur, it will probably take over $10,000,000 in emergency funds to supplement the presently available $8,000,000 in local revenue. This would allow us to mount a partially effective emergency program. Manpower, equipment and materials still will have to come principally from local sources once funds become available. None of the supplemental funds will be spent on permanent long range source reduction programs. The funding will be stopped once the emergency is terminated by control or by the onset of winter and associated decreases in vector activity.

Let us turn now, even though briefly, to a consideration of malaria - a disease for which we have even less of a basis for predictions. Malaria used to be endemic in California. The vectors *Anopheles freeborni* and *Anopheles punctipennis* were identified and procedures for their control evolved. The disease disappeared in areas where control programs were mounted but an enigma was that the disease also disappeared in areas where no purposeful control was done. We have assumed that reductions in the degree of effective contact between the *Anopheles* and human hosts was produced both by vector control and by social changes such as improved housing and the increased availability of alternate sources of bloodmeals for the vectors such as domestic animals. The disease was declared eradicated from the United States some years ago. It is ironic that with the resurgence of malaria in many parts of the world, the State Department of Health Services has reported 225 diagnosed cases in California in 1978. This is over 30% of the 694 cases reported in the United States. Fortunately, it has been possible to trace each case in California to a recent exposure while traveling or in residence outside the United States. By definition, this disease, even though commonly diagnosed, is still eradicated from our state. However, the above observations have been of little comfort to agencies such as the Sutter-Yuba Mosquito Abatement District when 44 of the cases occurred in their area in 1978 alone. That District had little choice each year when cases were reported but to follow up each case with the assumption it could be a locally transmitted infection and that there was a potential source for additional local transmission. This is a costly and demanding obligation. We do not have the information regarding levels of vector population, human sources of infection, susceptibles exposed, and environmental factors, as we do for encephalitis, that will allow us to predict if malaria will become endemic again. We have had to assume that it could as it did in the past. We do have extensive records of the population levels of *Anopheles* throughout the state thanks to your accumulated light trap records. These records will be a valuable resource to determine the levels of vector population associated with secondary transmission if it occurs, but that will be of little consolation.

CONSIDERATIONS OF SELECTIVE CONTROL MEASURES.–Most agencies represented in this audience have been forced to the decision that the limitations in funds for mosquito control will force a redirection of programs. There probably will be a decrease in larvaciding. Emphasis will be given to maintenance and extension of source reduction, utilization of self perpetuating biological control agents (such as *Gambusia*) and public education. When necessary, temporary procedures such as adulticiding will be used to reduce unusually large numbers of adults that invade urban centers or to abate an epidemic. The above may even be the priority order in program continuations.

A few comments may assist with regard to selection of a method that can have maximum use to abate an impending or in-progress epidemic of encephalitis or malaria. The most immediate target for control in such circumstances is the adult female vector population that has lived long enough to become infected with, complete incubation of, and transmit the pathogen by their bite. This is a very small proportion of the population. We now have knowledge of adult female vector populations that we didn't before. The mark-release-recapture studies reported by Milby (1979) reveal that from 23-46% of the *Cx. tarsalis* female population dies each day during the summer. In earlier studies we demonstrated that only about 25% of the *Cx. tarsalis* that were infected with encephalitis viruses could transmit when they fed, and it was assumed they died before they completed incubation of the virus (Reeves et al. 1961). Studies by Dr. Kramer and associates (1979) will report that a proportion of *Cx. tarsalis* that become infected with WEE virus never will transmit infection no matter how long they live.

All of the above findings, as well as experience in control of epidemics of SLE in recent years, means that an emergency control program is best aimed at reduction of the adult female vector population. The killing of the older, infected and transmitting females will accomplish the most rapid and effective cessation of transmission of infection to susceptible hosts. Acceptance of this approach also identifies new areas for research. Namely, what are the most effective current methods for adulticiding, what is the state of resistance of adult female vectors to the array of available insecticides, is there a difference in susceptibility of different aged females to insecticides, what will be the most effective timing for successive adulticide applications to suppress pathogen transmission, and can we expect cases to stop within an incubation period after effective adulticiding? We do not have answers to all of the above questions but acceptance of this approach to emergency epidemic control may depend on the availability of such knowledge.

FUTURE SURVEILLANCE OF VECTOR POPULATIONS AND VIRAL ACTIVITY.–We believe that the preceding considerations leave no question there is a necessity to maintain surveillance of mosquito populations in your Districts. You have population indices on a weekly and annual basis that extend in most Districts for over a 20-year period. These data have been analyzed with reference to viral activity, the occurrence of encephalitis cases, and complaints of discomfort. There is a high correlation between the levels of mosquito population and these problems. If such records are continued, you can make your own predictions each week as the records accumulate. We intend, as long as you and the Vector Biology and Control Section continue to amass the records, to utilize our computer and statistical resources for their epidemiological analysis, and to provide our findings as a guide for your actions. Similarly, a collaborative effort of the University with the Virus Laboratories of the State Department of Health Services will provide critical information on viral activity in vector populations and sentinel birds and the occurrence of clinical cases. It is going to be difficult to maintain these programs at the local and state levels due to decreases in funds. However, to make epidemic predictions and decisions on the need for control solely on the basis of information other agencies provide such as water availability and temperature or the number of clinical cases of encephalitis and malaria reported by physicians and veterinarians, would be to ignore and not to apply the scientific and professional advances made since our last major epidemics of encephalitis and malaria in 1952 and 1954. We also believe that carefully documented records of the levels of mosquito populations and disease occurrence will be the type of quantitative evidence that will convince lawmakers, budgetary decision makers, and residents that mosquito control deserves the highest level of community support.

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SURVEILLANCE FOR ARTHROPOD-BORNE VIRUSES AND DISEASE BY THE CALIFORNIA DEPARTMENT OF HEALTH SERVICES, 1978

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This is the ninth annual report summarizing the Department's recent arbovirus surveillance efforts. A revival of interest in determining vector infection levels began in 1969, and reports of that and subsequent years' activities have been published in Mosquito News (Sudia et al. 1971) and in the Proceedings of this Association's annual conferences (Emmons et al. 1972, 1973, 1974, 1975, 1976, 1977 and 1978). Several other review articles have also been prepared (Emmons and Grodhaus 1976, Workman et al. 1976, and Emmons 1979). The Department's work is correlated with that of the many local mosquito abatement districts, University of California research units, county health departments, and other agencies, private veterinarians and physicians who have interests and concerns in prevention of arbovirus encephalitis.

During 1978 at least 367 patients were tested for western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE), as well as for the more likely causes of encephalitis (mumps, herpes, enteroviruses, etc.). This surveillance effort is increasingly being participated in by the larger local health department laboratories which have developed viral diagnostic capabilities (Table 1). A large proportion of the sera from cases not yet identified as to etiology will subsequently be tested by Dr. W. C. Reeves' research group (14th year of this study) to search for possible relationship of encephalitis to mosquito-borne viruses other than WEE and SLE viruses. There were 10 human brain samples and 1 cerebrospinal fluid sample from fatal cases of encephalitis which were tested in suckling mice during 1978, but none yielded arboviruses.

Only 1 case of SLE was detected in 1978: a 29 year old man from La Mesa, San Diego County, who was most likely infected by mosquito bites at a country club golf course in nearby Lakeside the weekend of September 30/October 1. A prior trip to Mexicali and El Centro 2 weeks earlier was beyond the maximum incubation period. Onset of illness was October 20, and fever, progressive headache, vomiting, stiff neck and some disorientation characterized the disease course. He was hospitalized from October 24 until November 1, and recovered without apparent sequelae. Serum samples taken October 24 and November 7 showed rising SLE antibody titers, confirming the diagnosis: complement-fixing (CF) antibody titers were 1:4 and 1:16 in the San Diego Laboratory (<1:8 and 1:32 in the state Viral and Rickettsial Disease Laboratory); indirect fluorescent antibody (IFA) titers were 1:32 and 1:128; hemagglutination-inhibition antibody titers were 1:20 and $\geq$1:160 (courtesy of the School of Public Health, U.C., Berkeley), and the plaque-reduction neutralizing antibody titers were 1:128 and 1:1,024, respectively. Mosquito collections made at sites nearby the apparent site of his exposure, during the summer period prior to and subsequent to the case occurrence, did not yield viruses. However, possible vector species (*Culex tarsalis, Culex pipiens*) were present.

There were 35 suspect cases of encephalitis in equines reported to the Department from 18 counties in the state, and 12 were found to be positive or presumptive-positive WEE cases by serologic tests (Table 2). This is a significant increase in the total number and the percent positive over the findings of the past several years (only 6 confirmed cases from 1972-1977), and correclates with the marked increase in WEE virus activity during 1978. Of 10 equine brain samples from fatal cases of suspected encephalitis which were tested in suckling mice, all were negative for arboviruses. In addition, 7 brain samples from squirrels dying of suspected encephalitis were tested, but no viruses were isolated. Such squirrel brains have sometimes yielded WEE virus in past years.

In total 1,798 mosquito pools (including 77,914 mosquitoes) were collected and tested in suckling mice during the year (Tables 3 and 4). There were 187 viruses isolated, including 87 WEE, 39 SLE, 39 Turlock, and 22 Hart Park (Table 5). The large reservoir of WEE and SLE viruses, the increase in equine cases of WEE, and the prospects for abundant water and potential mosquito breeding sites during the 1979 summer, indicate the continuing need for surveillance and control efforts to prevent involvement of an increasingly susceptible human population.

The other mosquito-borne virus disease of interest this year was dengue, which was proven or presumptive positive in at least 37 cases, the largest number ever recorded in modern times in the state. These included travelers returning from Tahiti (28), Puerto Rico (3), Colombia (1), El Salvador (2), Djakarta, Indonesia (1), the Seychelles (Indian Ocean) (1), and "Southeast Asia" (1). In the absence of *Aedes aegypti* or other suitable vectors in California, this disease is of less interest to vector control agencies than another "exotic" disease - - malaria - - which can be spread by native *Anopheles* species. At least 226 cases of imported malaria were recorded during 1978.

There were only 6 cases of Colorado tick fever, a low number indicating that the virus was at a low ebb in its natural tick-rodent cycle, or perhaps simply less interest by physicians in having their clinical suspicion verified by specific laboratory tests.

ACKNOWLEDGMENT.—The assistance and cooperation of many staff members of the Viral and Rickettsial Disease Laboratory, the Vector Biology and Control Section, and others in the California Department of Health Services, of local mosquito abatement districts, county health departments, the California Department of Food and Agriculture, other agencies and private physicians and veterinarians in carrying out the surveillance program are gratefully acknowledged. The contribution of Dale V. Dondero in conducting the mosquito virus-testing portion of the study and summarizing the results is especially noted.

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Table 1.–Humans tested serologically for mosquito-borne arbovirus disease by the Viral and Rickettsial Disease Laboratory Section, California State Department of Health Services and by County Health Department laboratories, by county and month of illness onset, California 1978.

County	Totals	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Unknown
Totals	367	1	2	4	8	36	45	48	57	50	63	23	1	29
Alameda County	2					1					1			
Berkeley	6					1	1		2	1				1
Butte	4								1		2			1
Contra Costa	2				1		1							
El Dorado	6						1		1		1		1	2
Fresno*	27					3	3	10	3	8				
Humboldt-Del Norte	5						1	2			1			1
Imperial	1								1					
Kern	1					1								
Kings	1													1
Lake	4							2			1			1
Los Angeles County*	68						1	2	20	14	26	5		
Madera	1											1		
Marin	2								1					1
Mendocino	2								2					
Merced	8			1		1					1	3		2
Napa	3									1				2
Nevada	2					1						1		
Orange*	16					3	6	1	1	1		3		1
Placer	6	1							2	2	1			
Sacramento*	27					1	6	4	4	6	2	4		
San Bernardino*	11						4	5	1		1			
San Diego*	75				2	12	10	11	10	11	17	2		
San Francisco	7		1		1	2	1				2			
San Joaquin	6								2	1	1	2		
San Luis Obispo	4				1				1	2				
San Mateo	2					1		1						
Santa Clara	10				2	1		2	2		1	1		1
Santa Cruz	8					1	2	2		1	2			
Shasta	11					2	1	3		1		1		3
Solano	5					2	3							
Sonoma	4			1		1	1							1
Stanislaus	2				1					1				
Sutter	2			1					1					
Tehama	1													1
Tuolumne	1							1						
Ventura	10			1		1	2		2		3			1
Yolo	5		1			1	1	2						
Unknown	9													9

*Most or all sera tested by County Health Department laboratory.

Table 2.–Positive and presumptive positive cases of western encephalitis in equines – California, 1978.

Case No.	County of Occurrences	Age and Sex	Date of Onset	Vaccination History	Serological Results Date	CF[a]	IFA[b]	HAI[c]
1	Riverside (Palo Verde)	8 yr. G	06-26-78	Unknown	06-26-78 07-08-78	<1:8 1:32	<1:8 1:64	1:20 ≥1:160
2	Yuba (Marysville)	2 yr. F	07-11-78	None	07-11-78 07-25-78	1:256 1:1024	1:256 1:2048	≥1:160 ≥1:160
3	Butte (Chico)	2 yr. M	07-21-78	WEE (1977)	07-17-78 07-31-78	<1:8 ≥1:64	1:8 ≥1:2048	≥1:160 ≥1:160
4	Sutter (Live Oak)	3 yr. M	07-25-78	None	08-03-78 08-15-78	1:512 1:512	1:2048 1:2048	≥1:160 ≥1:160
5	Yolo (Davis)	1 yr. F	08-20-78	None	08-20-78 09-01-78	<1:8 ≥1:64	<1:8 ≥1:1024	1:40 ≥1:160
6	Yolo (Davis)	3 yr. F	09-04-78	None	09-04-78 09-15-78	<1:8 ≥1:64	<1:8 ≥1:1024	<1:40 ≥1:160
7	San Joaquin (Lodi)	4 yr. G	09-20-78	Unknown	09-25-78 10-17-78	1:256 1:256	1:512 ≤1:2048	≥1:160 NT
8	Tehama (Corning)	4 yr. F	09-26-78	Unknown	09-26-78 10-20-78	<1:8 ≥1:64	<1:8 ≥1:1024	≥1:160 NT
9	Sacramento (Galt)	2 yr. F	09-28-78	None	10-03-78	1:128	1:512[d]	≥1:160
10	Yolo (Woodland)	7 yr. F	10-02-78	Unknown	10-04-78 10-24-78	1:64 1:128	1:512 1:512	NT ≥1:160
11	Stanislaus (Oakdale)	1 yr. F.	10-13-78	WEE)June EEE)1978	10-13-78 10-26-78	1:32 ≥1:128	1:256 1:2048	≥1:160 ≥1:160
12	Fresno (Sanger)	3 yr. G	10-26-78	None	10-26-78 11-08-78	1:16 ≥1:128	1:512 ≥1:4096	≥1:160 ≥1:160

[a]Complement Fixation Test.

[b]Indirect Fluorescent Antibody Test.

[c]Hemagglutmation Inhibition Antibody Test. (School of Public Health, University of California, Berkeley.)

[d]Horse expired; convalescent sample could not be obtained.

NT = not tested.

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Table 3.– Number of mosquitoes (pools) tested by county and species, by the Viral and Rickettsial Disease Laboratory Section, California State Department of Health Services, 1978.

County	*Culex tarsalis*	*Culex erythro-thorax*	*Culex pipiens* complex	*Culex peus*	*Culex thri-ambus*	*Culiseta inornata*	*Culiseta* spp. unident.	*Culiseta particeps*	*Culiseta incidens*	*Aedes vexans*	*Aedes melanimon*	*Aedes dorsalis*	*Aedes sierrensis*	*Aedes nigro-maculis*	*Anoph. fran-ciscanus*	*Anoph. free-borni*	*Culi-coides* spp.	TOTALS
Butte	95(4675)										20(1000)							115(5675)
Contra Costa	4(167)										4(175)							8(342)
Fresno	29(1328)																	29(1328)
Glenn	46(2287)																	47(2299)
Imperial	139(5257)	2(20)	28(727)								1(12)							169(6004)
Kern	25(1291)																	25(1291)
Kings	26(1420																	26(1420)
Lake	4(162)												1(55)					5(217)
Madera	17(788)																	17(788)
Merced	35(1760)										4(179)			2(145)				41(2084)
Orange	7(330)	1(100)														1(6)		9(436)
Placer	9(427)									1(55)								10(482)
Riverside	486(21,245)	58(2321)	26(606)	22(588)		12(100)	1(21)	4(12)	5(24)			1(8)						597(24,925)
San Bernardino	246(11,803)	8(234)								2(76)								256(12,113)
San Joaquin	4(169)																	4(169)
San Diego	54(1032)	4(18)	2(8)	1(1)	1(1)										2(23)		1(23)	65(1106)
Sacramento	19(834)										1(12)							20(846)
Shasta	15(592)																	15(592)
Stanislaus	24(1006)										1(50)							25(1056)
Solano	26(1195)																	26(1195)
Sutter	14(619)																	14(619)
Tehama	40(1988)																	40(1988)
Tulare	109(5238)		1(10)											6(367)				116(5615)
Yolo	7(260)										2(22)							9(282)
Yuba	2(71)																	2(71)
Mohave, AZ	67(3243)		1(50)			1(1)				2(98)								71(3392)
Yuma, AZ	24(1006)	12(571)																36(1577)
Baja California, Mexico	1(2)																	1(2)
TOTALS	1556(70,195)	85(3264)	58(1401)	23(589)	1(1)	13(101)	1(21)	4(12)	5(24)	5(229)	33(1450)	1(8)	1(55)	8(512)	2(23)	1(6)	1(23)	1798(77,914)

Table 4. Number of mosquitoes (pools) tested, by species and month, by the Viral and Rickettsial Disease Laboratory Section, California State Department of Health Services, 1978.

	April	May	June	July	August	September	October	November	December	TOTALS
Culex										
tarsalis	77(3496)	267(11898)	316(14514)	205(9345)	417(18537)	212(9563)	61(2841)		1(1)	1556(70195)
erythrothorax	5(213)	7(88)	24(954)	9(318)	26(1081)	8(352)	6(258)			85(3264)
pipiens complex	6(130)	14(291)	13(360)	12(248)	5(68)	7(303)			1(1)	58(1401)
peus		12(255)	2(62)		2(51)	5(210)	1(10)		1(1)	23(589)
thriambus									1(1)	1(1)
Culiseta										
inornata		6(52)				1(1)	6(48)			13(101)
spp. unidentified			1(21)							1(21)
particeps		4(12)								4(12)
incidens		5(24)								5(24)
Aedes										
vexans		4(174)		1(55)						5(229)
melanimon		1(12)	16(699)	2(76)	1(50)	13(613)				33(1450)
dorsalis	1(8)									1(8)
sierrensis			1(55)							1(55)
nigromaculis			3(171)		3(196)	2(145)				8(512)
Anopheles										
franciscanus						2(23)				2(23)
freeborni					1(6)					1(6)
Culicoides spp.									1(23)	1(23)
TOTALS	89(3847)	320(12806)	376(16836)	229(10042)	455(19989)	250(11210)	74(3157)		5(27)	1798(77914)

Table 5.–Viral isolates from mosquito pools tested during 1978 by the Viral and Rickettsial Disease Laboratory Section, California State Department of Health Services.

Identifying Number	County	Place	Date Collected	Species	Number in pool	Agent Isolated
RV-1513	Riverside	Mecca	4/19	*Cx. tarsalis*	50	Hart Park
IV-670	Imperial	Laguna Dam	4/19	" "	50	Turlock
IV-701	Imperial	Laguna Dam	5/11	" "	50	Turlock
SD-302	San Diego	San Diego	5/16	" "	15	Hart Park
SB-652	Mojave, AZ	Bermuda City	5/24	" "	50	Turlock
SB655	Mojave, AZ	Bermuda City	5/24	" "	50	Turlock
SB-656	Mojave, AZ	Bermuda City	5/24	" "	50	Turlock
SB-672	Mojave, AZ	Bemuda City	5/24	" "	50	Turlock
SB-688	San Bernardino	Needles	5/24	" "	50	Turlock
SB-710	Mojave, AZ	Bermuda City	5/24	" "	50	Turlock
RV-1638	Riverside	Blythe	5/26	*Cx. tarsalis*	50	Turlock
RV-1640	Riverside	Blythe	5/26	" "	50	WEE
RV-1645	Riverside	Blythe	5/26	" "	50	WEE
RV-1647	Riverside	Blythe	5/26	" "	50	Turlock
RV-1648	Riverside	Blythe	5/26	" "	50	Turlock
RV-1653	Riverside	Blythe	5/26	" "	50	Turlock
RV-1654	Riverside	Blythe	5/26	" "	50	Turlock
RV-1664	Riverside	Blythe	5/26	*Cx. pipiens*	14	Turlock
V4-1881	Tulare	Pixley	5/31	*Cx. tarsalis*	41	Hart Park
V4-1882	Tulare	Waukena	5/31	" "	50	Hart Park
V4-1884	Tulare	Waukena	5/31	*Cx. tarsalis*	50	Hart Park
V4-1885	Tulare	Waukena	5/31	" "	50	Hart Park
V4-1886	Tulare	Waukena	5/31	" "	71	Hart Park
IV-736	Imperial	Calexico	6/8	" "	26	WEE
IV-737	Imperial	Calexico	6/8	*Cx. pipiens*	5	WEE
IV-745	Imperial	Brawley	6/8	*Cx. tarsalis*	50	WEE
IV-746	Imperial	Brawley	6/8	" "	16	WEE
IV-784	Imperial	Laguna Dam	6/8	" "	50	Turlock
IV-789	Imperial	Laguna Dam	6/8	" "	50	WEE
IV-792	Imperial	Laguna Dam	6/8	" "	50	Turlock
IV-799	Imperial	Laguna Dam	6/8	*Cx. tarsalis*	50	WEE
IV-803	Yuma, AZ	Yuma Test Station	6/8	" "	50	Turlock
IV-743	Imperial	Seeley	6/9	" "	50	WEE
SD-306	San Diego	Bonsall	6/15	" "	20	Turlock
SD-308	San Diego	Oceanside	6/15	" "	16	Hart Park
SD-309	San Diego	Oceanside	6/15	" "	34	Hart Park
V4-1895	Kern	Old River	6/19	" "	36	Hart Park
V4-1899	Kern	Lost Hills	6/19	" "	50	Hart Park
SB-762	San Bernardino	Needles	6/22	" "	50	Turlock
SB-765	San Bernardino	Needles	6/22	" "	50	Turlock
SB-770	San Bernardino	Needles	6/22	*Cx. tarsalis*	50	WEE
SB-772	San Bernardino	Needles	6/22	" "	50	WEE
SB-773	San Bernardino	Needles	6/22	" "	50	WEE
SB-779	San Bernardino	Needles	6/22	" "	50	WEE
IV-809	Imperial	Seeley	6/26	" "	22	WEE
V1-1566	Glenn	Willows	6/28	" "	50	Hart Park
V4-1908	Tulare	Tipton	6/28	" "	50	Hart Park
V4-1909	Tulare	Tipton	6/28	" "	96	Hart Park
V4-1911	Tulare	Tipton	6/28	" "	50	Hart Park
V4-1912	Tulare	Tipton	6/28	" "	50	Hart Park

Identifying Number	County	Place	Date Collected	Species	Number in pool	Agent Isolated
V4-1918	Tulare	Alpaugh	6/28	*Cx. tarsalis*	17	Hart Park
RV-1675	Riverside	Blythe	6/29	" "	50	WEE
RV-1677	Riverside	Blythe	6/29	" "	50	WEE, SLE
RV-1679	Riverside	Blythe	6/29	" "	50	WEE
RV-1681	Riverside	Blythe	6/29	" "	35	WEE
RV-1683	Riverside	Blythe	6/29	" "	50	WEE
RV-1684	Riverside	Blythe	6/29	*Cx. pipiens*	21	WEE
RV-1687	Riverside	Blythe	6/29	*Cx. erythrothorax*	50	WEE
RV-1689	Riverside	Blythe	6/29	" "	50	SLE
RV-1690	Riverside	Blythe	6/29	" "	44	WEE
RV-1800	Riverside	Blythe	6/29	*Cx. tarsalis*	50	WEE
RV-1804	Riverside	Blythe	6/29	" "	50	SLE
RV-1807	Riverside	Blythe	6/29	" "	50	WEE
RV-1810	Riverside	Blythe	6/29	" "	50	WEE
RV-1813	Riverside	Blythe	6/29	" "	50	WEE
RV-1815	Riverside	Blythe	6/29	" "	50	WEE
RV-1817	Riverside	Blythe	6/29	" "	50	WEE, SLE
RV-1818	Riverside	Blythe	6/29	" "	50	WEE
RV-1819	Riverside	Blythe	6/29	" "	50	WEE
RV-1820	Riverside	Blythe	6/29	" "	50	WEE, SLE
RV-1821	Riverside	Blythe	6/29	*Cx. tarsalis*	50	WEE
RV-1822	Riverside	Blythe	6/29	" "	50	WEE
RV-1825	Riverside	Blythe	6/29	" "	50	WEE
RV-1826	Riverside	Blythe	6/29	" "	50	WEE
RV-1827	Riverside	Blythe	6/29	" "	50	WEE
RV-1828	Riverside	Blythe	6/29	" "	50	WEE
RV-1829	Riverside	Blythe	6/29	" "	50	WEE, SLE
RV-1830	Riverside	Blythe	6/29	" "	50	WEE
RV-1831	Riverside	Blythe	6/29	" "	50	WEE
RV-1832	Riverside	Blythe	6/29	" "	50	WEE
RV-1834	Riverside	Blythe	6/29	*Cx. erythrothorax*	50	WEE
IV-817	Yuma, AZ	Yuma Test Station	7/6	*Cx. tarsalis*	25	SLE
IV-822	Imperial	Seeley	7/6	" "	50	SLE
IV-824	Imperial	Seeley	7/6	" "	37	SLE
VI-1591	Tehama	Woodson Bridge	7/10	" "	50	Turlock
V4-1927	Merced	Merced	7/11	*Cx. tarsalis*	50	Turlock
V4-1931	Merced	Merced	7/11	" "	50	Hart Park
V2-3002	Sacramento	Elk Grove	7/18	" "	44	Turlock
RV-1582	Riverside	Coachella Valley	7/19	" "	50	SLE
RV-1584	Riverside	Coachella Valley	7/19	" "	50	SLE
RV-1585	Riverside	Coachella Valley	7/19	*Cx. tarsalis*	50	SLE
RV-1588	Riverside	Coachella Valley	7/19	" "	50	Turlock
RV-1845	Riverside	Blythe	7/19	" "	50	SLE
RV-1846	Riverside	Blythe	7/19	" "	50	SLE
RV-1849	Riverside	Blythe	7/19	" "	32	SLE
RV-1850	Riverside	Blythe	7/19	" "	50	WEE
RV-1851	Riverside	Blythe	7/19	" "	50	SLE
RV-1852	Riverside	Blythe	7/19	" "	35	WEE
RV-1855	Riverside	Blythe	7/19	*Cx. erythrothorax*	50	SLE
RV-1865	Riverside	Blythe	7/19	*Cx. tarsalis*	50	WEE

Identifying Number	County	Place	Date Collected	Species	Number in pool	Agent Isolated
RV-1868	Riverside	Blythe	7/19	*Cx. tarsalis*	50	WEE
RV-1870	Riverside	Blythe	7/19	" "	50	WEE
RV-1879	Riverside	Blythe	7/19	" "	13	SLE
RV-1881	Riverside	Blythe	7/19	" "	50	WEE
RV-1882	Riverside	Blythe	7/19	" "	12	WEE
RV-1883	Riverside	Blythe	7/19	" "	50	SLE
RV-1885	Riverside	Blythe	7/19	" "	50	SLE
RV-1886	Riverside	Blythe	7/19	" "	50	SLE
RV-1888	Riverside	Blythe	7/19	" "	16	WEE
RV-1890	Riverside	Blythe	7/19	*Cx. erythrothorax*	35	WEE
SD-324	San Diego	San Diego	7/20	*Cx. tarsalis*	18	Turlock
V1-1640	Butte	Gray Lodge	7/26	" "	50	Turlock
V2-2787	Sutter	East Nicolaus	7/27	" "	50	Turlock
V2-2800	Placer	Lincoln	7/27	" "	65	Turlock & Hart Park
SB-795	San Bernardino	Needles	8/2	" "	50	WEE
SB-796	San Bernardino	Needles	8/2	" "	50	WEE
V1-1656	Shasta	Persimmon Orchard	8/3	" "	58	Turlock
IV-837	Yuma, AZ	Yuma Proving Ground	8/7	" "	22	SLE
V2-3007	Sacramento	Elk Grove	8/8	" "	50	WEE
V2-3008	Sacramento	Elk Grove	8/8	" "	50	WEE
IV-834	Imperial	Bard	8/8	" "	10	SLE
IV-835	Imperial	Laguna Dam	8/8	" "	59	SLE
V4-1962	Tulare	Tipton	8/8	" "	60	WEE, SLE
RV-2001	Riverside	Blythe	8/10	" "	50	SLE
RV-2002	Riverside	Blythe	8/10	" "	50	SLE
RV-2003	Riverside	Blythe	8/10	" "	50	SLE
RV-2004	Riverside	Blythe	8/10	" "	50	SLE
RV-2005	Riverside	Blythe	8/10	" "	50	SLE
RV-2006	Riverside	Blythe	8/10	" "	50	SLE, WEE
RV-2007	Riverside	Blythe	8/10	" "	50	SLE
RV-2016	Riverside	Blythe	8/10	*Cx. tarsalis*	50	SLE
RV-2017	Riverside	Blythe	8/10	" "	50	SLE
RV-2019	Riverside	Blythe	8/10	" "	5	SLE
RV-2022	Riverside	Blythe	8/10	" "	50	SLE
RV-2023	Riverside	Blythe	8/10	" "	50	SLE
V2-3011	Sacramento	Elk Grove	8/14	" "	50	Hart Park
V4-1968	Fresno	Firebaugh	8/15	" "	57	Turlock
V2-3022	Sacramento	Elk Grove	8/21	" "	43	Hart Park
V1-1664	Butte	Gray Lodge	8/22	" "	50	WEE
V1-1667	Butte	Gray Lodge	8/22	" "	50	WEE
V4-2034	Tulare	Tipton	8/22	" "	50	WEE
V2-3025	Stanislaus	Newman	8/24	" "	50	WEE
V2-3029	Stanislaus	Crows Landing	8/24	" "	50	WEE
V2-3031	Stanislaus	Crows Landing	8/24	" "	50	WEE
V2-3034	Stanislaus	Crows Landing	8/24	" "	50	WEE
V2-3038	Stanislaus	Crows Landing	8/24	" "	50	WEE
V2-3040	Stanislaus	Hills Ferry Road	8/24	" "	50	WEE
V2-3044	Sacramento	Elk Grove	8/29	" "	42	Turlock
V4-2040	Kings	Hanford	8/30	" "	50	WEE
V4-2044	Kings	Hanford	8/30	" "	50	Hart Park

Identifying Number	County	Place	Date Collected	Species	Number in pool	Agent Isolated
V4-2046	Kings	Lemoore NAS	8/30	*Cx. tarsalis*	50	WEE
SB-801	San Bernardino	Needles	8/30	" "	50	Turlock
SB-806	San Bernardino	Needles	8/30	" "	50	Turlock
SB-813	San Bernardino	Needles	8/30	" "	50	Turlock
SB-870	San Bernardino	Needles	8/30	" "	50	WEE
SB-886	San Bernardino	Needles	8/30	" "	50	WEE
SB-889	San Bernardino	Needles	8/30	" "	50	Turlock
IV-859	Imperial	Laguna Dam	8/30	" "	16	WEE
V3-3040	Solano	Dixon	9/6	" "	50	Turlock
V4-2063	Merced	Gustine	9/12	*Ae. nigromaculis*	95	WEE
RV-2035	Riverside	Riverside	9/14	*Cx. tarsalis*	50	SLE
V1-1704	Butte	Gray Lodge	9/20	" "	50	WEE
V1-1706	Butte	Gray Lodge	9/20	" "	50	WEE
V1-1708	Butte	Gray Lodge	9/20	" "	50	WEE
V1-1717	Butte	Gray Lodge	9/20	" "	50	WEE
V1-1720	Butte	Gray Lodge	9/20	" "	50	WEE
V1-1721	Butte	Gray Lodge	9/20	" "	50	WEE
RV-2043	Riverside	Blythe	9/22	" "	50	SLE
RV-2065	Riverside	Blythe	9/22	" "	50	Turlock
RV-2111	Riverside	Blythe	9/22	" "	50	Turlock
SB-929	San Bernardino	Needles	9/22	*Cx. tarsalis*	52	WEE
SB-933	San Bernardino	Needles	9/22	" "	50	WEE
SB-936	San Bernardino	Needles	9/22	" "	50	WEE
SB-937	San Bernardino	Needles	9/22	" "	50	Turlock
SB-938	San Bernardino	Needles	9/22	" "	50	WEE
SB-940	San Bernardino	Needles	9/22	" "	50	WEE
V4-2073	Kern	Old River	9/26	" "	50	WEE
V4-2074	Madera	Chowchilla	10/3	" "	50	WEE
V4-2079	Madera	Madera	10/5	" "	50	WEE
V4-2104	Tulare	Allensworth	10/12	" "	20	WEE

COMPARATIVE VECTOR COMPETENCE OF SAN JOAQUIN AND IMPERIAL VALLEY POPULATIONS OF *CULEX PIPIENS QUINQEFASCIATUS* SAY AND *CULEX TARSALIS* COQUILLETT FOR ST. LOUIS AND WESTERN EQUINE ENCEPHALITIS VIRUS

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ABSTRACT

Studies were done to assess the susceptibility to laboratory infection with St. Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) viruses of field populations of *Culex pipiens quinquefasciatus* Say and *Culex tarsalis* Coquillett collected in the San Joaquin or Coachella and Imperial Valleys of California. Adult females used in experimental infections were obtained by either CO_2 light traps or reared from pupae collected from principle breeding sources. In the laboratory females were infected by pledget feeding using serial ten-fold dilutions of virus suspended in a defibrinated rabbit blood sucrose mixture. Relative to virus endemicity, regional isolates of SLE virus were routinely used for infecting test populations from the same geographical area. Only a single strain of WEE virus, however, was used in our evaluations.

Preliminary results indicated no major differences in susceptibilities between either mosquito species from the San Joaquin or Coachella and Imperial Valleys for SLE virus. Populations of *Cx. tarsalis* tested from the latter regions were apparently two-fold more susceptible to infection with SLE virus than sympatric *Cx. pipiens quinquefasciatus* and slightly more susceptible than *Cx. tarsalis* from the San Joaquin Valley. In all tests *Cx. pipiens quinquefasciatus* was highly refractory to infection with WEE virus regardless of geographic location.

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ARBOVIRUS SURVEILLANCE AND CONTROL IN MINNESOTA

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INTRODUCTION.–Mosquito borne virus activity in Minnesota has been widely recognized since the severe western equine encephalitis epidemic during the summer of 1941 when 794 human cases of encephalitis were diagnosed, 324 cases of which were subsequently proven to be western encephalitis by serology or autopsy findings (Eklund 1946).

Mosquito-borne arbovirus activity in Minnesota is an annual problem, usually of low incidence involving California (LaCrosse) Encephalitis (CE), western encephalitis (WEE) and rarely St. Louis encephalitis (SLE).

CE virus is generally confined to east central and southern Minnesota where the vector *Aedes triseriatus* develops in basal tree holes in oak woodland areas. The restricted flight behavior of this species confines the virus to these wooded areas. The virus is an occasional cause of cases of clinical encephalitis in children.

The vector WEE, *Culex tarsalis*, occurs in the central and northwester 2/3rds of the State (Barr 1958) where its population typically comprises less than one percent of all mosquitoes, except in years of above normal spring and summer rainfall. During periods of heavy rainfall, normally intermittent *Aedes*-producing depressions become saturated and retain water, making a favorable habitat for the development of *Cx. tarsalis*. During such years, this mosquito can represent up to 11 percent of the mosquito population.

SLE occurs infrequently in Minnesota in contrast to other regions of the Midwest where epidemics may occur in years of high rainfall. *Culex pipiens*, however, does not develop high populations in Minnesota and rarely bites man, as indicated by adult biting collections taken over the years. Only occasional isolated rural SLE cases have been reported.

WESTERN EQUINE ENCEPHALITIS.–In 1975, unusually heavy spring and summer rainfall created favorable conditions for the development of high *Culex tarsalis* populations. In May, equine encephalitis cases were diagnosed in the Alexandria area and subsequent flooding in the Red Valley resulted in a widespread outbreak of WEE during the months of June through September. WEE virus activity was widespread across Minnesota, North Dakota, South Dakota and Manitoba that year with 65 cases of western encephalitis confirmed in humans, and approximately 700 suspect equine cases.

The following year (1976) brought extreme drought and no human cases were reported. During 1977, above normal rainfall again occurred and WEE virus activity was again reported in Minnesota with nine human cases confirmed and 105 suspect equine cases.

In November, 1977, the Minnesota Arbovirus Surveillance Advisory Committee was formed to develop a coordinated approach to arbovirus surveillance, and serve in an advisory capacity to the Minnesota Department of Health and the Metropolitan Mosquito Control District. The cooperative efforts included staff of the following: Department of Entomology, University of Minnesota, Department of Veterinary Medicine, University of Minnesota; Livestock Sanitary Board; State Departments of Agriculture and Health, State of Minnesota and the Metropolitan Mosquito Control District. In 1978, veterinarians and physicians across the state were requested to increase case reporting, nestling bird bleeding was initiated and increased mosquito surveillance statewide was initiated. Although very low WEE virus activity occurred in 1978 east of the Rocky Mountains, the extensive surveillance system did detect four of the five WEE cases in humans reported in the United States in 1978.

CALIFORNIA (LACROSSE) ENCEPHALITIS.–Historically LaCrosse Encephalitis cases in children occur annually in southeastern Minnesota and the adjacent Wisconsin region. Increased physician awareness resulting from improved communications with physicians via an Arbovirus Surveillance Newsletter distributed by the Minnesota Department of Health, increased the reporting of clinical encephalitis cases. The increased reporting coincided with the above normal rainfall in endemic regions contributing to an increased public awareness of mosquito-transmitted disease. A total of 24 cases of LaCrosse Encephalitis were confirmed in Minnesota, and an additional 40 cases at the Gunderson Clinic in LaCrosse, Wisconsin in 1978.

ST. LOUIS ENCEPHALITIS.–The extensive 1975 Midwest outbreak of SLE virus resulting in 1,815 cases of SLE did not spill over into Minnesota; only two cases were confirmed between 1975 and 1978, both occurring in the drought year, 1976.

Interstate discussions on arboviral encephalitis were held in LaCrosse, Wisconsin on November 14 and 17, 1978 to develop unified recommendations for the surveillance and control of LaCrosse Encephalitis. Sixty-five participants from five states attended the meeting and broke into four committees to respond to assigned charges dealing with Laboratory Support, Human Disease Surveillance, Insect and Animal Surveillance and Disease Control. Policy recommendations from each of the committees are currently being compiled.

Interstate discussions on WEE are planned for fall 1979 in Fargo, North Dakota, with policy recommendations for prevention and control to be published in the spring of 1980.

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TOXICITY OF CHEMICALS USED IN CALIFORNIA RICE FIELDS TO THIRD INSTAR HYDROPHILID LARVAE[1]

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ABSTRACT

Seven pesticides registered for use in rice fields were tested against third instar larvae of *Hydrophilus triangularis* Say and *Tropisternus lateralis* (Fabricius) in the laboratory. Flit MLO®, MCPA, and parathion proved to be most toxic, propanil and carbaryl relatively less toxic, and $CuSO_4$ and molinate nontoxic. Under field conditions, environmental factors at the time of application could modify each chemical's effect.

The larvae of water scavenger beetles, especially *Hydrophilus triangularis* Say and *Tropisternus lateralis* (Fabricius), have been shown to prey upon midge larvae and mosquito larvae in California rice paddies (Veneski and Washino 1970; Zalom et al. 1978). Gerhardt (1954; 1955) found that mosquito larvicides had a negative effect on rice field predators. Later, Lewallen (1962) reported that other potential larvicides adversely affected *T. lateralis* larvae. In Japan, Ito et al. (1962) noticed that insecticides used in field applications against rice pests lowered the number of predators with a subsequent increase in the pest populations. A number of pesticides are registered and applied to California rice paddies during the season. This study analyzes effects of many of those chemicals (Table 1) on third instars of two species of hydrophilids.

METHODS.–Third instar larvae of *H. triangularis* and *T. lateralis* were captured in untreated paddies at the rice research foundation field station near Biggs, Butte County, California, on June 3, 17 and 24, 1978, and transported in coolers to a greenhouse at Davis. To avoid cannibalism during the experiment, the larvae were kept in separate glass jars, each containing 100 ml of distilled water. The jars were kept in a large pan containing water to maintain uniform temperature. The 25 replicates of each treatment for both species were arranged within randomized complete blocks.

After all insects were established in the containers, enough pesticide was introduced to equal the recommended rate for rice field use at a 6″ water depth. Each insect was monitored every 15 min for 2 hr, every half hour for the next 4 hr, and every 3 hr for the remainder of the 24 hr period or until death was determined following lack of response to prodding.

Results for each treatment were compared to the control by a 2 x 2 chi-square contingency test.

RESULTS.–The test results (Table 2) indicate that under our experimental conditions, all of the compounds screened except for molinate and copper sulfate significantly ($P<0.005$) increased the mortality of third instar larvae of both hydrophilid species when compared to controls. The larvae of the smaller species, *T. lateralis*, were affected faster and had a higher percent mortality than the larvae of *H. triangularis*.

It is interesting to note that Flit MLO®, MCPA, and ethyl parathion were similar with respect to percent mortality and killing rate, each proving to be highly effective against these predators. The oil (Flit MLO) apparently coated the water surface interfering with the ability of hydrophilid larvae to obtain atmospheric oxygen. A similar, milky coating was seen following MCPA application, indicating the solvent in the EC formulation could have been responsible for the observed mortality. It is doubtful that these results would be duplicated in field trials with MCPA as the herbicide is applied only after the rice plant canopy is established and many broadleaf weeds have emerged (∿40 days post flood) which would tend to confine much of the material to the aerial parts of plants.

Propanil and carbaryl killed far fewer larvae than the other group of pesticides. Although the percent mortalities of both were significantly greater than the control ($P<0.005$), they were also significantly less than Flit MLO, MCPA and parathion ($P<0.0005$). Further, the mean time to death was 3 to 6 times longer for the propanil and carbaryl treatments.

DISCUSSION.–Several factors must be considered when analyzing the effects of chemicals applied for the control of aquatic organisms in rice fields. These include the toxicity to the organisms at full application rates, water depth, the release of paddy water before and after application, the extent of the plant cover at the time off application, the extent of the plant cover at the time of application, the relative density of the organisms at that time, and the environmental persistence of the material. Unless a pesticide reaches an individual at sufficient strength, the organism may survive even though it is considered highly toxic to the organisms.

Table 2 lists the relative toxicity of various registered chemicals to third instar larvae of *H. triangularis* and *T. lateralis* which have been shown to be the most efficient predatory stage of each species (Zalom et al. 1978). In the same paper, the authors show peak larval abundance to occur from mid May through early July. Therefore, the most damaging time of application of a toxic compound, with respect to the larvae of the two hydrophilid species, is from the initial flooding to early June, before a dense plant canopy forms. Of the pesticides considered, only Flit MLO and parathion appear to meet most of the criteria including potential use at the time of peak hydrophilid larval abundance. The other pesticides should affect hydrophilid larvae to a lesser extent due to lack of toxicity to the larvae ($CuSO_4$ and molinate), relatively minor toxicity to the larvae (propanil and carbaryl), or treatment after the plants cover the water (MCPA).

[1]Coleoptera:Hydrophilidae.

Table 1.–Pesticides tested for hydrophilid larval mortality.

Pesticide	Type	Target Organism(s)	Total Acreage Treated In 1977
$CuSO_4$	Inorganic Copper	Tadpole Shrimp/Algae	6097
Flit MLO®	Oil	Mosquitoes	--
MCPA	Phenoxy	Broadleaf Weeds/Sedges	303278
Molinate	Carbothioate	Barnyardgrass/Broadleaf Weeds/Sedges	255905
Parathion	Organophosphate	Mosquitoes/Tadpole Shrimp/Rice Leafminer	153246
Propanil	Analide	Barnyardgrass/Broadleaf Weeds/Sedges	13763
Carbaryl	Carbamate	Western Yellow-Striped Armyworm/Tadpole Shrimp/ Rice Leafminer	1183

Table 2.–Third instar larval mortality of two species of hydrophilids following pesticide application.

		H. triangularis		*T. lateralis*	
Pesticide	Dosage	% Mortality	$\bar{x}$ Time to 50% Mortality Occurring in 24 hrs (± Standard Deviation)	% Mortality	$\bar{x}$ Time to 50% Mortality Occurring in 24 hrs (± Standard Deviation)
$CuSO_4$	15.0 lbs.	4	-	4	-
Flit MLO®	2.0 gal.	92**	5.43±4.50	96**	2.26±3.55
MCPA	1.0 lb.	72**	3.36±2.70	85**	1.88±1.56
Molinate	3.0 lbs.	0	-	0	-
Parathion	0.1 lb.	88**	3.81±1.08	92**	2.78±1.22
Propanil	4.0 lbs.	28**	14.57±6.58	40**	13.20±6.81
Carbaryl	1.0 lb.	24**	22.00±3.10	36**	13.67±7.37
Carbaryl	2.0 lb.	44**	21.55±4.80	68**	12.56±6.31
Untreated	Water	0	-	0	-

**$P<0.005$; 2x2 X^2 contingency test.

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ALKANAMIDES – NEW MOSQUITO LARVICIDES

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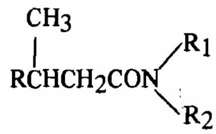
The larvicidal activity of various types of substituted aliphatic carboxylic acids and esters against larvae of the southern house mosquito *Culex pipiens quinquefasciatus* Say was previously reported (Hwang et al. 1974a, b, 1976, 1978) and reviewed (Hwang and Mulla 1976). These carboxylic acids and esters are related to autoregulating compounds produced by larvae of this species and consist of 2-alkylalkanoic acids, 3-methylalkanoic acids, and their esters. The larvicidal activity of these types of compounds was known to be strongly influenced by the position and the size of substitutents as well as by the length of the carbon chain.

A number of insecticidal isobutylamides of unsaturated, aliphatic, straight-chain C_{10-18} acids have been isolated from plants of the families Compositae and Rutaceae. These isobutylamides possess quick knockdown and killing effects on flying insects. Examples of this type of insecticidal amide are spilanthol and affinin. Spilanthol was isolated from *Spilanthes oleraceae* Jacq. and identified by Jacobson (1957) to be *N*-isobutyl-2,6,8-decatrienamide. The same worker also reported that pure spilanthol was highly toxic to *Anopheles* larvae as well as to adult houseflies. Affinin, isolated from *Heliopsis longipes* (A. Gray) Blake and later shown to be the same compound as spilanthol (Acree et al. 1945a, b, Crombie et al. 1963), had knockdown effects on females of *Aedes aegypti* (L.) (Jacobson 1971). These studies indicate that some alkanamides possess insecticidal activity.

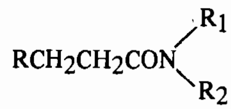
To expand our knowledge of the biological activity of derivatives of the above-mentioned substituted carboxylic acids and to develop new types of effective, environmentally acceptable mosquito larvicides, we recently synthesized and evaluated straight-chain and branched-chain alkanamides, some of which, with certain structural requirements, were found to be larvicidally active. In this report, we briefly discuss our preliminary findings on the larvicidal activity and the structure-activity relationship of the alkanamides.

The alkanamides investigated here were readily synthesized as follows: A carboxylic acid, either straight-chain or branched-chain, was dissolved in an excess amount of thionyl chloride. The solution was heated under reflux for 1-2 hours. The excess thionyl chloride was removed by evaporation to give an acyl chloride. Into a cooled aqueous solution of ammonia, a primary amine such as methylamine or isolbutylamine, or a secondary amine such as dimethylamine, the acyl chloride was added dropwise. The resulting mixture was stirred at room temperature for several hours. An excess amount of water was added to the mixture which was then extracted three times with ether. The combined ether extracts were washed with water and dried. Evaporation of ether yielded a crude alkanamide which was purified by recrystallization from ethanol or acetone.

The synthesized alkanamides included 3-methylalkanamides and straight-chain alkanamides which are represented by the general structures as follows,



3-Methylalkanamides

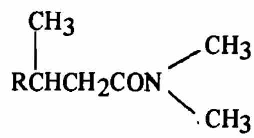


Straight-chain alkananmides

wherein R is a long-chain alkyl and R_1 and R_2 are hydrogen or short-chain alkyl such as methyl or isolbutyl. When R_1 and R_2 are both hydrogen, the amides are primary. When R_1 is an alkyl and R_2 is hydrogen, the amides are secondary. When both R_1 and R_2 are alkyls, the amides are tertiary.

These alkanamides were bioassayed against first instars of *Cx. p. quinquefasciatus* according to the procedure of Hwang et al. (1974a). Briefly, 20 first-instar larvae were placed in 200 ml water in Pyrex custard dishes and fed with a mixture of ground rabbit pellets and yeast. Serially diluted acetone solutions of the test compounds were added to the dishes, which were then kept at 27±1°C under a photoperiod of 14 hr. The bioassays were run in duplicate and repeated at least twice. Mortalities were assessed every other day until adult emergence. The larvicidal activity was expressed in terms of LC_{50} and LC_{90} in ppm. When both values of a compound were larger than 10 ppm, it was considered to be inactive. When the values fell between 10 and 1 ppm, it was moderately active. If LC_{50} was smaller than 1 ppm, and LC_{90} was smaller than 3 ppm, it was considered to be active.

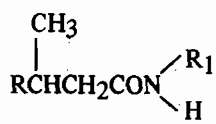
The first amides in this study were those in the group of 3-methylalkanamides. They can be considered as derivatives of 3-methylalkanoic acids previously investigated (Hwang et al. 1978). The tertiary amides of this type possess the following structure:



R=*n*-$C_{16}H_{33}$ or *n*-$C_{17}H_{35}$

N,N-dimethyl-3-methylnonadecanamide (R=*n*-$C_{16}H_{33}$) showed moderate activity, however, *N,N*-dimethyl-3-methyleicosanamide (R=*n*-$C_{17}H_{35}$) was inactive.

The secondary amides of 3-methylalkanamide type can be expressed as follows,



R=*n*-$C_{11}H_{23}$, *n*-$C_{15}H_{31}$, or *n*-$C_{17}H_{35}$

R_1=CH_3 or *iso*-C_4H_9

All secondary 3-methylalkanamides, including *N*-isobutylalkanamide and *N*-methylalkanamide, did not possess any larvicidal activity in the range of concentrations tested.

The primary 3-methylalkanamides possess the structure:

$$\overset{CH_3}{\underset{|}{}}\ RCHCH_2CONH_2$$

$R{=}n\text{-}C_{11}H_{23}, n\text{-}C_{14}H_{29}, n\text{-}C_{15}H_{31}, n\text{-}C_{16}H_{33}$, or $n\text{-}C_{17}H_{35}$

They invariably demonstrated a high level of larvicidal activity. These included 3-methyltetradecanamide ($R{=}n\text{-}C_{11}H_{23}$), 3-methylheptadecanamide ($R{=}n\text{-}C_{14}H_{29}$), 3-methyloctadecanamide ($R{=}n\text{-}C_{15}H_{31}$), 3-methylnonadecanamide ($R{=}n\text{-}C_{16}H_{33}$), and 3-methyleicosanamide ($R{=}n\text{-}C_{17}H_{35}$).

Conclusively, the secondary and tertiary 3-methylalkanamides were generally inactive as larvicides whereas the primary 3-methylalkanamides manifested excellent activity.

The second type of amides was a group of straight-chain alkanamides. Contrary to the primary 3-methylalkanamides, the primary straight-chain alkanamide, which possess the following structure,

$$RCH_2CH_2CONH_2$$

$R{\text -}n\text{-}C_{13}H_{27}$ or $n\text{-}C_{16}H_{31}$,

did not show any larvicidal activity against *Cx. P. quinquefasciatus*.

The secondary straight-chain alkanamides are represented by the structure:

$$RCH_2CH_2CON\begin{matrix}\nearrow R_1\\ \searrow H\end{matrix}$$

$R{=}n\text{-}C_{13}H_{27}$ or $n\text{-}C_{15}H_{31}$

$R_1{=}CH_3$ or *iso*-C_4H_9

These secondary amides, including *N*-isobutylalkanamides and *N*-methylalkanides, were all inactive.

The tertiary straight-chain alkanamides have the following structure:

$$RCH_2CH_2CON\begin{matrix}\nearrow R_1\\ \searrow R_2\end{matrix}$$

$R{=}n\text{-}C_{13}H_{27}$ or $n\text{-}C_{15}H_{31}$

$R_1{=}R_2{=}CH_3$

These amides, including *N,N*-dimethylhexadecanamide and *N,N*-dimethyloctadecanamide, exhibited a high level of larvicidal activity.

In conclusion, the primary and secondary straight-chain alkanamides were inactive whereas the tertiary straight-chain alkanamides demonstrated good activity.

If the structure of the alkanamides is represented by the following general formula,

$$\overset{R_1}{\underset{|}{}}\ RCHCH_2CON\begin{matrix}\nearrow R_2\\ \searrow R_3\end{matrix}$$

the structural requirements for the alkanamides to possess good larvicidal activity are:

1. R should be a long-chain alkyl from C_{11} to C_{17}.
2. When R_1 is a methyl, R_2 and R_3 should both be hydrogens.
3. When R_1 is hydrogen, R_2 and R_3 should both be methyls.

We have thus presented preliminary results showing that some types of alkanamides possess good larvicidal activity. The use of the amides as larvicides has some advantages. The alkanamides are simple and easily synthesized by simple methods as previously described. Particularly, the starting materials for preparing straight-chain alkanamides are inexpensive, commercially available, straight-chain fatty acids and dimethylamine. Because of using inexpensive starting materials and simple synthetic methods, our findings may thus provide inexpensive larvicides for mosquito control. Furthermore, the chemical structures of these alkanamides point out that they are hydrolyzable in nature to produce naturally abundant, nontoxic fatty acids and amines or ammonia. The amides will therefore have minimal environmental impact when used as larvicides for the management of mosquito populations.

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LARVICIDAL ACTIVITY OF ODD-NUMBERED BRANCHED-CHAIN FATTY ACIDS AGAINST *CULEX PIPIENS QUINQUEFASCIATUS*

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ABSTRACT

Even-numbered, branched-chain fatty acids are known to possess larvicidal activity against first instars of *Culex pipiens quinquefasciatus* Say. To ascertain whether odd-numbered, branched-chain fatty acids would have the same biological activity, 2-hexylundecanoic acid, 2-ethylheptadecanoic acid, 2-ethylnonadecanoic acid, and methyl 2-hexylundecanoate were synthesized and bioassayed. The synthesis of these fatty acids were carried out by treating an even-numbered fatty acid with mercuric oxide and bromine in carbon tetrachloride, condensing the resulting odd-numbered alkyl bromide with a deithyl alkylsodiomalonate in anhydrous ethanol, and saponifying and thermally decarboxylating the thus-obtained dialkyl malonic ester. Although 2-hexylundecanoic acid did not show good activity against mosquito larvae, its methyl ester, methyl 2-hexylundecanoate, possessed considerable larvicidal activity. 2-Ethylheptadecanoic acid and 2-ethylnonadecanoic acid were highly active with LC_{50} of 0.54 and 0.75 and LC_{90} of 1.2 and 1.5 ppm, respectively.

During the course of our investigations on larvicidal branched-chain fatty acids, we found that even-numbered 2-ethyl, 2-butyl, and 2-hexyl-substituted fatty acids from C_{14} to C_{18} generally exhibited good larvicidal activity against first instars of *Culex pipiens quinquefasciatus* Say (Hwang et al. 1974b). Methyl esters of these fatty acids also showed a high level of larvicidal activity (Hwang et al. 1976). The even-numbered, 2-substituted fatty acids were synthesized from readily available even-numbered alkyl bromides. Because the availability of odd-numbered alkyl bromides was scarce, odd-numbered, 2-substituted fatty acids were not studied at that time.

As the structure-activity relationship of these substituted fatty acids had been gradually elucidated, it became necessary for us to determine whether odd-numbered, 2-substituted fatty acids would manifest the same biological activity as their even-numbered homologues. Here we report the synthesis and larvicidal activity of some odd-numbered, 2-substituted fatty acids against *Cx. p. quinquefasciatus*.

MATERIALS AND METHODS.–Synthesis.--The odd-numbered, 2-substituted fatty acids were synthesized as follows. An even-numbered fatty acid was treated with mercuric oxide and bromine in carbon tetrachloride (Cristol and Firth 1961). Under this condition, the fatty acid underwent a Hunsdieker-type reaction to give an odd-numbered alkyl bromide. The alkyl bromide was allowed to react with diethyl alkylsodiomalonate in anhydrous ethanol to yield diethyl dialkylmalonate, which upon saponification and subsequent thermal decarboxylation produced the desired odd-numbered, 2-alkylalkanoic acid. Esterification was effected by treating a fatty acid with ethereal diazomethane.

All melting points and boiling points were uncorrected. All substituted acids synthesized were racemates; however, the prefix *dl* is omitted for convenience. Elemental analysis data were within ± 0.2%. All spectrometric data were in accordance with the structures.

1-Bromononane.--A mixture of red HgO (82.3g, 0.38 mol) and decanoic acid (86 g, 0.5 mol) in CCl_4 (250 ml) was heated under gentle reflux. Into the boiling mixture, a solution of Br_2 (79.9 g, 0.5 mol) in CCl_4 (50 ml) was added dropwise. The mixture was kept refluxing during the addition and for an additional hour after the addition. The mixture was filtered, and the filter cake was washed with CCl_4. The solvent was evaporated. To the residue, ether (500 ml) was added. The ether solution was washed consecutively 3 times with aq. Na_2SO_3 solution, 6 times with 5% aq. KOH solution, and 3 times with H_2O. After drying over Na_2SO_4, the ether was evaporated. The residue was distilled under reduced pressure to give pure 1-bromononane (48 g), bp 108-9°C (20 mm).

Diethyl Hexylnonylmalonate.--Soldium (2.2 g, 0.097 g-atom) was added into absolute EtOH (50 ml). The solution was cooled to 40-50°C. Into this sodium ethoxide solution, diethyl hexylmalonate (23.6 g, 0.097 mol) was added with stirring. 1-Bromononane (19.5 g, 0.097 mol) was then added dropwise into the resulting clear solution. The reaction mixture was refluxed with stirring for 5 hr and distilled to remove EtOH. Water was added into the residue, and the resulting mixture was extracted 3 times with ether. The combined ether extracts were washed once with dilute HCl and 3 times with H_2O. After drying over Na_2SO_4, ether was evaporated. The residue was distilled in vacuo to give pure diethyl hexylnonylmalonate (26.6 g), bp 168-72°C (1.0 mm).

2-Hexylundecanoic Acid *(1)*.--A mixture of diethyl hexylnonylmalonate (25.1 g, 0.07 mol) and 50% aq. KOH solution (200 g) was heated under reflux with stirring for 10 hr. Enough H_2O was added into the mixture to dissolve the acid salt. The resulting solution was washed once with ether and acidified with HCl. The separated oily substance was extracted 3 times with ether. The combined ether extracts were dried over Na_2SO_4. Evaporation of ether gave crude hexylnonylmalonic acid, which was heated to about 180°C until evolution of CO_2 ceased. The remaining liquid was distilled in vacuo to give pure 2-hexylundecanoic acid (17 g), bp 169-73°C (0.85 mm).

Methyl 2-Hexylundecanoate *(2)*.--2-Hexylundecanoic acid (2g) was dissolved in ether (30 ml). Into this solution, an excess amount of diazomethane in ether was added. Evaporation of the solution gave a residue, which upon distillation in vacuo yielded pure methyl 2-hexylundecanoate, bp 117-21°C (0.25 mm).

1-Bromopentadecane.--According to the method previously described for 1-bromononane, 1-bromopentadecane (85.8 g), bp 153-5°C (2.4 mm), was obtained from hexadecanoic acid

(128.2 g, 0.5 mol), HgO (82.3 g, 0.38 mol), and Br_2 (79.9 g, 0.5 mol) in CCl_4 (490 ml).

Diethyl Ethylpentadecylmalonate.--According to the method described for diethyl hexylnonylmalonate, diethyl ethylpentadecylmalonate (20.2 g), bp 150-8°C (0.1 mm), was obtained from Na (2.3 g, 0.1 g-atom), diethyl ethylmalonate (18.8 g, 0.1 mol), and 1-bromopentadecane (29.1 g, 0.1 mol) in EtOH (100 ml).

2-Ethylheptadecanoic Acid *(3)*.--According to the method for 2-hexylundecanoic acid, 2-ethylheptadecanoic acid (8.5 g), bp 158-63°C (0.1 mm) and mp 43-4°C (lit. mp. 44-6°C, Breusch and Ulusoy 1953), was obtained by treating diethyl ethylpentadecylmalonate (20.2 g) with 50% aq. KOH (200 g) and subsequently decarboxylating the resulting ethylheptadecylmalonic acid.

1-Bromoheptadecane.--From octadecanoic acid (142.2 g, 0.5 mol), HgO (82.3 g, 0.38 mol) and Br_2 (79.9 g, 0.5 mol) in CCl_4 (600 ml), 1-bromoheptadecane (96.3 g), bp 154-60°C (0.4 mm), was obtained.

Diethyl Ethylheptadecylmalonate.--From Na (2.3 g, 0.1 g-atom), diethyl ethylmalonate (18.8 g, 0.1 mol), and 1-bromoheptadecane (31.9 g, 0.1 mol), in EtOH (100 ml), diethyl ethylheptadecylmalonate (22 g), bp 180-5°C (0.15 mm), was obtained.

2-Ethylnonadecanoic Acid *(4)*.--By treating diethyl ethylheptadecylmalonate (20 g) with 50% aq. KOH (200 g) and subsequently decarboxylating the resulting ethylheptadecylmalonic acid, 2-ethylnonadecanoic acid (14.4 g), mp 53-4°C (lit. mp 54-5°C, Breusch and Ulusoy 1953), was obtained.

BIOASSAY PROCEDURE.–The bioassay tests were conducted according to the procedure of Hwang et al. (1974a), which is tersely described here. Twenty first-instar larvae of *Cx. p. quinquefasciatus* were placed in Pyrex custard dishes containing 200 ml tap water and fed with a mixture of ground rabbit pellets and yeast. The larval dishes were placed in a room kept at 27±1°C and under a photoperiod of 14 hr. The testing compounds were dissolved in acetone and serially diluted. Mortalities were assessed every other day until adult emergence. The larvicidal activity was expressed in terms of LC_{50} and LC_{90} in ppm.

RESULTS AND DISCUSSION.–The larvicidal activity of the odd-numbered, 2-substituted fatty acids are shown in Table 1. 2-Hexylundecanoic acid *(1)* showed weak activity with LC_{50} and LC_{90} at 10 and 20 ppm, respectively. A similar level of activity was previously found for its immediate even-numbered homologues, 2-hexyldecanoic acid and 2-hexyldodecanoic acid (Hwang et al. 1974b).

In our earlier paper (Hwang et al. 1976), we reported that the larvicidal activity of even-numbered 2-alkylalkanoic acids could be increased by esterification of the acids to their corresponding esters. We therefore hypothesized that this would also hold true for the odd-numbered 2-alkylalkanoates and proceeded to make esters of some of these acids. Esterification of 2-hexylundecanoic acid *(1)* produced methyl 2-hexylundecanoate *(2)*, which showed increased activity with LC_{50} and LC_{90} at 0.8 and 5.0 ppm, respectively. This methyl ester similarly displayed the same level of larvicidal activity as its immediate even-numbered homologues, methyl 2-hexyldecanoate and methyl 2-hexyldodecanoate (Hwang et al. 1976).

Table 1.–Larvicidal activity of odd-numbered, 2-substituted fatty acids against first instars of ***Cx. p. quinquefasciatus***.

Compd	R_1 (structure: R_1/R_2 >$CHCO_2R_3$)	R_2	R_3	Activity (ppm) LC_{50}	LC_{90}
1	n-C_6H_{13}	n-C_9H_{19}	H	10	20
2	n-C_6H_{13}	n-C_9H_{19}	CH_3	0.8	5.0
3	C_2H_5	n-$C_{15}H_{31}$	H	0.54	1.20
4	C_2H_5	n-$C_{17}H_{35}$	H	0.75	1.51

2-Ethylheptadecanoic acid *(3)* and 2-ethylnonadecanoic acid *(4)* exhibited a high level of larvicidal activity. The former displayed LC_{50} and LC_{90} of 0.54 and 1.20 ppm, respectively, and the latter of 0.75 and 1.51 ppm, respectively. They were more active than their immediate even-numbered homologues, 2-ethylhexadecanoic acid, 2-ethyloctadecanoic acid, and 2-ethyleicosanoic acid (Hwang et al. 1974b).

We have therefore produced experimental results proving that odd-numbered, 2-substituted fatty acids are as larvicidally active as their even-numbered homologues. In some cases, the former compounds are more active than the latter. The discovery of such simple, even- and odd-numbered, substituted fatty acids and their esters provides a novel approach to mosquito control using simple and relatively innocuous materials.

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STATUS OF DEVELOPMENT OF ALTERNATIVE CHEMICALS FOR CONTROL OF RESISTANT PESTS

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It is generally acknowledged that the possibility of discovering compounds which would be immune to resistance is beyond our immediate reach. However, within certain new or novel groups of pesticides, progress has been made toward the synthesis of compounds which are highly active and which are affected only slightly by known resistance mechanisms. Since these compounds are not immune to resistance, the challenge in continued effective pest control assumes a dual orientation: (a) the synthesis of compounds with high activity and minimal cross-resistance influences; and (b) utilization of such compounds in ways which do not lead to resistance. During the first three decades of control by synthetic insecticides, the challenge was totally in the hands of the synthetic chemist; now it must be shared at least equally by the pest manager.

Recent developments in the area of alternative chemicals for control of resistant arthropods are discussed below:

(1) Pyrethroids.–Considerable emphasis has been placed on the synthesis of pyrethroids possessing greater intrinsic toxicity to insects and greater photostability. Because insecticidal activity was found to be greatly influenced by the geometrical features and optical form of the acidic and alcoholic components of the molecule (Elliott et al. 1974, Elliott 1977), much effort has been devoted to methods for preparing pyrethroids in as pure a stereoisomeric state as possible. One product of this effort is decamethrin (NRDC 161), an exceptionally potent insecticide (Elliott et al. 1974) (LD_{50} on *Musca domestica* = 0.0032 γ/♀, LC_{50} on *Culex 5-fasciatus* = 0.0001 ppm). Further work has revealed another equally toxic pyrethroid, (*S*)-α-cyano-3-phenoxybenzyl (1*S*)-*cis* -3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate (Elliott et al. 1978a, 1978b). A small number of pyrethroids are now marketed for field use in various countries, and there is little doubt that this class of insecticides will play an important role in pest control during the next 5-10 years.

Despite the brief time since their introduction, cases of cross-resistance to certain pyrethroids have been detected in several insect species. These include a 30x cross-resistance to permethrin in *Aedes aegypti* (Prasittisuk and Busvine 1977), 10x to bioresmethrin in the same species (Chadwick et al. 1977), 10x to cypermethrin in *Boophilus microplus* (Nolan et al. 1977), 2,000x to permethrin in *Spodoptera exigua* (Holden, 1978, personal communication) and others. Furthermore, intensive field use of bioresmethrin against house flies has induced a 105x level of resistance to this insecticide (Keiding 1976). Laboratory selection of *Culex 5-fasciatus* with *trans*-permethrin has produced the extremely high level of >4,000x resistance in larvae (Priester and Georghiou 1978).

There is evidence indicating that pyrethroid resistance is polyfactorial (Priester and Georghiou 1979, Farnham and Sawicki 1976) and that its components include a factor causing reduced sensitivity in nerve tissue (probably *Kdr*, which is also a factor in DDT resistance), as well as factors controlling oxidative and hydrolytic detoxification.

Of considerable concern is the potential magnitude of resistance and the extent of cross-protection imparted by the *Kdr* component. In studies on a bioresmethrin-selected strain of *Musca domestica*, we found that cross-resistance at levels of 14-100x extends to all of the 16 pyrethroids we have tested (DeVries and Georghiou 1978). There is thus a distinct possibility that high resistance to one pyrethroid, once established, might strongly affect the field usefulness of many, if not all, other members of this class of chemicals.

(2) Carbamates with high activity on "insensitive" AChE.–A mechanism of resistance to OPs and carbamates which has caused considerable concern in recent years involves the selection of individuals possessing forms of acetylcholinesterase that are far less sensitive to inhibition by these chemicals than normal forms. Until recently, such "target site insensitivity" was known to occur only in certain tetranychid mites and the cattle tick. However, it now occurs in several insect species (e.g. green rice leafhopper, house fly, *Anopheles albimanus*). Its significance lies in the high degree of protection and relatively broad spectrum of cross-resistance which it confers. In *Anopheles albimanus* it is known to affect all carbamates and a large number of OPs (Ayad and Georghiou 1975, Ariaratnam and Georghiou 1974).

A potentially significant development is the recent discovery by Japanese workers that acetylcholinesterase of the green rice leafhopper, which is "insensitive" to *N*-methylcarbamates, is readily inhibited by *N*-propylcarbamates regardless of the ring substituents of the latter (Yamamoto et al. 1977). It has also been shown that *N*-methyl- and *N*-propylcarbamates demonstrate considerable synergistic activity against this insect (Takahashi et al. 1977, 1978). Unfortunately, *N*-propylcarbamates are not toxic to resistant *An. albimanus* and they do not synergize *N*-methylcarbamates in this species (Georghiou 1978, unpublished).

(3) Carbamates with higher lipophilicity and greater mammalian selectivity.–Work by Fukuto and his associates at UC Riverside has led to the discovery of modified carbamates which display high insecticidal activity while being less toxic to mammals than the parent compounds. These chemicals are mostly *N*-substituted biscarbamoyl sulfides (Fahmy et al. 1974) and *N,N'*-thiodicarbamates (Fahmy et al. 1978) of *N*-methylcarbamates. Because of their higher lipophilicity, they penetrate faster through the insect cuticle and thus display greater toxicity. This is especially marked in mosquito larvae and it results in lower levels of resistance than are found toward the parent carbamate. Some of these modified carbamates are being developed commercially.

(4) Insect development inhibitors.–(a) Diflubenzuron (Dimilin). Although high resistance to this chitin synthesis in-

hibitor has been induced in house flies by direct selection (Oppenoorth and van der Pas 1977, Pimprikar 1977, Pimprikar and Georghiou 1978), prolonged selection of ***Culex tarsalis*** failed to induce resistance. In field tests this chemical has shown high effectiveness against OP-resistant mosquito larvae and is being tested further under limited licensing.

(b) Methoprene (Altosid). Prospects for development are clouded by a limited market and the demonstration of cross-resistance and induced resistance in house flies, mosquitoes, and several other species. Research is being continued on anti-juvenile hormones of plant origin (Bowers et al. 1976), and other pioneering work of potential significance involves the identification of toxic natural products which regulate plant-host selection by pest species (Berenbaum 1978).

It is apparent that considerable research is currently being pursued toward the discovery of new and novel chemicals for insect control. Coupled with research on methods for more sophisticated utilization of insecticides, this brings renewed hope that procedures for effective curtailment of resistance may be available in the near future.

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INSECTICIDE SUSCEPTIBILITY OF MOSQUITOES IN CALIFORNIA: STATUS OF ORGANOPHOSPHORUS RESISTANCE IN LARVAL *CULEX TARSALIS* THROUGH 1978, WITH NOTES ON MITIGATING THE PROBLEM

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INTRODUCTION.–The development of resistance is a continuing problem in California, with the main reason presumed to be insecticidal pressure exerted on the populations under control. The purpose of this status report is twofold: (1) to update the record of the severity of resistance in each control agency, and (2) to summarize the status of resistance to the represented chemicals in each control agency.

METHODS AND MATERIALS.–Test results were obtained as described by Gillies and Womeldorf (1968). All data were processed through a computerized probit analysis program. Tests were considered invalid if there were fewer than three data points, lack of mortality above or below 50%, mixed species, pupation or statistically significant heterogeneity.

RESULTS.–To demonstrate the severity of the resistance problem, Table 1 shows the highest LC_{50} records for each mosquito control agency. Malathion, parathion, fenthion and chlorpyrifos are included to represent the organophosphorus (OP) larvicides. Table 1 lists the data for each agency by chemical, year of test, LC_{90}/LC_{50} ratio, and the LC_{50} and its 95% confidence limits. The inclusion criteria caused some test data to be left out, so that not all chemicals or agencies are listed although tests were performed in some cases. The failure thresholds are as follows:

Parathion and fenthion - - $LC_{50} \geqq 0.005$ and $LC_{90} \geqq 0.01$;
chlorpyrifos - - $LC_{50} \geqq 0.0025$ and $LC_{90} \geqq 0.005$.
malathion - - $LC_{50} \geqq 0.1$ and $LC_{90} \geqq 0.2$.

To summarize the status of resistance in each control agency to the represented chemicals, letters have been used in Table 2. The letter S means that normal control should be achieved at the legal dosage rate. The letter B indicates that resistance has progressed to the extent that erratic control should be expected if applications were done under less than perfect conditions. The letter R depicts that development of resistance has reached the point that field failures should occur even under perfect application conditions. The determination of the resistance status in each control agency was based on the examination of all test data from 1973 through 1978, knowledge of the tested sources, pesticide usage patters and the agency's resistance history. This admittedly subjective view of the resistance status is intended to be a guide to the probable effectiveness of the listed chemicals. Currently, chlorpyrifos is the only OP expected to achieve successful control of most *Culex tarsalis* populations.

DISCUSSION.–Information extracted from annual pesticide use reports shows that in the last five years usage of OPs declined 50%, yet a comparison with our 1973 report (Gillies et al. 1974) indicates that *Cx. tarsalis* in several agencies continued to show increased resistance levels. Although fewer mosquito populations are being treated with OPs, resistance continues to increase in those populations still under OP control. Therefore, it is a misconception to assume that reduced total pesticide use is synonymous with reduced insecticidal pressure.

To prevent or delay further resistance development, larvicides must be applied in such a way that susceptible mosquitoes are given a chance to survive and serve as a source to dilute the resistance pool. This can be done by not treating the entire source, but deliberately leaving part of it completely untreated. The untreated portion will provide susceptible individuals to mix genetically with the resistant survivor from the treated portion.

The difficult task will be to establish a threshold of allowable numbers of mosquitoes from the epidemiological standpoint. If the virus level of encephalitis is known to be low, higher populations of *Cx. tarsalis* can be allowed. Conversely, of course, if there is a great deal of virus activity, fewer *Cx. tarsalis* can be tolerated.

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

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

Table 2.–Insecticide resistance status of *Culex tarsalis* to organophosphorus larvicides 1973 - 1978.

	MALATHION	PARATHION	FENTHION	TEMEPHOS	CHLORPYRIPHOS
Alameda County	-	-	R	-	-
Contra Costa	-	-	S	-	-
North. Salinas Valley	B	B	B	-	R
San Mateo County	R	B	R	B	S
Santa Clara County	-	-	B	S	-
Burney Basin	S	B	S	-	-
Butte County	-	B	R	-	-
Colusa	B	B	B	-	S
Corning	B	S	B	-	S
Los Molinos	S	S	B	-	S
Pine Grove	-	S	B	-	S
Shasta	B	B	R	-	S
Tehama County	B	B	B	-	S
Eastside	-	-	R	-	R
Merced County	-	-	R	-	R
San Joaquin	R	R	R	B	S
Turlock	-	-	R	-	B
Consolidated	S	-	-	-	-
Delano	-	R	R	B	B
Delta	S	-	R	S	-
Fresno Westside	R	R	-	B	B
Kern	B	R	R	R	B
Kings	R	B	R	B	B
Madera County	-	-	R	-	-
Tulare	R	R	R	B	B
Westside	R	B	B	-	S
Coachella Valley	B	R	-	B	R
Goleta Valley	-	-	B	-	-
Imperial County	R	R	R	S	B
Inyo County	-	-	-	S	-
Los Angeles Co. West	S	-	B	S	-
Orange County	B	S	R	-	-
San Bernardino County	-	-	-	-	S
San Diego County	B	B	B	B	S
Southeast	-	S	B	R	-
Ventura County	-	-	B	-	S

S = Normal Control
B = Anticipate erratic control if treatment conditions are less than optimum.
R = Anticipate control failures.

AN EXPERIMENTAL SOLID FABRICATION OF METHOPRENE (ALTOSID®) AND ITS APPLICATION IN AN URBAN FLOOD CONTROL CHANNEL

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INTRODUCTION.–The larvicidal efficacy and residual characteristics of methoprene (Altosid®) are well documented in both laboratory and field studies on various mosquito species (Schaefer and Wilder 1972 and 1973; Schaefer et al. 1974). Since the 1973 EPA experimental-use sanction of Altosid, and at this writing, this insect growth regulator (IGR) has been available from Zoecon Corporation only as SR-10, the microencapsulated liquid, and Briquet, the solid controlled release formulation. However, recipe and directions for the making and use of Altosand (a granular, sustained release formulation) from SR-10 are available from Zoecon.

Of these available formulations, only the Briquet may be used in limited slow-flow situations, usually ditches with shallow depths and narrow widths having "minimal" flow. It was designed primarily for catch basins where it has been used successfully (Schoeppner 1978). In larger flow situations, e.g., streams and urban flood control channels, its applicability is costly and efficiency questionable.

At the Southeast Mosquito Abatement District, our 1000-plus miles of flood control channels represent extensive breeding sources. They are routinely sprayed at considerable expense on a periodic cycle of 7 to 10 days with larvicide oil and occasionally organophosphorus materials using a force of 3 vehicles and a 4-man crew. Our concern was to improve the cost-effectiveness of this spray program without sacrificing our control success. We felt that could be best accomplished with a controlled release compound capable of one or more months larviciding activity. We chose not to use organophosphorus granules because of their relatively poor residual, nonspecific toxicity, and difficulty of application. We knew that application of the Altosid Briquet was not economically feasible, but felt a larger form of this material, placed strategically at points of water inflow into the channels might work. With this thinking and the cooperation of Zoecon's Robert Lucas, Sales and Market Development Manager, the Altosid "pie" or "disc" was created and preliminarily tested by the authors.

MATERIALS AND METHODS.–In mid-May, 1978, the District received from Zoecon three buckets of 4% Briquet material, tailings from the Briquet manufacture (Fig. 1). This material weighed a total of 5.9 kgs. The buckets were processed into 12 discs of various weights, ranging from 198.0 to 722.9g($\bar{X}$ = 492.8 ± 154.3g) tagged for identification, enclosed in ¼-inch mesh hardware cloth, and placed on May 30 at strategic flow and inflow locations along a flood control channel (Figs. 2 and 3).

Bouton Creek, the selected flood control channel, is a steep-sided cement-lined, slow-flow channel approximately 4.5 to 9m wide and 1.5km long that flows easterly and terminates as part of a marine estuary system (Fig. 4). Water velocity averages 0.3m/sec, but flow is heterogeneous due to accumulated debris and some plant growth. During our investigation, pH fluctuated between 7.05 and 7.4. Because of its estuarine nature, Bouton Creek is subject to tidal flows and backwashing.

By mid-May, mosquito breeding in this channel is extensive, with *Culex pipiens quinquefasciatus* the predominant species. *Culex tarsalis*, *Culex peus*, and *Culiseta incidens* also occur throughout the warmer months. Larvae are usually confined to the edges of the channel, as in most flood control channels,

Figure 1.–One of three buckets of 4% Altosid Briquet material received from Zoecon. This material was tailings from the company's Briquet manufacturing process.

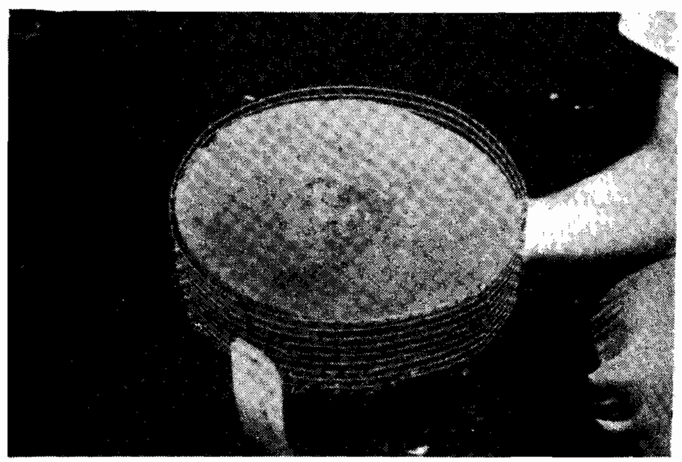

Figure 2.–Example of one of the discs obtained from cutting the buckets into approximately equal quarters. Hardware cloth enclosures were used to minimize disc movement, but later removed and found to be unnecessary.

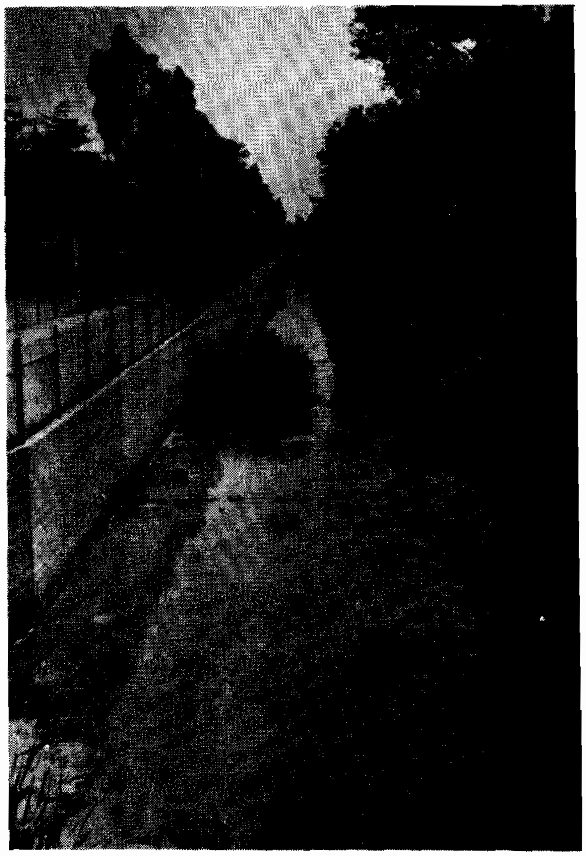

Figure 3.–Bouton Creek, the flood control channel selected for this study. Seen is upper Bouton with tule growth and channel debris. An Altosid disc (not seen) was later placed between the tule clumps in the middle of the photograph.

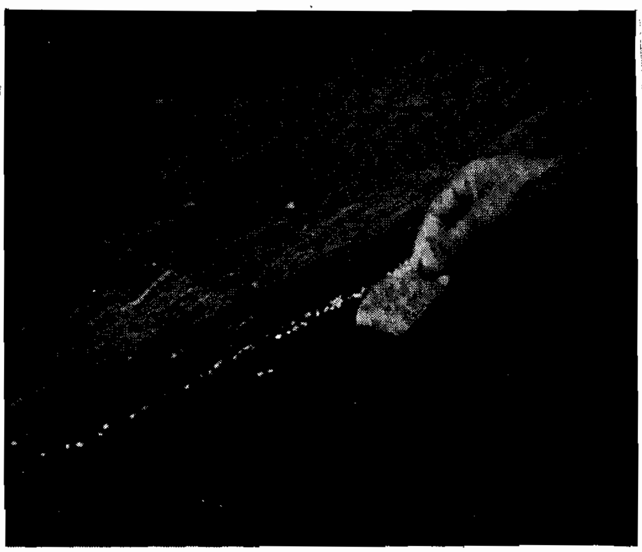

Figure 4.–Illustration of hand sampling for mosquito pupae. The capture device was a piece of 14-inch mesh fiberglass screen cut U-shaped. Pupae were randomly collected along the entire channel length and returned to the laboratory for observation.

but where algal mats or debris accumulate larvae also concentrate. In addition, several pestiferous chironomid midge species occur in the channel. Bouton Creek usually generates several to many complaints (7 complaints logged for 1976-77) during the peak mosquito season, and is considered to be within a moderate complaint area of mostly residences.

Discs were placed in the channel beginning upstream according to weight and site. Heavier discs placed first were followed by lighter discs along the channel at active outfalls and constricted flow sites to maximize exposure to water, permitting optimum material release and distribution downstream. The 12 discs extended a distance of approximately one kilometer. In addition, 300 Briquets were used in the channel in areas believed more or less isolated from channel flow and water exchange. Three days after placement of the discs, and at periodic intervals for a period of 56 days (May 31 to July 25), the upper and lower halves of the entire channel were alternately sampled for mosquito pupae. Pupae were collected by hand dipping with a piece of 14-inch mesh fiberglass screen, returned to the laboratory, and observed for subsequent emergence. We had intended to monitor the Briquets for positional changes and longevity, but this became too laborious following a shift (presumed due to tidal activity) in slightly more than 50% of the Briquets after the first sampling week. One standard New Jersey light trap was installed 15 days after disc placement (June 15) at a residence directly behing the channel's midpoint to monitor emerging adult populations. (Traps were not available prior to this date.)

RESULTS AND DISCUSSION.–The results of this study are summarized in Figures 5 and 6. The pupal mortality is illustrated in Figure 5. These data reflect absolute mortality of pupae. They do not include the characteristic IGR related postemergent physical and physiological aberrations observed in some of the adults which prevent them from flying, feeding, and reproducing. The fewest pupae (37) were collected on the last day of pupal sampling. The greatest number of pupae (411) were collected on day 13 of the study. The average number of pupae collected was 197±104. Numbers of collected pupae reflected the availability of mosquito immatures. The highest rate of pupal mortality (98%) occurred 13 and 14 days following placement of the discs, and apparently no less than 80% mortality occurred during the first 30 days of treatment. These results exceeded our expectations for a minimum of one month of control.

Confirming these highly satisfactory results were data collected from the light trap (Fig. 6). Although pretreatment emergence data are not available, numbers of adults are presumed higher for that period. The 14.8 average females per trap night value after 20 days posttreatment probably represents a diminished population, but the degree is speculative. The light trap data indicate a definite time lag between the onset of peak pupal mortality and fewest trapped females per night, 0.25 first collected on the 42 post-treatment day. The surviving adults emerging prior to treatment might explain this observation.

The longevity of the discs was a function of their weight and placement. Two discs of approximately equal weight, about 500g, were monitored for material loss at different flow exposures. Where water moved at about 0.5m/sec over one disc, it eroded completely within 32 days. Water flowing at slightly less than 0.15m/sec completely dissolved the second disc in 79 days.

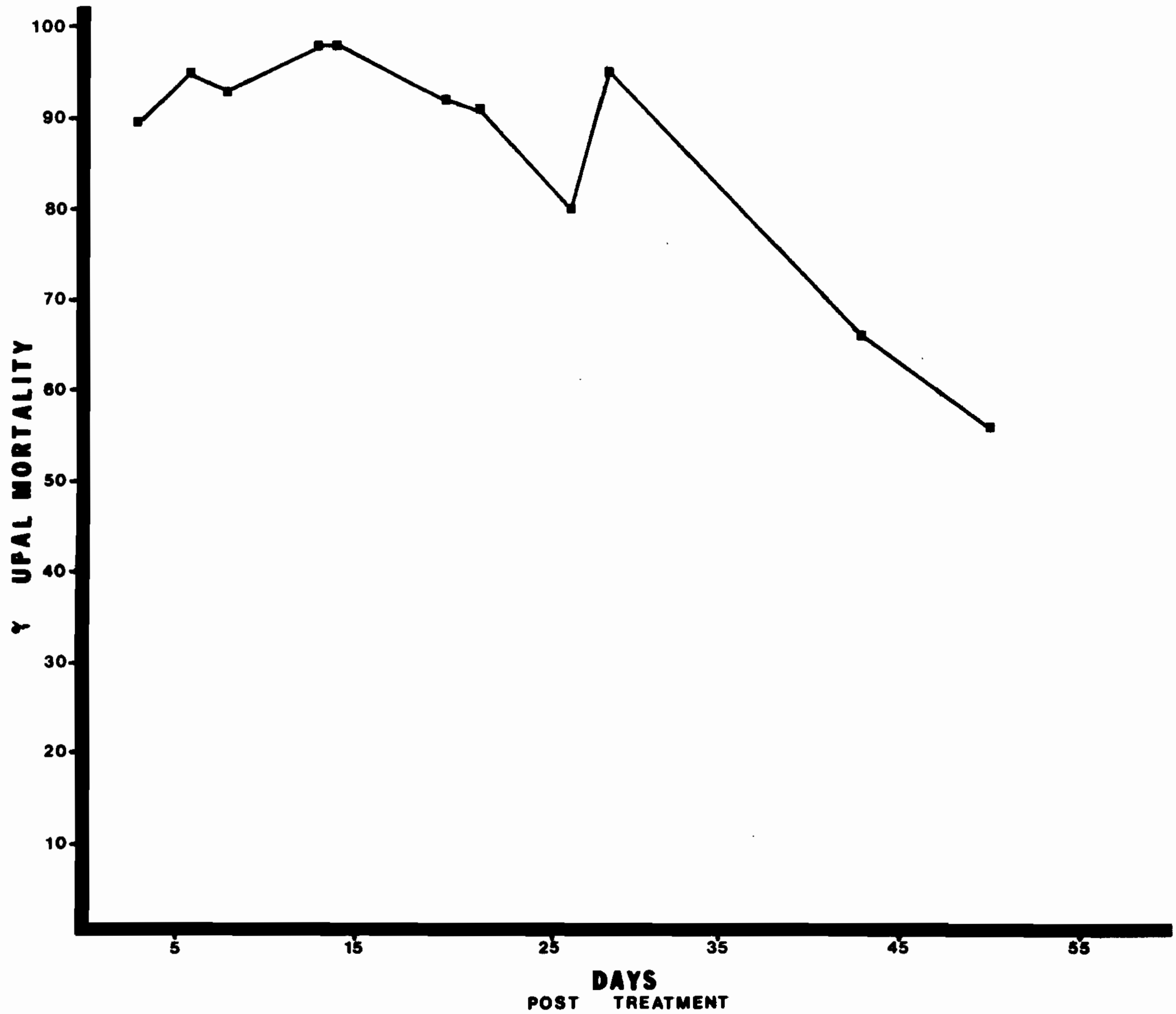

Figure 5.–Illustration of the percent mortality of various species of mosquito pupae collected from an urban flood control channel experimentally treated with a new solid formulation of Altosid. *Culex pipiens quinquefasciatus* was the predominant species.

Although uniform spatial arrangement of the discs was considered, the various weights and sizes of the discs made this impractical. However, future use of uniform weight and size discs may demand keeping particular distances between them.

Of all the observations made during this study, two are particularly interesting: 1) no mosquito or midge complaints or service requests were logged for the Bouton Creek vicinity; and 2) no adult midges were observed or trapped despite the abundant larval midge populations observed in the channel. If midges were controlled by Altosid in this form, it represents a bonus we intend to investigate further.

As a result of this study, the California Department of Food and Agriculture has registered an Altosid disc formulation to meet a special local need (SLN). It is registered as California SLN 780183.

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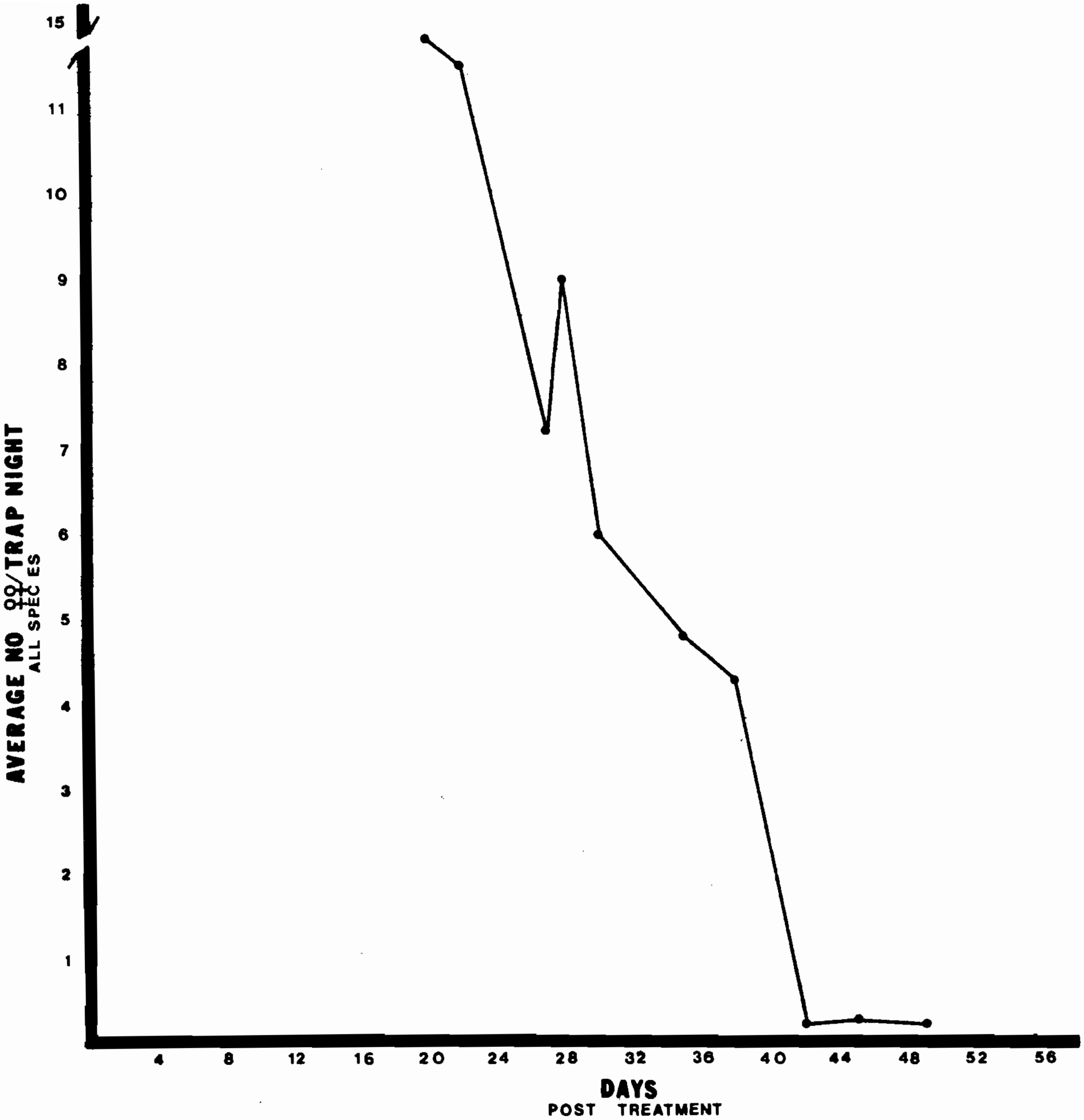

Figure 6.–Graph of all female mosquito species captured per trap night.

PERSISTENCE OF BAY SIR-8514 IN A POND TREATED FOR THE CLEAR LAKE GNAT

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ABSTRACT

A 0.5% sand granule formulation was used to treat a farm pond with SIR-8514 at 5ppb. The active ingredient was released from the sand granules in the upper and mid-levels of the pond; none was measured in the deepest water (14 ft) 4 hrs after treatment. At 24 hrs the average concentration in pond water was 4.6 ppb. The residues in water then steadily declined to 0.3ppb at 21 days and were below detectable limits (<0.2 ppb) at 28 days. No residues were ever detected in any of the sediment samples. Both mosquitofish and bluegill sunfish accumulated SIR-8514 residues from the water to about 400ppb in tissues within 48 hrs of the treatment; these then steadily decreased and no detectable residues in fish tissues were present at 28 days.

EFFECTS OF BAY SIR-8514 ON THE CLEAR LAKE GNAT AND NONTARGET ORGANISMS

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ABSTRACT

An application of BAY SIR 8514, yielding an initial concentration of 5 ppb in an experimental pond, resulted in a >95% reduction in the emergence of adult Clear Lake gnats for 4 weeks. Emergence of adult chironomids declined and remained below the 95% reduction level for 6 days.

Major temporary reductions in populations of cladocerans and copepods occurred after the treatment. Damselfly numbers also declined and did not return to pretreatment levels until 8 weeks posttreatment. Less severe changes occurred in populations of water striders, broad-shouldered water striders, water boatmen, velvet water bugs, rotifers, dinoflagellates, and aquatic snails following the application of BAY SIR 8514.

PRELIMINARY STUDY OF *GAMBUSIA* PRODUCTION AND HOLDING PONDS: COMPARING SECONDARY EFFLUENT WITH FRESHWATER

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INTRODUCTION.–Our objective at Orange County Vector Control District is to increase the capacity for holding large numbers of *Gambusia affinis* (Baird and Girard) during the winter and early spring. *Gambusia* are used extensively in our biological control program for stocking flood channels, ornamental aquatic habitats, depressions, ponds, etc., primarily during the spring and summer months. In addition, gravid *Gambusia* are separated from various fish populations, using a minnow grader, then placed in culture cages for our mass rearing project (Reynolds 1977). *Gambusia* are not available from the field in substantial numbers during the winter due to environmental conditions. Therefore, a majority of these fish must be collected during late summer months when they are abundant in the field, then confined in large holding ponds until they are needed. Two types of ponds are being evaluated in this study, one utilizing well water and the other secondary effluent.

METHODS AND MATERIALS. Our evaluation is not a true comparison since the pond using well water has a 20 mil PVC liner, no shoreline vegetation, supplementary feeding and effective algae control; while the secondary effluent ponds have no liner, heavy shoreline vegetation, no supplementary feeding and algae control only to reduce the formation of heavy mats. In addition, make-up water to maintain the water level in the ponds was 13°-16°C for well water and 18°-21°C for secondary effluent during the summer.

On October 9, 1975, a small holding pond was constructed at our District. The dimensions were 49 feet in length, 18 feet in width, with a depth sloping from two feet to one inch (Figure 1). The deep end of the pond has a 1½ inch drain complete with an overflow pipe and shut-off valve to maintain a constant water level (Figure 2). A skiploader tractor with a scraper was used to excavate the basic outline of the pond. Final contour of the pond was obtained using shovels and rakes. A pond liner was required due to extremely sandy soil. Three types of materials were considered for the pond lining: compacted clay, shotcrete cement and a plastic liner. A 20 mil PVC liner was chosen on the basis of cost, soil type, ease of application, seining, cleaning and aquatic weed control. PVC plastic will decompose rapidly (approximately six months to two years) when exposed to the ultraviolet rays of direct sunlight, but when it is underwater or covered with 6 to 8 inches of soil at the shoreline, it should last for approximately seven to ten years. There are other types of plastic sheeting that are extremely resistant to ultraviolet light such as CPE (30 mil) or Hypalon® (Dupont, 36 mil) but they are more expensive (Win Bachelder, personal communication).

Before placing the liner into the pond area, a 1½ inch drain and overflow system was installed at the deep end. After the liner was set in place, the edges of the liner were folded back about five feet and a one foot deep trench was dug around the perimeter of the pond. Edges of the liner were placed into this trench and covered with sand to prevent lifting during heavy winds and to stabilize the berm. A ¾ inch inflow pipe was installed to fill the pond with an estimated 6,600 gallons of water from our District well. Five pounds of *G. affinis* were stocked in this pond on August 4, 1977. (*Gambusia* fry were stocked in this pond during 1976 to observe winter survival potential, but no specific data were recorded.) On April 17, 1978, Aquazine® was applied to the pond for algae control at 1 ppm. A blend of 50% Purina Trout Chow® and 50% wheat bran was fed daily to the fish at a rate of 10% of the estimated biomass.

The secondary effluent ponds were built on a site seven miles from our District in cooperation with and adjacent to the Orange County Sanitation District, Plant No. 1, in Fountain Valley. On September 14, 1976, a skiploader tractor with a scraper was used to excavate two 25 ft x 20 ft x 2 ft ponds and one 50 ft x 20 ft pond (pond No. 1). The latter pond had a depth sloping from two feet to one inch. These ponds were

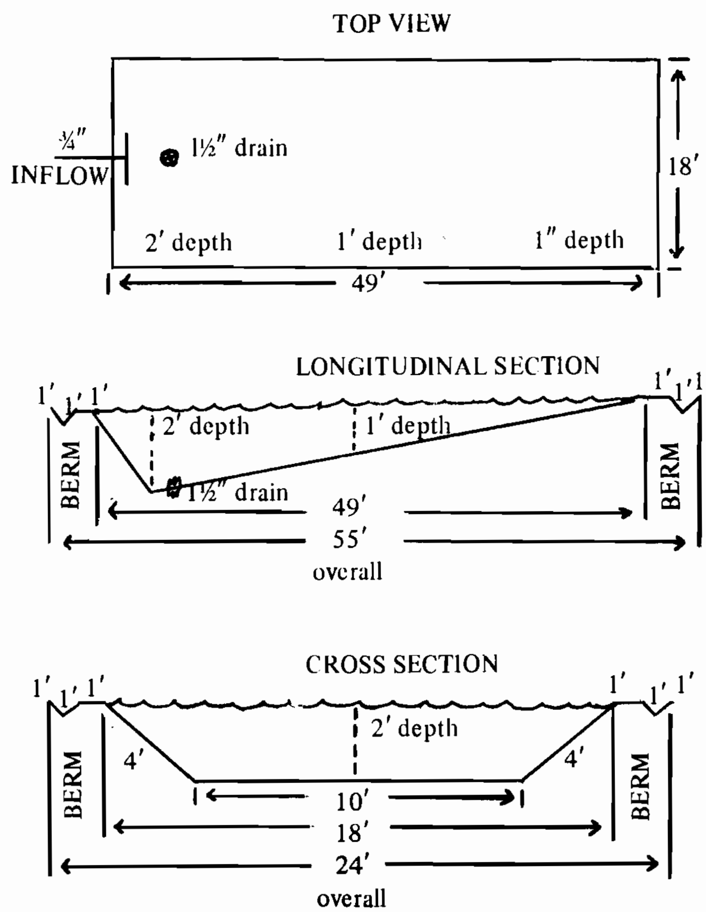

Figure 1.–Dimensions of low cost *Gambusia* pond.

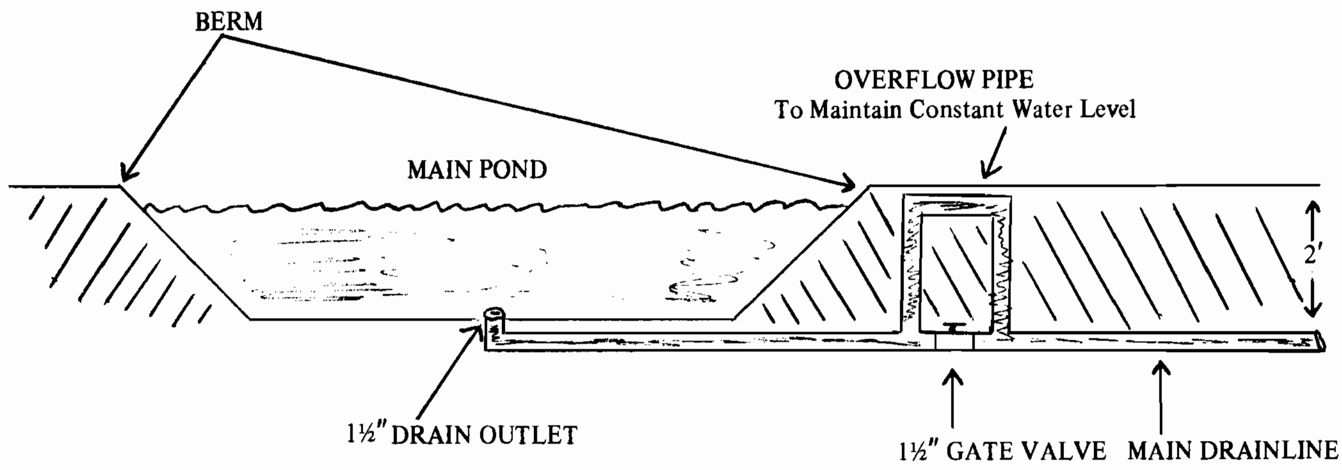

Figure 2.--Cross section of deep end of pond showing drain and overflow pipe arrangement.

filled on September 23 with secondary effluent water. Pond No. 1 was filled with an estimated 7,500 gallons.

After allowing the water to settle, a small number of *Gambusia* were stocked in these ponds on November 11. None of the *Gambusia* survived. The pond water was tested and found to have a high BOD and nitrate count. *Gambusia* were placed in buckets of pond water, one with aeration and one without. Only the *Gambusia* in the aerated bucket survived. This led us to believe that a high level of ammonia nitrogen was present in the pond water and the aeration process was stripping it sufficiently to allow *Gambusia* to survive. In relating this to our pond situation, we designed our ¾ inch PVC inflow pipe in the shape of a T and perforated the leading edge of the pipe to aerate the secondary effluent water as it dropped into the pond. In addition, we filled one 25 ft x 20 ft pond, siphoned this pond into the next 25 ft x 20 ft pond and finally siphoned that pond into our 50 ft x 20 ft pond (pond No. 1). This allowed more complete aeration and settling of the secondary effluent before it reached pond No. 1. Also, periodic treatments with potassium permanganate at 4 ppm were used in these ponds to help satisfy the BOD and increase oxygen levels. On January 6, 1977, small numbers of *Gambusia* were again stocked in pond No. 1. This time they survived. From March to June of 1977, five additional ponds (100 ft x 20 ft) were excavated at the Orange County Sanitation District. They had a depth sloping from two feet to one inch. None of the secondary effluent ponds was lined or sealed as we had an abundant supply of secondary effluent from the Orange County Sanitation District. Inflow pipes (¾ inch) were installed at the deep end of the ponds. Each pond was filled with an estimated 15,000 gallons of water. No drain or overflow system was provided. Nylon siphon hoses (¾ inch) were placed between each pond to maintain uniform water levels in all ponds. During May of 1977, the Sanitation Plant No. 1 installed an activated charcoal filtration system that greatly improved the water quality.

On August 2, 1977, five pounds of *Gambusia* were stocked in pond No. 1 and another five pounds in the adjacent pond No. 2. Also, on September 29, 1977, two 100 ft x 20 ft ponds (Nos. 3 and 4) were each stocked with five pounds of *Gambusia*. Two additional 100 ft x 20 ft ponds (Nos. 5 and 6) were each stocked with five pounds of fish on September 29, 1977, but they will not be harvested until 1979 (Table 1).

No supplementary fish food was given to the *Gambusia* in these ponds. Some of the invertebrate aquatic organisms observed in abundance during the summer months that most probably were utilized as a food source included Ostracoda, Hemiptera (Corixidae and Notonectidae), Cladocera (Daphnidae) as well as immature Odonata and Diptera (Chironomidae). Also, numerous miscellaneous insects and spiders dropped into the ponds from nearby heavy shoreline vegetation.

RESULTS AND DISCUSSION.–Harvest results to date from the ponds studied are presented in Table 1. The 49 ft x 18 ft PVC lined pond at the Orange County Vector Control District with well water was harvested on April 18, 1978: 6.5 pounds of *Gambusia* were obtained. A preliminary data check indicates the entire original population stocked (five pounds) had survived the winter and shown a weight gain. However, when we look at the relationship between the number of fish stocked and the number of fish harvested, we get a more accurate account. The fish did experience a weight gain of 30% but the survival rate was only 59.5%. If we compare these results with a May 26, 1978 harvest from the 50 ft x 20 ft secondary effluent pond No. 1, we see a harvest of 6.1 ponds of fish for a weight gain of 22% and a survival rate of 50.9%. Unfortunately, the results obtained from secondary effluent pond No. 1 (50 ft x 20 ft) and pond No. 2 (100 ft x 20 ft) are obscure if not invalid. During heavy winter rains, the siphon hose between pond Nos. 1 and 2 apparently became clogged and allowed pond No. 2 to overflow into pond No. 1. This also permitted *Gambusia* to move freely between the ponds. After the water level dropped, a great deal more *Gambusia* were observed in pond No. 1 than pond No. 2. Thus, when these ponds were harvested on May 26, 1978, the yield of *Gambusia* from each pond was computed by totaling the actual harvest from each pond and dividing by two. Since there was little or no fry production from the time *Gambusia* were stocked in these ponds (August 2, 1977) until they were harvested (May 26, 1978), these estimates should approximate the actual yield from pond Nos. 1 and 2 had there not been an overflow problem. Nevertheless, when we examine yield data

Table 1.–Stocking and harvest results of study comparing secondary effluent with freshwater ponds.

EVALUATION	FRESHWATER	SECONDARY EFFLUENT			
	District PVC Pond 50′ x 20′	Pond No. 1 50′ x 20′	Pond No. 2 100′ x 20′	Pond No. 3 100′ x 20′	Pond No. 4 100′ x 20′
Lbs. Stocked	5.0	5.0	5.0	5.0	5.0
Date	8/4/77	8/2/77	8/2/77	9/29/77	9/29/77
Lbs. Harvested	6.5	6.1[2]	6.1[2]	21.0	10.0
Date	4/18/78	5/26/78	5/26/78	9/21/78	9/21/78
Net Gain (Lbs.)	1.5	1.1	1,1	16.0	5.0
% Weight Gain	30.0	22.0	22.0	320.00	100.0
Est. No. of Fish Stocked[1]	6,560	6,560	6,560	6,560	6,560
Est. No. of Fish Harvested[1]	3,900	3,338	3,338	11,676	5,560
% Survival of Initial Stock	59.5	50.9	50.9	-	-
% Increase in No. of Fish	-	-	-	44.0	-

[1] Estimated by weighing 1 lb. of *Gambusia*, counting the number of fish in 1 lb. then multiply this by total weight of fish stocked or harvested.

[2] Due to overflow problems, this figure was computed by totaling the actual harvest from each pond and dividing by two (see text).

from the freshwater pond and secondary effluent pond Nos. 1 and 2 to get an indication of the overwintering ability of *Gambusia* in these media, we find a similar weight gain and survival rate, although it should be noted that the secondary effluent ponds showed a slight reduction in the categories, possibly due to an increased disease incidence. The average weight gain and survival rate of *Gambusia* in these three ponds were 24.7% and 53.7% respectively (Table 1). Actually, the 53.7% survival rate is quite good if we compare this with 25.2%, 7.8% and 0.1% survival rates experienced in ponds with heavy bird predation (Coykendall 1977). There was light predation observed during the year in the secondary effluent ponds by the following birds: Great Blue Heron (*Ardea herodias*), Green Heron (*Butorides virescens*), Snowy Egret (*Leucophoyx thula*), Pied-billed Grebe (*Podilymbus podiceps*) and Black-necked Stilt (*Himantopus mexicanus*).

In order to observe the prolonged effect of holding *Gambusia* in secondary effluent ponds and to obtain an estimate of fry production during the summer, five pounds of *Gambusia* were stocked in pond Nos. 3 and 4 on September 29, 1977. Almost a year later, on September 21, 1978, 21 pounds of fish were harvested from pond No. 3 and 10 pounds from pond No. 4. The wide disparity in harvest weights for these ponds may be due to factors such as bird predation, disease, food availability, water quality, pond structure or other factors that affect the fish population to a varied degree. Data for these two ponds show an average weight gain of 210%, while the average increase in number of fish was only 31.4% (Table 1). Weight gain per individual fish may be more important than an increase in numbers. Research by Barney and Anson (1921), Krumholz (1948), Hubbs (1971, Wu et al. (1974), and Stearns (1975) has shown that fertility increases with size. However, Wu et al. (1974), determined that in *Gambusia affinis* the weight of a fish was the most accurate predictor of brood size; the more a female weighed, the larger was her potential brood. Length was the second most important factor.

These studies have great importance in relation to our *Gambusia* stocking program. They indicate possible initial stocking rates in terms of the weight or number of *Gambusia* to be used in a source for effective mosquito control. However, at the present time, we cannot ensure effective mosquito control in random sources by prescribing a set initial stocking rate. If the excellent research we have had in the past continues, we may be provided these answers in the future. Until then, we must agree with Wu et al. (1974) that there are many factors which potentially influence the productivity of a population of *Gambusia affinis*.

Table 2.–Cost of vinyl-lined pond for holding and rearing of *Gambusia*.

Tractor Rental (one-half day)	$ 40.00
Plastic Pipe and Fittings	21.13
38.10 mm (1½″) Brass Gate Valve for Drain Line	19.00
38.10 mm (1½″) Drain Line Fitting	9.54
(vinyl liner must be cut to fit drain line in place)	
20 ml Vinyl Liner (55′ x 25′)	302.50
$0.22 per square foot (1,375 square feet)	
Freight	25.50
Sales Tax	18.15
TOTAL COST	$435.82

Labor costs of District personnel not included. (2 men/2 days)

Costs of Plastic Liners for this size (55′ x 25′):	
PVC (20 mil)	$0.22 per sq. ft.
CPE (30 mil)	0.39 per sq. ft.
Hypalon® (36 mil)	0.55 per sq. ft.

We were unable to study the summer fry production potential of *Gambusia* in our 49 ft x 18 ft PVC lined freshwater pond last year. Due to curtailment of all seasonal personnel, brought about by Proposition 13, there was a shortage of manpower for seining *Gambusia* in our District biological control program. This caused a reduction in the number of *Gambusia* available for field stocking. The fish in this pond were seined and stocked in the field on April 18, 1978 (Table 1), then the pond was used as a centralized holding pond for *Gambusia* seined from the field. Therefore we were unable to compare summer production from this pond with summer production from secondary effluent ponds No. 1 and No. 2.

Our preliminary conclusion is that in our ponds *Gambusia* apparently can overwinter equally well in freshwater and secondary effluent. Also, we found an average weight gain of 210% for *Gambusia* in our 100 ft x 20 ft secondary effluent ponds during the study period. We will continue this study in 1979 and attempt to eliminate some of the problems encountered during our preliminary study.

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EVALUATING THE FUNGUS *LAGENIDIUM GIGANTEUM* FOR THE BIOLOGICAL CONTROL OF THE CLEAR LAKE GNAT, *CHAOBORUS ASTICTOPUS*, IN AN AGRICULTURAL POND IN LAKE COUNTY, CALIFORNIA

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Two 960 square meter agricultural ponds in Lake County, California, were selected to assay the effectiveness of *Lagenidium giganteum* against the Clear Lake gnat, *Chaoborus astictopus.* The two ponds were similar in supporting high larval gnat densities as well as in other characteristics including mean depth, water temperature, pH and zooplankton. One pond was inoculated with the fungus and the other left untreated as a control. The fungal inoculum was transported to the facillities of the Lake County Mosquito Abatement District two days before the inoculation date. A passage was made to susceptible mosquito larvae on that day, and after a suitable incubation period, the fungus was introduced into the test pond on two consecutive days. A week after the pond was inoculated, a 38% suppression of the larval gnats was observed in the treated pond as compared to a 50% increase in the control pond.

Although the fungus could not be re-isolated from the gnats collected from the treated ponds, the sentinel mosquito larvae from the same pond did show evidence of infection for 3 weeks after inoculation. The suppression in the gnat population occurred at a dosage range 52% and 17% of that calculated for minimal and maximal suppression of the gnats from a dosage-response-curve from previous studies. Laboratory experiments were conducted in which different densities of larval gnats were exposed to constant fungal dosages. At higher larval densities gnat larvae became infected earlier and in greater numbers as compared to the lower densities. The results suggested that the probability of the fungus infecting gnat larvae increases with increasing gnat densities.

PREDATION BY THE MISSISSIPPI SILVERSIDE *(MENIDIA AUDENS)* IN CLEAR LAKE, CALIFORNIA[1]

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ABSTRACT

The introduction of Mississippi silversides into Clear Lake was followed by a 95% decrease in the Clear Lake gnat (*Chaoborus astictopus*) population during the years when no insecticide treatments occurred. However, dissections have not revealed significant numbers of chaoborus in the stomachs of silversides. Differential digestion rates are not sufficient to account for the paucity of chaoborus in the stomachs if the silversides were controlling the population. The vertical and horizontal distribution of first instar chaoborus larvae in Clear Lake is similar to the distribution of the silversides during the peak feeding periods for the fish, but other zooplankton are preferred to the early instars as prey. Large silversides prefer late instar chaoborus to other Clear Lake prey, but the late instar larvae are in the benthos and are unavailable as prey during the periods when the silversides are feeding.

INTRODUCTION.–Clear Lake is a large (> 16,000 ha), natural inland water in the Coast Range of northern California. The lake is shallow (mean depth = 6.5 m), polymictic and eutrophic (Goldman and Wetzel 1963). Clear Lake provides a favorable larval habitat for *Chaoborus astictopus*. During the summer months > 3 billion adult chaoborus may emerge from the lake each night (Lindquist and Deonier 1942b). Although the chaoborus is a non-biting insect, it is strongly attracted to lights, and the adults can provide such a nuisance that they can have a severe effect on the recreation-oriented economy of Lake County. The chaoborus have been a problem since the earliest descriptions of the region (Herms 1937). The chaoborus were controlled from 1949-1957 with DDD (Hunt and Bischoff 1960), and the methyl parathion treatments initiated in 1962 resumed control of the chaoborus (Hazeltine 1963). However, the problems of insecticide resistance, insecticide cost and environmental concerns prompted an increase in the biological control research at the Lake County Mosquito Abatement District in the early 1960s (Cook 1965). A small (< 150 mm SL) atherinid fish, the Mississippi silverside, was introduced into Clear Lake in 1967 with the hopes that it would become established and provide effective gnat control (Cook and Moore 1970). Within a few years, the silversides became the most numerous fish in the lake (Moyle 1976). The present experiments were undertaken to study the effectiveness of the Mississippi silverside as a control agent of chaoborus.

MATERIALS AND METHODS.–The littoral distribution of Mississippi silversides was monitored with a 9.2 x 1.0 meter (2.0 mm apertures) beach seine. The vertical and horizontal distribution of silversides was monitored with paired 1.27 cm (stretched measure) gill nets suspended from the water surface to various depths in Clear Lake and placed at various distances from shore.

Benthic (late instar) larvae and pupae of chaoborus were monitored with a standard Ekman dredge (sampling an area of 232 cm^2). The Ekman sediment sample was washed in a sieve (19.6 brass wire meshes per cm), and the organisms were identified and enumerated.

The vertical distribution of late instar planktonic chaoborus was monitored with a 15 l plankton trap (Schindler 1969). The distribution of early instar planktonic larvae was determined by sampling with a plankton pump. All plankton samples were preserved in a 4% formaldhyde solution containing rose bengal dye, and samples were examined at 30X.

During some studies silversides were measured to determine fish standard lengths (SL). During the "stomach" analyses, only items anterior to the pylorus were identified and enumerated.

RESULTS AND DISCUSSION.–When the methyl parathion treatments of Clear Lake were terminated in August of 1975 due to the development of resistance by the chaoborus (Apperson et al. 1978), the expected major increase in the abundance of chaoborus did not occur. Silversides were considered as a possible cause for the low chaoborus density. However, preliminary studies in 1975, conducted by R. A. Elston, H. W. Li, and the Lake County Mosquito Abatement District suggested that the silversides were not controlling the chaoborus population, because: (1) the silversides were most abundant near the shore while the chaoborus occurred primarily in deep offshore areas; (2) the silversides inhabited the euphotic zone while the chaoborus were largely benthic; (3) the silversides were primarily diurnal feeders while the chaoborus were present in the water column only at night; (4) the silversides were visual feeders, but the chaoborus larvae were rather transparent; and (5) the chaoborus apparently constituted < 0.5% of the diet of the silversides.

However, continued monitoring (58 Ekman samples per month) of the chaoborus population suggested that some change in Clear Lake was adversely affecting the chaoborus population. Studies in 1977 and 1978 indicated that after the silversides had become established the chaoborus density was often 95% lower than the density in non-treatment years prior to the introduction of silversides (Figure 1). No other important change(s) in the biological, physical, or chemical conditions of Clear Lake could be established, so additional silverside-chaoborus studies were initiated.

A laboratory study was conducted to determine whether Mississippi silversides were obligately diurnal, visual feeders. When fourth instar chaoborus larvae were introduced into a 2.0 liter vessel containing Mississippi silversides under conditions of complete darkness (light intensity below detection limit of 1.0 x 10^{-5} lux as measured by an IL 700 radiometer), a silverside was able to consume up to 18 larvae during 24 hr

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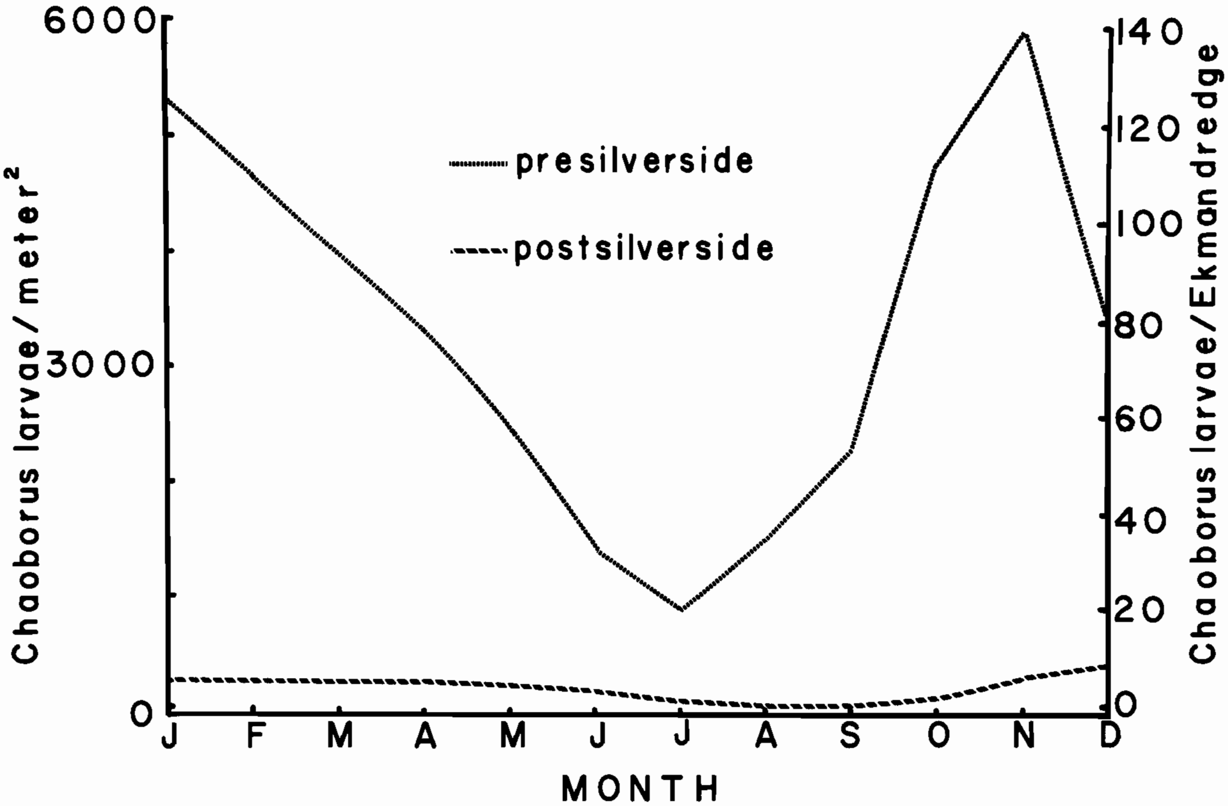

Figure 1.--Abundance of benthic larvae of *Chaoborus astictopus* in Clear Lake, California before (mean monthly density for years 1959-1960) and after (mean monthly density for years 1977-1978) the establishment of *Menidia audens*.

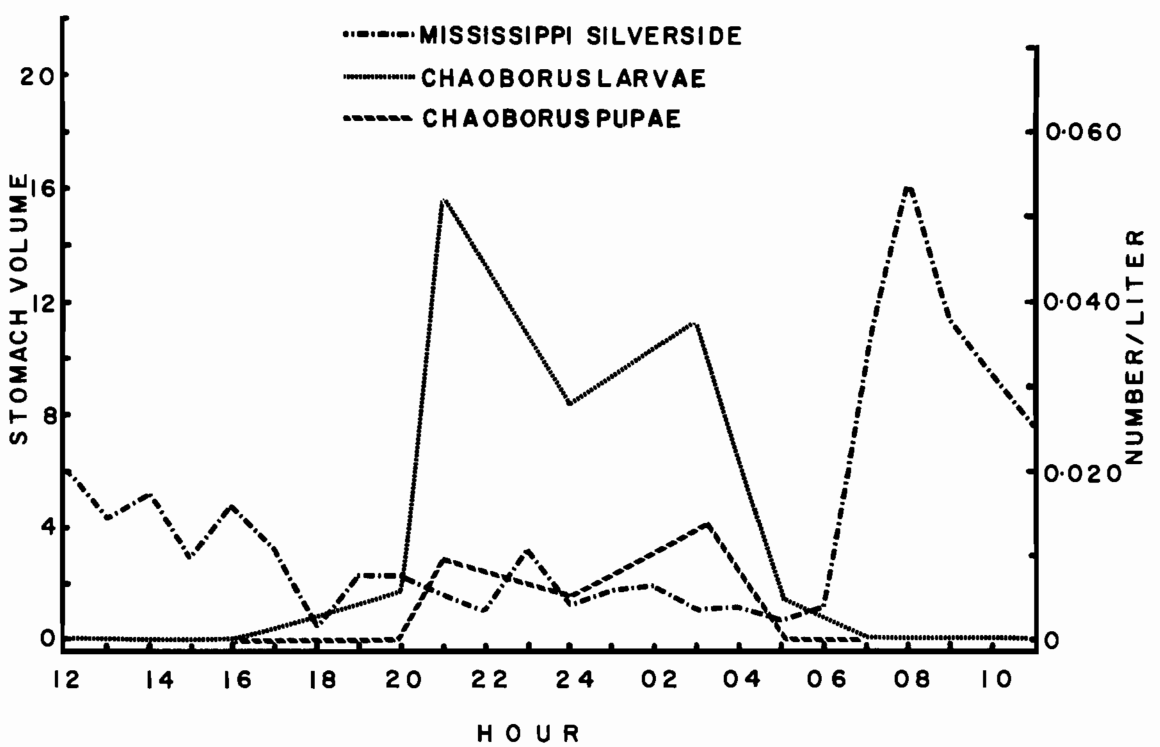

Figure 2.–Diel density (number per liter) of larvae and pupae of *Chaoborus astictopus* in water column in Clear Lake, and diel variation in volume (relative displacement) of stomach contents of Mississippi silversides. Silverside data adapted from Elston and Bachen (1976).

of darkness. So under some circumstances silversides did feed during non-day-light conditions, and the fish were able to ingest prey without visually locating them.

Field studies were undertaken to determine whether non-visual or nocturnal feeding of silversides on chaoborus could be important in Clear Lake. Figure 2 indicates that the chaoborus were most abundant in the water column at night when the feeding activity of silversides was at a minimum. During the peak feeding period of the silversides, most of the late instar chaoborus larvae and pupae were in the sediment at the bottom of Clear Lake and were apparently not available as prey for these fish. Adult chaoborus also were unavailable during the peak silverside feeding periods, since Lindquist and Deonier (1942a) have shown that the chaoborus are nocturnal in their emergence and oviposition activities.

A study of the horizontal distribution of chaoborus in the Clear Lake benthos was conducted by running a transect across the northern arm of Clear Lake and sampling at each successive water depth (four samples at each water depth). The benthic larval chaoborus density in deep (>3.5 m) water areas was 28 times the density in shallow (<3.5 m) water.

Several diel (24 hr) studies of the distributions of silversides and chaoborus in Clear Lake were conducted during the summer of 1978. It was established that silversides undergo both vertical and horizontal migrations which could bring them into the proximity of chaoborus larvae. During the night, silversides were located primarily at the water surface, but during daylight hours the fish were most abundant at depths of 50 to 200 cm.

Horizontal migrations of silversides also occurred. During the day, over 95% of the adult silversides were located within 50 meters of shore. However, at dawn and dusk these silversides migrated offshore and significant numbers were caught as far as 1000 m from shore.

Zooplankton sampling during these diel studies indicated that the early instar chaoborus larvae were most abundant at 0 to 50 meters from shore, which is the same region where the maximum silverside densities occurred. Since a first instar larva has never been found in the dissected stomach of any of the hundreds of silversides from Clear Lake, laboratory experiments were conducted to determine whether first instar larvae were too small and transparent to be seen or were otherwise unpalatable or unsatisfactory and therefore never consumed by the fish. It was found that a silverside (20-30 mm SL size class) consumed as many as 1500 first instar chaoborus larvae in a one hour period when the firsts were the only available prey.

The paucity of larvae in the stomachs of field-collected silversides even though the maximum densities of both silversides and first instars occurred in shallow water areas suggested that rapid digestion of firsts might result in underestimation of the number of first instars, compared to other prey types, consumed by silversides. To study this possibility, fish in the laboratory which had fed to satiety on first instar chaoborus were transferred to a zooplankton-free aquarium and then some fish were terminated (the spinal cord was cut to prevent regurgitation) at various time intervals. The number of identifiable chaoborus per fish were then enumerated and com-

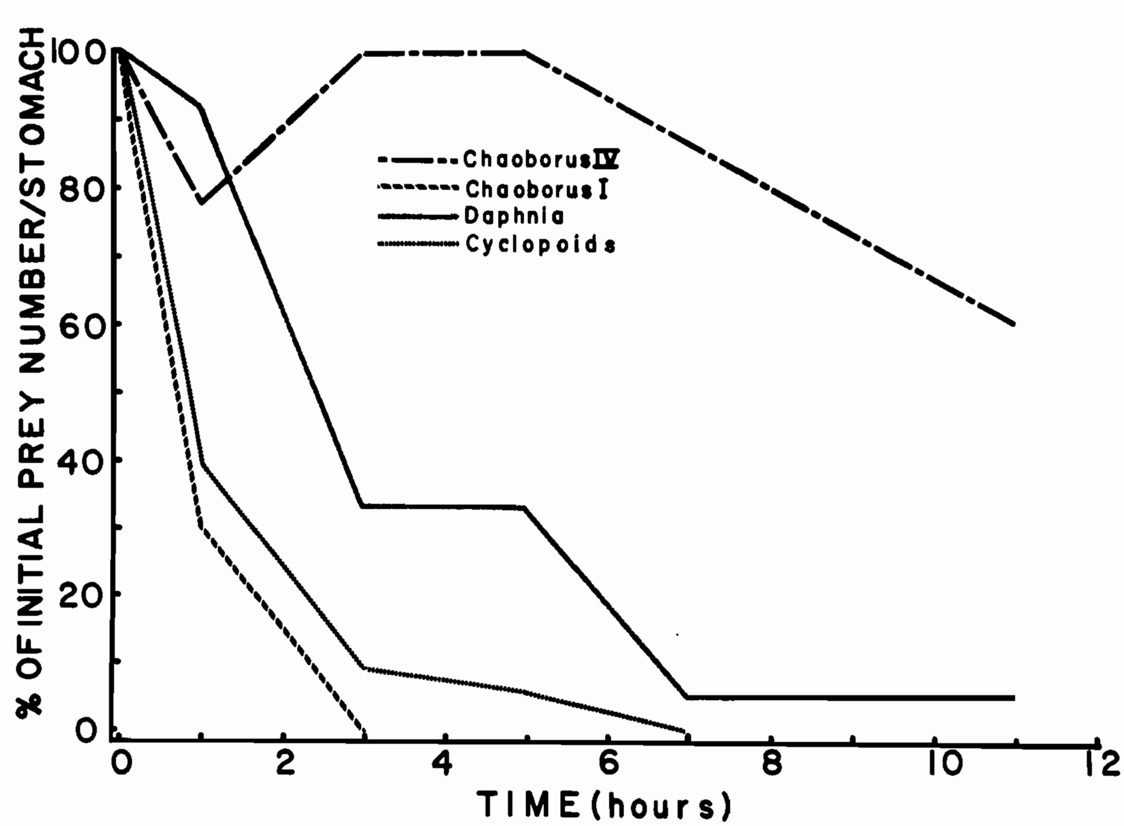

Figure 3.–Digestion of prey organisms by Mississippi silversides.

pared to the number of organisms consumed immediately following feeding. Concurrently, silversides which fed on either *Daphnia* or Cyclopoid copepods (the prey which comprised the majority of the diet of silversides in the lake), or on fourth instar chaoborus larvae, were similarly terminated and dissected. The results of this study (Figure 3) indicated that first instars were the most rapidly digested prey items. However the first instars were not digested significantly more rapidly than the microcrustaceans. The fourth instars were digested relatively slowly, so fish from Clear Lake which contained microcrustaceans in their guts would also have had identifiable fourth instars if the fish had been feeding on the fourth instar larvae.

The digestion rates were not sufficiently different to account for the disparity in the number of first instar chaoborus larvae consumed in the lab and the number found in silverside stomachs during field studies, so it is possible that first instars were non-preferred items when other food was available. To examine this possibility, mixed prey laboratory studies were conducted, and it was found that other zooplankton (especially the more densely pigmented water flea, *Daphnia*) were highly preferred to first instar chaoborus even when the first instars were the most numerous of all the prey types available.

Additional mixed prey laboratory studies indicated that third instar chaoborus larvae were preferred to other Clear Lake zooplankters. Fourth instar larvae were preferred by large (60 mm SL) silversides but were not eaten by small (30 mm SL) silversides.

Overall, the data indicate that the early instar chaoborus larvae, which are available, are not preferred prey items and are rarely consumed by the silversides. The late instar chaoborus are preferred to other Clear Lake zooplankton, but do not have sufficient temporal and spatial overlap with the silversides to permit much predation. So, if the silversides are controlling the chaoborus population in Clear Lake, an indirect mechanism may be involved.

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CYCLOPOID COPEPOD PREDATION ON *CHAOBORUS ASTICTOPUS*

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ABSTRACT

The cyclopoid copepods, *Cyclops vernalis* (length ca. 0.6-0.9 mm) and *Mesocyclops leuckarti* (ca. 0.9-1.3 mm), were effective predators on first instar *C. astictopus* (Clear Lake gnat) larvae during petri dish experiments in the lab and in tube studies submerged in Clear Lake. Smaller cyclopoids, *Tropocyclops prasinus* (ca. 0.4-0.6 mm), even in proportions of 10 cyclopoids per 1 chaoborid, did not eat first instars. *C. astictopus* eggs were not eaten by cyclopoids. Second instar *C. astictopus* were not easily eaten by, nor could they easily consume *C. vernalis*. It appears that the ratio of chaoborid head size to cyclopoid width affects cyclopoid predation. In 1 liter jar experiments, fourth instar *C. astictopus* readily ate *T. prasinus* and *C. vernalis*, but not the larger *M. leuckarti*. Given the widespread occurrence of cyclopoids and their high densities in lakes such as Clear Lake, reciprocal predation should be considered as a possible regulatory mechanism in systems where both cyclopoids and chaoborids occur.

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ADVANCEMENTS IN THE USE OF FLATWORMS FOR BIOLOGICAL MOSQUITO CONTROL

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ABSTRACT

Studies in an increasing number of localities with diverse climates show the usefulness of flatworms for biological mosquito control. Mass production techniques are developed for one species, *Dugesia dorotocephala* (Woodworth).

Flatworms (Platyhelminthes:Turbellaria) hold a unique potential in the biological control of juvenile mosquitoes largely because they can be mass produced easily (Legner and Tsai 1978); unlike predaceous insects, some can be confined at high densities without cannibalism.

Flatworms have been shown experimentally to devastate laboratory mosquito cultures, killing far more larvae than they consume (Jenkins 1964; Legner and Medved 1972, 1974; Medved and Legner 1974, I. N. McDaniel, personal communication). They have been reported equally effective in natural habitats (Legner and Medved 1974; Yu and Legner 1976). Among those habitats in which flatworms could be usefully deployed against mosquitoes are rice fields, roadside ditches, water sloughs, agricultural and construction drain depressions, catch basins, canal seepages, treated sewage effluents, and snowmelt and rainwater pools.

Other attributes making these organisms especially amensemi-dormant "winter" eggs or embryos (Borradaile and Potts 1958), their slow rate of dispersal which causes them to be effective near the place of treatment, and their effectiveness in shallow water with emergent vegetation (Yu and Legner 1976).

The extraordinary regenerative capabilities of flatworms were known since the early 1800's, some investigators considering them almost immortal under the edge of a knife (Bronsted 1969). Their ability to devour mosquitoes among other prey was soon discovered (Lischetti 1919); and Stage and Yates (1939) reported that *Dugesia tigrina* (Girard) effectively consumed *Culex* and *Aedes* larvae. The Turbellaria are nearly all free-living, non-parasitic worms (Borradaile and Potts 1958, Kenk 1972). Among the six orders are species that have great potential as predators of mosquitoes in freshwater habitats. Some genera in the order Rhabdocoela such as *Microstomum*, retain nematocysts in their ectoderm which are derived from Coelenterates on which they have fed (Kepner and Barker 1924). Such structures are useful in warding off predators, but also may be useful in killing mosquitoes.

Recent studies on the destructive capacity of *Dugesia dorotocephala* showed this species to be very effective against eggs, larvae and pupae of *Culex peus* Speiser, *Cx. pipiens quinquefasciatus* Say, *Cx. tarsalis* Coquillett in California at the application rate of 25 mature worms / m^2 of water surface (Legner and Medved 1972, 1974; Medved and Legner 1974; Yu and Legner 1976). In Florida additional mosquitoes killed by this predator were *Anopheles quadrimaculatus* Say, *An. crucians* Wiedemann, *Culex nigripalpus* Theobald, *Aedes taeniorhynchus* (Wiedemann), *Culex erraticus* (Dyar and Knab), *Cx. salinarius* Coquillett, *Aedes sollicitans* (Walker), *Psorophora confinnis* (Lynch-Arribalzaga), and *P. ciliata* (Fab.) (Levy and Miller 1978). Field studies with *Dugesia tigrina* to control feral *Culex restuans* Theobald and *Cx. pipiens pipiens* L. were performed by L. Learned in North Dakota (L. Learned, personal communication), and several exceptionally potent species of *Mesostoma* spp. were found in California rice fields (Collins and Washino 1978, Case and Washino 1979). In Maine, *Mesostoma macroprostatum* Hyman offers good potential in the control of univoltine *Aedes* spp. (I. N. McDaniel, personal communication). Over 75% control was achieved through inoculation of 3-6 eggs of this rhabdocoel turbellarian; and once established, the worms continued to cycle with the mosquitoes year after year! They both hatched at the same time in early spring and the worms produced eggs and died off at about the same time that the mosquitoes emerged. These worms normally had one generation per year. R. D. Sjogren (personal communication) reports over 40 natural populations of *Phagocata velata* (Stringer) from central Minnesota, with some densities exceeding 50/200-ml mosquito dipper samples.

Ecological Impact. - - Flatworms are natural components of aquatic communities, and no record has been found of their being considered pests in nature. However, extremely high application rates (exceeding 2,000/m^2 of water surface) of *Dugesia dorotocephala* were found lethal to *Tilapia* fish fry in experimental indoor and outdoor aquaria (Mulla and Tsai 1978). The latter report appears to have confused a nest brooder *Tilapia zillii* Gervais, with the mouth brooder *T. mossambica* Peters, as the authors state that *T. zillii* were used "on the 2nd day after leaving their parents' mouth cavity" (see Legner 1978). In another study the abundance of natural insect predators in experimental ponds was unaffected by *D. dorotocephala* applications of 25 worms/m^2 of water surface (Legner 1977). Although it may be expected that very young fish fry can be damaged by high application rates of flatworms (Mulla and Tsai 1978), one would not expect the need for such applications in environments where such fish are abundant.

Adaptability. - - Flatworms are quite tolerant of varying water qualities (Legner, Tsai and Medved 1976), but are adversely affected by Cl^- concentrations exceeding 1500 ppm (Levy and Miller 1978b). Certain pesticides were found to stimulate planarian reproduction (Levy and Miller 1978a). One planarian, *D. dorotocephala*, is highly active under temperatures fluctuating between 20° and 24°C (Medved and Legner 1974).

Mode of Attack. - - Some species of Turbellarians also apparently paralyze their prey prior to ingestion of body fluids (Case and Washino 1979, Legner and Yu 1975). A sticky mucus produced abundantly by epithelial glands in the head region serves as a "pseudomandible" which holds a mosquito long enough for toxins to exert an effect (Legner and Yu 1975). Only soft body contents of mosquitoes are consumed, the cuticle being discarded; and all developmental stages of mosquitoes are attacked (Legner and Yu 1975). Some microturbellarians in rice fields are thought to produce very potent toxins which enable killing of prey much larger than themselves (Collins and Washino 1978, Case and Washino 1979), their potency appearing unique (Coward and Piedilato 1972, Riser and Morse (eds.) 1974).

Propagation. - - Culture techniques for flatworms have been available for decades, with only small modifications required for mass propagation. The density of the planarian, *D. dorotocephala* was increased 88 times in 95 days through the development of a special biological filtration system using *Elodea densa* (Planchon) Caspary (Legner and Tsai 1978). Other procedures including the provision of various foods, host-predator densities, photoperiods, etc. are available for maximizing planarian yields (Jennings 1957; Legner, Tsai and Medved 1976; McConnell 1967; Tsai and Legner 1977; Wulzen 1923, 1924, 1927). *Artemia salina* (L.) has been the best alternate food to mosquito larvae (Tsai and Legner 1977).

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CONSIDERATIONS IN THE MANAGEMENT OF *TILAPIA* FOR BIOLOGICAL AQUATIC WEED CONTROL

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ABSTRACT

Tilapia zillii **continues to provide good to excellent biological aquatic weed control in the irrigation system of California's lower deserts. However, maximizing the effectiveness of this fish depends on sound management practices.**

Studies through 1978 on the performance of *Tilapia zillii* (Gervais) as a biological weed control in irrigation canals of California's lower desert sustained the present importance of this herbivorous fish in the suppression of noxious aquatic weeds, ***Potamogeton pectinatus*** L. and ***Myriophyllum spicatum*** var. ***exalbescens*** Jepson (Legner 1978; Legner and Pelsue 1977; Legner, Fisher and Medved 1973; Hauser, Legner and Robinson 1977; Hauser et al. 1976). Furthermore, aquaria studies indicated that ***Hydrilla verticillata*** Royle might be similarly suppressed should this weed become wide-spread in the irrigation system (Legner 1978; Legner, unpublished data). Particular behavioral characteristics of *T. zillii* that appear responsible for the high level of weed control are (1) annual winter die-back of fish populations that minimize territorial behavior in the following year, thereby guaranteeing a high density of fish suitable for weed control; (2) the ability of fish to survive temporary canal outages and corresponding high water temperatures exceeding 33°C, by burrowing into the mud; (3) a gregariousness which results in a tendency for a significant proportion of the fish population to remain in the vicinity of stocking sites even in the swiftest canals so that weed control is effected throughout the system; and (4) the fish is able to significantly reduce nonpreferred weed species, ***Myriophyllum spicatum***, during summer when this weed can become especially abundant. ***Tilapia zillii*** is now one of the principal game fishes in the lower Sonoran Desert of California where specimens averaging 15-25 cm occur in ca. 1/3rd of the creel during a major portion of the year. Larger specimens are increasingly found in lakes such as the Salton Sea where adjacent warm water springs permit winter survival. A corresponding increase in largemouth bass populations in the midst of high *T. zillii* densities seems correlated with direct feeding on *Tilapia* fry.

Despite the high degree of weed control witnessed through the use of *T. zillii*, the simple management formula is not always followed. Success depends on the annual spring stocking of adequate numbers of young *T. zillii* so that on-site reproduction may add to the total effective fish biomass by late summer, a period when aquatic weeds can show their maximum growth rate (Hauser, Legner and Robinson 1977). However, as irrigation districts do not currently produce more than 10% of their own *Tilapia*, supplies must be secured commercially. One large supplier produces his fish in thermal waters in Idaho and trucks them south in April and May. Probably because of diminished winter photoperiod in the far north, spawning is not very successful after October. Therefore, fish orders must be placed no later than September. Several times during the past 5 years orders were placed so late in the year that only 20% of the numbers desired by irrigation districts could be supplied. In 1977 because no orders were placed before November, no fish were supplied.

Special attention must be given to fish tolerances to water temperature. Even though *T. zillii* can escape high temperatures by burrowing into benthic mud when canal water levels drop, its ability to survive in such an environment has its practical limits. Often little or no thought is given to fish survival and a high percentage of the population can die before higher water levels are restored. Similarly, when water temperatures decline in winter to the lower tolerance limits (Hauser 1977), large numbers of fish could be harvested and protected over the winter for use in spring stocking. The cost for such operations using irrigation district personnel could be considerably less than new fish purchases. However, there has been no routine fish harvest integrated into the management scheme.

There are currently three species of *Tilapia* in residence in south California (Legner and Pelsue 1977), all of which continuously inhabit warmer drains in the low desert. These drains have been recognized as a reliable source for on-site bred fish, and irrigation districts regularly transfer fish from the drains to the canals to increase aquatic weed control. However, no attention is paid to removal of the *T. mossambica* and *T. hornorum* which often comprise the greater percentage of the drain harvest. Both of these species by virtue of their competitively superior mouth brooding habits out-reproduce *T. zillii*, the only effective herbivore for canals (Legner and Medved 1973, Legner, unpublished data).

Probably the greatest drawback to maximizing the use of *T. zillii* for aquatic weed control is the opinion that has developed among irrigation district personnel that the white amur, *Ctenopharyngodon idella* Valenciennes, offers the ultimate solution to the aquatic weed problem. Although on first consideration this species seems to possess several superior attributes for effective aquatic weed control, such as lower water temperature tolerances and larger size (Bailey 1972, 1975, 1978; Beach et al. 1976; Haller 1976; Rottman and Anderson 1978), to date there has been no demonstration of this species' ability to effectively reduce aquatic weeds in rapidly flowing water. Other problems of dispersion in the canal system at desirable densities and survival in high water temperatures when canal levels drop in summer must be resolved before further confidence may be placed on the white amur as a substitute for *T. zillii*.

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HABITAT AND FOOD SELECTION BY THE MOSQUITOFISH, *GAMBUSIA AFFINIS*

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ABSTRACT

Field studies were conducted to determine preferences of the mosquitofish, *Gambusia affinis* (Baird and Girard) for habitat and food. The fish preferred a shallow (< 8 inches) open water, but fry and immature fish tended to aggregate in a shallow densley vegetated area. Mosquitofish preyed upon various aquatic organisms as well as some terrestrial organisms; almost all organisms collected from the ponds were found in the gut contents. The most important food item was microcrustaeans (Cladocerans), accounting for ca. 82% of the gut content by number. Forage ratio was 1.04 and selective index was 0.02.

The mosquitofish, *Gambusia affinis* (Baird and Girard) is a native North American fish whose natural inhabitancy is in low land ponds, lakes and streams of Central North America from southern Illinois to Alabama and Texas (Moyle 1976). It has been introduced widely over the world for mosquito control (Herm and Gray 1944, Krumholz 1948, Dees 1961). The fish has been introduced into California in 1922 (Lenert and Stuart 1926) and has since been well established over the State. At present it is the only biological agent which is widely used by mosquito control agencies (Washino and Hokama 1967, Bay 1969, Hoy and Reed 1970, Hoy et al. 1972).

This investigation was undertaken to determine preferences of the mosquitofish for habitat and food under natural conditions.

MATERIALS AND METHODS.--Mosquitofish used were obtained from our stock pond located at the laboratory's backyard. Originally the fish were collected from the West Parlier sewage pond several years ago. Tests were conducted in a small pond (0.024 acre) at the Tracy plot. The description of the plot and facilities reported elsewhere (Miura et al. 1978).

Habitat Selection.--In order to examine distribution pattern, relative to certain topographical features, a small pond "D" was quartered; 2 adjacent quadrants were deepened ca. 1 foot and vegetation (reed grass) was removed from a deeper quadrant and an adjacent shallow quadrant. Thus, making 4 different habitat choices -- deep and open water, deep with emerged vegetation, shallow and open water, and shallow with emerged vegetation.

About 500 fish were placed in the pond and population density at each habitat was examined by means of trapping for a month. Maximal and minimal water temperatures were also recorded for each quadrant during the study period (August 7 to September 15, 1978).

Food Habits.--Pond "B" was used to study food habits of mosquitofish. The population densities of the fauna in the pond was censused by dipping with a 450ml dipper around the pond during August and September 1978 (Miura and Takahashi 1975).

About 300 various sized fish were placed into the pond on September 1, and they were recaptured on the 6th day post stocking. To secure undigested food items in the guts, fish were trapped during daytime (feeding period) and preserved in 75% alcohol in the field and they were brought to the laboratory.

All specimens were sorted into 4 groups -- <2, 2-2.5, 2.5-3, and >3cm; each gut was dissected out of the fish and stored in 70% alcohol. The gut contents were then examined under the binocular microscope (40X), each food item was identified as far as possible.

Food habits of immature fish were studied by utilizing 3 different methods, e.g., the number, weight and frequency of occurrence method (Haynes 1950).

The Forage Ratio by Hess and Tarzwell (1942) was used to express the relationship between abundance of prey in the pond and the degree of predation by mosquitofish. The ratio was calculated as follows:

$$\text{Forage Ratio (F.R.)} = \frac{N'}{N}$$

$$\text{where } N' = \frac{\text{no. of any prey in the guts}}{\text{total no. of preys in the guts}}$$

$$N = \frac{\text{no. of same prey in the pond}}{\text{total no. of preys in the pond}}$$

Ivlev's selection index (1961) was utilized to study food selection behavior:

$$\text{Selection Index (S. I.)} = \frac{U - V}{U + V}$$

where U = no. of any prey in the guts (in %)
V = no. of the prey in the pond (in %)

Since mosquito larvae were not found in the ponds during the field study, a special feeding experiment with mosquito larvae was conducted in the backyard of the laboratory. Two tanks (ca. 40 gal capacity; 112 x 76cm surface area) were filled with pond water which contained naturally occurring crustaceans, mayfly nymphs and other organisms, *Culex tarsalis* larvae were also placed in the tanks. Piror to stocking mosquitofish, the organisms in the tanks were censused twice and then ca. 30 mature mosquitofish were introduced. Twenty-four hours after introduction of the fish, they were recaptured and the gut contents were examined.

RESULTS AND DISCUSSION.--Habitat Selection.--Results of the habitat selection study is shown in Table 1. About 38% of mosquitofish trapped were obtained from the quadrant of shallow and opern water surface. This 38% catch is significantly different only from the 10.5% catch obtained from the quadrant of deep water with emergent vegetation (t=3.22; p<0.01), but it is not significant from those of 2 other quadrants. This finding agrees with the report by Reed and Bryant (1972) that mosquitofish distribution in rice fields was dependent upon water depth - - as depth increased from 2 to 8 inches, the percentage of catch also increased. Norland and Bowman (1976) also reported that the traps in the perimeter of the rice fields contained more fish than did the center traps, but in a paddy with a sparse rice plant stand, more fish were found in the center than in the perimeter.

Table 1.--Distribution of mosquitofish in a small pond (0.024) acre) with 4 different habitat choices.

	Deep water (14-20 inches)		Shallow water (<8 inches)	
Entry	Vegetation	No vegeta.	Vegetation	No vegeta.
Fish (%)[1]	10.46	24.63	26.07	38.85[2]
Mean Water Temp. (F)	77.4	78.6	75.6	80.8
Range (temp)	100-60	100-62	100-53	110-53

[1]Total fish trapped = 7841, N = 35 trappings.

[2]Any 2 means not underscored by the same line are significantly different at the 5% level.

About 26% were collected in the shallow with emergent vegetation and the majority of fish trapped from this quadrant were fry and small immature fish (<2cm). This phenomenon is probably due to the avoidance behaviior from cannibalism by larger fish (Dees 1961), rather than due to the feeding behavior.

Temperature gradients have an influence on distribution in nature (Winkler 1975), however, no temperature influence was noticed in this study (p>0.05).

Food Habits.--Although the ponds are semi-natural and only irrigated during spring and summer months, the fauna was very rich. Among organisms collected insects dominated by the number of species (Table 2) water beetles accounted for most of the insect species and were followed by Diptera. Springtails were common in the vegetation, but they were impossible to collect with the dipper. By the numbers of species, crustaceans were few, however, by the number of individuals, they were the richest organisms in the pond.

Mosquitofish swallow their food without chewing them, therefore, it is relatively easy to identify the food items in the guts. The species composition of the fauna and the gut contents are shown in Table 3. They were diverse feeders, almost all organisms collected from the ponds including some terrestrial organisms were found in the guts. Apparently they do not prey selectively upon the specific organisms, rather they preyed upon whatever was available, e.g., planktonic crustaceans

Table 2.–List of organisms collected in the fish ponds at the Tracy Experimental Plots.

ORGANISM	NOTES
Cladocera	
Ceriodaphnia spp.	Most abundant
Simocephalus spp.	Most abundant
Moina spp.	Most abundant
Chydorinae	Abundant
Podocopa	
Cyprois spp.	Common
Eucopepoda	
Cyclops spp.	Common
Collembola	
Isotomidae	Common in vegetations
Sminthuridae	Common in vegetations
Ephemeroptera	
Callibaetis spp.	Abundant
Odonata	
Enallagma civile	Common
Anax walsimghami	Few
Pantala hymenaea	Common
Hemiptera	
Belostoma sp.	Few
Corisella sp.	Common
Notonecta unifasciata	Abundant
Buenoa scimitra	Common
Coleoptera	
Copelauts spp.	Common
Eretes sticticus	Common
Hygrotus sp.	Few
Laccophilus spp.	Abundant
Rhantus spp.	Few
Thermonectus basillaris	Common, sometimes abundant
Berosus spp.	Common
Hydrophilus triangularis	Common
Helophorus sp.	Few
Tropisternus lateralis	Most abundant
Tropisternus ellipticus	Common
Diptera	
Chironomus attenuatus	Abundant
Chironomus stigmaterus	Abundant
Goeldichironomus sp.	Abundant
Brachydeutera argentata	Common
Culicoides variipennis	Few
Hydracarina	
Hydrachna sp.	Few

(cladocerans) were the most abundant in the ponds, so they were abundant in the guts. Movements of the potential prey also appeared to influence the rate of predation; mayfly mymphs (*Callibaetis* spp.) were fairly abundant in the ponds, but they probably could out-dart the fish. No mayfly nymphs were found in the gut contents.

Table 4. shows the composition of the food items of 4 different size groups. The small fish preyed predominantly upon smaller organisms and as they grow, their prey became larger. The food habit of mosquitofish did not change with size, except that larger fish ingested larger prey.

Figure 1 illustrates the results obtained from the feeding study of mosquitofish on selected aquatic organisms. Before

Table 3.–Summary of food habit study, showing the relationship of the gut content of fish and the organisms in the pond where the fish were collected (in %).

Organism	Pond[1]	Fish guts[2]	Forage ratio	Selection index
		AQUATIC		
Crustacea	78.28	81.60	1.04	0.02
water fleas	(69.81)			
seed shrimp	(2.20)			
copepods	(6.27)			
Collembola		3.14		
springtails	——[3]	(3.14)		
Ephemeroptera	3.27	0	0	-1.
mayfly (N)	(3.27)	(0)		
Odonata	1.23	.27	.220	- .64
dragonfly (N)	(.15)	(02)		
damselfly (N)	(1.08)	(.25)		
Hemiptera	3.13	.44	.14	- .75
giant water bugs	(.68)	(.01)		
water boatmen	(0)	(.11)		
back swimmers	(2.45)	(.32)		
Coleoptera	2.29	.65	.28	- .56
Laccophilus (L)	(1.61)	(.08)		
Tropisternus (L)	(.36)	(.18)		
Hydrophilus (L)	(.02)	(.03)		
Misc. beetle (L)	(.30)	(.36)		
Diptera	11.77	7.43	.63	- .23
Chironomid (L.P.)	(11.27)	(5.53)	.49	- .34
Culicoid (L.P.)	(.44)	(.92)		
Ephydrid (L.P.)	(.06)	(.98)		
Hydracarina	——	.22		
water mites		(.22)		
Cyprinodontes		.01		
mosquitofish		(.01)		
		TERRESTRIAL		
Thysanoptera		.58		
thrips	——	(.58)		
Ephemeroptera		.04		
mayfly (A)	——	(.04)		
Homoptera		2.64		
aphids	——	(.24)		
leaf hoppers	——	(2.40)		
Hymenoptera		.80		
ants (A)	——	(.10)		
chalcidfly (A)	——	(.70)		
Coleoptera	——	.12		
rove beetles		(.12)		
Diptera		1.91		
Chironomids (A)	——	(1.07)		
culicoids (A)	——	(.46)		
misc. fly (A)	——	(.38)		
Araneida		.05		
spiders	——	(.05)		

[1]N = 360 dips during August 7 - September 1, 1978.
[2]N = 96 fish guts.
[3]denotes no datum.

Table 4.–The change in composition of the food with increase in size of the fish (in %).

Organism	Size groups (cm)			
	<2 (25 M&F)[1]	2-2.5 (17 M&F)	2.5-3 (25 M&F)	3< (29 F)
Crustaceans	77.84	82.36	83.62	82.53
Sptingtails	12.57	0	0	0
Dragonfly (N)	0	0	0	.08
Damselfly (N)	0	0	.29	.69
Giant water bug (N)	0	0	0	.02
Water boatmen	0	0	.36	.08
Back swimmers	0	.27	.66	.34
Laccophilus (L)	0	.18	.07	.08
Tropisternus (L)	.06	.27	.22	.19
Hydrophilus (L)	0	0	0	.11
Misc. beetles (L)	.13	.36	.82	.12
Chironomids (L.P.)	2.60	6.62	1.75	10.88
Culicoids (L.P.)	0	.98	2.11	.57
Ephydrids (L.P.)	.51	1.97	1.60	.15
Water mites	.76	0	0	.11
Fish fry	0	0	0	.04
Thrips	1.33	.54	.44	0
Mayfly (A)	0	0	.14	0
Aphids	.06	.18	.44	.27
Leaf hoppers	2.60	2.86	3.35	.73
Ants	0	0	.07	.34
Chalid fly (A)	.25	1.16	.58	.80
Rove beetle (A)	0	0	.07	.04
Chironomid (A)	.25	1.25	1.89	.88
Culicoids (A)	.70	.63	.51	0
Misc. fly (A)	.19	.36	.51	.45
Spiders	.12	0	.07	0

[1]Numbers in parentheses are sample sizes; F - females, M = males.

Table 5.–The relationship of the gut content of *Gambusia* fish and organisms in the environment where the fish and organisms were artificially introduced (number method, in %).

	Container	Fish guts	Forage ratio	Selective index
Mosquito (L.P.)	42.10	39.79	0.945	- .028
Crustaceans	53.74	57.52	1.070	+.034
Mayfly (N)	.92	0	0	-1
Backswimmers	.37	0	0	-1
Beetle (L)	.55	0	0	-1
Chironomid (L.P.)	1.11	1.34	1.207	+.094
Ephydrid (L.P.)	.65	.60	.923	- .040
Aquatic mites	.28	0		
Thrips		.30		
Beetle (A)		.30		
Fish		.15		

L = larvae N = nymphs
P = pupae A = adults

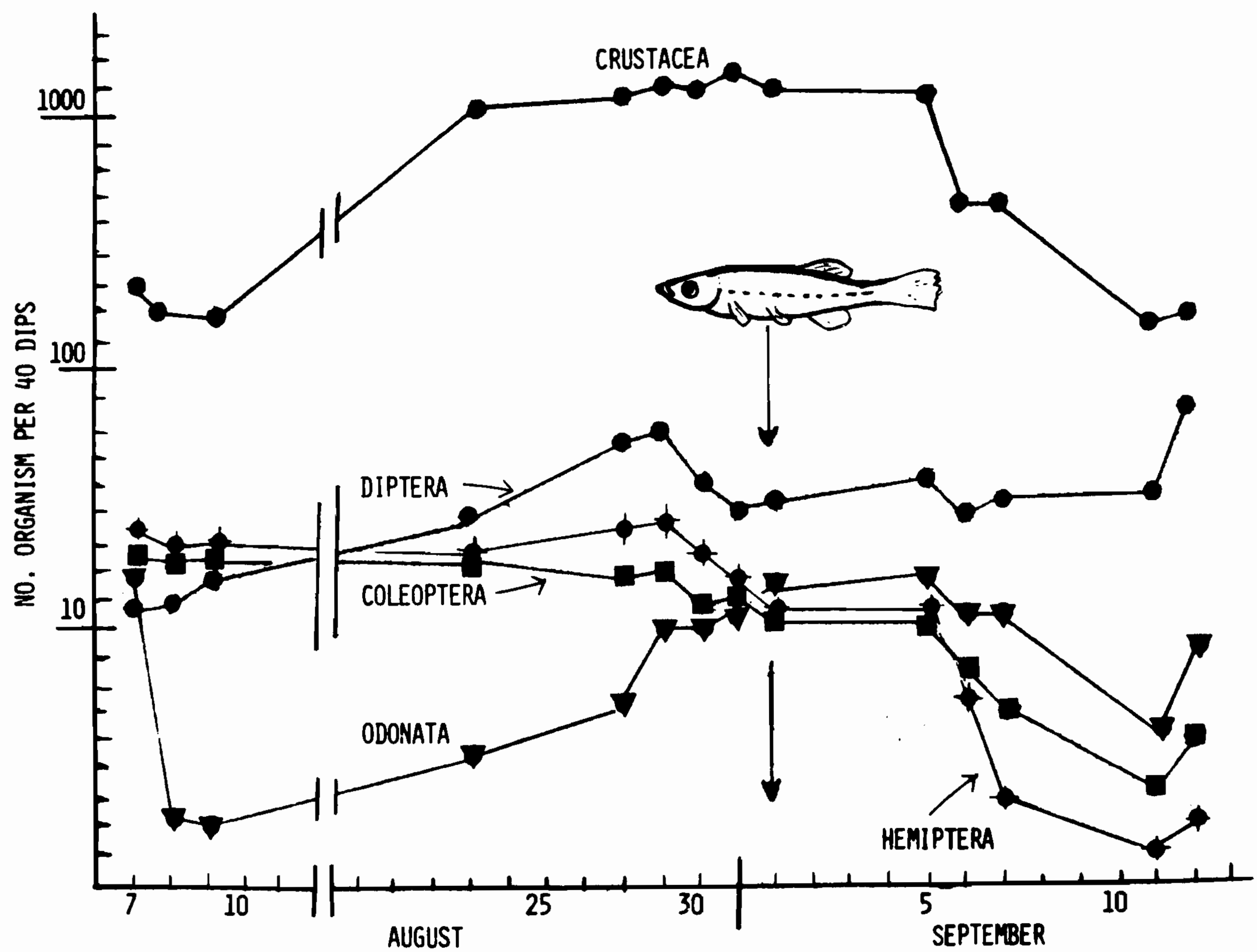

Figure 1.–The effects of mosquitofish predation on some selected aquatic organisms.

stocking the fish, crustacean population density fluctuated between 200 and 2000/40 dips, but on the 12th day post-stocking, the density was depressed to a 200/40 dips level. Population densities of Hemiptera and Odonata nymphs and Coleoptera larvae were also slightly affected. Diptera larvae and pupae are the important food item (Table 3), but overall population trend was not altered. Those findings are generally in agreement with the results reported by Bay and Anderson (1966) and Farley and Younce (1977).

The results obtained from the feeding study in the artificial containers are shown in Table 5. Mosquitofish did not prey selectively upon mosquitoes; the density of mosquito population was fairly high compared with other insect population densities, but F. R. was less than 1, and S.I. was negative. Crustaceans were the most abundant in the containers, so were they in the guts. Forage ratio and S. I. for Chironomid larvae and pupae were surprisingly high. This might be attributed to the relatively clean bottom condition of the containers.

The number method is most widely used for analysis of gut contents (Hess and Tarzwell 1942, Washino and Hokama 1967, Ahmed et al. 1970). However, this method merely indicates the number of individuals of each prey in the guts, it does not show the volume or frequency of occurrence of the various food items. In order to obtain a better understanding of the food habits of immature fish, gut contents were analyzed by the 3 different methods and the results were shown in Figure 2. The results which are assessed by the number method show that crustaceans formed ca. 78% of the food, collembola 12.5%, terrestrial organisms 5.5% and Diptera 3%; assessed by the occurrence method showed that crustaceans formed 96%, collembola 44%, terrestrial organisms 14% and Diptera 40%; assessed by the weight method, crustaceans formed ca. 40%, collembola 30%, terrestrial organisms 15% and Diptera 10%. Thus, comparing the data obtained from the 3 different methods, one can obtain the precise food habit of the mosquitofish.

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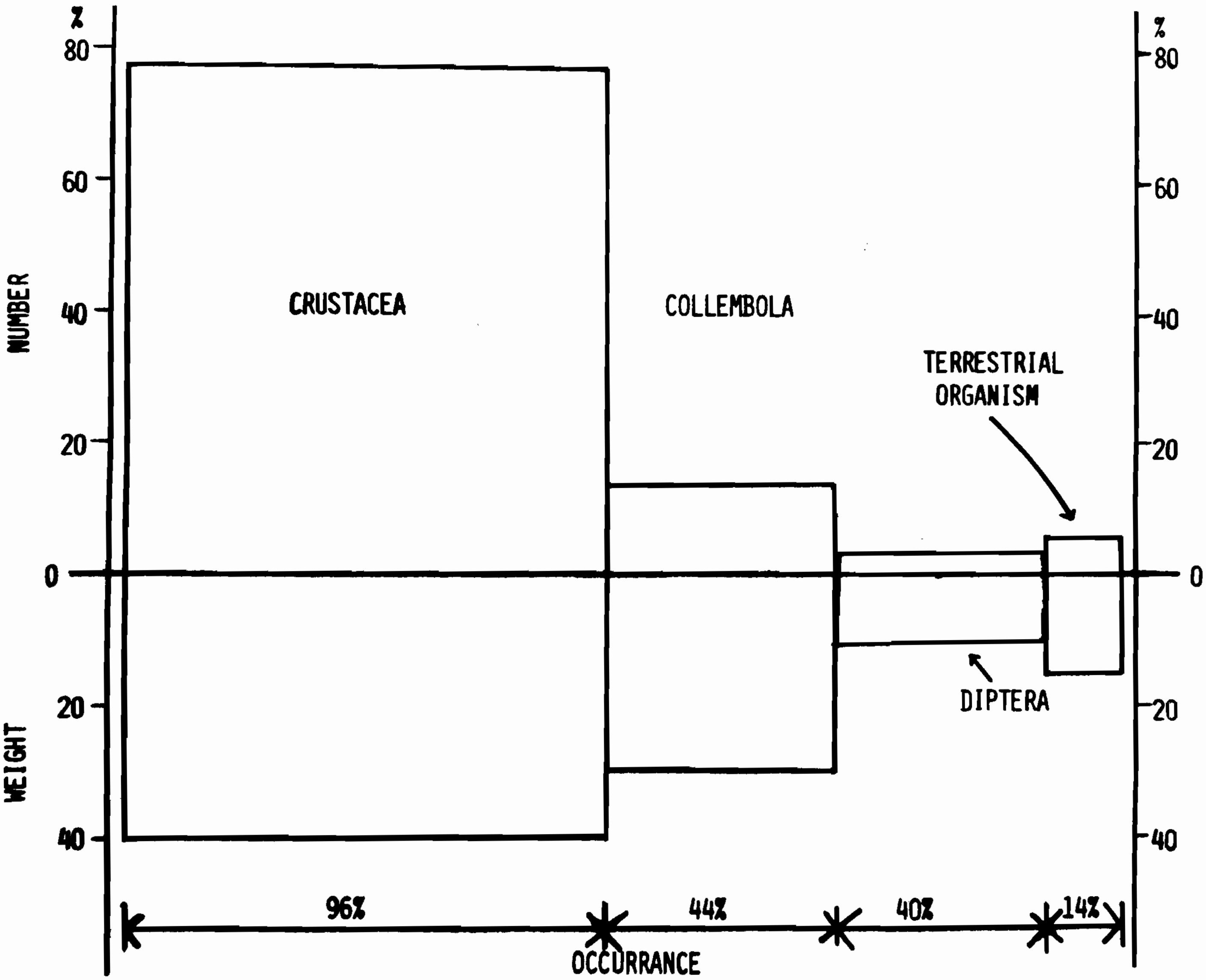

Figure 2.–Major food organisms of immature mosquitofish assessed by the number, weight and frequency of occurrence method (in %).

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TOLYPOCLADIUM, A NEW FUNGAL PATHOGEN OF MOSQUITO LARVAE WITH PROMISE FOR USE IN MICROBIAL CONTROL

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In 1972 Sanders reported a hyphomycetoous fungus causing epizootics in larval populations of *Aedes sierrensis* at Novato, California. Mortalities of up to 92% were recorded in some treehole populations. The pathogen was identified as *Beauveria tenella*. A subsequent paper describing laboratory evaluation of this fungus against several species of California mosquitoes and field trials against *Aedes sierrensis* larvae was published by Pinnock, et al. (1973). The results of this study were very encouraging.

We now have evidence, however, that *Beauveria tenella* was probably not involved in these epizootics, but that at least two different hyphomycetus fungi are in fact responsible for disease outbreaks observed at Novato. These are *Beauveria bassiana* and a *Tolypocladium* sp.

Beauveria bassiana was first collected in 1973-74 when it was found infecting larval populations of *Ae. sierrensis*. This was only the fourth time this species have ever been isolated from mosquitoes and the first time it has been reported causing epizootics in mosquito populations (Clark et al. 1968).

A species belonging to the genus *Tolypocladium*, most similar to *T. cylindrosporum*, was collected this past year and its isolation from *Ae. sierrensis* larvae represents the first published report of a member of this genus being an insect pathogen.

Several factors explain to a great extent how the misidentification could have been made in the first place, and why it has been so difficult to resolve this matter.

To begin with there is a striking similarity between these two species in macroscopic as well as certain microscopic characters: both fungi produce hyaline mycelia and conidia and both produce bulbous conidiophores. Another complicating factor may have been the presence of both pathogens in a single host, i.e. mixed infections. Nevertheless, conidia resembling those of *B. tenella* have been consistently absent, and an identification of *B. tenella* was almost undoubtedly without basis.

Secondly, all cultures used in the published work were freeze-dried as blastospores, and none of this material proved to be viable when reconstituted in 1974. As a result there were no cultures from this period to examine during later studies.

The 3 years of drought from 1974-77 added another complication that precluded the collection of new isolates, since no epizootics were observed during that time as a result of the extended dry season.

From 1974 until this past year, only *Beauveria bassiana* could be found infecting larvae in the field. Last year when the drought finally ended, fungal epizootics were discovered occurring in a number of treeholes. Isolations from diseased larvae revealed a fungal species that superficially resembled *Beauveria*, but showed significant morphological differences. At the same time, a single vial of freeze-dried conidia of "*Beauveria tenella*" that had been collected in 1972 was located. This culture proved to be viable and was identical to these new field isolates. This coupled with photomicrographs taken in 1972, led to the conclusion that what was called *Beauveria tenella* in 1972 was probably this different species either alone or in combination with *B. bassiana*.

The problem was then to determine the identity of these isolates. There was little doubt that this was an unknown insect pathogen. Cultures were sent to the Centraalbureau voor Schimmelcultures in Baarn, Netherlands, where the strains subsequently were identified as a *Tolypocladium* species near *T. cylindrosporum*.

The genus *Tolypocladium* was erected by Gams (1971), but little was known about the group other than the fact that most isolates had been taken from the soil. This represents the first description of a species within the genus as an insect pathogen, although some other isolates are known from insects (Samson, in press). These isolates from *Ae. sierensis* are currently being compared with known species of *Tolypocladium* to determine their specific status.

Figure 1. illustrates the conidiogenous cells and conidia of these 3 species. The characteristic that distinguishes the genus *Beauveria* from all other Hyphomycetes is the typical zig-zag shape of the rachis which elongates sympodially as conidia are produced. The characteristic that distinguishes *B. bassiana* from *B. tenella* is that *B. bassiana* produces spherical conidia whereas *B. tenella* produces elliptical conidia, although intermediate forms exist. The species of *Tolypocladium* are characterized by conidia which are produced in slimy heads by phialides. Colony morphology and conidiophore structure are very similar to those of *Beauveria*, but the genus is clearly distinct by its different conidium ontogeny. From all available evidence, it appears that *B. tenella* was never present. (Note: In 1972 the genus *Beauveria* was revised by de Hoog at which point the name *B. tenella* was superceded by *B. brongniartii*, the current proper name for this species.)

The first problem after isolation was, of course, to determine whether this species was merely a saprobe growing on dead larvae or a true pathogen. Over the past year bioassays against *Ae. sierrensis* larvae using these isolates have left no doubt that this is a highly invasive and extremely virulent pathogen of mosquito larvae. Figures 2 and 3 illustrate this point.

The inoculum used in the bioassay in Figure 2 was a blastospore suspension. Blastospores can be easily and inexpensively produced in shake culture using readily available nutrient media. Notice that at concentrations of 5 x 10^5 blastospores/ml at 25°C 94% mortality is reached within one day and 100% within 4 days.

The assay represented in Figure 3 was conducted using the conidial form. The conidia are produced on aerial sporulating structures on agar. Here mortality develops more slowly than

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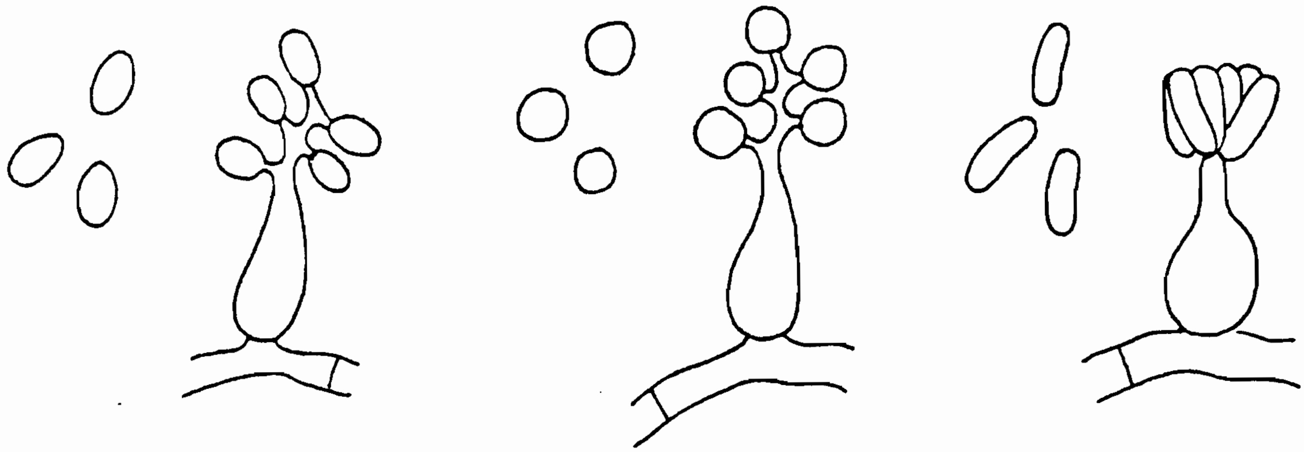

1 *Beauveria brongniartii* — 2 *Beauveria bassiana* — 3 *Tolypocladium* sp.

Figure 1.–Comparison of conidiogenous cells and conidia of ***Beauveria brongniartii*** **(=*tenella*)**, ***Beauveria bassiana***, and a *Tolypocladium* sp.

1. ***Beauveria brongniartii*** **(=*tenella*)** - identified in 1972 as infecting *Aedes sierrensis* larvae; evidence now indicates larvae were infected not with *B. brongniartii* but a *Tolypocladium* species.
2. ***Beauveria bassiana*** - isolated from diseased *Aedes sierrensis* larvae in 1973-74; present at epizootic levels.
3. *Tolypocladium* sp. - isolated from larvae in 1977; present at epizootic levels; strong evidence that it is same species that was described infecting *Aedes sierrensis* in 1972 and incorrectly identified as *Beauveria tenella*; species status not yet established; currently under study.

with blastospores. The LT_{50} for 5 x 10^6 conidia/ml is around 3 days here versus about 1 day for blastospores. Lower concentrations however, tend to produce higher final mortalities than do the blastospores. The reason for this is probably that the conidia, being a much more resistant stage, will survive longer, thus increasing the length of exposure to infection.

A small percentage of larvae infected with *Tolypocladium* usually remains at the surface after death (Fig. 4). When this happens, conidia are produced above the surface on a dense tuft of mycelium, but sporulation does not occur on dead larvae that remain submerged. In spite of this apparent limitation, the fungus generally seems able to survive from season to season and produce periodic outbreaks of disease. Nevertheless, the implication is that if *Tolypocladium* is to be used as a microbial control agent for mosquito larvae, it would probably have to be as a microbial insecticide.

Dissections and histological work have indicated that infection occurs primarily through the cuticle. The head and thorax as well as the saddle area appear to be the favored sites of infection. Penetration has also been noted at various points along the abdomen. There appears to be no preference for intersegmental penetration over penetration through more heavily scleritized portions of a segment. Invasion can occur through the midgut wall, but it does not appear to be the principle route of infection in *Ae. sierrensis* larvae.

Both spore stages can be easily and inexpensively, produced on artificial media, so that inoculum necessary for laboratory evaluation and small scale field trials can be readily produced.

As for the mass production of conidia in quantities sufficient for use in microbial control, the conidia of both *Beauveria* and *Tolypocladium* can be produced inexpensively on a semi-solid bran medium similar to that used in the commercial production of *Bacillus thuringiensis.* The technology for mass production of fungal pathogens has reached its highest level of development in Russia and eastern Europe. In Russia a particular strain of *Beauveria bassiana* has been used for ten years to control the Colorado potato beetle, *Leptinotarsa decemlineata*. The conidial preparation called Boverin, is produced in factory-sized fermentation facilities and applied at a rate of 30-45 x 10^{12} conidia/hectare (Ferron 1978). The production of such large quantities of conidia appears to be cost effective to the point that chemical pesticides such as DDT and carbaryl used in combination with *Beauveria* initially, have been abandoned in favor of these higher concentrations of spores.

A great deal of work remains to be done before the potential of this pathogen can be fully evaluated. More needs to be learned of the response of *Tolypocladium* to various environmental conditions. Host range determinations must be made for other mosquito species as well as non-target organisms. Effect on mammals must be critically examined and all safety questions resolved.

But at this point, all the signs are very encouraging; *Tolypocladium* is a virulent, naturally occurring pathogen of mosquito larvae, that can be cheaply and easily produced. All that is needed at this point is the interest and the funding to conduct the necessary research.

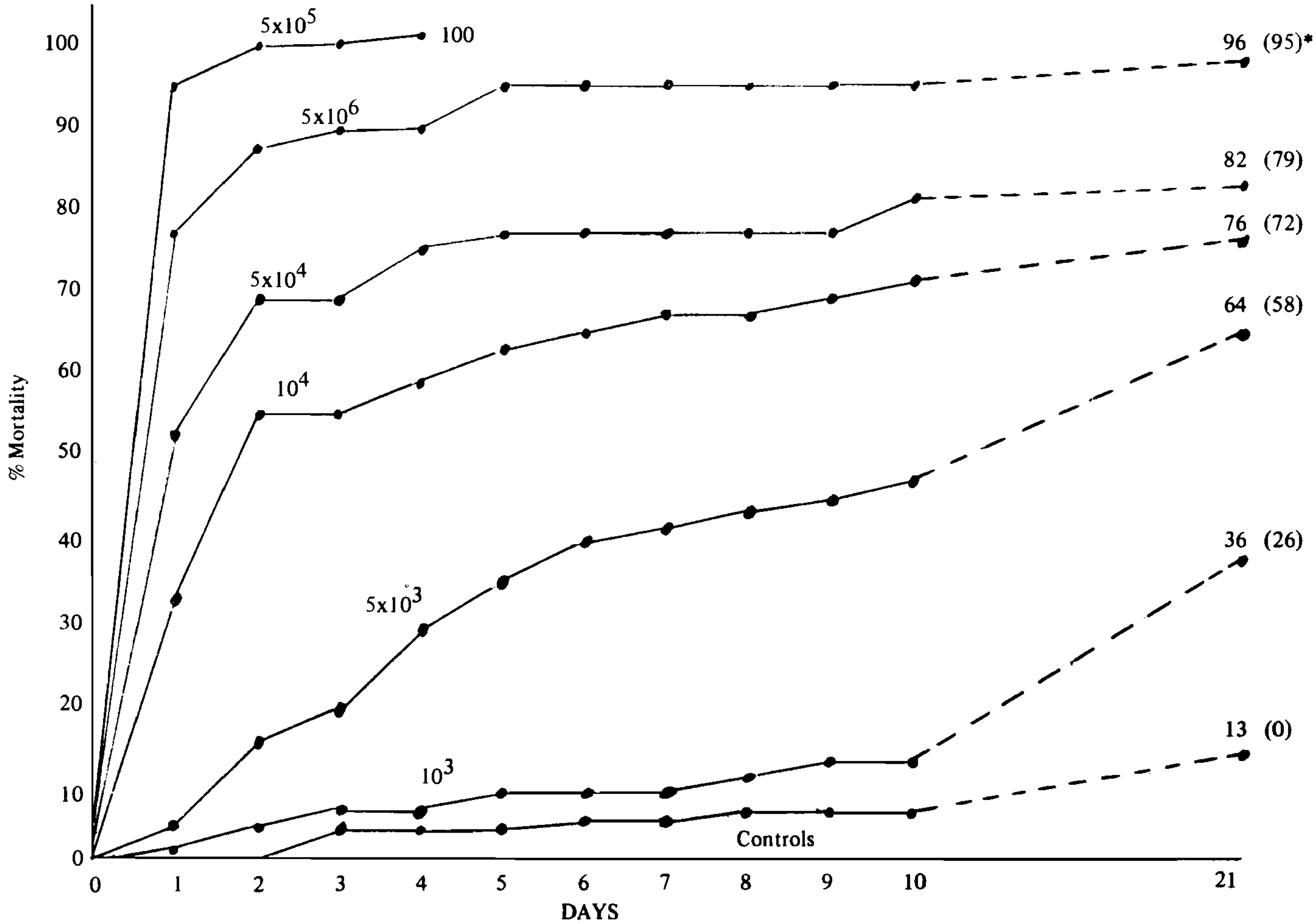

Figure 2.–Cumulative mortality in *Aedes sierrensis* larvae[1] subjected to varying concentrations[2] of ***Tolypocladium*** sp. blastospores at 25°C[3].

[1] 2nd instars; 50 larvae per treatment; 5 replicates of 10 larvae each.
[2] concentrations read as conidia/ml of water in test container; 85% viable.
[3] 12 hr light/12 hr dark photoperiod.
*corrected mortalities using Abbott's formula.

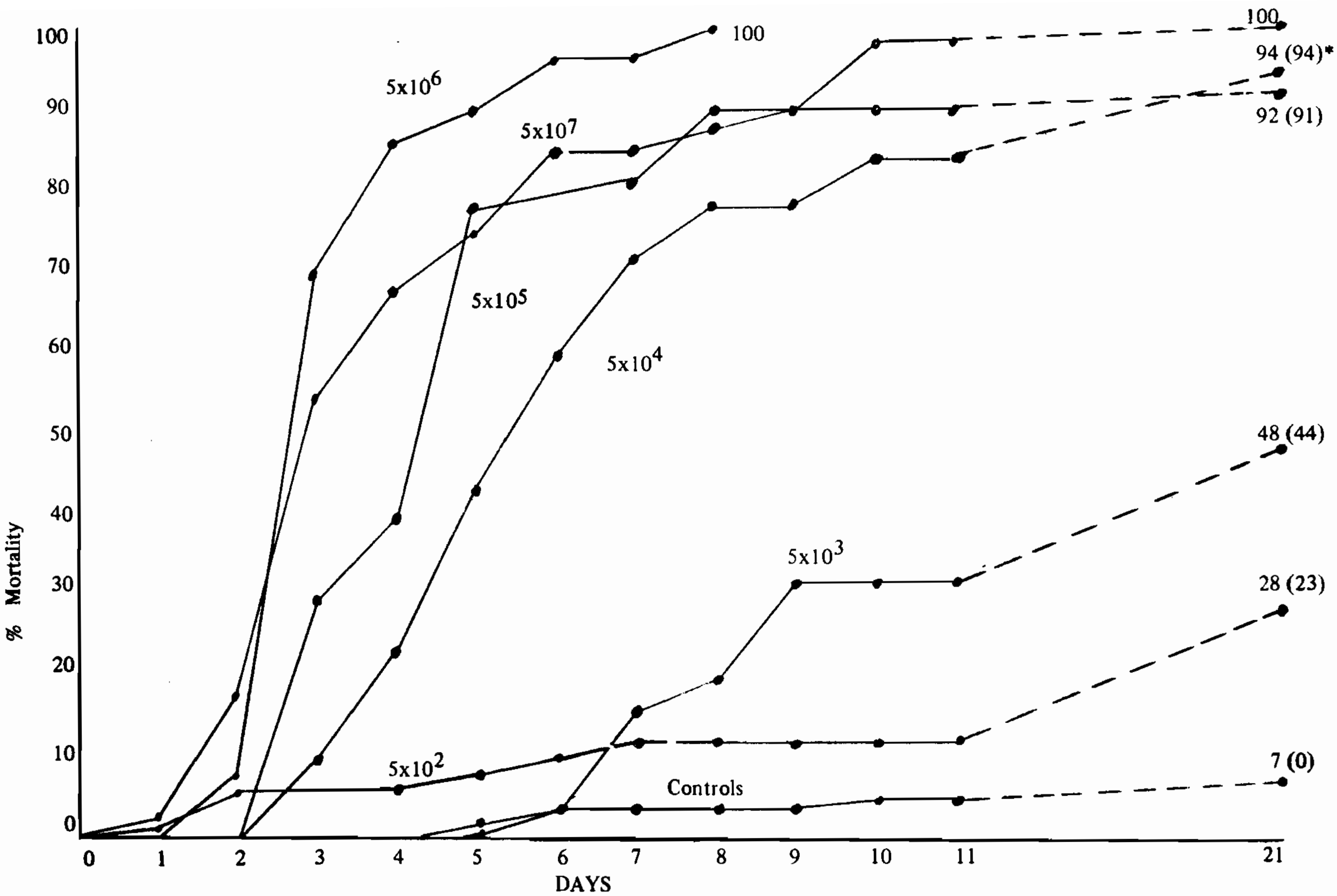

Figure 3.--Cumulative mortality in *Aedes sierrensis* larvae[1] subjected to varying concentrations[2] of *Tolypocladium* sp. conidia at 25°C[3].

[1]2nd instars; 50 larvae per treatment; 5 replicates of 10 larvae each.

[2]concentrations read as coidia/ml of water in test container; 93% viable.

[3]12 hr light/12 hr dark photoperiod.

*corrected mortalities using Abbott's formula.

Figure 4.–*Aedes sierrensis* 4th instar larvae infected with *Tolypocladium* sp.

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EARLY SEASON APPLICATION OF *ROMANOMERMIS CULICIVORAX*

PROVIDES CONTINUOUS PARTIAL CONTROL OF RICE FIELD MOSQUITOES

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ABSTRACT

An early season application of postparasites of the mosquito parasitic nematode, *Romanomermis culicivorax* demonstrated that the nematodes could mature to adults, mate and lay eggs in the rice field environment. Preparasites hatching from these eggs provided continuous partial control of larval *Anopheles freeborni* and *Culex tarsalis* throughout the rice growing season. Control of *An. freeborni* was comparable to that obtained in the past by direct applications of preparasites and exceeded that previously achieved for *Cx. tarsalis*. Infection was observed up to 20 feet from the original point of application.

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LAGENIDIUM GIGANTEUM AND MOSQUITOES ASSOCIATED WITH IRRIGATED PASTURES

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In Hanford, California, McCray et al. (1973) introduced zoospores of *Lagenidium giganteum* into an irrigated pasture to test the efficacy of the fungus as a biological control agent against *Aedes nigromaculis*. The infection pattern observed in the field larval population was sufficiently high (i.e., minimal infection rate of 83 per cent) to warrant further evaluation. Two subsequent field studies were initiated in California pastures to confirm and possibly extend the initial observation. In the first study conducted in Linda, Yuba County, California (Christensen et al. 1977), a maximum of 45 per cent infection rate was observed in native aedine larvae during the second irrigation cycle after inoculation. Contamination of the untreated control check adjacent to the treated check occurred however, and made evaluation difficult. The cause of the contamination presumably was due to small animals which continuously entered the barbed wire fencing erected on the levee surrounding the experimental checks. The second study was initiated in an experimental irrigated pasture in East Nicolaus, Sutter County, California, and constitutes the basis for this report.

The East Nicolaus study site consisted of a 176,000 ft^2 irrigated pasture which was divided into 17 approximately equal checks. Four of these checks were utilized for the study, 2 for treatment, and 2 for untreated controls. To minimize the chances of contamination, the selected checks which measured approx. 855 ft^2 each, were widely separated from one another.

Each check was inoculated at a rate of over 10,000 infected *Culex tarsalis* per check on day 1; over 5,850 infected larvae inoculated on day 2. Infection rate was monitored with 10 sentinel cages, per check; 5 with *Cx. tarsalis* and 5 with aedine larvae. In addition, the native mosquito population was dipped to estimate possible population changes and extent of fungal infection.

The infection rate was high in both native and sentinel larvae of *Cx. tarsalis*, but inconsistent and low for aedine species. These results support the earlier conclusion that *L. giganteum* is a virulent pathogen of *Cx. tarsalis* larvae, but appears to be a rather inconsistent agent against pasture aedine larvae. The modification of the experimental design was successful in excluding contamination into the untreated control checks as experienced in the previous study.

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STATUS OF RESEARCH ON THE GENETICS OF *CULEX TARSALIS* DIRECTED TOWARDS INTEGRATED CONTROL[1]

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The research which I will describe has been supported in part since 1975 by State Research Funds appropriated through the efforts of the California Mosquito and Vector Control Association. The major objective has been to determine whether genetic manipulations can contribute to an integrated program to control *Culex tarsalis*. This work represents a joint effort of staff from the Department of Environmental and Biomedical Health Sciences and the Department of Entomological Sciences on the Berkeley Campus of the University of California. Our so-called "genetic branch" is part of an extensive project aimed at studies on and the control of *Cx. tarsalis*, a principal vector of western equine (WEE) and St. Louis encephalitis (SLE) viruses in western North America. One of our greatest assets in the program is that multi-disciplinary personnel collaborate in each project.

Genetic studies of *Cx. tarsalis* began when Plapp and his co-workers (1961) in the early 1960's found that DDT and malathion resistance in *Cx. tarsalis* were heritable traits. Unfortunately very little basic genetic information was added to that significant find until Barr and Myers (1966) isolated white-eye and yellow larval mutants, and Calman and Georghiou (1970) determined the chromosomal relationships of these mutants. Our research group picked it up from there for 2 reasons: *Cx. tarsalis* continued to be the most important vector of WEE and SLE viruses in our part of the country, and no effective single weapon or integrated program was available to adequately control this species in routine programs, or in the face of recurring outbreaks of these viral infections (Reeves and Milby 1979).

Genetic control advocates the involvement of the insects themselves in their own destruction and therefore it is often referred to as "autocidal" control. It is a highly selective means of biological control and is directed solely at a target species. The perfection of any genetic-control system is technologically complex, so while categorized as applied research, it necessarily includes the development of extensive basic research and knowledge.

While several approaches are possible for genetic control, our primary thrust has been directed at the use of translocations for field introductions. These induced aberrations when released into a native population can contribute to its reduction over more than 1 generation. In addition they can act as transport systems for other desirable genetic factors that can either act as lethal "time bombs" at a later time and contribute to the reduction of the population, or replace some of the genome of the population.

Translocations are abnormal chromosomes that have been induced by ionizing radiation or other mutagenic agents. Two non-homologous chromosomes are broken and, in the process of becoming re-attached, exchange positions. Such an exchange creates a false or pseudolinkage of genes. It also interferes with subsequent meiotic divisions in the progeny that have inherited the interchange. The results are that 4 of the 6 possible sperm types have an unbalanced set of chromosomes and are inviable. This leads to 50% or more reduction in viable progeny, and half of these potentially carry the interchange to the next generation. From these heterozygous translocations we can construct homozygous translocations, in which the single heterozygous chromosome becomes established as a pair. These breed true, but when mated with normal adults again produce heterozygotes which as indicated cause a population reduction in subsequent generations.

If we are to obtain and identify these interchanges we must obtain basic genetic information on the species, and this requires that we have genetic markers. The latter are mutations, or heritable variations from the normal, and if they are easily recognized and not lethal in themselves, they become important tools to establish other genetic information. In the last few years we have established 14 mutant strains as laboratory colonies, and with these have gone on to construct 14 different multiple-marker lines, that is, laboratory colonies each of which have at least 3 markers, 1 on each of the 3 linkage groups. Both single-mutation stocks and multiple-marker lines are essential to our work for various reasons: they are used in studies on the basic genetics of the species; they serve to genetically identify the position of chromosomal breaks and reattachments; they have application in mapping chromosomes; and they can be used as markers when inserted into stocks for field and laboratory experiments.

Since visible genetic markers are incorporated into translocation lines for genetic identification of the interchanges, it is important that we assess the relative fitness of mutant stocks. Both laboratory tests and outdoor pond tests indicated that the presence of carmine eye-color does not significantly decrease mating ability (Ainsley et al. 1978). The study also indicated that fitness of the stock can be improved substantially if outcrossed and backcrossed frequently.

We can also identify chromosomal abnormalities cytologically by making slides of the mitotic and meiotic processes. When a reciprocal translocation has been induced, the disjunction after a crossover never terminates completely, and we can identify an interchange by a chain of 4 chromosomes and a bivalent set. Also by observations of the length of the chromosomes, we can determine which of the 3 pairs are involved. We recently used multiple marker stocks and cytological observations to establish that in *Cx. tarsalis* the longest chromosome carries the sex-determining gene (McDonald et al. 1978b). We

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know that sex is determined by a single gene or locus in culicine mosquitoes. As mentioned above, translocations cause pseudolinkage, or the abnormal linking of characters that are carried on specific chromosomes. For example, if we have a translocation that involves linkage groups 1 and 2, is sex-linked, and does not include the smallest chromosome, the latter could not be holding the sex-determining linkage group. Likewise in a second translocation, not sex-linked, the interchange is between the medium and the shortest chromosomes. Thus the longest chromosome must be the carrier of the sex-determining factor. Genetic studies with markers known to be on specific linkage groups confirm this observation. What makes this new finding important or unique is that in other culicine mosquitoes, the sex-determining factor is on the shortest chromosome. Another exception to this generality is that in *Culex tritaeniorhynchus* there is a sex locus on both the shortest and the longest chromosomes (Baker and Sakai 1976).

We have made significant progress in other cytological work as we can now isolate and identify for mapping the large salivary-gland chromosomes in *Cx. tarsalis*. In Diptera these chromosomes, as a rule, are 100 times longer than the metaphase mitotic chromosomes, and can be easily studied. Even more important than their size is that they have a distinctive pattern of transverse banding that allows accurate mapping throughout their length. We have now identified the 3 chromosomes in *Cx. tarsalis* as distinct entities and recognize much of their banding sequence.

Returning to our studies of autocidal systems, the interchange that we have studied the most over the past few years is a double sex-linked heterozygous translocation - - in other words it includes all 3 chromosomes in the 2 interchanges (Terwedow et al. 1977). There is virtually no crossing over between the points of the breaks and the sex gene, and about 99.3% of all males fathered by this line inherit the linked combination of the 3 chromosomes. There is only a 28% hatch from crosses that involve these males. The line competed well against other populations in laboratory and quonset cage tests, and was used in our pilot release studies in the summers of 1977 and 1978 (Asman et al 1979). We gave these field studies a top priority during the past 2 years. We have learned what this specific interchange can do in a field situation and have become cognizant of a number of problems associated with the programming of a field introduction.

The site where this translocation was field-tested is known as Poso West. This is an isolated field area 10 miles north of Bakersfield in Kern County that is within reasonable distance of our field station. The breeding site is stable as it is waste waters from oil wells, and the natural mosquito population is almost solely *Cx. tarsalis*. Earlier studies in 1976 had provided detailed estimates of population densities throughout the summer as well as information that allowed us to project the numbers of genetically modified males that should be released in the pilot study (Nelson et al. 1978). The actual numbers to be released were ascertained in a computer simulation model based on the release of males that carried the specific translocation we were releasing. The first study in 1977 indicated that the release stock could be mass produced, that the males could survive in the field as immatures and adults, and that the development time of their progeny in the field was similar to that of the native field population. The first pilot study also showed where more research was needed, and where improved techniques relevant to release strategies and field monitoring were necessary. In 1977, the natural population was larger than in previous years, and consequently the number of released males (76,000) was in too low a ratio to the field population to compete effectively with the native males and have an impact on the wild population. The number released was approximately 24,000 less than planned. In addition, an estimated 11,000 females which did not carry the interchange were inadvertently released. The 1977 translocation stock was released by putting pupae into emergence ponds at the field site. One of the difficulties in the mass-rearing of this stock, other than the low percent hatch of egg rafts and the need to cull expanding lines for eye color, was that a manual sexing process was used that did not remove all the small-sized female pupae. A major current effort is to develop a genetic sexing mechanism that can be used in future mass-production programs.

Based on information gained in 1977, a second release was initiated in 1978. In that release we focused on 2 objectives carried forward from the previous year: to reduce the population significantly with an insert of males that carried the double sex-linked interchange; and to recover the translocation from egg rafts or progeny derived from the field. Our second mass-rearing production program began in January of 1978. Quality control tests of the release material were made on 5 occasions, and the summarized data indicated that 99.4% of the males released were indeed carrying the interchange. The actual number to be released was again ascertained by computer simulations based on the population estimated at the field site over the past 3 summers. In 1978 we released male adults rather than pupae, and thus guarded against insertion of females. We allowed male pupae to emerge in the laboratory, and any females that had been inadvertently included were removed.

In the 1978 releases 180,000 translocated males were released between mid-March and the first week in June. A monitoring system was established to ascertain if the release males were mating with field females. Females collected from the field were taken into the laboratory. Those which gave low egg-hatch rafts were reared, male progeny were outcrossed to marker stocks, and the progeny from these crosses that again came from low-hatch rafts were further tested genetically. In 22 cases the tests confirmed that the translocation-carrying males released at the site had mated with native females. Thus we met 1 more objective in 1978; we found that the genetically-modified males could mate with field material in the field. Unfortunately, parallel tests in large outdoor cages, in which the release males competed against field males for field females, revealed that the release stock had become less competitive than it was in 1977. We now recognize that selection factors related to laboratory conditions had changed the release stock genetically. Thus the impact of our release was again not sufficient to cause an on-going reduction of the field population. In 1978, the population in June at Poso West was 40 percent less than in 1976 and 1977 even though the drought of 2 years was over. In fact, 1978 was a year of excessive rainfall in the release area. Analyses of data have demonstrated that there was a 4-week delay before the population reached a peak as compared to the times of peak populations in the 2 previous years. However, when the peak in 1978 occurred it was approximately twice that observed in 1977, which in turn was considerably higher than in 1976. At the peak in 1978 it was estimated that there were 450,000 females in the study area. Since our releases ended in early June, the insert could well have contributed to the reduction of the population up to that time.

Over the past 3 years mark-release-recapture studies carried on by our associates (Milby 1979) have allowed us to estimate the size of the population at Poso West, the daily survival of native females and of the introduced experimental strain, and the ratio of released to wild males during the release period. In order to accomplish this 29,217 males and 50,164 females were marked with fluorescent dusts and released this past year. These were all from the field, taken either as adults or collected as pupae and allowed to emerge in the laboratory. Recovery efforts totalled a collection of over 400,000 females and 55,000 males which included 5,500 marked females and 81 marked males. Our limited ability to recapture marked males has revealed an area for additional research. It appears that the marking of males may have an adverse effect on their survival, movement, and mating capacity. From mark-recapture studies we also determined that the released males did not move within the study area as readily as did males from the natural population. We are investigating the use of genetic markers rather than dusts for future mark-release-recapture studies.

In the past 2 years we perfected a method for the rapid isolation and identification of homozygote translocations (McDonald et al. 1978a). Again this was possible because we had developed multiple-marker stocks (Asman 1976). To illustrate the advantage of the new method, we have 2 homozygote lines - - one isolated in 21 generations by the older standard procedure, and the second isolated in just 5 generations by the newly devised scheme. Since both of these lines are autosomal (non-sex linked), both sexes can carry the translocations. These homozygote lines are now being expanded and will be evaluated for their mating and other characteristics in our large quonset cages this spring and summer. An advantage of homozygote over heterozygote interchanges is that they can transport desirable genotypes, and can conceivably replace a field population. By population genetics we know that if fitness is the same for both the native and a released population, the least frequent population will be displaced in subsequent generations. Thus we hope to utilize the replacement mechanism as a bonus, when we introduce a homozygote that itself carries autocidal properties.

Another desirable genotype that our colleague Dr. Hardy has isolated relates to vector competence in *Cx. tarsalis* for WEE virus (Hardy et al. 1978). A refractory strain was isolated after 17 generations of selection. We determined that the resistance of this line to WEE virus was recessive and possibly was controlled by more than 1 gene. This strain is a candidate for production of translocations that might serve to carry resistance to viral infection into a population as well as cause a population reduction. We presently have 3 wing mutants (fringe wing, wide wing, and gabled) that also have some potential to be semi-lethals if introduced with a homozygote translocation. All 3 mutants have approximately 30% mortality when emerging from the pupal case because of abnormal wing structure. We have initiated another line of study to isolate a temperature-sensitive conditional lethal. This type of lethal would be desirable as a "time bomb" insertion. We are still hopeful that a non-diapausing factor can be selected, as it would be desirable for field introduction with a translocation homozygote.

A most useful development for the testing of experimental populations in semi-outdoor conditions has been to modify quonset-hut frames to make large outdoor screened cages that can be used for a variety of experiments. We consider these to be "half-way" testing units. All populations under consideration for field releases are first evaluated in competition tests in the quonset cages. In addition to using these cages to test potential release material, they have been utilized for a number of experiments relative to the biology and ecology of our species.

For the past 2 years we have studied the feasibility of using the sterile-male technique as a support system to the insertion of translocations for control of *Cx. tarsalis*. The procedures followed were suggested by the International Atomic Energy Agency (IAEA Proceedings 1974). The first phase is finished as we have established sterility curves, done mating competition tests in small laboratory cages, and then extended the tests into field-cage trials. A dose of 5 krads from a Co^{60} source when delivered in approximately 33 minutes produced highly sterile and competitive males. In the competitive mating experiments in the large outdoor cages, we found a 43% hatch when irradiated and non-irradicated males competed in a 1:1 ratio, as compared to a 92% hatch in the unirradiated control cage, and 3.0% hatch in the irradiated control cage. The low proportion of eggs that hatched indicates that in special circumstances the sterile-male technique might reduce *Cx. tarsalis* populations. In other tests we found that changing the sex ratio, which would be the case in field releases of sterilized males, did not significantly alter the mating competitiveness of sterile males (Ainsley and Asman 1979). This summer we hope to do a small-scale release of sterile males in the field.

We are convinced that the single most important factor that has limited success in the sterile-male and other programs for genetic control of insects has been a deficiency of basic biological information on the species in question. For this reason we have continuously carried out studies on the behavior of *Cx. tarsalis*. In addition to the remarks Dr. McDonald (1979) will make on mating behavior, observations by our associates suggested that marked laboratory reared females tended to be trapped earlier than marked field females of comparable age. The results of a subsequent study showed that a significantly higher proportion of laboratory reared females took blood meals on the first night following "release", regardless of their insemination status. Other studies indicated that female *Cx. tarsalis* usually are monogamous, and that a male can inseminate up to 8 females. We have also noted that if normally autogenous females are retained as virgins 6-7 days prior to mating they do not oviposit autogenous rafts; however, when subsequently mated and blood-fed they oviposit anautogenous rafts.

When we realized that our laboratory-reared heterozygous translocated stock had become less fit with time, we initiated a new direction of study. Improvement of the fitness of all colonies and stocks that are to be involved in field releases is now a chief priority. To achieve this objective we must establish an effective quality-control program that insures that specific attributes that are essential for normal behavior in the field have not been lost during or after colonization. If such traits are lost they will have to be re-estalbished. We have changed our programs for laboratory rearing and mass-production of release materials. Representatives of our heterozygous line have been moved into a large outdoor cage facility for their colony maintenance. We hope this procedure will select for mating competitiveness in a more natural environment and will extend flight behavior. We are currently attepting to carry a field population collected from our release site through the winter in an outdoor quonset cage. No artificial heat or light are being provided, and temperatures have been unusually low this winter. Females, assumed to be in diapause, took refuge in old rubber tires provided for that purpose. We await the onset of blood feeding and oviposition this spring.

A final study that should be mentioned is an effort to identify genetic variations that could contribute to negative-competitive fitness. This approach involves a genetic allozyme analysis by electrophoretic studies of the wild-type populations as well as each laboratory colony that will be involved in competitive and field release studies (Houk et al. 1979). The isolation of electrophoretic markers also would contribute to formal genetic studies, release experiments, and the study of reproductive behavior.

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EFFECTS OF LABORATORY COLONIZATION ON THE REPRODUCTIVE ABILITIES OF A FIELD-COLLECTED *CULEX TARSALIS* POPULATION[1]

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ABSTRACT

The insemination, oviposition and egg hatch of newly-colonized and laboratory populations of *Culex tarsalis* were compared. The effect of laboratory colonization on these reproductive characteristics of a newly colonized population was followed for four generations. The results indicated a substantial and continuing reproductive disadvantage to the newly-colonized population during the initial colonization process.

INTRODUCTION.–The establishment of laboratory colonies of *Culex tarsalis* is the first step if there is to be a program for genetic control of this species. The mosquitoes to be released in the field must be vigorous and competitive and so the laboratory colonies must be vigorous, especially with regard to reproduction and behavior.

The procedures used to colonize and maintain insects are important as they will determine the vigor of the released population (Bush 1978; Mackauer 1976; McDonald 1976). When a field population is brought into the laboratory it must adapt to colonization. Some of the adaptation is physiological and represents the population's temporary response to changing conditions Some of the adaptation is genetic and is inherited by the progeny of the initial population. Many adaptive changes are brought about by inadvertent selection, unplanned and undirected by the scientist. These changes may lead to genetic decay, loss of vigor, or production of a laboratory ecotype unsuitable for field release (Coppel and Mertins 1977).

We do not know the best way to produce laboratory colonies of *Cx. tarsalis* and maintain the desired vigor. The experiments described here are initial attempts to determine and document the effect of laboratory colonization upon three important reproductive characteristics: insemination, oviposition, and egg hatch.

MATERIALS AND METHODS.–The first studies were to determine these reproductive characteristics of laboratory and field-collected populations, when inbred or outcrossed, and are reported in greater detail elsewhere (McDonald et al. 1979). Subsequent studies were made of reproductive characteristics of a field-collected population in the first four generations of colonization. The mosquito strains used in the studies were Knights Landing (KL), a laboratory population from Yolo County, California established six years prior to the experiment, and Poso West Wild (PWW), a population collected in late Septembeı, 1977, as pupae and allowed to emerge in the laboratory. Generally 200 newly-emerged mosquitoes of each sex were placed in a colony cage measuring 60 cm on each side. The cage was provided with water and sugar but no oviposition site. After three to five days anesthetized chicks were offered to provide a bloodmeal. Within one and one-half days individual engorged females (100 total for each population) were placed in a cotton-stoppered shell vial with a sugar wick. A layer of water at the bottom provided an oviposition site. Egg rafts were examined three days after oviposition and embryonation and hatch were determined. The females were frozen after a six to seven day oviposition period and spermathecal examinations to determine insemination status were made at a later date. The numbers of eggs remaining in the ovaries in Christophers stage V were recorded.

RESULTS.–The first level of observations related to ovarian development and fertilization (Table 1). Nearly all engorged females from populations of KL and PWW either inbred or outcrossed developed eggs. The few which did not were excluded from further analysis. The females were classified in three categories: those that retained all eggs without ovipositing (retaining), those that laid non hatching rafts of unembryonated eggs (sterile), and those that laid hatching rafts (fertile). The inbred KL population had few retaining and sterile females, and mainly fertile females. More of the outcrossed KL females were retaining or sterile, and fewer were fertile. A great proportion of outcrossed PWW females were retaining or sterile, and fewer were fertile. The inbred PWW population had the highest percentages of retaining and sterile, and fewest fertile females. Only 18% of inbred PWW females would have contributed to the next generation of a laboratory colony because they retained eggs or laid sterile rafts. The reason for the lower success of PWW females can be explained in large part by the low insemination rates and low rates of oviposition by inseminated females. The percent of insemination was highest for the inbred KL, lowest for the inbred PWW, and intermediate for the outcrosses. The percent oviposition of inseminated females was lower for PWW females than for KL females.

The second level of observations related to the fate of the eggs which the females developed (Table 2). Developed eggs included all eggs which developed to Christophers stage V or beyond, including those retained as well as those oviposited. The number of developed eggs was greater for the two populations with KL females than those with PWW females. The inbred KL population scored the highest percentages for

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Table 1.—Observations on ovarian development and fertilization of gravid ♀♀ *Culex tarsalis* from laboratory colonies and field populations.

Population (♀♀ x ♂♂)	No. ♀♀ observed	PERCENT OF FEMALES retained all eggs	laid non-hatching rafts	laid hatching rafts	inseminated	inseminated that oviposited
KL x KL	98	3	5	92	96	98
KL x PWW	98	12	23	65	78	96
PWW x KL	96	51	15	34	47	73
PWW x PWW	100	55	27	18	28	71

KL = Knights Landing laboratory colony.
PWW = field-collected Pso West, Kern County, California.

developed eggs laid, embryonated, and hatched. The inbred PWW population had the lowest percentage of developed eggs laid, embryonated, and hatched, and the outcross populations were intermediate. The sources of reproductive failure for the inbred PWW population were eggs not laid or eggs laid that were not embryonated. Essentially all embryonated eggs hatched in all populations. The overall reproductive loss for the inbred PWW population was great as only 12% of developed eggs hatched compared with 77% for the inbred KL population.

The PWW population was followed for four generations (Table 3). A high percentage of the females retained eggs or gave sterile rafts in all generations. The percentage of fertile females remained low for three generations, and rose in the

Table 2.—Developed eggs from *Culex tarsalis* in laboratory cages.

Population (♀♀ x ♂♂)	No. eggs developed	Percent of Eggs Developed: ovi-posited	embryon-ated	hatched
KL x KL	18,213	96	80	77
KL x PWW	19,740	87	59	57
PWW x KL	14,407	45	28	28
PWW x PWW	15,425	40	13	12

KL = Knights Landing laboratory colony.
PWW = field-collected Poso West, Kern County, California.

Table 3.—Observations on ovarian development and fertilization of gravid ♀♀ from the new *Culex tarsalis* PWW colony.

Generation	No. ♀♀ observed	PERCENT OF FEMALES retained all eggs	laid non-hatching rafts	laid hatching rafts	inseminated	inseminated that oviposited
1	100	55	27	18	28	71
2	50	80	8	12	54	22
3	62	50	29	21	48	57
4	17	29	18	53	76	77

Table 4.—Developed eggs from newly-colonized PWW *Culex tarsalis* ♀♀ in laboratory cages.

Generation	No. eggs developed	Percent of Eggs Developed: ovi-posited	embryon-ated	hatched
1	15,425	40	13	12
2	9,191	19	11	10
3	8,586	37	10	9
4	3,442	78	57	55

fourth. Insemination rates increased gradually over the four generations. The proportion of inseminated females that oviposited fluctuated, and the mean value for the four generations was 57%.

Egg laying was especially depressed the first three generations of the PWW colony, and most eggs were not embryonated, but almost all embryonated eggs hatched (Table 4). Overall all factors increased in the fourth generation.

Our next goal is to develop procedures to reverse deficiencies associated with colonization. We will begin such efforts by maintaining colonies in field cages or outcrossing them to field material at different intervals before they are released in field tests. Selective breeding for desirable reproductive characteristics may also be undertaken.

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THE EFFECTS OF SEX RATIOS AND THE RATIO OF STERILE Co^{60} IRRADIATED *CULEX TARSALIS* MALES TO FERTILE MALES ON MATING COMPETITIVENESS

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INTRODUCTION.–The release of sterile males for control of pest populations increases the proportion of males in a target population. A successful release requires the establishment of a high ratio of sterile to fertile males (Richardson 1978). However, mating behaviors might be induced which increase mating competitiveness of fertile males in a target population. Such behavioral changes may be induced in either the females or males of a wild population in response to high sex ratios or large ratios of sterile released males to resident fertile males (Crystal 1978; Ehrman and Parsons 1976), Mating competitiveness of some male phenotypes in several Diptera is known to have an inverse relationship to their frequency in populations. In these cases, rare male phenotypes mate more successfully than the more frequent ones (Ehrman and Parsons 1976). The existence of a mating system which favors wild males inversely to their proportion in the total male population would seriously impair the efficiency of the sterile male release method for pest population control.

Experiments reported here evaluated the mating competitiveness of sterile males in small laboratory cages in which the sex ratio or ratio of sterile to fertile males was changed.

METHODS.–Stocks - - PWC stock was initiated from collections at Poso West in 1976. The ble stock was obtained by outcrossing the original recessive black eye mutant stock (Asman 1975a) to PWC stock twice, each time recovering the ble phenotype from among the F_2 progeny.

Mating Trials - - Newly emerged PWC males were sterilized by exposure to 5 Kr of gamma radiation from a Co^{60} source at the rate of 160 r/min. Three sets of mating trials were done. The first set of trials consisted of 100 sterile PWC males, 100 fertile PWC males, and 100 fertile PWC virgin females. In the second set of trials the sex ratio was changed to 50 sterile PWC males, 50 fertile PWC males, and 100 fertile PWC virgin females. The ratio of sterile to fertile males was changed in the third trial, which consisted of 150 sterile PWC males, 50 fertile ble males and 100 fertile PWC virgin females. The ble males were used as fertile males in this trial due to a shortage of PWC stock. The ble stock was chosen because it had been successfully outcrossed to the PWC stock twice just before the mating trials were started in October 1978. The first 2 sets of mating trials were carried out in 60 cm x 45 cm x 60 cm tall cages, and the third trial was carried out in a 60 cm cube cage.

Each cage was provided with a water-filled oviposition cup and sucrose cubes. Restrained chicks were left in the cages one night as a source of blood meals. The light cycle consisted of 15 hours of intense light and 1 ½ hours each of simulated dusk and dawn. Egg rafts were collected daily, separated into individual vials, and examined after at least 3 days for egg hatch and embryonation. Since females as a rule only mate once (Asman 1975b), the sire of each raft was uniquely determined from the hatch and embryonation rates of each egg raft (= family). If the hatch rate was greater than 50%, the sire was assumed to be fertile. If the hatch rate was less than 50% and there were unhatched-embryonated eggs, the sire was assumed to be sterile. If only unhatched-unembryonated eggs were present, the egg raft was assumed to have been laid by an unmated female.

RESULTS.–As in previous mating trials with sterile *Cx. tarsalis* males, there was a clear separation in the distribution of high and low hatch egg rafts (Figure 1). If it is assumed that egg rafts with less than 50% hatch were sired by sterile males, then 23 of 61 (38%) and 36 of 83 (43%) egg rafts (= families) from mated females were sired by sterile males when the sex ratio was 2:1 and the ratio of sterile to fertile males was 1:1 (Table 1). When the sex ratio was lowered to 1:1, 33 of 70 (47%) and 20 of 38 (53%) egg rafts from mated females were sired by sterile males. When the ratio of sterile to fertile males was increased to 3:1, 27 of 43 (63%) sired egg rafts were sired by sterile males.

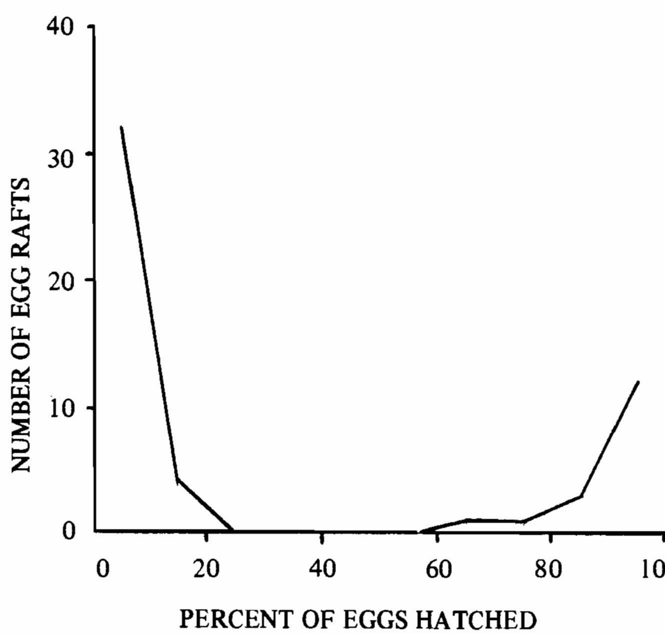

Figure 1.–Distribution of % egg hatch in combined data from 4 mating trials with equal numbers of fertile and sterile males.

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Table 1.—*Culex tarsalis* laboratory mating trials - effects of sex ratio and ratio of sterile to fertile males.

Sex ratio (males:females)	Ratio of sterile to fertile males	Replicate	Number of Egg Rafts		
			<50% hatch	<50% hatch	Not sired
		A	23 (38%)*	38	2
2:1	1:1	B	36 (43%)	47	0
		Totals	59 (41%)	85	2
		A	33 (47%)	37	2
1:1	1:1	B	20 (53%)	18	1
		Totals	53 (49%)	55	3
2:1	3:1		27 (63%)	16	8

*% low hatch egg rafts among total sired egg rafts.

DISCUSSION. Changing the sex ratio did not significantly alter the mating competitiveness of sterile males. This is in agreement with previous experiments (Ainsley and Asman, unpublished data). Since the proportion of egg rafts sired by sterile males was not significantly different than the proportion of sterile males in the mating trials with 1:1 or 3:1 ratio of sterile to fertile males, the mating competitiveness of the sterile males, s, was not significantly affected by increasing the ratios of sterile to fertile males. Additional experiments are planned to confirm this tentative conclusion.

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MATING COMPETITIVENESS OF STERILIZED VERSUS NORMAL MALE *AEDES SIERRENSIS* RELEASED WITH FEMALES INTO FIELD CAGES

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ABSTRACT

In two experiments at the University of California Russell Tree Farm Field Station, laboratory-reared mosquitoes were released into tents having screened sides and natural turf floors. The tents were stocked with oviposition boxes and rotting fruit (for carbohydrate meals), and the mosquitoes periodically obtained blood meals from domestic rabbits or chickens. In the first experiment (June 28 through July 26) one tent received 500 females, 500 normal males and 500 sterile males, a second tent received 500 females and 1,000 sterile males, and a third tent had 500 females and 1,000 normal males. In the second experiment (August 24 through October 4) the three tents were again stocked with 500 females, but one tent received 500 normal and 500 sterile males, a second tent received 200 normal and 800 sterile males, and a third tent was stocked with 100 normal and 900 sterile males. Resultant eggs were collected from tents each week and bleached to determine the sterile:fertilized ratio.

Observations of released mosquitoes revealed that both normal and sterile males mated with virgin females within 5 minutes after release of the females. Bleached eggs from the various tents showed that the mating competitiveness of irradiated, sterile males was about as expected at a 1:1 ratio (an average of 42 percent sterile eggs), somewhat less than expected at a 4:1 ratio of S:N males (from 42 to 65 percent sterile eggs in different weeks), and as expected at a 9:1 ratio of S:N males (from 84 to 94 percent sterile eggs in different weeks). In the experimental control tents having either all normal or all sterile males, the results were as expected (3 percent sterile for the former and 96 percent sterile for the latter).

CHEMICAL ECOLOGY OF MOSQUITOES AUTO AND TRANSSPECIFIC REGULATING CHEMICALS IN NATURE

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The chemical ecology of mosquitoes is an important but complex field of research that has been barely studied. In the chemical factory of nature mosquitoes are bombarded by a multitude of molecules which shape the various life processes of the organisms. Both adults and immature mosquitoes respond in a variety of ways to chemical stimuli in nature. Some of these chemical stimuli are of mosquito origin regulating their own life processes, while others are produced in their environment by other organisms. To fully understand mosquito biology, ecology and behavior, a knowledge of these auto and transspecific regulating principles is of utmost importance. Here, I would like to attempt to bring together the current knowledge and our own research information pertaining to the interaction between chemical stimuli (mostly produced in nature) and the immature mosquitoes and allude to the ecological and practical implications of such systems. In this context the autoregulating factors and oviposition modifying principles of larval and egg origin will be discussed. The transspecific substances produced by microbial flora in organic infusions and the chemical principles produced by other species of organisms cohabiting with mosquitoes will also be discussed in general. There are many physico-chemical factors which elicit or modify a variety of responses in adult mosquitoes. Some of these factors will be discussed by the next speaker. Due to lack of time I will not elaborate on the chemical ecology of adult mosquitoes.

A–AUTOREGULATING AGENTS OF LARVAL ORIGIN. Mosquito larvae prevailing in a confined habitat produce all kinds of factors and metabolites, some of which either are toxic or delay the development of the young larvae. The elaboration of such toxic and growth retarding factors have been reported for a number of species of mosquitoes. Under overcrowded conditions of larvae and with the provision of unlimited amount of food, larvae of *Aedes aegypti* required prolonged periods for development and contained large amounts of undigested food in the gut, despite the fact that food intake was normal (Shannon and Putman 1934). Overcrowding of mosquito larvae was reported to result in retarded growth and high mortality of the larvae, small and nonuniform size of the resulting adults and lower fecundity (Terzian and Stahler 1949). Klomp (1964) interpreted these observations as a case of mutual interference due to intense bodily contact under overcrowded conditions. However, the basic problem of overcrowding and the elucidation of the chemical factors were not investigated until the initiation of detailed studies on the ecology of overcrowding in our laboratories at the University of California, Riverside.

Ikeshoji and Mulla (1970a) were the first to show the elaboration of larval factors produced under overcrowded conditions, characterizing these factors biologically and entomologically. They showed that chemical factors produced by overcrowded 3rd stage larvae of *Cx. quinquefasciatus* were toxic to the younger larvae of this species and *Ae. aegypti*, *An. albimanus* and *Cx. tarsalia*. The stability of fractions from thin layer chromatographs was also assessed after identification of the active zones. Subsequently we studied the toxic and growth retarding components of larval elaborate obtained under highly overcrowded conditions (Ikeshoji and Mulla 1970b). Physiological effects of these larval factors and their bacteriostatic effects were established.

In a systematic manner Ikeshoji and Mulla (1974a) isolated and chemically identified the toxic and growth-retarding larval factors. Two straight and two branched long-chain hydrocarbons were n-heptadecane, and n-octadecane, while the branched chain hydrocarbons were 7-methyloctadecane and 8-methylnonadecane. Additionally, some minor constituents tentatively identified as 2-ethyl long chain fatty acids were also tentatively identified. These biologically active principles were elaborated by the larvae when they were overcrowded, having an "ego space" of less than 9 mm^2 per larva.

Further studies on the authentic compounds showed that the branched chain hydrocarbons showed greater activity against the larvae then the straight chain n-octadecane (Ikeshoji and Mulla 1974b). Addition of 3-methyloctadecanoic acid enhanced the activity somewhat. The branched chain hydrocarbons markedly delayed larval development.

Once the active principles in larval cultures (under overcrowded conditions) were identified, systematic research on the synthesis and larvicidal activity of these ecologically, important factors was spearheaded in our laboratory. In the first phase of this, several analogs of the branched chain fatty acids were prepared and evaluated (Hwang et al. 1974a) including 2- and 3-substituted fatty acids. Some of these substituted acids showed high level of activity against 4 species of mosquitoes while others had little or no activity. This field of research was expanded by synthesizing and evaluating others: branched fatty acids, branched and straight chain hydrocarbons, alkanoates, halogenated acids and their esters and finally the alkanamids (Hwang et al. 1974b, 1976ab, 1978, Hwang and Mulla 1976b, Mulla and Hwang 1975). A detailed review of the overcrowding factors, their ecological and physiological significance was recently published (Hwang and Mulla 1976a).

Thus it is for the first time that the chemistry of the toxic and growth retarding factors elaborated by larvae has been elucidated, and closely related as well as distant analogs and homologs of these have been synthesized and studied against mosquitoes. These natural products play an important role in the autoregulation of mosquitoes. In the very near future we will be initiating field studies on the activity of the most active factors and their analogs. Both acute and chronic effects as well as developmental impact of these factors will be elucidated in nature. It is hoped that with proper formulation techniques some of these naturally occurring chemicals can be proven to be good mosquito control agents. I would like to emphasize that the level of activity of these simple natural products will not compare with some of the highly effective synthetic insecticides. However, their potential activity is subtle and looks much better than some of the mosquito control agents used at the present time.

B–OVIPOSITION MODIFYING FACTORS. An important area of research dealing with the chemical ecology of mosquitoes is the relationship of self and trans-specific chemical agents regulating oviposition behavior of a variety of mosquitoes in various habitats. Once the general suitable and optimum breeding source is located by means of physical and chemical stimuli (Clements 1963), then the gravid mosquito is further directed for ovipositing by close range physical and chemical stimuli to the most optimum habitat where the young progeny are likely to thrive. Therefore, breeding sources which seemingly appear identical are not equally suitable for oviposition and thus survival of mosquito larvae.

Mosquito breeding activity is highest in habitats that are subjected to intermittent flooding and wetting and drying. Rarely do we see heavy breeding (except in a few situations) in permanent bodies of water and certain types of temporary sources. The absence of mosquito breeding in these sources is generally believed to be due to the presence of ample and diverse fauna of biotic regulating agents. Although this may be true to a certain extent, there is no evidence to fully support this hypothesis under most breeding source conditions. To prove or disprove this notion one has to really document the presence and ovipositional activity of gravid mosquitoes. Is it that the mosquitoes are not attracted for oviposition to permanently flooded sources or is there ample oviposition but no survival of the ensuing larvae. These types of basic biological and ecological questions have not been made the subject of any systematic and detailed investigations. Now that basic chemical and ecological investigations on the complex ovipositional behavior of diverse species of mosquitoes have been initiated at the University of California at Riverside and other institutions, hopefully, it will be possible in the near future to provide plausible answers to some of these perplexing questions.

Evidence to date point out that gravid mosquitoes do not deposit their eggs at random, but rather they can discriminate between preferred and suboptimum sites. At close range this preference is mediated through the presence of volatile and nonvolatile chemical stimuli acting on chemoreceptors which in turn result in positive or negative ovipositional responses. Practically all the studies conducted to date have been directed toward the elucidation of oviposition attractants (including stimulants). It has been only in the last year or so that we have successfully isolated and identified chemical stimuli which act as oviposition repellents (including deterrency), inducing negative ovipositional responses in *Culex* mosquitoes (Hwang et al. 1979, Kramer and Mulla 1979).

1. Soil and Organic Pollution Factors. - - The biological, physical and chemical nature of a breeding source undergoes a dynamic and continuous change during the postflooding period. Although these changes are not clearly known, we know that there is no or little oviposition by our *Culex* species during the first few days of postflooding. The breeding source becomes attractive to ovipositing females 7-10 days after flooding. The attractancy of the breeding source to gravid females for oviposition declines drastically after prolonged period of flooding. This initial attractancy or no attractancy of breeding sources was believed to be due to emanation of chemical factors produced by the degredation of organic plant and animal matter in the aquatic medium or in the soil. It is through the production and certain concetrations of these species or group specific volatile substances that mosquitoes can discriminate between brdding sources. Some researchers as early as 6 decades ago pointed out to the selective discrimination of breeding sites by ovipositing females (Baettie 1932, Gerhardt 1959, Herms and Freeborn 1921, Macan 1961, Muirhead-Thomson 1940, 1941).

In nature and in laboratory, mosquitoes are either attracted or repelled by certain infusions of organic matter acted upon by microorganisms. Hay, and grass infusions induce more oviposition than distilled water in *Cx. quinquefasciatus (= fatigans)* (DeZulueta 1950, Jobling 1935) and grass infusion was also found to be attractive to *Cx. tarsalis* females (Gjullin et al. 1965) and *Ae. albopictus* and *Ae. polynesiensis* (Gubler 1971). Rice straw infusions were found attractive to *Cx. p. pallens* (Hayashi 1962, Ikeshoji 1966, Oda 1967).

Decomposition of certain organic matter in water yield volatile chemiclas which attract and induce gravid female mosquitoes to oviposit. In this regard horse manure infusions were highly attractive to *Cx. fatigans* and *Ae. aegypti* (Manfield 1951, O'Gower 1963), and chicken manure infusions were highly attractive to *Cx. p. pallens* (Hayashi 1962). Water in breeding sources which are rich in organic matter is attractive for many species of mosquitoes. The tree-hole mosquito *Ae. triseriatus* preferentially oviposit in tree-hole water (Bentley et al. 1976, Wilton 1968), while *Ae. aegypti*, *Ae. albopictus* and *Ae. vittatus* prefer water with floating timber (Kochhar et al. 1972). *Cx. quinquefasciatus* and *Cx. tarsalis* prefer to oviposit in log pond water (Gjullin and Johnson 1965). Woodcreosote, a constituent of many trees, is shown to act as ovipositional attractant for *Cx. quinquefasciatus* (Gjullin 1961) and a number of other species of mosquitoes.

In general the production of bioactive compounds in mosquito breeding sources affecting oviposition has been linked with the action of bacteria and possibly other microorganisms. The bacterial involvement was suggested and documented by several authors (Gerhardt, 1959, Kramer and Mulla, 1979, Murphey and Burbutis 1967). Hazard et al. (1967) isolated *Aerobacter aerogenes* from a hay infusion attractive to *Cx. p. quinquefasciatus* and *Ae. aegypti* and those in the family Pseudomonadeceae were shown to be responsible for the production of oviposition modifying chemicals (Ikeshoji et al. 1967, Maw 1970). As a follow up of these studies, Ikeshoji et al. (1975) identified the bacteria *Pseudomonas aeruginosa* to be responsible for the production of oviposition attractants for *Ae. aegypti* and *Cx. p. molestus* on a substrate of capric and pelargonic acids. They suggested that the attractants are the intermediate breakdown products and metabolites from these fatty acids.

Although a number of studies on the attractancy and stimulancy of organic infusions and bacterial cultures have been conducted, very few or no detailed studies on oviposition repellents have been carried out. DeZulueta (1950) and Muirhead-Thomson (1941) noted that *Anopheles darlingi* and *An. minimus* are repelled by infusions characterized by high organic content. Ikeshoji et al. (1967) postulated that bacteria may also be capable of producing not only attractants but also mosquito oviposition repellents. This aspect of the chemical ecology has been neglected by researchers until our recent studies elucidating the repellent nature of some organic infusions to *Culex* species (Kramer and Mulla 1979) and the isolation and identification of the authentic repellent compounds produced in some organic infusions (Hwang et al. 1979). These repellents are effective in certain ranges of concentration, producing no repellency at below these concentrations.

2. Egg Pheromones. - - Another group of chemicals that can influence oviposition behavior of a number of mosquitoes are those produced during oviposition and these volatiles emanated from the eggs or egg rafts are called oviposition pheromones. These egg associated phermones have been detected in *Cx. tarsalis* (Osgood 1970), *Cx. p. quinquefasciatus* and *Cx. p. pipiens* (Dadd and Kleinjan 1974, Starratt and Osgood 1973). Existence of these pheromones has also been suggested for *Ae. albopictus* and *Ae. polynesiensis* (Gubler 1971). The oviposition pheromones of *Cx. tarsalis* have been identified chemically as a mixture of 1, 3 diglycerides (Starratt and Osgood 1972). The active fractions of oviposition pheromones from *Cx. p. quinquefasciatus* and *Cx. p. pipiens* were not distinguishable from each other by spectrometric and thin layer chromatographic methods (Starratt and Osgood 1973).

3. Oviposition Factors of Larval and Pupal Origin. - - Still a third group of chemicals that modify mosquito oviposition behavior are those related to larval and pupal presence. These factors significantly affect oviposition. The presence of larval factors have been shown for a number of *Aedes* species (Bentley et al. 1976, Kalpage and Brust 1973, McDaniel et al. 1976, Soman and Reuben 1970), and in *Culex* species (Dadd and Kleinjan 1974). Pupal produced factors have also been reported for a number of species of *Aedes* (Kalpage and Brust 1973, Soman and Reuben 1970). These factors have also been reported for *Culex* species (Andreadis 1977, Dadd and Kleinian 1974, Hudson and McLintock 1967). To date the chemical nature of these pheromones has not been elucidated.

It should be pointed out that egg associated pheromones and larval and pupal produced pheromones for one species may not work for another. Although cross activity has been shown for some species, there is complete discrimination in response to these pheromones by other species.

C--ALGAE AND ALGAL TOXINS.- Planktonic and filamentous algae are most common cohabitants with immature mosquitoes. Some of these exert profound regulating pressures on mosquitoes. To date, no in-depth studies have been undertaken to exploit the potential of algae as biological control agents for mosquito vectors.

We have recently found 2 species of green algae (*Chlorella ellipsoidea* and *Rhizoclonium hieroglyphicum*) which are as important biological control agents for mosquitoes. The fillamentous species grow as a thin mat at the bottom of aquatic habitats and has many desirable features in using nutrients from eutropic water, keeping the water clean and precluding the growth of undesirable planktons and macrophytes. It decimates noxious aquatic insects, as well as immature mosquitoes.

The green alga *C. ellipsoidea* which has been recently found in container habitats during the course of our studies on mosquitoes in a large urban cemetry, exert significant regulating pressure on mosquitoes. Supernates and suspensions of this alga have been found to induce immediate and delayed mortality in mosquitoes. Containers devoid of this alga, when inoculated, became positive (70%+), remained so for weeks, and eliminated Culicine mosquitoes. This alga has broad tolerance to a variety of factors and is readily culturable, requiring inexpensive and unsophisticated equipment and facilities. It is possible that these agae, their supernates or the chemical factors they produce may be employed in future mosquito control programs.

With these encouraging results, a systematic research program has been implemented to study: (a) Biological activity of these 2 and other algal species against various species of mosquitoes, (b) Determine the occurrence of these algae, as well as other biologically active species in a variety of breeding sources, (c) Evaluate the potential of algae as biocontrol agents against culicines under field conditions, (e) Develop simple mass-culturing procedures for the most promising entities for use in field situation, (f) Isolate and chemically identify the transspecific chemicals produced by algae which regulate populations of immature mosquitoes.

This area of research has been very promising and productive. Research information on the biological characterization of the two algae, their toxins and fractionation will be presented by Mr. M. S. Dhillon in this conference. About 300 mg of crude extract from some 50-60 litres of active suspension of *C. ellipsoidea* has been obtained. The extract shows high level of activity and contains principles which produce quick and delayed mortality in the immature stages as well as inducing marked morphogenetic changes during emergence. The adults resulting from larvae treated with sublethal dosages show bizzare morphological aberrations and even those adults which seem normal in appearance are incapable of flight.

The crude extracts of bioactive principles of algae is now ready for chemical separation, purification and identification. Dr. Y.-S. Hwang is engaged in this chemical research and hopefully will be able to at least place the active compounds in chemical groupings and finally have the chemicals identified. Once the chemical nature of the algal toxins is known, it will then open a new area of research to study the nature of ecological interaction between algae and mosquitoes.

D--SUMMARY.--The current status of our knowledge regarding the chemical ecology of the immature mosquitoes is reviewed and the information is interpreted within the limits of available data. There is ample evidence that certain chemical principles associated with eggs, larvae and pupae of mosquitoes act as auto promoting and regulating principles. Egg assocated pheromones and some factors elaborated in larval and pupal cultures provide stimuli for positive response in gravid mosquitoes. On the other hand larval factors produced under overcrowded conditions are toxic and retard development of the very young progency, thus providing an autoregulating mechanism for population in overutilized habitats. During the course of our research we have made siginificant contributions to an understanding of this complex ecological phenomenon. The active principles involved in overcrowding ecology have been chemically identified, analogs synthesized and their activity determined. Field trials with the most active principles will be initiated in the near future.

Although many workers have noted the relationship between organic infusions and mosquito oviposition, very little is known regarding the chemical nature of the transspecific chemicals produced in these infusions. These chemicals have been tentatively identified for only one or two species of mosquitoes. In the past 2-3 years we have conducted systematic research on mosquito oviposition attractants and repellents. The active principles involved in negative mosquito oviposition behavior have been identified chemically and their repellency effects fully documented. The repellent factors were identified as short chain fatty acids, and since most are commercially available, their role and potential in field ecology of mosquitoes will be investigated. Although we have been successful in chemically identifying oviposition repellents produced in organic infusions, the attractant principles have not been identified as yet. Studies in this direction are in progress now.

Another important area in the chemical ecology of mosquitoes is a study on algae and algal toxins as biocontrol agents. Our progress in this area of research has been quite promising and we are now working towards the chemical identification of various toxins produced by algae. These toxins show various modes of action in mosquito larvae and from the studies conducted thus far, it appears that probably 2 or more compounds are involved in shaping the interaction between the algae in question and mosquitoes.

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MOSQUITO CONTROL PROBLEMS ON WILDLIFE AREAS – A CASE HISTORY:

GRAY LODGE, BUTTE COUNTY, CALIFORNIA

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Each fall 10 million ducks and a million or more geese migrate down the Pacific flyway into California and are in need of food, rest and water. In 1906 a survey by the US Department of Agriculture showed that almost 3½ million acres of wetlands were existent at that time within the State. It is currently estimated that less than 500,000 acres now remain. Of this, about 225,000 acres are in private duck clubs, 200,000 acres in Federal wildlife refuges, and about 50,000 acres in wildlife area operated by the California Department of Fish and Game.

The extreme importance of the wetland habitat to the continued survival and well being of the waterfowl making this annual migration is only too obvious. However, the importance and function of the wetland habitat provides for more than just the natural preservation purposes for the birds; there are also major economic considerations. In addition to the benefits of the hunting provided, a prime purpose of the refuge system is the protection of adjacent cropland from depredation, the significance of which has been well demonstrated in the past.

Both the U. S. Fish and Wildlife Service and the California Department of Fish and Game have recognized that the flooding of these various wetlands has led to significant problems with mosquito production, resulting in the adoption of policies relating to when and how mosquito control should be carried out. One statement in such developed policies is: "Since mosquito control programs may adversely affect fish and wildlife, they should be planned and executed with full consideration given to the protection of fish and wildlife values". I agree with this policy, but I also believe that the converse should be stated. This is that "since wetlands management practices may adversely affect human health and comfort they should be planned and operated with full consideration given to the protection of human health and welfare." Such consideration has not always been provided and probably will not be fully accepted as a game management philosophy for many years, if ever. Happily, though, progress is being made toward the realization that mosquito prevention is a very critical part in the operation of some wetland waterfowl habitats.

The GrayLodge Wildlife Area, located in the middle of the Sacramento Valley has been a source of varying degrees of controversy regarding its contribution to the mosquito population of the area for more than twenty years. I cannot tell you that we have found a sure fire, quick and easy solution in its management that will serve to stop mosquito production on Wildlife Management Areas because I don't believe that there is one. However, we have been able to get started on a program which I believe has already produced some tangible results.

In 1931 the State of California purchased the 2500 acre GrayLodge Gun Club located in southern Butte County just north of the Sutter Buttes. This was operated as a sanctuary until 1953 when public hunting was first allowed. Between 1952 and 1955 additional land purchases increased the acreage to approximately 6800 acres and subsequent additions have brought it up to over 8000 acres. Part of the area is maintained as so-called permanent ponds (600+ acres) and several hundred acres are summer irrigated for millet production. Milo, corn and other cereal crops are also produced as food for wildlife. Agriculture in this part of the Sacramento Valley is primarily rice and irrigated pasture. Along the west and southwest boundary of GrayLodge are thousands of acres of privately owned duck clubs in an area known as the Butte Sink.

Three mosquito abatement districts share the burden of the mosquito control problems in this area. Most of the wildlife area lies within the Butte County MAD and much of the adjacent duck club area is in the Colusa MAD. The Sutter-Yuba MAD shares a small portion of the refuge plus sizable acreages of duck club, pasture and rice in the adjoining properties.

Four species of mosquitoes, *Culex tarsalis*, *Anopheles freeborni*, *Aedes melanimon*, and *Aedes nigromaculis* are produced abundantly during summer. The major mosquito problem, however, is during the late summer and fall, created by flooding of approximately 5000 acres of refuge land and 12-14,000 acres of nearby duck club property prior to the arrival of migrating waterfowl.

In the late 1950's residents of the nearby community of Pennington complained to the Sutter-Yuba MAD about the mosquito problem in their area. The MAD asked the Vector Control Section of the State Department of Health Services for assistance in determining the source of these mosquitoes. The study that was carried out as a result of this request indicated a probability that the GrayLodge Wildlife Area was a major contributor to the mosquito population. Obviously, in light of the type of agriculture and the expanse of duck club land, the Wildlife Area was not the only source. A variety of recommendations were made concerning water management, land preparation and other mosquito control techniques. Data collection and various studies continued over the years but GrayLodge continued to be a major mosquito producer while much of the surrounding area came under increasingly intensive control. A token effort at *Gambusia affinis* production and use was initiated but no real progress was made in either direct chemical or biological control or in land or water management practices which would affect mosquito production.

In 1975 the mosquito abatement districts, the Department of Fish and Game and the State Health Department were again stimulated by demands from residents of the community of Pennington for relief from the hordes of mosquitoes invading the area. Initial investigations were begun in 1976 but progress was slow. In January of 1977 a planning meeting was held between the MADs, Fish and Game, and the Vector Control Section. Three MAD managers, eight Fish and Game staff and seven Vector Control Section biologists and engineers attended. During the meeting it became clear that no simple solution was available but it was also evident that all the agencies including the Department of Fish and Game were determined this time to tak positive action toward the following two goals: (1) to provide immediate control over fall-emerging *Aedes* and (2) to develop a plan of long range action to reduce mosquito production on GrayLodge.

Jim Leiby, Chief of Operations for Department of Fish and Game, suggested the formation of a task force to develop the needed plan. Named to this task force were Trev Wright, Regional Wildlife Management Supervisor, Gene Kauffman, Manager of the Sutter-Yuba MAD, Bill Hazeltine, Manager of the Butte County MAD and me as representative for the California Department of Health Services. Ken Whitsell, John Cowan, Dick Husbands, and Reuben Junkert served as advisors and contributed greatly to the final plan. April 1 was set as the date for completion of a draft to be presented to the full committee.

During the following three months the task force met several times and developed a series of twenty recommended actions covering four categories: 1) management practices such as vegetation control, irrigation, field design, water control structure design and maintenance; 2) investigational activities including Gambusia
including *Gambusia* usage, pesticide strategies, and field design; 3) chemical control including pre-flood application of granular insecticides, adulticiding, and selective larviciding; and 4) population monitoring and assessment of program success. On April 11, 1977, these proposals were presented to the full committee and after considerable discussion and some revision, were accepted. Following this a cooperative agreement between the California Department of Fish and Game, California Department of Health, Butte MAD and Sutter-Yuba MAD was formulated. This agreement detailed the specific responsibilities of each agency in carrying out the recommendations included in the plan.

During 1977 action was initiated on most of the recommendations with varying degrees of success. Two very direct control activities which produced encouraging results involved the use of a pre-flood applications of Dursban granules and the use of *Gambusia affinis* for *Aedes* control. The Department of Fish and Game purchased three tons of 2% chlorpyrifos granules which were applied to 3000 acres of the GrayLodge Wildlife Area scheduled for fall flooding. Application began on August 18 and was completed on August 24. Results of this treatment were generally good but a number of conditions such as dense vegetation, high levees and difficulties in marking swaths contributed to incomplete control in certain fields. A considerable amount of "mop up" treatment was done by the Butte MAD.

A very successful demonstration of the effectiveness of *Gambusia* against *Aedes* larvae was carried out by the Sutter-Yuba MAD. A 100-acre field divided into two sections by a levee was stocked with *Gambusia* at 2 and 4 pounds/acre shortly after it was flooded in late August. An adjacent field used as a control produced *Aedes melanimon* at up to 500 larvae per dip. Larvae in the *Gambusia*-stocked field were restricted to 3-4 per dip in small isolations along the edges of the field.

Judging by the subjective observations of neighboring residents and the data from light traps operated on and around the Wildlife Area, mosquito populations were dramatically reduced. These two techniques seemed to offer a practical means of reducing mosquito production to acceptable levels on a temporary basis while attempts were being made to develop land preparation and water and vegetation management practices compatible with wildlife propagation and mosquito prevention.

Proceeding along this line a plan was developed for 1978 which included variations on the methods of use of the granular insecticide and further tests on the use of *Gambusia* under a variety of conditions of field preparation.

Studies by Bob Coykendal and Gene Kauffman indicated that fish stocking rates of 1 pound per acre should give adequate control of *Aedes* in this habitat. They prepared a detailed proposal for producing the needed fish in ponds on GrayLodge.

Near the beginning of the 1978 season we were fortunate enough to be able to enter into a cooperative agreement with the Department of Fish and Game to finance a grant to Gordon Hanna. He spent the summer and fall at GrayLodge analyzing the effectiveness of some of the techniques proposed in our 1978 plan.

The data that he has accumulated this past year reinforces our belief that a combined program of chemical, biological, water and land management practices can reduce mosquito production on GrayLodge and still be compatible with wildlife management objectives.

DYNAMICS OF *GAMBUSIA AFFINIS* IN FRESNO COUNTY RICE FIELDS

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ABSTRACT

Research on three projects was carried out in 1978. Two of the projects, the effects of different stocking regimes on the seasonal distribution patterns of *Gambusia affinis* in rice fields and the diel and seasonal movement patters of the fish were continuations of studies begun in 1977. The third study, a comparison of *G. affinis* populations from two stocking rates, was begun in 1978.

SEASONAL DISTRIBUTION. Fish were stocked by the following methods:

1. The total field allotment divided into equal portions and stocked in the even numbered paddies (2, 4, 6, etc.).
2. The total field allotment divided into three groups and stocked in the second paddy, the center paddy and in a paddy approximately half way between the center paddy and field outlet.
3. The total field allotment stocked into the second paddy.

Fish trapping indicated there was no significant differences in total fish production among fields stocked by each of the three stocking methods. There were, however, significant differences in the distribution of the fish. Fields stocked in the even numbered paddies and those stocked in three places had fairly even fish distribution throughout the fields (as indicated by trapping). Where even distributions did not exist, no consistent pattern could be seen. Fields which were stocked in only one place in the field (second paddy) consistently had very high fish populations in the first few paddies but the numbers dropped dramatically toward the drain end of the field. The data indicates that the bulk of the fish stocked in a rice field do not move very far from the original stocking point. The progeny of the original stock do spread throughout the field, but development of large populations of fish away from the stocking area is delayed due to the lag time between the progeny inhabiting the new areas of the field and their reaching sexual maturity and reproducing. The result is that field-wide mosquito control potential is diminished when fish are stocked in only a single stocking point within the field.

DIEL AND SEASONAL MOVEMENTS.–Results of 1977 and 1978 studies using outflow traps to collect mosquitofish leaving the rice field outlets indicate a large migration of fish beginning 40 to 50 days after stocking and lasting 25 to 35 days. The migration was evident in four of the five fields studied in 1977 and seven of the nine fields studied in 1978. Outflow trap catches ranged from several hundred to several thousand fish per day and varied with the day and the field. The maximum catch recorded was 7,000 fish in a 24 hour period. During the height of the migration, several hundred fish were caught where no water was flowing through the outlet box; the fish had to jump over the weir board and into the trap.

The mosquitofish leaving the fields during the migration period were predominantly young unfertilized females ranging in length from 17 to 35 mm (mean = 23.7 mm, standard deviation = 2.89 mm). The movements do not correlate with temperature fluctuations, lunar cycles, or any other external factor, and may represent an inherent distribution mechanism. The primary result of the migration is a lower than expected total fish population in the field late in the season.

Diel movements were studied by trapping throughout the day and night. It was found that *G. affinis* movements are confined almost exclusively to the daylight hours. Hourly trapping through the daylight hours indicates that the activity cycle is roughly bell-shaped with a peak between 1200 and 1400 hours. Trap counts were near zero at dawn and dusk.

STOCKING RATE EVALUATIONS. Fish populations (as measured by trapping) were monitored in ten fields stocked at 0.25 pounds per acre and ten fields stocked at 0.125 pounds per acre. Population curves for both stocking rates were sigmoid which is expected for fish introduced into a virtually non-limiting environment, and then nearing carrying capacity late in the summer. Trap counts indicated mean mature *G. affinis* populations in June were twice as high in the 0.25 pound per acre fields as in the 0.125 pound per acre fields. However, mean populations in August were nearly the same in fields stocked at both rates. This result is also expected since the *G. affinis* populations in the 0.25 pounds per acre fields neared the carrying capacities of the fields more quickly than those stocked at 0.125 pounds per acre. This study is a part of a larger investigation to determine stocking rates which give adequate *G. affinis* populations at critical times during the mosquito season.

ECOLOGICAL IMPACT OF MOSQUITO CONTROL RECIRCULATION DITCHES ON SAN FRANCISCO BAY MARSHLANDS: PRELIMINARY CONSIDERATIONS AND EXPERIMENTAL DESIGN

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Public awareness and concern about the fate of coastal marshes and wetlands has increased greatly during the past two decades. This has paralleled the increase in knowledge regarding the role of these marshes in nutrient cycling, filtration, and primary production (Correll 1978). Marshlands are particularly vulnerable to capricious development because many of the real values of tidal marshes are not easily recognized, or accrue some distance from the marsh itself (Gosselink et al. 1974). For example, trapping of eroded sediments and production of particulate organic matter for filter feeders are essential but not easily observed benefits.

Until recently the marshlands of the San Francisco Bay were treated with little regard to their importance as a natural resource. In fact, over the past century, activities such as filling for urban development and diking to form salt ponds have eliminated over 60% of the original salt marsh area bordering San Francisco Bay (Nichols and Wright 1971). Because of the importance of these marshlands to life within the bay and beyond, both regulatory agencies and conservation groups have shown considerable interest in protecting the remaining lands.

Some of this interest has been directed to the physical control practices for salt marsh mosquitoes. Until fairly recently, post-World War II mosquito control practices in the United States consisted of a preventive schedule of pesticide applications. Evidence suggests that such methods were not only insufficient, but were both expensive and environmentally unsound (Provost 1977). This influenced local Mosquito Abatement Districts to return to a previously used method of source reduction, recirculation ditches. Ditching techniques have been expanded and improved (Figure 1) since J. B. Smith first suggested them in 1904 (Provost 1977). Most of the refinement of ditching techniques has been done in Atlantic coastal marshes, which comprise a much more extensive area than west coast marshes.

The purpose of this project is to evaluate the impact of recirculation ditches on various aspects of salt marsh ecology. The approach taken in developing the experimental design for the study has been to analyze the most fundamental and definable relationships, those directly subject to the physical changes caused by recirculation ditches, and to examine their influence on basic biological processes such as productivity and diversity of marsh biota.

Critical studies on the ecological impact of ditching have previously focused on plant succession (Miller and Egler 1950), primary production (Shisler and Jobbins 1977), food and shelter for birds, mammals, and invertebrates (Bourn and Cottam 1950), and general hydrography (Connell 1940). Impact on these biological components has been attributed to both lowering of the water table and accidental impoundment of water behind turf lines (Chapman 1960). However, all of

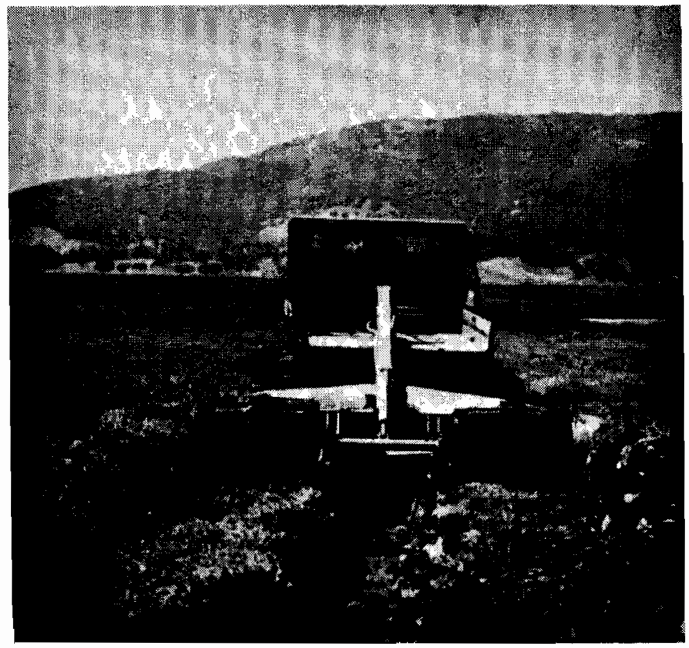

Figure 1.—Spryte ditch digger cutting a recirculation ditch through the Petaluma marsh.

these studies were limited to the Atlantic coast where soil and vegetation types are often very different from those found on the Pacific coast. This project has not merely repeated, but rather has expanded such studies of impact on non-target organisms to west coast marshlands.

This study is a joint effort between several faculty and students of the University of California, Berkeley and personnel of the California Mosquito and Vector Control Association Coastal Region Mosquito Abatement Districts. The extensive cooperation offered by the Mosquito Abatement Districts of the Coastal Region and by Dr. E. I. Schlinger, Dr. R. Garcia, and Mr. M. Barnby, of the University of California, Berkeley, has been invaluable to this project.

CHOICE OF STUDY SITES.—The San Francisco Bay, like all estuarine environments, is characterized by increasing salinity toward the ocean. These salinity differences, along with relative height above mean low water, greatly affect plant community composition (Chapman 1960, Hinde 1954). The two major "high" marsh communities in the San Francisco Bay Area are those dominated by 1) pickleweed, *Salicornia virginica* L. and 2) saltgrass, *Distichlis spicata* (L.) and wire-grass, *Juncus balticus* Willdenow. We have chosen marshes along the Petaluma River and along Albrae Slough in South San Francisco Bay as sites representing the first marsh type. The second type is represented by a tidal marsh at the base of Potrero Hills in northern Suisun Bay (Figure 2).

EXPERIMENTAL DESIGN.–Pilot studies, field sampling programs, and data analysis have been designed to extend over a four-year period (Figure 3) and include examination of invertebrate and vertebrate fauna, marsh vegetation, and physical and chemical factors.

Arthropod Diversity - - As the dominant marsh animals, arthropods are a major source of food for both migratory and resident birds, including the rare and endangered California Clapper Rail and threatened Suisun Song Sparrow. Furthermore, arthropods have proven to be the most efficient and reliable indicators of environmental perturbation in other ecosystems (e.g. Kaesler et al. 1974, Wilhm 1972). We have just completed a one-year bi-weekly census of the terrestrial arthropods occupying both marsh types.

The terrestrial arthropod fauna of the salt marshes under examination is dominated by brine flies (family Ephydridae), leafhoppers (Family Cicadellidae), plant hoppers (family Delphacidae), mites, and spiders (both comprising several families). In previous studies of terrestrial salt marsh invertebrates, both specific populations (e.g. Nixon and Oviatt 1973) and whole communities (e.g. Cameron 1972, Davis and Gray 1966) have been examined. In this study, community structure is being analyzed by means of the Sequential Comparison Index

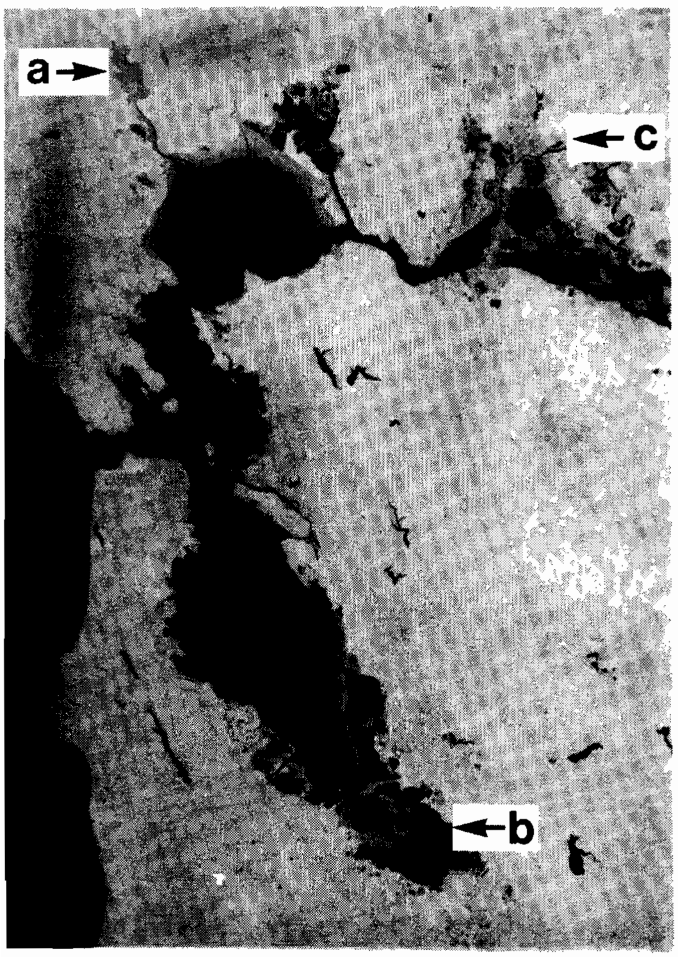

Figure 2.–Satellite photograph of San Francisco Bay Area showing location of study sites: a) Petaluma marsh; b) Albrae Slough marsh; and c) Suisun marsh.

(SCI Cairns et al. 1968), an approach not previously used for salt marsh communities. However, correlation analysis between calculated SCI indices and more traditional methods of analysis such as numbers of species (Figure 4) and diversity indices (Figure 5), indicate the suitability of the SCI for this type of analysis.

Community structure is being compared between ditched and natural sites at 1, 3, and 10 meters distance. Further studies will analyze the distribution and seasonal trends of the dominant species.

Vegetation Analysis - - The particular phenology of pickleweed, that of little or no senescence until late fall (Mahall and Park 1976) permits determination of annual growth with just two sampling dates. A pilot study was done to assess the required number of samples that must be taken to estimate standing crop at a desired degree of precision (Figure 6). Based on these results, an experimental design was developed in which standing crop was measured first in early March, 1978 to determine biomass of the perennial component, and again in late September, 1978 to measure annual growth. Samples were taken at 1 and 10 meters distance from the channel margin along ditches of 5 and 8 years of age and along a natural channel. Similar procedures are planned for the next two years to augment this past year's results.

A very different approach has been used on the *Distichlis-Juncus* tidal marsh. Because of the relatively higher diversity, seasonal succession, and year-round senescence of plants common to this marsh, sampling for annual biomass would be impractical. Instead, a quadrat sampling technique was used to determine occurrence and composition of species in relation to distance from ditches (Figure 7). Percent composition of species present was determined within 0.25 m^2 quadrats on transects perpendicular to the ditches. Samples were taken every 3 meters from 0-30m along 14 transects. This sampling program will be repeated seasonally for the next two years.

Aerial Photography - - By incorporating black and white, color, and infrared photographic techniques into the experimental design, initial reconnaissance of the ditched area in each of the marshes examined (e.g. Figure 8) was greatly facilitated. This approach is also being used to examine the recovery rates from vehicle activity associated with ditching (Figure 1).

Physical Factors - - Certain key physical features integrally related to salt marshes are also being examined. Measures of tidal regime, salinity, water table height, increases in channel volume, and rate of siltation, are either currently being taken, or are in the planning stages. Such measures are intended to complement biological studies as needed.

Fish Distribution and Feeding Analyses - - Studies on species composition and densities of fishes found in ditched and unditched areas of the Albrae Slough marsh were recently completed and have been described in detail elsewhere in these Proceedings (Balling et al. 1979). Trapping studies have shown that twice as many species used the ditched as compared to the unditched areas. Three resident fish dominated most samples and appeared year round. These fish, the mosquitofish, *Gambusia affinis* (Baird and Girard), the rainwater killifish, *Lucania parva* (Baird and Girard), and the stickleback, *Gasterosteus aculeatus* L., were also the dominant species in several other marshes sampled around the Bay. Population densities, especially of *G. affinis*, were almost 3 times greater in ditched than in unditched areas. Based on these studies,

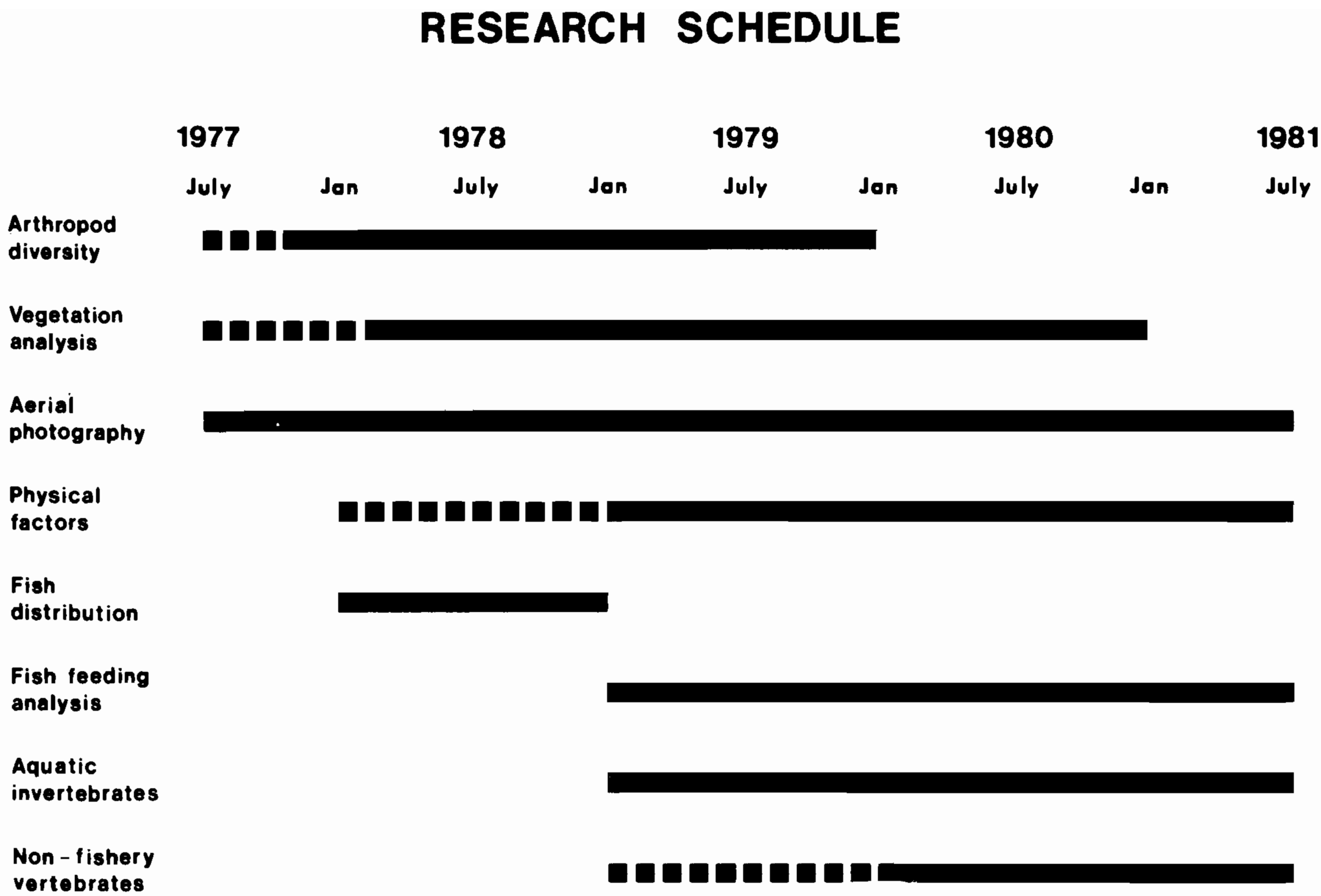

Figure 3.–Four-year research schedule for evaluating the ecological impact of mosquito control recirculation ditches on San Francisco Bay marshlands.

ditching does not appear to have a detrimental effect on salt marsh fishes. Analysis of fish feeding habits in relation to mosquito and other prey item distribution is underway.

Aquatic Invertebrates - - The distribution and abundance of invertebrates in potholes and ditches have a direct effect on the distribution and abundance of foraging fish and waterfowl. We are currently examining ecological features of selected invertebrate populations as a function of food availability, predators, physiographic characteristics, and salinity and tidal regimes.

Non-fishery Vertebrates - - Bird utilization of various sub-habitats of the marsh, i.e. sloughs, ditches, potholes and open marsh, is a vital concern. It is important to be able to predict the potential shift in species composition when a marsh with many small potholes becomes one with many small ditches. Bird counts within the above sub-habitats will be conducted this year in the Petaluma marsh which has a large mosaic of such environments.

CONCLUSION.- Recirculation ditches have proven to be a highly effective, low maintenance technique for control of salt marsh mosquito populations, yet the impact of recirculation ditches on west coast marsh ecology is largely unknown. An appropriate experimental design that analyzes the biological relationships directly linked to the physical changes in habitat caused by recirculation ditches may provide key information that clarifies the many unanswered questions regarding mosquito control practices in these ecologically important habitats.

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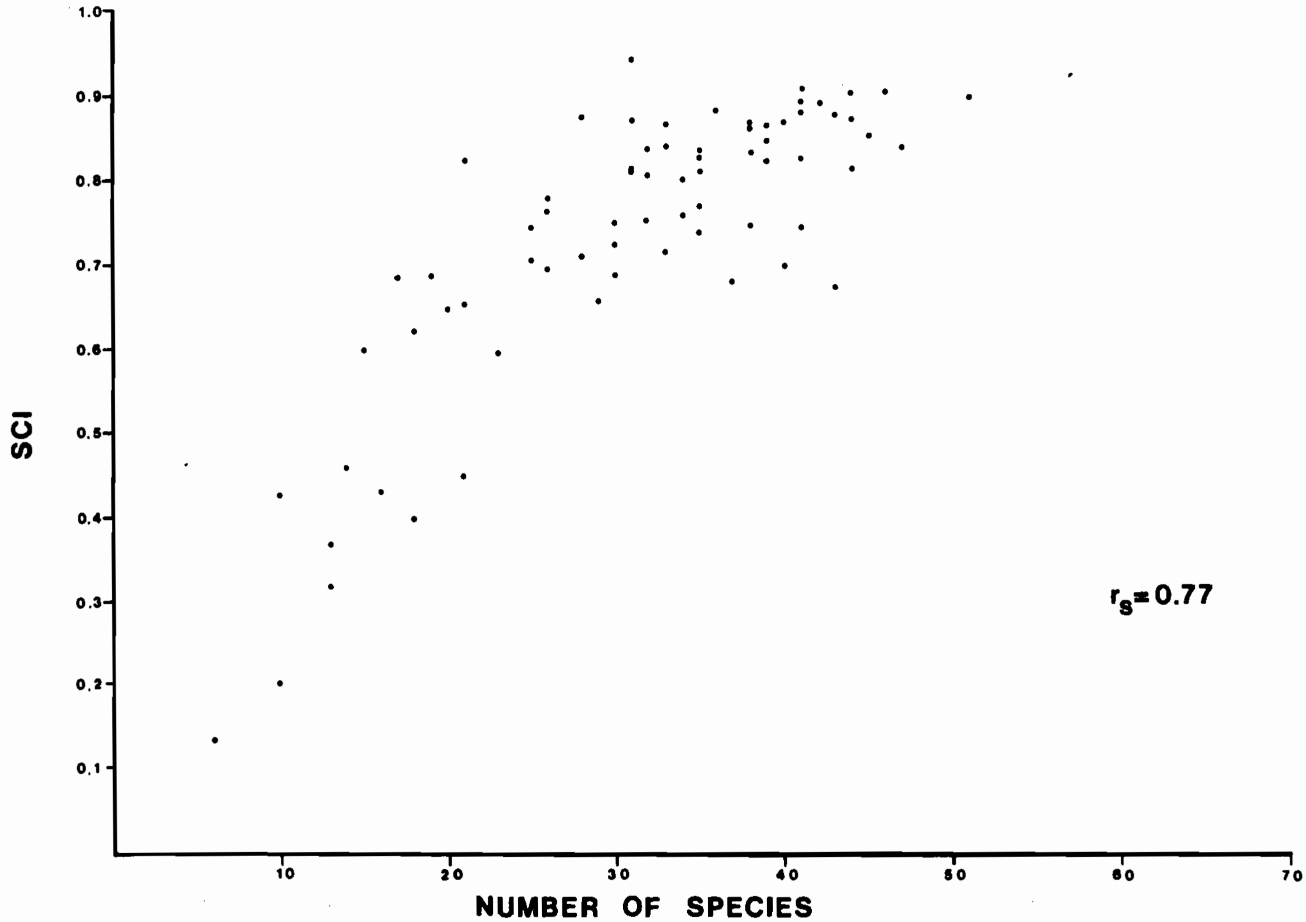

Figure 4.–Spearman rank correlation analysis of Sequential Comparison Indices and number of species based on 75 D-vac collections of arthropods from Petaluma marsh.

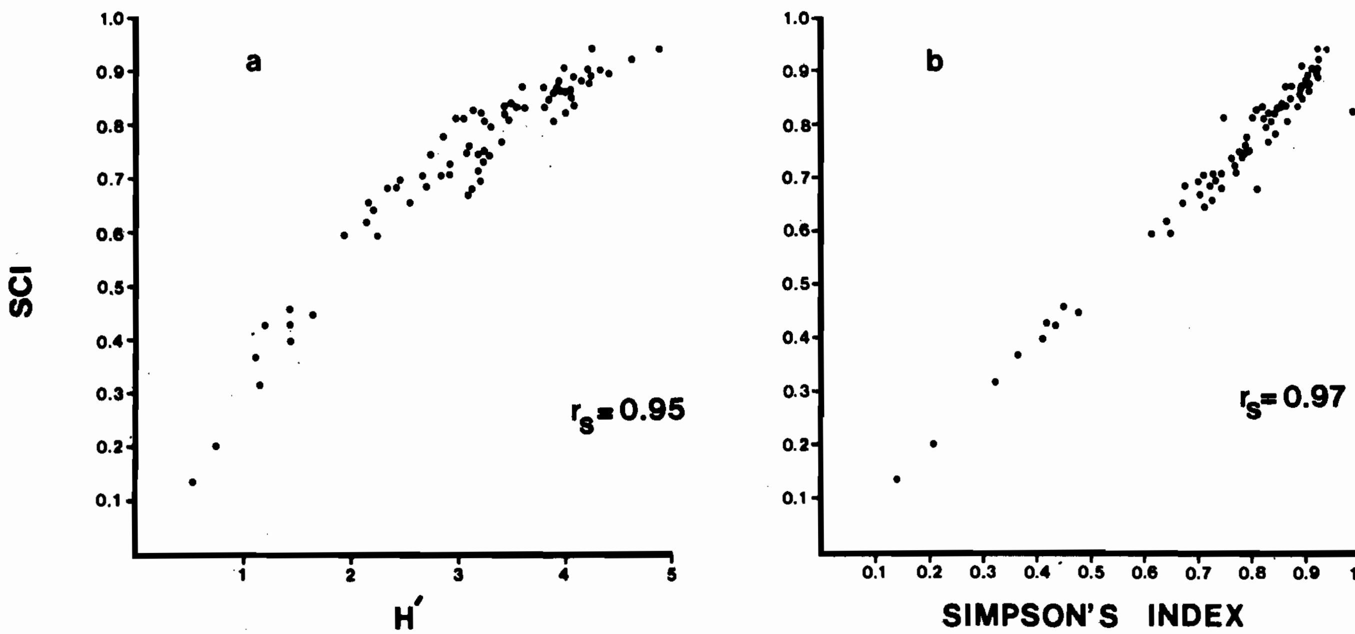

Figure 5.–Spearman rank correlation analysis of Sequential Comparison Indices and more widely-used diversity indices [H′ (Information Theory) and Simpson's Index, see Peet 1974 for formulae] based on collections described in Figure 4.

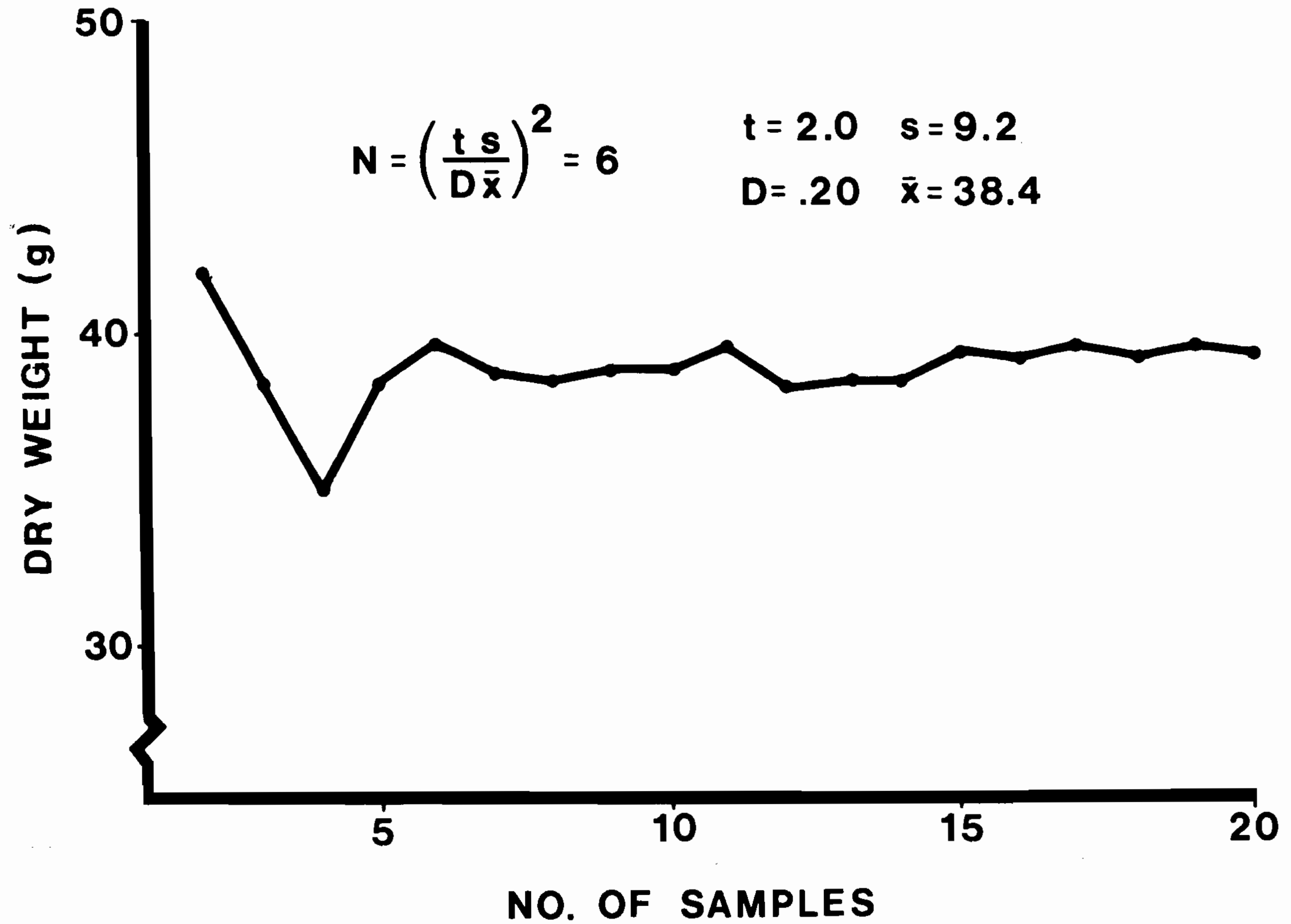

Figure 6.--Required number of samples to estimate pickleweed biomass in the Petaluma marsh based on a desired precision of 95% confidence limits within ± 20% of the mean.

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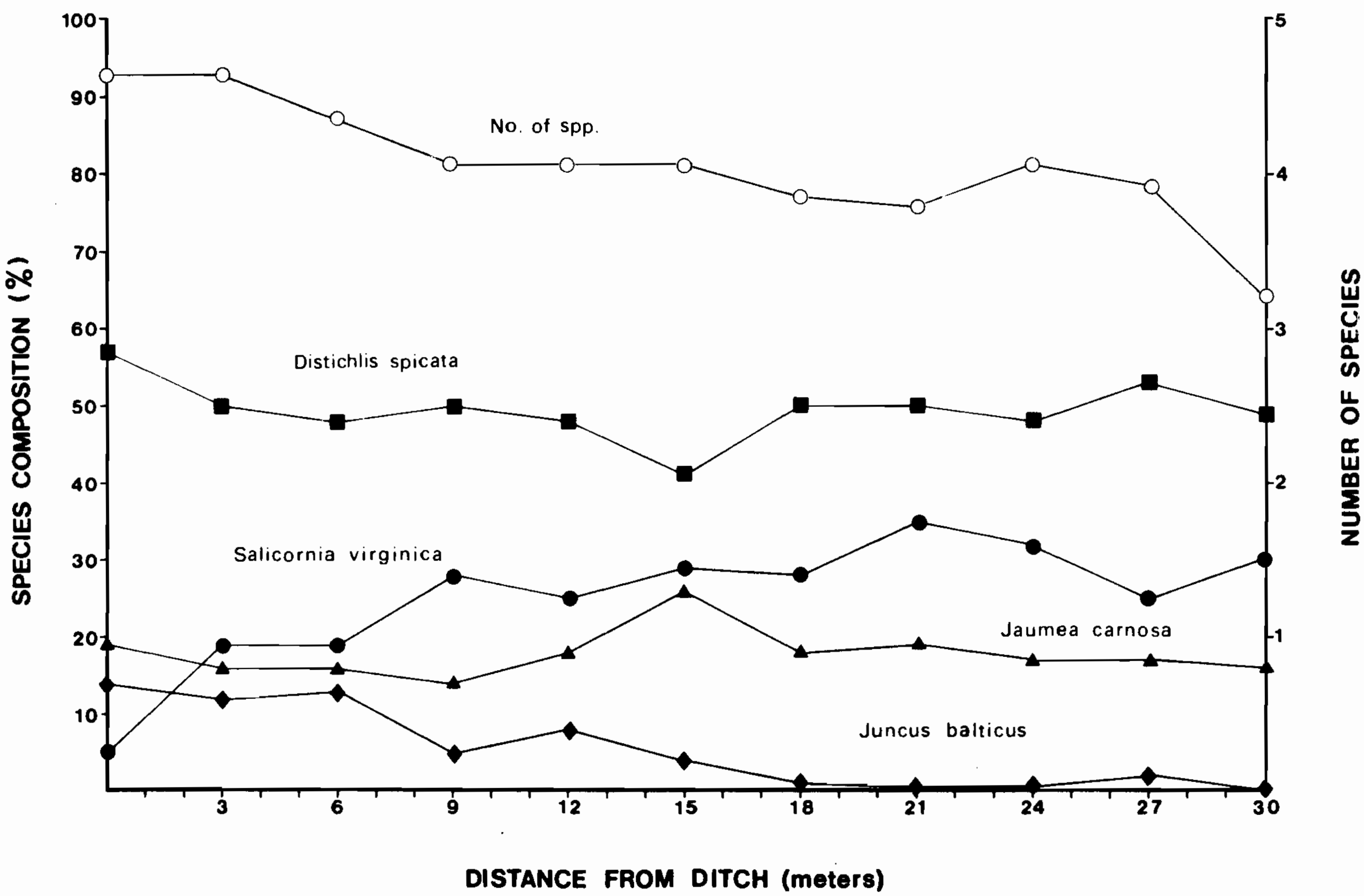

Figure 7.–Mean number of species and percent composition of vegetation in the Suisun marsh as a function of distance from recirculation ditches; means based on 14 transects, 8 September, 1978.

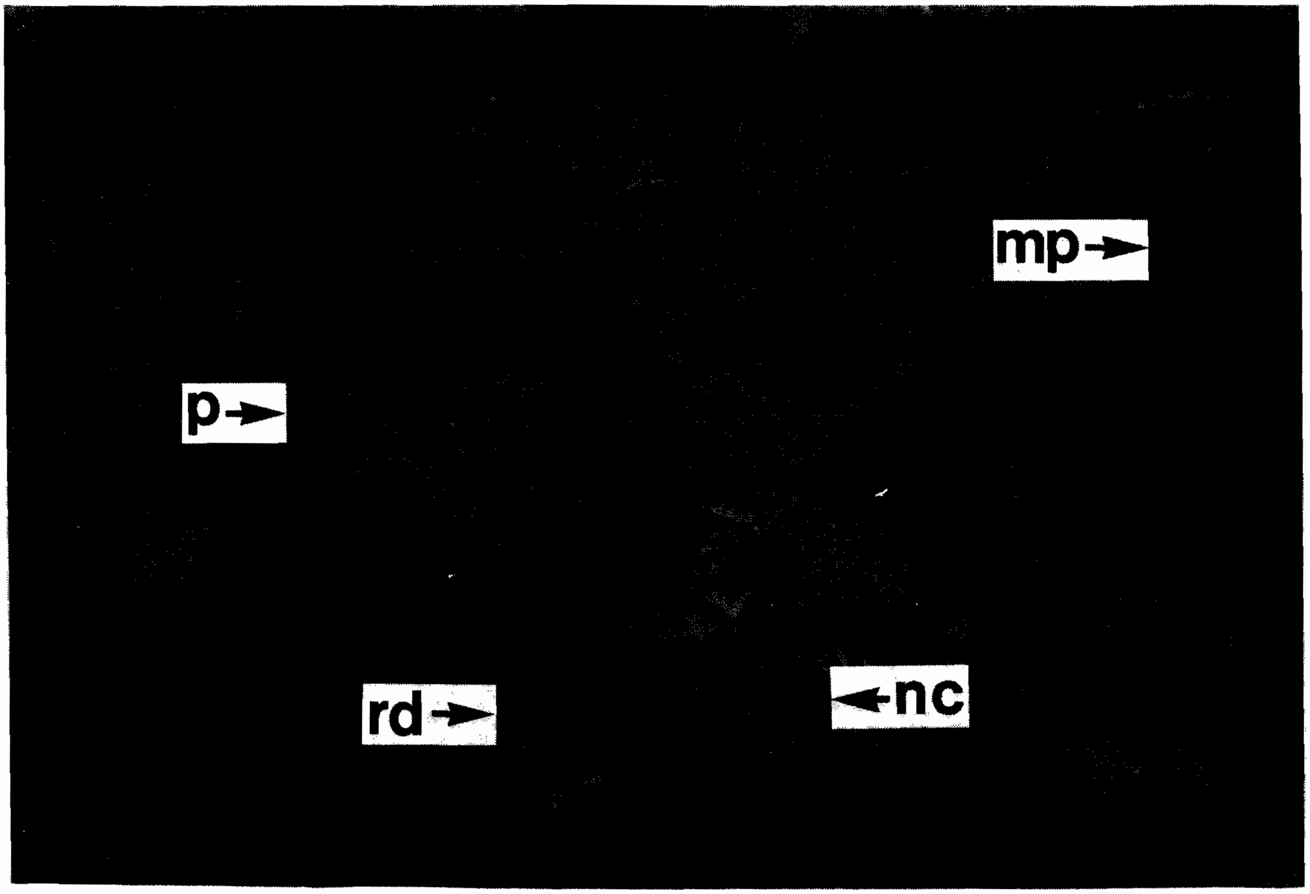

Figure 8.—Aerial photograph of Petaluma marsh showing recirculation ditches (rd) ,draining potholes (p) and flowing into natural channels (nc). Unditched marsh ponds (mp) are not mosquito breeding sites because of wind-action and natural predators.

COMPARATIVE DISPERSAL OF FIELD AND LABORATORY STRAINS OF *CULEX TARSALIS*[1]

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ABSTRACT

Mark-release-recapture studies were made of genetically modified and field populations of ***Culex tarsalis*** Coquillett at an isolated area in Kern County, California. Recapture data indicated that dispersal and survival were superior for field mosquitoes.

[1]This research was supported in part by Research Grant Al-03028 from the National Institute of Allergy and Infectious Diseases, and by General Research Support Grant I-S01-FR-05441 from the National Institutes of Health, U. S. Department of Health, Education, and Welfare.

A COMPARISON OF THE EFFECTIVENESS AND EFFICIENCY OF THREE LARVAL SAMPLING DEVICES

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INTRODUCTION.—Christensen and Washino (1978) compared 3 immature mosquito samplers: the standard aquatic dip net, a 50 square inch plastic tub and the standard one pint dipper. When *Culex tarsalis* and *Anopheles freeborni* were sampled, the dip net had the highest mean number of larvae per unit sample, followed by the tub, and then the dipper. However, the tub was found to be the most precise device since the variation about its mean was the least of the three methods.

As a continuation of that work, this study focuses on the useful and practical aspects of various sampling devices when sampling for *Culex* and *Anopheles* species in rice fields.

The following three sampling devices were tested with respect to their efficiency and effectiveness: the dipper, the tub and the area sampler. Since efficiency is defined as producing a desired effect with a minimum of effort, expense, or waste, an efficient sampling device is one that catches numerous larvae and pupae with a minimum of effort. An effective device is one capable of catching mosquitoes; the more effective the device, the greater the number of mosquitoes it will catch.

MATERIALS AND METHODS. The dipper was the standard one pint dipper and the volume of water sampled was approximately 390 ml. At each sampling site, the total number of mosquitoes caught in 3 dips constituted a dipper sample.

The tub was made of polyurethane and was 34.5 cm long, 26 cm wide, and 11.5 cm high. The long edge was pushed down and the water allowed to flow into it until it was ¾ full (i.e., contained about 4430 ml of water). The number of mosquitoes caught in a tub ¾ full was considered a tub sample.

The area sampler was a polyvinylchloride cylinder 69 cm high and 20 cm in diameter. After being pushed into the floor of the rice field, the water within it was to be removed using a dipper. However, since it was virtually impossible to remove all the water, a decision was made to limit the water sample to 5 dips. Because the dipper was being removed at an angle, some of the water spilled out and only about 225 ml of water was sampled per dip (i.e., a total of 1125 ml of water was sampled).

The larval concentrator originally developed by Husbands (1969) was modified so as to stand unsupported when pushed into a rice field, thus allowing greater manual freedom (Figure 1). Polyvinylchloride cement was used to join the following polyvinylchloride pieces: a reducer (2.5 cm x 3.8 cm), a T (10.2 cm long x 8.5 cm wide), a pipe (1.7 cm long x 3.2 cm diameter), an elbow (6.5 cm long x 6.5 cm wide), a pipe (94.5 cm long x 3.2 cm diameter), and a cap (4 cm high x 4 cm diameter). A 50 ml plastic, conical tube was split in half and the top half was fitted snugly into the reducer using a rubber ring.

To make a small sieve, a 2.3 cm diameter hole was burned into the cap of a conical tube and the mouth of a synthetic organdy bag (15 cm long x 4.5 cm wide) was glued to the periphery of the hole. This sieve was then screwed onto the conical tube of the concentrator and a funnel placed in it to facilitate pouring of water samples. The 11 cm diameter mouth of the funnel was covered with a wire screen to help filter out large debris. Several hundred sieves were made so that many samples could be collected prior to processing in the laboratory.

Once the sample was collected, the sieve was removed and screwed onto an uncut, conical tube. A labelled cork was placed into the mouth of the sieve to identify the sample and

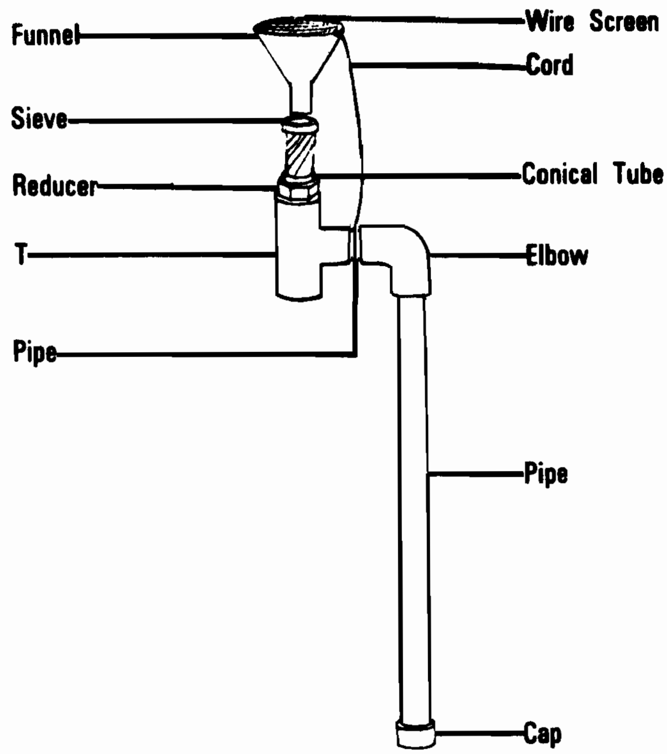

Figure 1.—Larval Concentrator.

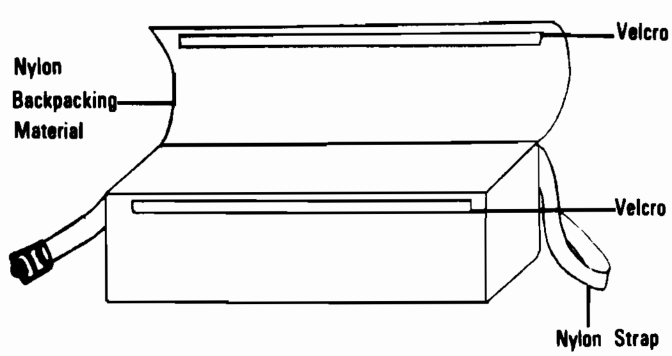

Figure 2.—Belly Pack.

to keep the humidity high so as to prevent dessication of the larvae and pupae. Previous experience had shown that it was much easier to count live mosquitoes than dead ones, and so efforts were made to keep them alive. Upon returning to the laboratory, the sieves were unscrewed and inverted into cups of water and the number of mosquitoes per sample determined.

A belly pack, capable of carrying 30 sampling tubes, was sewn out of nylon backpacking material (Figure 2). It was 37.5 cm long, 10 cm high, and 13 cm wide. Since the pack was strapped around the waist, it was easy to see and reach the sampling tubes.

Due to the enormous size of some checks in a rice field, a decision was made to sample only a portion of each. The area sampled was arbitrarily chosen to be 5645.8 m^2 and within it, there were 40 regularly spaced sites at which all three sampling devices were tested. To minimize any bias, the order and placement of each device was rotated as one went from site to site. For example, at site 1, the dipper was the first device to be tested and it was used to the left of the person. The tub was then tested in front of the person, followed by the area sampler being used to the right of the person. At site 2, the area sampler was now the first device to be tested and it was used to the left of the person. The dipper was then tested in front of the person, followed by the tub being used to the right of the person.

The devices were tested in checks known to have varying densities of immature mosquitoes. Checks 1 and 2 were considered to have a heavy infestation since previous sampling with a dipper produced a mean of .79 mosquitoes per dip. Checks 3 and 4 were considered to have a moderate infestation since there was a mean of .24 mosquitoes per dip, and checks 5 and 6 were considered to have a light infestation since there was a mean of .03 mosquitoes per dip.

When measured at ground level, there was an average of 10 rice stems per square foot in each of the checks. The height of the rice varied anywhere from .9 to 1.5 meters.

Each check was sampled three times and the number of mosquitoes summed over the three trials and divided by 40, giving the mean number of immature mosquitoes per site for each device.

In the previous calculations, each device sampled a different volume of water. To correct for this, the mean number of immature mosquitoes per site was divided by the volume of water sampled, to find the mean number of immature mosquitoes per ml of water per site.

RESULTS AND DISCUSSIONS.—Figure 3 summarizes the results for the 3 devices, irrespective of the volume of water sampled. The tub consistently caught the most mosquitoes, followed by the dipper, and then the area sampler. This is presumably due to the fact that the tub sampled the greatest volume of water, followed by the dipper, and then the area

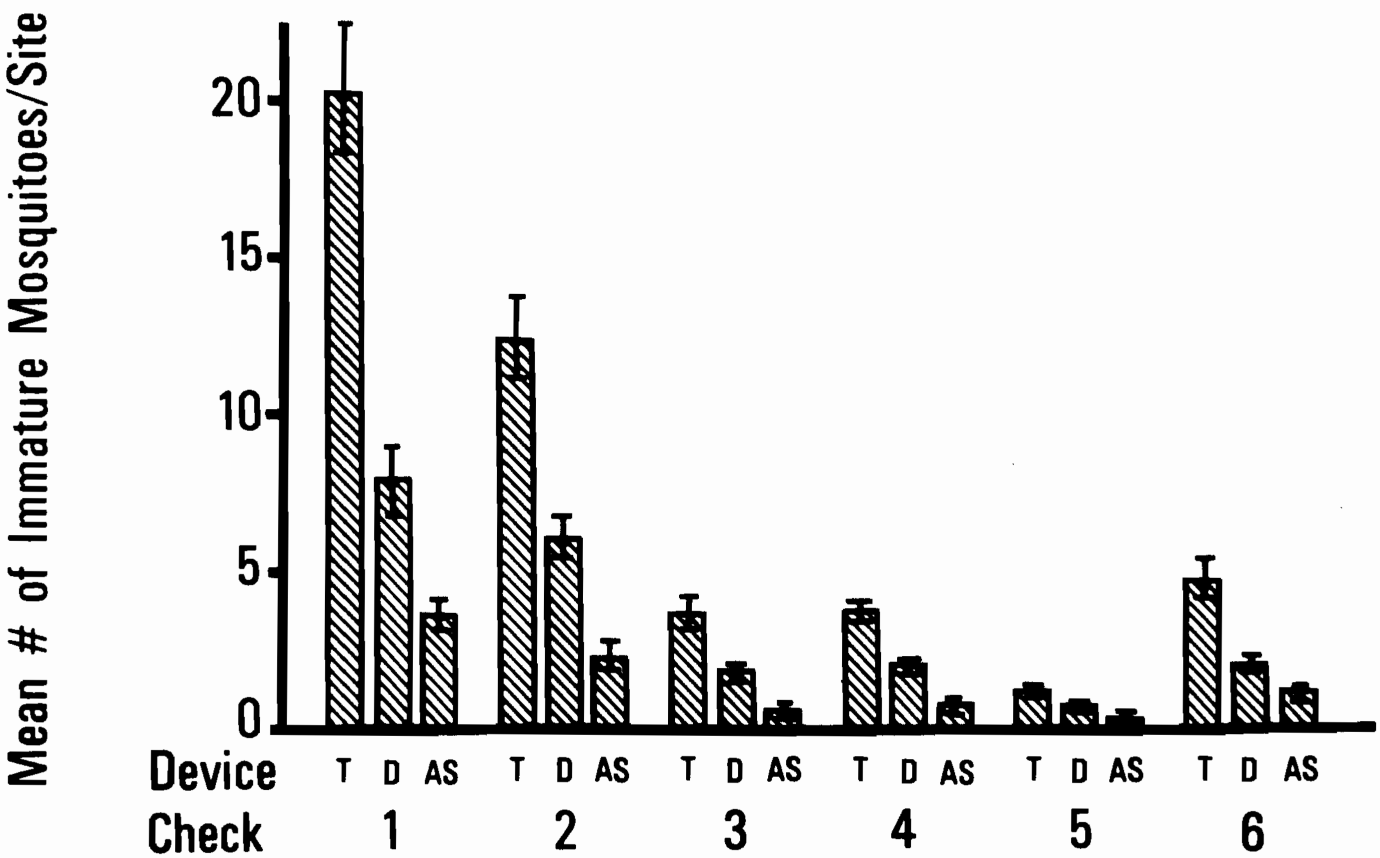

Figure 3.—Comparison of larval sampling devices when volumes are unequal (mean ± standard error). Let:T = Tub, D = Dipper, AS = Area Sampler.

sampler. The tub was the most efficient sampling device since it caught the most mosquitoes with a minimum of effort. The dipper would have required more effort to use since it would have taken 11 dips to sample an equivalent amount of water. The area sampler was the least efficient device since it not only caught the fewest mosquitoes, but it was also difficult to use due to its size and shape.

When the volume of water sampled was taken into account, the same data now appeared as in Figure 4. The dipper consistently caught the most mosquitoes, followed by the tub, and then the area sampler. Although there was an overlap of the standard errors in check 5, this was probably due to the small number of mosquitoes being caught. These results showed the dipper to be the most effective device since it caught the greatest number of mosquitoes.

Contrary to what was anticipated, the results also indicated that the density of mosquitoes did not affect the efficiency and effectiveness of the devices. Regardless of the mosquito density, the tub remained the most efficient device and the dipper remained the most effective device. However, the overlapping standard errors of check 5 in figure 4 suggests that the degree of effectiveness may not be as great when the density is very low.

In summary, the dipper should be used if the objective is to collect numerous immature mosquitoes regardless of the amount of effort and/or time involved. However, if the main concern is collecting the most mosquitoes with a minimum of effort and/or time, then the tub should be utilized. Also, the effectiveness and efficiency of a device will not be affected by the density of mosquitoes except when it is very low. Although the area sampler ranked low in effectiveness and efficiency, it is still the only practical means of measuring absolute changes in the mosquito population on a routine operational basis.

Although this study focused on only 3 devices, many other devices are available for sampling immature mosquito populations. Service (1976) describes many of them and explains various methods which have been used to estimate the size of larval populations (e.g., the mark-recapture method). Knight (1964) also discusses various devices and methods used to determine the absolute and relative densities of immature mosquito populations.

Croset et al. (1976) compared the ability of 3 sampling methods to estimate population size. The mark-recapture technique, the removal method, and the dipping method worked equally well since each estimated the population size within 20% of each other. Because the dipper was easy to use in the field and was as good as other methods for estimating population size, the authors felt that it would be a good tool for studying mosquito populations.

The one pint dipper and a 20 cm x 25 cm rectangular area sampler were tested by Hagstrum (1971) to determine if they would catch similar percentages of different life stages. The area sampler was drained 3 times with a suction device to insure that all the larvae were removed. When *Culex tarsalis* and *Culiseta inornata* were sampled, both devices collected similar proportions of the different life stages. Both devices also caught very few first instar larvae and this was attributed to

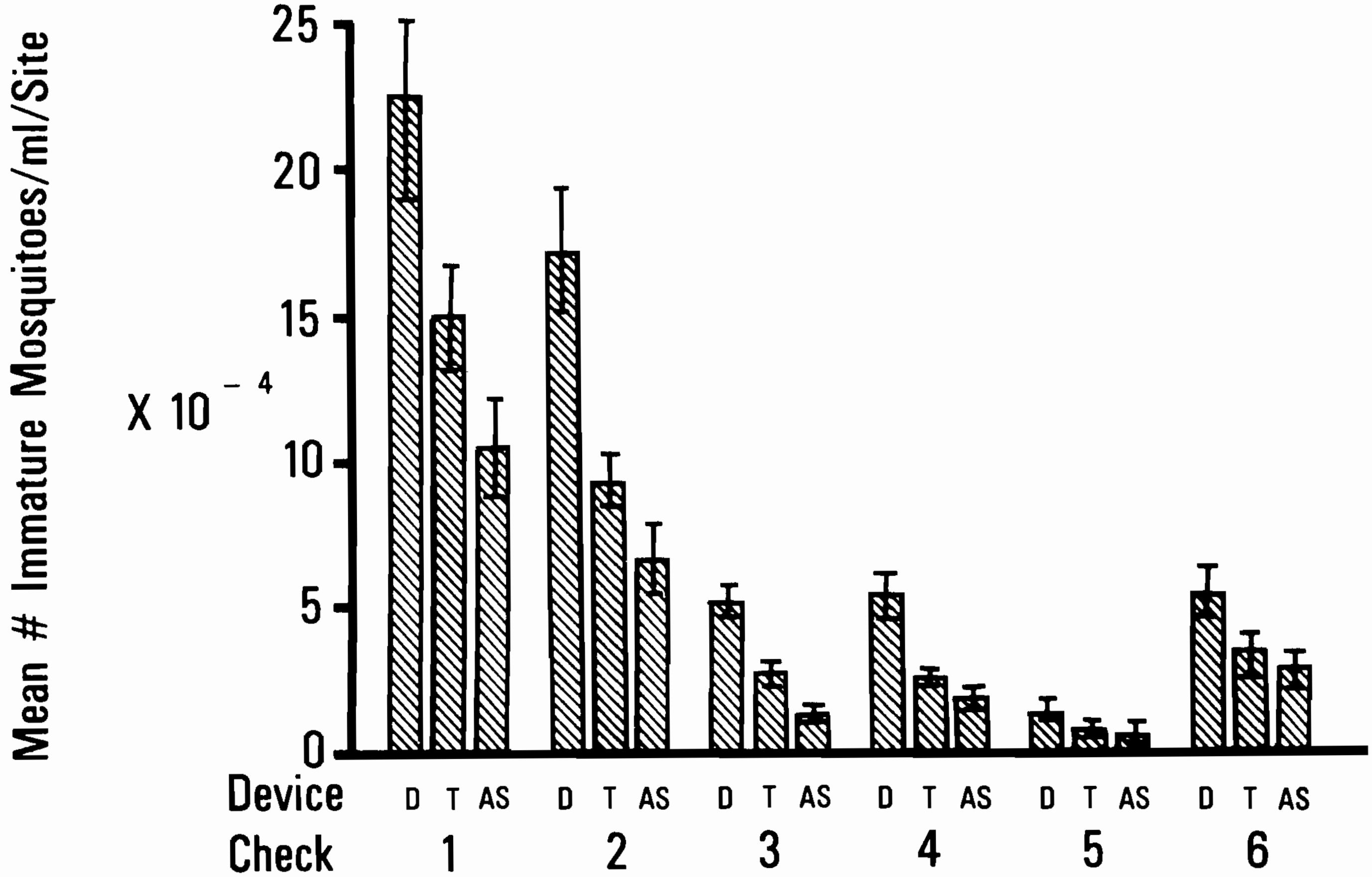

Figure 4.–Comparison of larval sampling devices when volumes are constant (mean ± standard error). Let: T = Tub, D = Dipper, AS = Area Sampler.

the fact that the first instar larvae were distributed in clumps. As a result, many of the samples contained no first instar larvae.

Wada and Mogi (1974) tested a dipper (15 cm diameter x 3 cm depth) in artificial rice ponds stocked with known numbers of *Culex tritaeniorhynchus summorosus*. The efficiency for each life stage, i.e., the probability of a larva in one m^2 being collected in one dip, was found. Using the dipping efficiency, the absolute density was calculated by equating the number of mosquitoes caught per dip to the number of mosquitoes found in one m^2 and the number of mosquitoes found in one hectare.

ACKNOWLEDGMENTS.–The authors would like to express their appreciation to K. G. Whitesell, W. Reilly, L. Davis, S. Hoffmann, and R. Patrock for their help in gathering the data. Special thanks also go to J. Brittnacher, F. H. Collins and W. Marks for their statistical and computer assistance and to C. Fukushima for his technical assistance.

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ATTRACTION OF FEMALE MOSQUITOES (*AEDES AEGYPTI* AND *ANOPHELES QUADRIMACULATUS* SAY) TO STORED HUMAN EMANATIONS: EFFECT OF CO_2 H_2O AND TEMPERATURE ADJUSTMENTS

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ABSTRACT

An apparatus has been assembled which permits storage of air containing human emanations in 150-liter Teflon bags. This stored air can be injected into one port and air contining no emanations into the other port of a thoroughly cleaned plexiglass dual-port olfactometer (OF), while monitoring the two flows for differences in temperature, relative humidity, and carbon dioxide level. Results of bioassays so conducted have led us to conclude that female mosquitoes (*Anopheles quadrimaculatus* Say) are attracted in significant numbers primarily by chemical emanations other than carbon dioxide and water, and that (L)-lactic acid is not the sole attractive chemical constituent of human emanations.

A glass arm box is used to collect human emanations from a stream of outside air or compressed air passed over an enclosed forearm. One of three pillow-shaped Teflon bags (90 x 150 cm) receives the emanations, and "clean" air is pumped into the other bag(s). Flow restrictors (6 mm I.D. Teflon tubes) allow a bag connected to an OF for bioassay to empty for 8 min at 10 liters/min without using a pump. Mounted on the front of each bag is a garden hose type valve; also, each bag is fitted with a septum for syringe injection of CO_2 or removal of air for CO_2 analysis while a bioassay is in progress. To test the systems for leaks, each was filled with 100 liters of Freon® 12 and air mixture, subsequent "sniffing" with a refrigeration-type Freon leak detector found none.

The three systems are contained in a 70°C fan-circulated oven on shelves: each bag is effectively immersed in oven air. Two bags are used only for emanations, the third is reserved for clean air; an emanation bag must be "washed" with clean air by 10 successive fillings and emptyings before mosquitoes no longer respond.

The collection systems, the olfactometers and the instrumentation made possible presentation to the mosquitoes of two streams of air, each at a desired (decreased or increased) level of % RH (± 2%), ppm CO_2 (± 2 ppm), temperature (±0.02°C) and flow rate (±0.1 liter/min). In addition, it was possible to include human emanations in one stream. Without exception, the mosquitoes responded to the emanation air, even when a biologically realistic excess (20 or 100 ppm) of CO_2 and/or H_2O had been added to the check air. We believe the experiments clearly demonstrate the preeminence of human produced chemicals other than CO_2 and H_2O in attracting female mosquitoes in an olfactometer from more than 1 m to less than 1 cm away.

Biological organisms, specifically mosquitoes, respond to hierarchy of stimuli having elements which probably, even necessarily, overlap. While recognizing the futility of attempting to disassociate one element from all others, we believe that we have at least simplified the mosquito attraction hierarchy.

THE MARK-RELEASE-RECAPTURE STUDY AS A RESEARCH TECHNIQUE[1]

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Mark-release-recapture studies have been used in mosquito research for almost 70 years. The earliest studies measured the distance and direction of adult mosquito dispersal from breeding areas. The emphasis was on qualitative, rather than quantitative, results. For example, in the Canal Zone in 1913, large numbers of marked *Anopheles* were released at their breeding site in a saltmarsh northeast of Gatun (Zetek 1915). Many of these were later recaptured indoors in the inhabited area of Gatun. In 1951 and 1952, marked *Aedes taeniorhynchus* were released on Sanibel Island off the southeast coast of Florida (Provost 1952, 1957). Recoveries were made as far as 20 miles away on the mainland, which included movements of 2 to 4 miles over water. Also in the early 1950s, marked *Aedes nigromaculis* were released on the west side of Kern County and recovered in the foothills north and east of Bakersfield, at a distance of 20 to 30 miles from their release point (Smith et al. 1956). Each of these studies specifically documented infiltration into densely populated areas from distant uncontrolled sources.

Attempts to determine the actual distance and direction of mosquito dispersal, as in studies done with *Culex tarsalis* and other species in Kern County in the 1940s (Reeves et al. 1948) and in the Sacramento Valley in the early 1960s (Bailey et al. 1965), were hampered as it was impossible to maintain a constant sampling effort as the distance from the release point increased. The Kern County study reported maximum recovery distances of 2.5 miles for *Cx. tarsalis* and *Culex quinquefasciatus*, 1 mile for *Culex peus*, 0.9 miles for *Anopheles franciscanus* and 0.6 miles for *Culiseta incidens*. The most distant recovery in the Sacramento Valley study was a *Cx. tarsalis* recaptured 15.75 miles downwind 2 days after release. Significant numbers of this species were recovered at distances of 7 to 10 miles.

Results of 2 other dispersal studies in California were reported at the 1951 conference of this organization. Recoveries of *Aedes nigromaculis* were made 1.5 miles upwind and 1.9 miles downwind from their release point in an irrigated pasture near Turlock (Thurman et al. 1951). Nine Mosquito Abatement Districts in the Bay Area cooperated in a study which involved the release of 2 million marked *Aedes squamiger* and *Aedes dorsalis* from a saltmarsh near Petaluma (Aarons et al. 1951). Although most recaptures were made within 2 miles of the release site, 1 *Ae. squamiger* female was recovered 24 miles southeast in Contra Costa County, and 2 others 38 miles south in San Mateo County. These studies provided new information of importance in control operations but their results could not be interpreted as the maximum flight range of the mosquitoes.

The directional sampling problem was resolved in part in a 1961 study, also in Kern County (Dow et al. 1965). Marked *Cx. tarsalis* were released outside the 4 corners of a 25 square mile recovery area. Mosquitoes from all 4 release points were recovered inside the area at distances up to 9.6 miles, and there was no apparent influence of prevailing winds.

Since the 1950s, as the resistance of mosquito populations to control by insecticides became increasingly widespread throughout the world, research efforts turned to the development of genetic control methods. In general, these involved the release of mosquitoes which would introduce partial or complete sterility into the native population. Models were developed to determine the ratio of released to native mosquitoes needed to achieve the desired degree of control. It then became necessary to estimate the actual size of the native populations in order to know how many genetically-altered mosquitoes should be released to achieve these ratios.

One of the earliest mark-release-recapture studies for this purpose was done with *Aedes aegypti* in a temple compound in Bangkok in 1966 and 1967 (Sheppard et al. 1969). Resting adults were collected indoors, given a unique mark and released in the room of capture. The marking system involved the application of small dots of paint at pre-determined spots on the wings and thorax. These dots could be read as binary numbers, which meant that individual mosquitoes, rather than groups, could be recognized on recapture. Previously marked mosquitoes were recorded and released again. Collections were made 3 days a week for 12 months. In addition to survival and population estimates, much information was gained on the movement of mosquitoes within the compound and fluctuations in population size throughout the year. However, this method would have little value for the study of non-domesticated mosquitoes.

In our population estimation studies of *Cx. tarsalis* at Poso West in Kern County (Nelson et al. 1978), we found a good correlation between routine light trap indices and population estimates for females. The ratio of total female population to light trap index was consistently about 500 to 1 throughout the summer months of 1976 and 1977. Flooding in early 1978 caused extensive alteration of the study area and a subsequent shift of the mosquito population with respect to established trap sites. The ratio of female population to light trap index changed to about 200 to 1 during the 1978 mosquito season. Despite this, we feel the method could be useful in an ecologically stable environment.

A major concern in population estimation studies is the choice of an appropriate model for the calculation of results. A study in Burma in 1967 estimated the size of a population of *Culex pipiens fatigans* by 2 different models, which produced widely differing estimates (MacDonald et al. 1968). In Delhi in 1971, Reuben and co-workers (1973) did a mark-release-recapture experiment with *Ae. aegypti*, and compared population estimates based on 6 different models. They found

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good agreement between the results of 4 of the models. One of these, Bailey's Corrected Lincoln Index, is computationally the simplest, and has subsequently become the method of choice in most population estimation experiments. A major advantage of this model is that it does not require the re-release of captured marked specimens. However, because of the short life span of mosquitoes, it is necessary to incorporate survival estimates into the model.

Another important concern is the choice of appropriate sampling methods. Different trapping techniques sample different components of the population. For example, shelter collections sample only the resting adults, bait traps are selective for blood-seeking females, and light traps attract the airborne segment of the population (which may consist largely of blood-seeking females). The biases associated with various methods have been discussed at length by Service (1976, 1977). The sampling methods used should be those which will provide the maximum number of recoveries of the type required for the purposes of the experiment. Recapture rates will reflect the intensity of recovery efforts. Large numbers of mosquitoes must be released to obtain sufficient recaptures, especially if the area to be sampled is large.

Males are especially difficult to sample. Inability to recapture a high proportion of marked males over a period of time has created problems for many research groups, including our own. We have used fluorescent dusts as markers in all our current mark-release studies of *Cx. tarsalis* (Nelson et al. 1978). Although marked adults of both sexes survived as well in laboratory cages as unmarked ones, we have rarely been able to recapture more than 1% of marked males released in the field. This is in contrast to average recoveries of 9% of marked females. It may be that males carry a heavier load of dust in relation to their body weight than females, and that this inhibits their behavior and survival in the field. If true, this introduces serious bias into the interpretation of male recapture data as a reflection of actual field behavior.

We tested the effect of marking on the mating ability of male *Cx. tarsalis* in large outdoor "Quonset hut" cages in September 1978. Adults were reared in the laboratory from pupae collected at Poso West. One thousand 1 to 3 day old virgin adults of each sex were released into each of 2 cages measuring 6 x 5.5 x 3 m. All males in 1 cage were marked with dust prior to release. Insemination rates were determined daily for a sample of 25 females collected by aspirator from each cage. Daily rates for the 2 groups of females are shown in Figure 1. The rates for females caged with marked males were initially very low, but the differences were slight after the 4th day.

Research efforts in genetic control have now progressed to the field trial stage in a number of locations. Mark-release-recapture studies are being used extensively to monitor the outcome of the trials. By marking the genetically-altered mosquitoes prior to release, it is possible to develop estimates not only of their survival in the field, but also of the ratio of released to native adults. In Kenya in 1974, the mating competitiveness of released translocated male *Ae. aegypti* was monitored by simultaneously releasing marked virgin females (McDonald et al. 1977). Upon recapture, these were allowed to oviposit, and scored on the basis of egg hatch as having mated with translocated or wild males in the field.

The limited success of such genetic field trials has led to more and more mark-release-recapture studies designed to provide knowledge of the behavior of both wild and colonized mosquitoes in the natural environment. In a 1969 study of *Culex nigripalpus* and *Culex pipiens quinquefasciatus* in Florida, marked virgin females of known age were released (Lea and Edman 1972). Those recaptured each day were examined for the presence of sperm to determine the age at which they became inseminated. Marked *Cx. pipiens fatigans* were released in the Delhi area during 1970 and 1971 to select the time of year when survival and dispersal would be optimal for the success of a genetic control effort (Yasuno et al. 1973). Another study in Kenya in 1973 compared the indoor resting behavior of domestic, peridomestic and feral populations of *Ae. aegypti* (Trpis and Hausermann 1975).

Studies which utilize some form of bait as part of the sampling scheme have produced data on the interval between blood meals. In a 1970 Tanzania study, a gonotrophic cycle of 4 days was estimated for marked *Ae. aegypti* which were fully fed when released (Conway et al. 1974). Nearly half of these females were estimated to have sought a secondary feed during the 4-day cycle. An earlier study in the same area related actual age to number of gonotrophic cycles completed for 2 species of *Anopheles* (Gillies and Wilkes 1965). A recent study of *Aedes sierrensis* in an orchard at Davis gave evidence that females reached maturity in 4 to 5 days, and required 7 to 14 days to complete a gonotrophic cycle under normal temperatures in spring (Bennett 1978).

At Poso West in June 1978 we marked and released over 7000 wild female *Cx. tarsalis* which had been collected in CDC light traps baited with dry ice the previous night. Recaptures of these females for 12 nights following release are graphed in Figure 2. The peak on night 1 can be attributed to the fact that, having failed to obtain a blood meal the previous night,

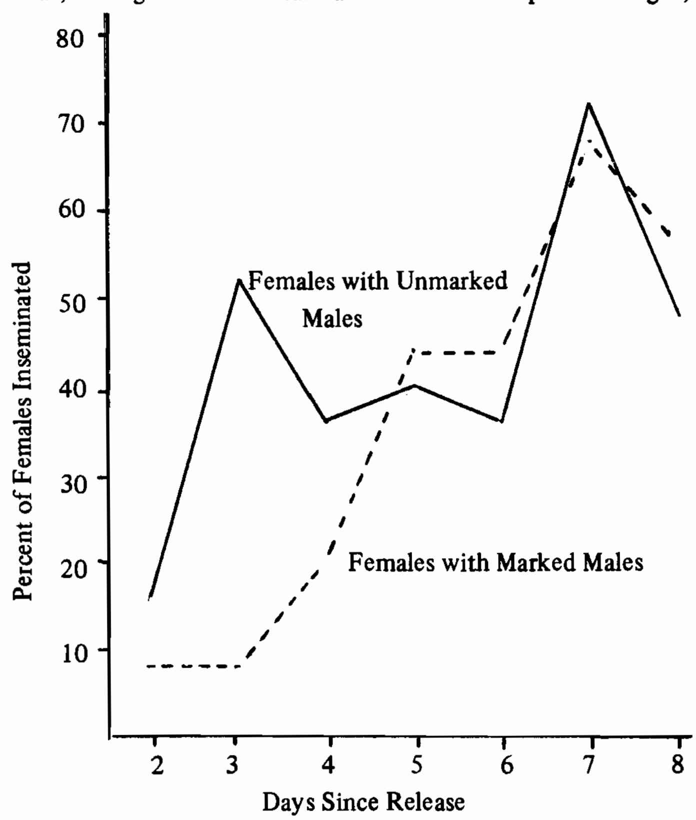

Figure 1.—Daily insemination rates of female *Culex tarsalis* in large outdoor cages with marked vs. unmarked males.

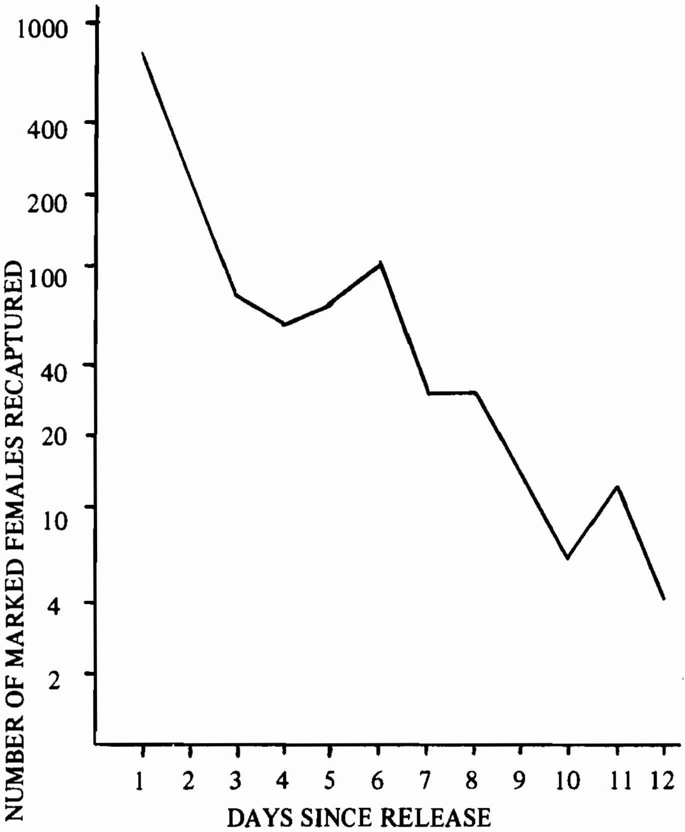

Figure 2.–Daily CO_2 light trap collections of marked female *Culex tarsalis*, Poso West, June 1978.

these females were still blood-seeking and thus attracted to our CO_2 light traps. Subsequent peaks occurred at 5-day intervals, on nights 6 and 11. Daily survival of these females was estimated to be 68%. Other data from the same study indicated that the first blood meal was taken when females were 5 days old. If true, this means that only 2% of emerging females live long enough to take a second blood meal, and thus could be capable of acquiring and subsequently transmitting encephalitis viruses.

Results from another mark-release study at Poso West which compared the field behavior of colonized vs. wild adults are being presented at this meeting by R. L. Nelson.

During the fall of 1978, we marked and released over 40,000 *Cx. tarsalis* adults at Poso West in an attempt to study overwintering behavior. Weekly shelter collections are small, but we have recovered adults as long as 6 weeks after release.

Many other mark-release-recapture experiments have been reported. At least 9 species of concern to mosquito control in California have had at least one study using mark-release-recapture techniques. It can be anticipated there will be many more. The examples in this paper were chosen to demonstrate the variety of study designs and purposes for which the technique has been used. It provides a valuable tool for the study of mosquito popula'ions in their natural habitats.

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MODIFICATION OF AN *AEDES AEGYPTI* SYSTEMS MODEL FOR *AEDES SIERRENSIS*

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Aedes aegypti, the yellow fever mosquito, has a worldwide distribution in the semitropics. Based on extensive field and laboratory studies Gilpin and McClelland (1979) have built a systems model of the growth and development of this species. We propose to adapt this realistic systems model to another species of prime interest and local importance, *Aedes sierrensis*.

Briefly, the *Ae. aegypti* model is based on physiological time, a concept well established in entomological studies. Starting from hatched eggs, larval weight gain is followed through development as a function of both food and larval density. Laboratory experiments consist of inputting various amounts of food and larvae and observing the resultant growth behaviors and pupation success. Experimental evidence indicates the existence of a minimum weight and time for pupation. When the initial food supply is exhausted, larval weight decreases due to basal metabolic activity. Death by starvation is put off via consumption of excess lipid reserves. The systems model, with the eventual incorporation of an adult stage, yields results which are in very good agreement with the growth behaviors actually observed, and permits predictions of population dynamics over many generations.

Having had success modelling *Ae. aegypti*, we hope to obtain similar results for *Ae. sierrensis*. By virtue of their close ecological and taxonomic affinity, we expect the developmental, physiological and ecological mechanisms on which the model is based to be similar for the two species. The most important difference between them appears to be the fact that *Ae. aegypti* is semitropical whereas *Ae. sierrensis* is found in a temperate zone. In accord with this, *Ae. aegypti* development is supended below 13.4°C (Gilpin and McClelland 1979), while McClelland has demonstrated growth for *Ae. sierrensis* as low as 3°C. By utilizing the 25°C *Ae. sierrensis* growth data of Gilpin and Langford (1978), and assuming that 25°C falls within the linear range of developmental dependence on temperature for this species, the model may be modified to account for the different temperature response by a scale conversion. McClelland has work in progress to test the validity of this assumption. In our preliminary attempts to adapt the model to *Ae. sierrensis* we have assumed all aspects of larval development to be the same for these two species, with the exception of the temperature difference, and a longer minimum pupation time (12 days at 25°C) for *Ae. sierrensis*.

The systems model simulates larval weight gain through time, and defines pupation to occur when the larval weight trajectory intersects a hyperbolic pupation window corresponding to the minimum weight and time requirements. Variance in larval weights is represented by the width of the trajectory band. Figure A shows the systems model simulation for the case of high food availability, which is in good agreement with experimental data for *Ae. sierrensis*. (c.f. Gilpin and Langford, Figure 2). Similarly, the model is in good agreement with the data for the case of moderate food availability. However, for the food stressed case (depicted in Figure B) the model fails to predict the slow trickle of pupation observed as long as day 40 in the laboratory studies.

We consider two hypotheses to account for this discrepancy. The model assumes minimum pupation weight to be a constant over time. If, however, it were a decreasing function of larval age, the lower edge of the pupation window would slope downwards, possibly intersecting the upper edge of the larval weight trajectory. We are convinced that any such effect is of small magnitude, and is insufficient to explain the degree of pupation observed. Our second, and more plausible hypothesis, is that interference among the larvae is much more pronounced for *Ae. sierrensis* than for *Ae. aegypti*. Gillett (1972) has reported active cannibalism among *Aedes* species, but Gilpin and McClelland (1979) found no evidence of any intraspecific interference among *Ae. aegypti* larvae. Larval competition among *Ae. aegypti* is assumed to exist only in the form of exploitation, for which the evidence is strong.

If, for example, cannibalism or any other aggression leading to death proves to be a significant factor in the development of *Ae. sierrensis*, the variance in the larval weights would be greater. The systems model could yield pupation behavior consistent with the empirical evidence, depending on the degree to which the thicker band of larval weights penetrate the pupation window. Further work is in progress to investigate this and other possibilities.

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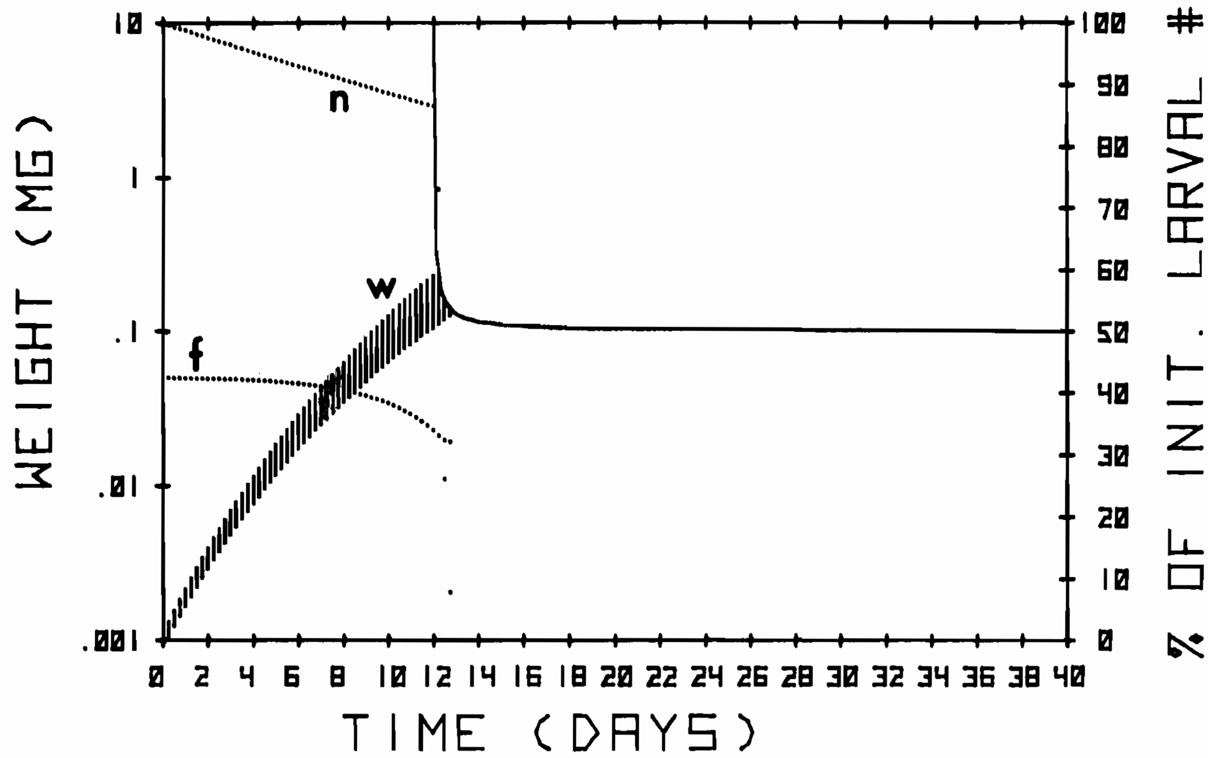

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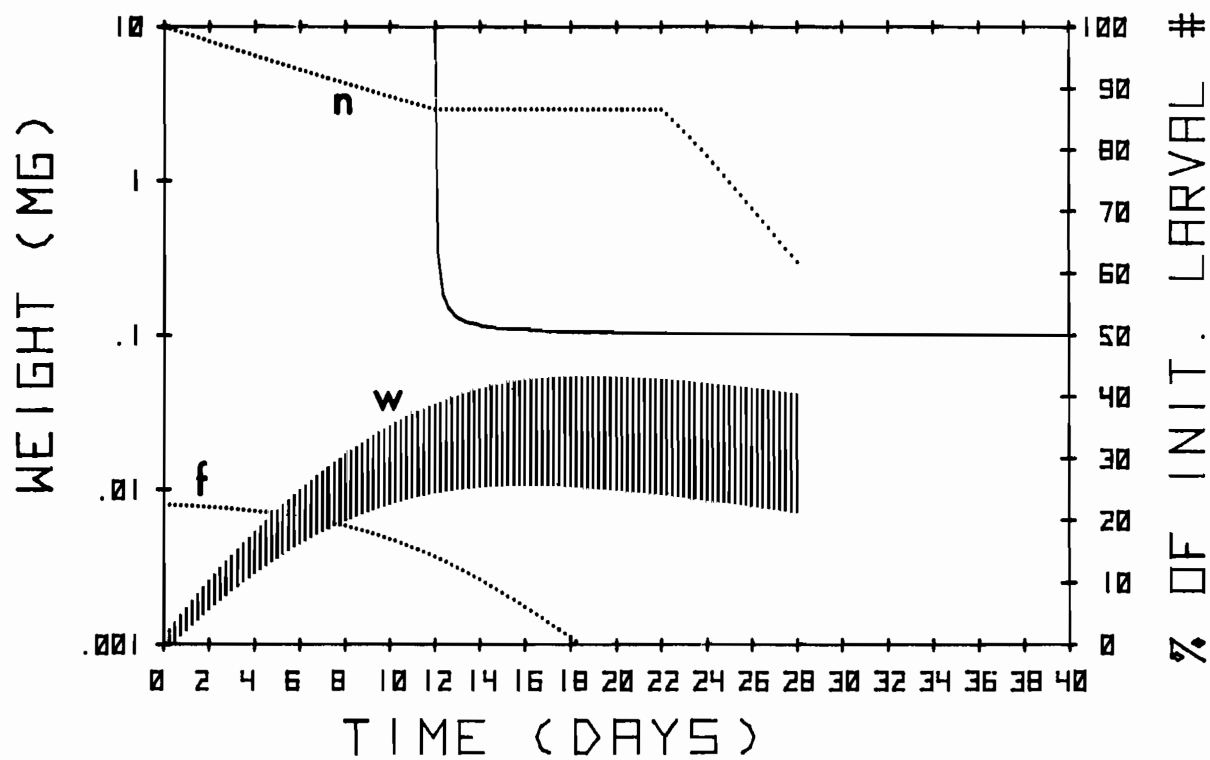

Figure A.–Systems model simulation of development for *Ae. sierrensis* in case of high food availability, initialized with 50 larvae and 50 mg of food. Larval weight in mg is plotted by the curve labelled w, and food supply in g by the curve labelled f, on the left hand ordinate. Curve n shows the number of larvae present as a percentage of the initial total, plotted on the right hand ordinate.

Figure B.–Simulation of food stressed case, initialized with 50 larvae and 8 mg of food.

SPECIES COMPOSITION AND ABUNDANCE OF FISHES IN DITCHED AND UNDITCHED AREAS OF A SAN FRANCISCO BAY SALT MARSH

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Recirculation ditches, used extensively prior to the introduction of DDT, have re-emerged as an important salt marsh mosquito control measure. These ditches connect natural tidal channels to marsh depressions (e. g. potholes) that serve as larval habitats. Thus, mosquitoes are eliminated by 1) the drainage of pools so no water remains at low tide (Smith 1904) or 2) an increase in accessibility for larvivorous fish (Connell 1940, Ferrigno and Jobbins 1968). Although alterations in aquatic habitat from ditching activities suggest the possiblity of adverse impacts on salt marsh fish communities, no information currently exists that relates this practice to Pacific Coast marsh fishes. In this study, three aspects of a fish community (species composition, population density, and population size structure) were examined to test the hypothesis that ditching has an adverse effect on salt marsh fishes.

This study is part of a multidisciplinary project designed to analyze the effects of recirculation ditches on salt marsh ecology (Resh and Balling 1979). The project is a joint effort between the University of California, Berkeley and the California Mosquito and Vector Control Association Coastal Region.

SITE DESCRIPTION. The study was conducted in a pickleweed (*Salicornia virginica* L.) marsh at the upper end of Albrae Slough, Fremont, Alameda Co., California. This area, located between large salt evaporation ponds, is subject to water salinity ranges of 16°/oo to 25°/oo. Temperatures of pools and channels isolated from daily tidal flushing fluctuate with ambient air temperatures.

Fish populations were compared in ditched and unditched areas. The ditched area, 551 m^2 of aquatic habitat, was formed in 1977 when shallow, hand-dug ditches were added which opened both pools and blocked channels to daily tidal flushing but did not drain them completely. The control, or unditched area, comprised 404 m^2 of aquatic habitat and consisted of a series of pools and channels subject to tidal action only during spring tides. Both areas were similar in substrate, marginal vegetation, water depth and invertebrate fauna.

METHODS.–Fish species composition in ditched and unditched areas was determined by periodic collections with G-type minnow traps (mesh size = 6 mm) and D-frame nets (mesh size = 1 mm) from January to August 1978. Fish populations in both areas were inventoried in August of 1978 to determine population densities. The single channel draining the ditched area was screened off at high tide and all fish were collected at low tide. Similarly, a screened ditch was added to drain the unditched area and fish were inventoried as above. Four weeks later the unditched area was again examined to determine the nature and extent of recolonization.

The size structure of ditched, unditched, and recolonization populations of the dominant species, the mosquitofish *Gambusia affinis*, was determined by examining total length-frequency distributions. Maturity was indicated by the presence of a gonopodium in the male (Krumholz 1948) and by convexity of the abdomen (indicating presence of eggs at this time of year) in the female.

RESULTS.–During the 8 months of sampling, 10 species of fish were collected in the ditched area, but only 5 were caught in the unditched area; none were unique to the unditched area (Table 1). The most abundant species were *G. affinis*, the threespine stickleback *Gasterosteus aculeatus*, and the rainwater killifish *Lucania parva*.

Table 1.–Species composition and densities of fishes collected in ditched and unditched areas of Albrae Slough marsh, Alameda Co., California.

Species	Ditched area individuals/m^2 (%total)	Unditched area individuals/m^2 (% total)
Gambusia affinis (Baird and Girard)	10.5 (94.9)	3.4 (91.7)
Gasterosteus aculeatus Linnaeus	0.4 (3.7)	0.2 (5.0)
Lucania parva Girard	0.1 (0.6)	0.1 (2.8)
Gillichthys mirabilis Cooper	<0.1 (0.2)	<0.1 (<0.1)
Clevelandia ios (Jordan and Gilbert)	<0.1 (0.5)	**
Leptocottus armatus Girard	*	**
Atherinops affinis (Ayres)	<0.1 (<0.1)	<0.1 (0.4)
Atherinopsis californiensis Girard	*	**
Dorosoma petenense (Gunther)	*	**
Engraulis mordax Girard	*	**

*Collected during sampling January to August, but not during August inventory.

**Collected only in ditched area.

Based on the August 1978 inventory, fish densities were 3 times greater in the ditched than the unditched area (Table 1). The difference was largely due to the respective *G. affinis* populations which comprised 94% of all fish collected.

Examination of the *G. affinis* male and female length-frequency distributions for ditched, unditched, and recolonization populations indicated that differences in the proportion of immature individuals were most pronounced. The percent immatures was 61% in the recolonization population, 41% in the ditched population, but only 20% in the unditched population.

DISCUSSION.–The three fish community parameters examined indicate that the ditched area has: 1) greater number of species, 2) greater fish density, and 3) greater proportion of immature individuals.

Our evidence suggests that moderation of water temperature and improvement of immigrant access are the prime factors that influence the observed differences in fish composition and abundance in these two areas. Increased tidal flushing that results from ditching reduces the extreme diel temperature fluctuations characteristic of unditched habitats. Such temperature moderation reduces environmental stress and may therefore increase fecundity and survival. In addition, cooler water species such as gobiids and sculpin can then occupy these ditched areas.

The high proportion of immatures in the ditched and recolonization populations, both affected by ditching activities, supports our contention that a substantial degree of migration by immature fish occurs as a result of these practices. This increase in accessibility also explains the presence of nonresident spawning or foraging fishes such as *Engraulis mordax* and *Dorosoma petenense* in the ditched area.

We conclude that our original hypothesis should be rejected. In contrast, evidence from our study suggests that the addition of shallow recirculation ditches in Albrae Slough positively affected this fish community through both temperature moderation and increased accessibility.

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A COMPARISON OF EGG HATCHING TECHNIQUES FOR *AEDES SIERRENSIS*

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ABSTRACT

Six of 17 hatching stimuli initially tested under a variety of conditions were used to determine which method produced the best egg hatch and to determine if different mosquito strains responded differently to the same hatching stimulus. The following 6 hatching stimuli used reduced the dissolved oxygen concentration in the water to below 1 ppm: aqueous solutions of cysteine hydrochloride, ascorbic acid, KCl-Na_2SO_3, and Bacto nutrient broth (Difco Laboratories, Inc.), as well as boiled water and water treated by bubbling nitrogen into it. A total of 6000 eggs from the following 5 strains of *Aedes sierrensis* were exposed to the above stimuli: established laboratory colonies at the University of California, Davis, the University of Notre Dame, the University of California Fresno Mosquito Laboratory, 1978 colonies established from field collections from Briones Regional Park, Contra Costa County, and Sunol, Alameda County, California.

The results revealed that. 1) the longer established laboratory strains had a 20% greater egg hatch overall in comparison to recently colonized strains, 2) KCl-Na_2SO_3 produced the best mean hatch for all strains (84%) followed, in order of decreasing success, by nitrogen-bubbled water, cysteine hydrochloride, Bacto-broth, boiled water and ascorbic acid (33%); KCl-Na_2SO_3 produced the lowest dissolved oxygen concentration of all media at 0.35 ppm, 3) considerable differences in hatching success existed between strains tested in the same medium, in cysteine hydrochloride 88% of Fresno eggs hatched while only 48% of Davis eggs hatched, in ascorbic acid only 33% of Fresno eggs hatched while 84% of Notre Dame eggs hatched.

AN ANALYSIS OF QUANTITATIVE METHODS OF SAMPLING MOSQUITO LARVA FOR ESTIMATION OF POPULATION DENSITY

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ABSTRACT

Mosquito larval samples were collected from various sources in San Mateo County, California, using standardized dipping methods between December of 1975 and March of 1976. The larval densities, as reflected by the sample means are compared and discussed in relation to source complexity. The number of dips required to achieve specified sampling accuracy was estimated and discussed for Searsville Lake, Woodside, California. It was found that as mean larval density decreases, the number of dips required to achieve desired sampling accuracy increases exponentially.

The dipping techniques of three operators were also compared. Even though the dips were taken from the same source under similar conditions, significant differences were found among the different operators, as well as between replicate samples taken by the same operator within the same source.

INTRODUCTION.–The taking of mosquito larval samples is important in estimating the relative density of mosquito larvae in any particular aquatic habitat. For a mosquito control program to be effective, the density of mosquito larvae in habitats of interest needs to be estimated with some degree of accuracy and reliability.

The characteristic feature of most methods of estimating relative population densities is that they depend upon a collection of samples that represent a constant but unknown proportion of the total population. This may require that a method be developed for determining the numerical relationship between the number of individuals in the sample and the total population size.

The primary purpose of this study was to evaluate the sampling characteristics and usefulness of standardized mosquito larval dip samples. Specifically, two areas of concern in the study were the following: to determine the minimum adequate sample sizes to be used in dip samples, and to evaluate the consistency of the samples collected by different people.

MATERIALS AND METHODS.–The dip count was the method by which the mosquito larvae were collected since it is the accepted standard unit of measurement used in mosquito control to estimate the relative density of the larval stages of the mosquito. The standard one pint sized enamel dipper (a metal cup having a 95 cm^2 orifice and a 5.2 cm depth) was used throughout this study.

Larval samples were collected twice weekly at the various mosquito sources selected for this study. Samples were composed of 10 or 25 dips. The sample means were then compared with each other using the analysis of variance, complete randomized design, and multiple range analysis as described by Woolf (1968). Other analyses such as correlation and regression analyses were utilized to measure the nature of relationships that occurred between variables of interest (Woolf 1968).

RESULTS. To determine the number of dips required in a sample to insure a desired level of sampling accuracy, 10 or 25 dip samples were taken a total of 18 times at Searsville Lake between late December 1975 and March 1976 (Table 1). The minimally adequate sample size (n) required to insure a desired accuracy of 25% (X - u = 0.25 x X) was estimated by the following formula (S. C. Williams, personal communication):

$$N_a = \left[\frac{S \times t_{0.05}}{\text{desired accuracy}}\right]^2$$

Where: N_a = minimum sample size required to insure a given level of sampling accuracy
S = standard deviation
$t_{0.05}$ = "t" from Students "t" table for 0.05 level of significance and degrees of freedom associated with the estimate of the standard deviation

The minimum number of dips required to achieve the desired accuracy, varied substantially from week to week although the samples were taken from the same source with the same dipping techniques (Table 1). During the last weeks of the study, the number of dips required to attain the desired level for sampling accuracy increased. This appeared to correlate with decrease in population density. During the earlier portion of the sampling period, fewer sampling dips were required since the population density was much higher.

The relationship between the minimal sample size required to achieve a desired sampling accuracy and the population density of mosquitoes (mean number of larvae per dip) was studied using correlation analysis. A significant negative correlation was found between these two variables. Furthermore, least squares analysis indicated that as the larval density decreases (sample mean decreased) the number of minimally adequate dips per sample increases at an exponential rate ($r = -0.79$; $p<0.01$) (Figure 1). For example (desired accuracy 25%), when the sample mean is 5, the number of dips required to be taken is 5 dips. When the sample mean is 3, the number of dips needed is 15. As the sample mean decreases to 1, the number of dips required rises to 45. A similar trend was observed for 50% desired sampling accuracy.

A comparison between the dipping styles of three operators was made at Hooper's marsh, Woodside, California. This portion of the study consisted of each operator (A, B and C) taking two 10 dip samples (n=10) from the same source under

Table 1.-Data characteristics of 18 larval samples taken at Searsville Lake between December of 1975 and March of 1976. Woodside, California.

Sample	Means	Standard Deviation	Kurtosis Coef.	Skewness Coef.	No. of Dips 25% Accuracy	No. of Dips 50% Accuracy
1	4.1	1.5	-0.59	0.21	10.0	2.5
2	4.4	2.6	-0.15	0.47	27.2	6.8
3	3.4	1.3	-0.65	0.95	11.2	2.8
4	4.0	1.0	-0.24	0.00	3.8	0.9
5	4.1	0.8	-0.12	0.32	2.3	0.5
6	1.6	1.1	-1.42	-1.12	36.5	9.1
7	2.6	1.4	-0.42	-0.89	21.7	5.4
8	1.2	1.0	-1.84	-2.61	47.8	11.9
9	1.4	1.0	-1.03	-1.83	27.4	6.8
10	1.8	1.4	-0.59	-0.42	41.9	10.4
11	1.1	0.8	0.32	0.30	38.8	9.6
12	1.7	1.2	-0.06	-0.06	18.1	4.5
13	1.1	1.0	1.67	0.25	52.8	13.2
14	1.3	1.3	1.38	0.77	59.9	15.1
15	0.9	0.8	1.02	-0.30	52.8	13.2
16	0.9	0.6	-0.43	-0.60	31.5	7.8
17	1.1	1.2	-0.11	0.21	76.9	19.2
18	0.8	0.5	-0.45	-1.38	32.0	8.0

Table 2.—Comparison of dip sampling characteristics of three operators. Data gathered on 15 March 1976 at Hooper's marsh, Woodside, California. Each operator took two samples of 10 dips each (n=10). Units are number of mosquito larvae per dip.

	Operator A		Operator B		Operator C	
Sample:	1	2	1	2	1	2
	3	6	0	0	1	0
	4	7	0	1	1	1
	5	7	0	1	2	1
	5	8	0	1	3	1
	5	8	1	2	4	1
	6	8	1	2	7	1
	6	8	1	4	8	1
	7	9	2	4	9	1
	7	10	3	7	13	3
	8	11	3	10	15	4
$\bar{X}$:	5.6	8.2	1.1	3.2	6.3	1.4
S.D.:	1.5	1.47	1.2	3.2	5.0	1.2
S.E.:	.47	.46	.38	1.0	1.6	.37

Table 3.—A measure of the repeatability of larval density estimates by the same operator. The results of three operators each sampling the same habitat, under the same sampling conditions, two times with 10 dip samples (n=10) are recorded. Sample means, range, and 95% C.L. are represented in the table. Data collected at Hooper's marsh, Woodside, California.

Operator	Mean	95% C.L.
A_1	5.6	6.5-4.60
A_2	8.2	9.1-7.20
B_1	1.1	1.8-0.35
B_2	3.2	5.2-1.20
C_1	6.3	9.4-3.20
C_2	1.4	2.1-0.67

Table 4.—A measure of the repeatability of larval density estimates by three different operators. The results of three different operators sampling the same habitat, under the same sampling conditions, and combining their two 10 dip samples (n=20). Sample means and 95% C.L. are recorded for each operator. Data was collected at Hooper's marsh, Woodside, California.

Operator	Mean	95% C.L.
A	6.9	7.8-6.0
B	2.2	3.3-1.0
C	3.9	5.7-2.0

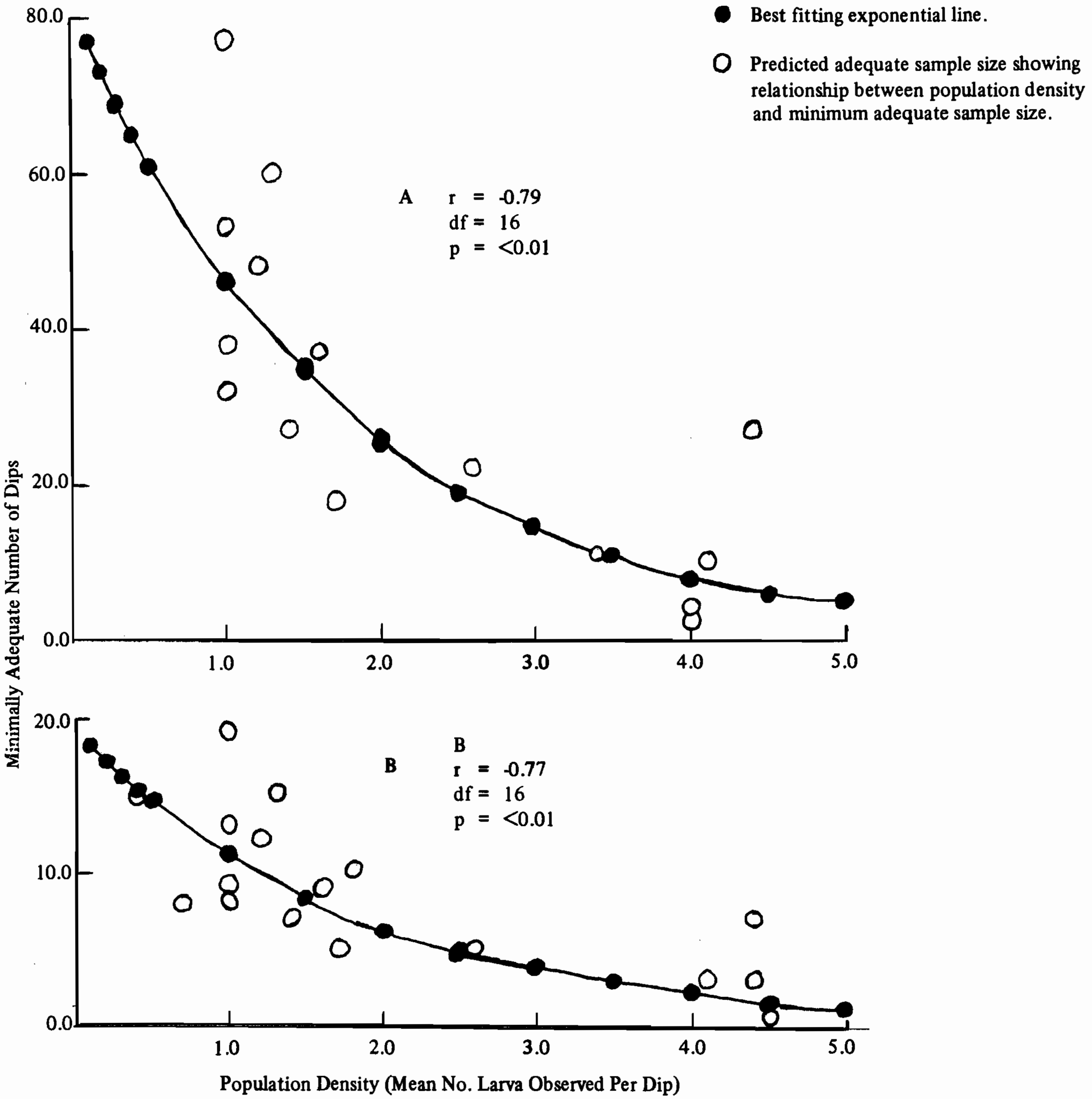

Figure 1.–The relationship between larval density and minimum adequate sample size required to achieve desired accuracy. Dip samples were taken at Searsville Lake, Woodside, California between December of 1975 and March of 1976. Best fitting exponential line is also indicated. (A) = best fitting line for 25% desired accuracy. (B) = best fitting line for 50% desired accuracy.

similar conditions (Table 2). An analysis (Figure 2) of the sample means of the three operators, using the "t" test (Table 3), indicated that the two samples collected by operator "A" differed significantly from each other ($p<0.05$). Operator "B" showed no significant difference ($p<0.05$) between the means of his two, 10 dip samples. Operator "C", like "A", had sample means which differed significantly from each other. Operator "B" was the only individual whose sample means showed no significant difference. A consistent sampling technique was observed in the dip samples of operator "B". Operators "A" and "C" had sample means which differed significantly from each other. When the two samples from each operator were combined (n=20) and analyzed (Table 4) against each other, the mean of operator "A" difference significantly from the means of operators "B" and "C" (Figure 3). No significant difference (p>0.05) was found between "B" and "C".

DISCUSSION AND CONCLUSIONS.—The most important information learned through this study is that when trying to estimate the larval population densities of mosquitoes in any source, to achieve desired sampling accuracy, a greater number of sample dips need to be taken when the population densities are low, rather than when the densities are high. It was also found that for the different types of sources sampled in the study, it would not normally be practical to try to estimate population densities to a high degree of accuracy (i.e., 25 percent of mean density) since so many sample dips would be required which would result in a greater amount of time having to be spent out in the field. It is believed, therefore, that in mosquito control, more emphasis should be placed on qualitative, rather than on quantitative findings. This is not to imply that population numbers should be ignored.

Qualitative sampling is useful to mosquito abatement districts when the kinds of mosquitoes in any particular source need to be known (potential vectors of disease). In most cases however, only the presence of absence of larvae are noted by field sampling carried out by mosquito control technicians. This type of information may be sufficient to initiate some type of control measure. If on the other hand, the effectiveness or lack of effectiveness of control procedures need to be evaluated, then quantitative sampling is useful. Selecting 25% desired accuracy may be important in trying to determine whether treatment or experimental pesticides have been effective in controlling the larvae, since with 25% desired accuracy one can tell whether the population has been halved, or doubled in size. To try to achieve 50% desired accuracy on the other hand, may be impractical since small changes in population densities may not be evident.

The information on the numbers and types of mosquitoes found in a source should be integrated with adult mosquito captures in light traps or sweep net, and with resting station counts, to help form a comprehensive mosquito control program.

Furthermore, conditions such as dipping techniques, operator bias, complexities within a source, complexities amoung sources, predators, depth of water, i.e., types of substrate, etc., all influence sampling in such a way that complete conformance with a standardized dipping technique has not been practiced at this time by mosquito control technicians.

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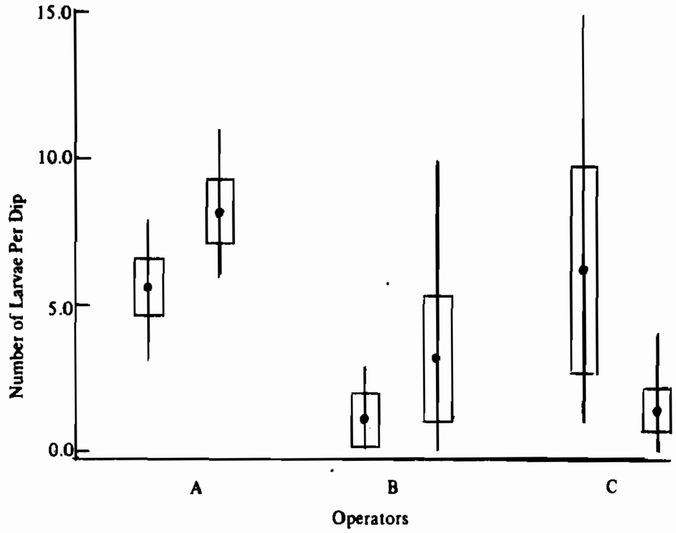

Figure 2.—A measure of the repeatability of larval density estimates by the same operator. The results of three different operators each sampling the same habitat, under the same sampling conditions, two times with 10 dip samples (n=10) are recorded. Sample means, range, and 95% C.L. are represented. Samples taken at Hooper's marsh, Woodside, California.

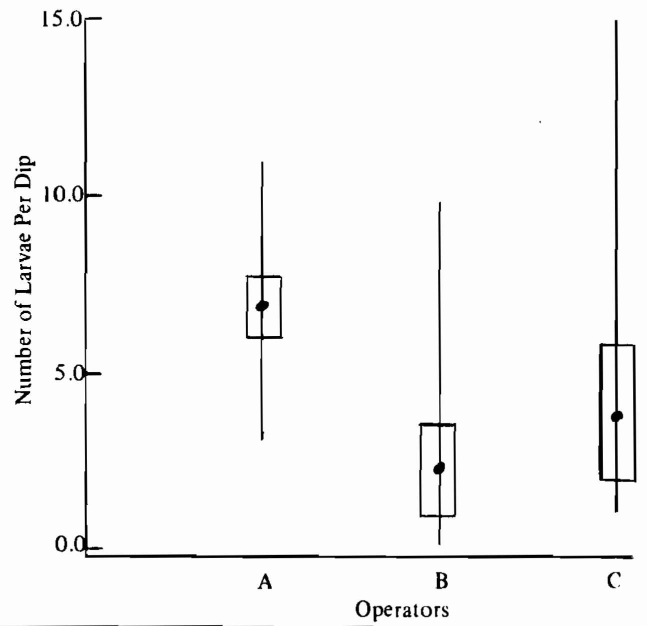

Figure 3.—A measure of the repeatability of larval density estimates by three different operators. Each operator taking two, 10 dip samples (n=20). Sample means, range, and 95% C.L. are shown. All samples taken at Hooper's marsh, Woodside, California.

GRAY LODGE WILDLIFE AREA (CALIFORNIA) – EXPLORATORY STUDIES OF MOSQUITO PRODUCTION AND CONTROL[1]

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ABSTRACT

Investigations were begun in August of 1978 to produce empirically based recommendations for mosquito control at Gray Lodge Wildlife Area, California. These recommendations will emphasize biological and physical control strategies and will be directed primarily against aedine species (especially *Aedes melanimon*). During the fall of 1978 experiments were established to test the relative efficacy of *Gambusia affinis* and chlorpyrifos. *G. affinis* shows promise as an effective control technique and future studies will concentrate on this strategy. Associations between habitat types and larval occurrence were identified to provide information toward the design of an adequate sampling system for future work.

There are approximately 231,000 acres maintained as State Wildlife Areas and National Wildlife Refuges in California alone. In addition there are several thousand acres in privately owned or leased "duck clubs". Incidental to the production and maintenance of habitat for waterfowl and other biota in these areas is the potential for the production of very large mosquito populations. As human populations inevitably encroach upon these historically generally remote localities, the problem is magnified.

In wildlife areas high emphasis is placed upon the use of alternatives to pesticides (especially those with known detrimental mammalian and avian toxicities). Biological and physical control strategies are given unusually high priority, especially those techniques deemed highly compatible with good wildlife management practices. Furthermore, increasing cost of pesticide use (in both material and application) together with ever increasing concern for low efficiency due to resistance, make this strategy a very expensive and risky one indeed, especially over large acreages.

There are more than a dozen published studies of tidal marsh wildlife refuges of the Atlantic seaboard (New Jersey, Delaware, and Florida) concerning physical control techniques. Most of this work has been reviewed by Provost (1977). A series of published studies have been conducted in marshes along the eastern shores of the Great Salt Lake in Utah. These studies concern primarily physical control, but include to some extent biological control, utilizing *Gambusia affinis affinis* (Baird and Girard) (Rees and Andersen 1966; Rees and Winget 1968 and 1969; Rees et al. 1974). Much has been discussed and recommmended concerning mosquito control compatible with wildlife management based upon these studies and the personal experience and insight of those charged with mosquito control. However, there is an extreme paucity of published empirical information concerning mosquito production and control in refuge areas pertinent specifically to California. Mortenson (1963) investigated mosquito production on duck clubs in Merced County, however these investigations, although valuable, were purely exploratory in nature. In 1958-59 a small study was conducted at Gray Lodge Wildlife Area by the California Department of Public Health, Bureau of Vector Control, in cooperation with the California Department of Fish and Game (Gerhardt 1960).

The present study was begun in August of 1978. Its prime objective is to ultimately provide empirically based recommendations for mosquito suppression to acceptable levels, emphasizing biological and physical control. Although these recommendations will be designed to be specific to this refuge, it is the prevailing hope that the studies will provide a model for mosquito control programs on fresh-water wildlife refuges and duck clubs throughout California. Recommendations will be based upon current studies at Gray Lodge as well as past pertinent research and ideas from other areas.

Although Gray Lodge produces very large numbers of *Culex tarsalis* Coquillett and *Anopheles freeborni* Aitken, the most immediate control problems are for *Aedes* spp., predominantly *Ae. melanimon* Dyar and to a lesser extent *Ae. nigromaculis* (Ludlow), during the spring and fall flooding seasons. A total of 7 control fields and 22 experimental fields (representative of the diverse environments encountered at the refuge) were established to test the effect of chlorpyrifos (Dursban®) and mosquito fish *Gambusia affinis affinis* (Baird and Girard) in suppression of larval populations.

Two different treatment types for chlorpyrifos were tested; entire field application of 2 lbs/acre of 2% granular chlorpyrifos and perimeter treatments (over a 60 foot wide swath of the margin of the field). The latter treatment was designed to test the hypothesis that application of the pesticide to the field perimeter is sufficient for adequate control because the bulk of the *Aedes* oviposition is located at the margin of the field.

The following two stocking rates of *G. affinis* were tested: 1 lb/acre and 3 lb/acre.

For the purposes of the entire-field chlorpyrifos and *G. affinis* studies, fields were divided into the following general habitat types:

a. Fields with "sparse emergent vegetation" (i.e., more than 50% open water when the field was fully flooded);

[1]This investigation was supported, in part, by research grants from the California Department of Fish and Game, and the Vector Biology and Control Section, California Department of Health Services.

b. Fields with "dense emergent vegetation" (i.e., less than roughly 30% open water);
c. Fields with little or no emergent vegetation (i.e., disced, mowed, or burned fields).

Results of these preliminary studies indicate that for fields with "sparse emergent vegetation" both the fish and chlorpyrifos treatments may be highly successful control strategies and it is possible that 1 lb/acre of *G. affinis* may produce generally the same level of control as the chlorpyrifos. For fields with "dense emergent vegetation", results were inconclusive for both chlorpyrifos and *G. affinis*. Although some reduction was evident, especially for the 3 lb *G. affinis* treatment, the reduction was not pronounced according to the sampling system employed this fall. No adequate comparison for fields with little or no emergent vegetation could be made this season because of the scarcity of this field type and the inadequacies of the sampling system. Studies for the spring and fall seasons of 1979 are presently being planned to further test *G. affinis* and to test field alternations to enhance *G. affinis* effectiveness.

Results of investigations of perimeter-treatment with chlorpyrifos indicate the possibility of substantial reduction of larval populations using this technique. However, based upon investigations this fall, larval populations are by no means restricted to edge environments (within 50 feet of the edge of the field pond, Table 1) and further study is necessary before drawing definitive conclusions.

A major objective of this first season's study was to obtain information necessary to design an adequate sampling system for future work. Several consistent features of larval distribution were found. Sampling experience indicated that one is more likely to dip larvae and pupae at the edge of the field pond, within 50 feet of the shoreline, than beyond this 50 foot margin. Quantitative comparison is illustrated in Table 1 for a control field which was relatively extensively sampled. Furthermore, sampling on all fields showed that one is much more likely to dip greater numbers of immatures in areas of emergent vegetation and areas of floating vegetation (Table 2). This information provides confirmation of earlier work (Rees and Andersen 1966, Mortenson 1963) and validation specific to Gray Lodge.

Areas with floating vegetation showed the highest values for percent positive dips and mean number of immatures per positive dip. These areas were often surrounded by broad expanses of open water and it is speculated that this larvae-floating vegetation association is due in part to habitat selection by larvae and in part to the greater ease of catching larvae in relatively sheltered, shaded environments.

Although the data are reported for only one field, they are representative of phenomena found to be generally consistent in the 31 fields included in the past seasons study.

ACKNOWLEDGMENTS.–The author gratefully acknowledges the very special cooperation and assistance of Eugene E. Kauffman, Manager of the Sutter-Yuba Mosquito Abatement District and the assistance in both personnel and equipment provided by the Sutter-Yuba MAD. The author is also indebted for the patience and expertise of Ernest E. Lusk of the Department of Health Services. This study is partially supported by a joint grant between the California State Departments of Fish and Game and Health Services.

Table 1.–A comparison of larval catches from sites of varying distance from the edge of a 230 acre, untreated field. (Samples were taken along 2 transects which ran the full width of the field. Total number of dips=617; total days sampled=5). Gray Lodge Wildlife Area, Butte and Sutter Counties, California. 1978.

Distance from edge of field pond								
0-50 ft			50-200 ft			>200 ft		
Total no. dips	% positive dips	Mean no. immat./dip	Total no. dips	% positive dips	Mean no. immat./dip	Total no. dips	% positive dips	Mean no. immat./dip
60	45	8.30	120	18	1.52	437	28	3.46

Table 2.–A comparison of larval catches from sites of varying amounts of vegetation and debris in a 230 acre, untreated field. Gray Lodge Wildlife Area, Butte and Sutter Counties, California. 1978.

"O" environment[1]			"E" environment[2]			"F" environment[3]		
No. dips	% positive dips	Mean no. immat. per positive dip	No. dips	% positive dips	Mean no. immat. per positive dip	No. dips	% positive dips	Mean no. immat. per positive dip
436	9	3.8	144	69	7.9	37	81	33.9

[1] "O" environment = open water with no emergent vegetation and no floating surface debris.

[2] "E" environment = edge (water-land interface), area within 25 ft. of edge, and areas where vegetation (most commonly grass and occassionally bulrush and cattail) emerged above the water surface.

[3] "F" environment = areas of floating surface debris (most commonly mowed grass).

[4] Positive dip = samples containing at least one larva or pupa.

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POTENTIAL OF VERTICAL DRAINAGE IN VARIOUS PASTURE SOIL STRATA

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ABSTRACT

Vertical drainage was shown to be an effective method to eliminate mosquito breeding sources within certain soil profile conditions. Drainage holes were effective where drilling penetrated through a highly impermeable stratum (e.g. clay or hardpan) into sand or loamy sand strata. For fields with no highly impermeable stratum within a meter of the surface, vertical drainage was not necessary and mosquito breeding could be eliminated by careful water management. Prolonged excessive irrigation and lateral, subsurface saturation from adjacent sources of water negated the benefits of drilling. A method was developed to obtain the rate of infiltration of standing water in a source area and to correlate this value with standards in order to determine both the probable cause for long-standing water and the possible benefit through vertical drainage implementation.

FACTORS AFFECTING THE DENSITY OF *CULEX TARSALIS* AND *ANOPHELES FREEBORNI* IN NORTHERN CALIFORNIA RICE FIELDS

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Two species of Typhloplanid microturbellarian commonly found in the rice fields of northern California (tentatively identified as *Mesostoma lingua* and *Rhynchomesostoma rostratum*) have been shown to prey on the pre-imaginal stages of *Culex tarsalis* and *Anopheles freeborni*, mosquitoes whose major breeding habitat is the flooded rice field (Collins and Washino 1978). Both microturbellarians are generalist predators (Hyman 1951), but because their densities in rice fields are frequently very high (*M. lingua* densities in excess of 1000/m^2 of surface area were observed in more than 30% of the fields studied in 1978), they probably exert an important effect on the mosquito population. Preliminary laboratory observations suggest that these microturbellarians prefer softer bodied prey such as oligochaetes and mosquito larvae over microcrustaceans and other aquatic insects, but the latter organisms are readily consumed when damaged or freshly killed. *M. lingua* has been maintained in the laboratory for approximately two years on a diet consisting almost exclusively of *Cx. tarsalis* larvae.

M. lingua is a 3-5mm flatworm which under laboratory conditions has been shown capable of rapidly subduing all larval instars of both *Cx. tarsalis* and *An. freeborni* by means of a sticky (and possibly toxic) mucus secretion. Body fluids of the prey are ingested through a medioventral, protrusable pharynx. This species has also been found in vernal pools in the central valley and Sierra Nevada foothills and in snow melt pools at elevations above 8000 feet in the Sierra Nevada. Preliminary studies suggest that it may be an important predator of mosquitoes in these habitats, particularly the various mountain *Aedes* species.

R. rostratum is a smaller (2-3mm) flatworm which, in California, has been found only in rice fields and associated irrigation and drainage canals. Although its density occasionally exceeds that of *M. lingua*, its distribution among fields is very irregular. Because of its smaller size, it is considerably less effective than *M. lingua* at capturing third and fourth instar mosquito larvae. Pupae, possibly because of their thicker cuticle and explosive movements, are practically immune to predation by both species of flatworm.

Previous studies in rice fields revealed that these flatworms rapidly invaded mosquito emergence707 cageds and caused significant mortality in sentinel *Cx. tarsalis* larvae. In fields where flatworms were abundant, mortality levels in excess of 90% usually occurred within 48 hours. Regression of sentinel mortality on the numbers of flatworms invading the emergence cages indicates that *M. lingua* was responsible for most of the mortality (see Table 1). This was often the only flatworm species present in emergence cages which sustained 100% sentinel mortality. As can be seen in the table, this interaction was present during both the 1977 and 1978 studies.

In 1977, rough estimates of the in-field flatworm and mosquito densities were obtained by means of dipper and aquatic sweep net samples. Analysis of these data revealed significant negative correlations (p<.05) between *M. lingua* vs. *Cx. tarsalis* and *R. rostratum* vs. *An. freeborni* (Collins and Washino 1978). To investigate the possibility of differential prey preference, predation rates were assessed in a laboratory setting. Individual *M. lingua* were placed in 100 ml containers with various numbers of third instar larvae of either *An. freeborni* or *Cx. tarsalis.* Surviving mosquito larvae were counted after a 24 hour interval. Control mortality was negligible. The results (see Table 2) indicate that under the conditions of the experiment there were no significant differences in the rates of predation by *M. lingua* on the two mosquito species at any of the prey densities examined. These results suggest that the lack of a negative in-field correlation between *M. lingua* and *An. freeborni* comparable to that observed between *M. lingua* and *Cx. tarsalis* is due to factors other than prey preference. Unfortunately, attempts to culture *R. rostratum* have not been successful enough to permit experiments involving this species.

Typhloplanid flatworms overwinter by means of heavily encapsulated, diapause eggs which begin development only after exposure to a low temperature and/or short day stimulus. By flooding soil samples containing such eggs, it has been determined that *M. lingua* can effectively overwinter in rice fields but cannot survive the summer dessication experienced in a field that has been rotated to an alternate crop.

To assess the rate at which flatworms re-colonize rice fields after a period of rotation, the 1978 study included the age of a field (the number of consecutive years in rice since the last rotation) among the variables under consideration. Thirty three sampling stations were established in fields ranging in age from first to thirteenth consective year in rice. Seasonal

Table 1.–Regression of sentinel survival on numbers of *Mesostoma lingua* and *Rhynchomesostoma rostratum* invading sentinel cages, % total R^2 indicated in parentheses. Rice field study, Sutter County, California.

Season	Total R^2	Due to *M. lingua*	Due to *R. rostratum*
1977	81.4	71.2 (87.5%)	10.2 (12.5%)
1978	79.6	62.2 (78.1%)	17.4 (21.9%)

Table 2.–Daily predation rates of adult *Mesostoma lingua* on third instar mosquito larvae. (1 adult flatworm/100 ml vessel; 16-8 day-night cycle; 21±1°C.)

	Prey density/vessel/day				
Prey species	1	2	3	4	5
Cx. tarsalis	.68±.08[1]	.82±.11	.92±.15	.95±.16	1.03±.15
An. freeborni	.76±.10	1.00±.14	1.00±.14	1.24±.16	1.19±.19

[1] Mean ± standard error.

Table 3.- Rice field study, Sutter County, California, 1978: Matrix of Spearman's correlation coefficients. (N = 33).

	M. lingua density	*R. rostratum* density	*Cx. tarsalis* density	*An. freeborni* density
Age of field	0.523[1]	0.015	-0.596[1]	0.467[1]
M. lingua density		0.159	-0.431[2]	0.144
R. rostratum density			0.162	-0.071
Cx. tarsalis density				-0.299[3]

[1] $p < .005$
[2] $p < .01$
[3] $p < .05$

Table 4.–Effect of location of sampling station on number of organisms captured by standard sample. Data from 33 rice field stations in Sutter County, California, 1978.

Location	Mean number of each species sampled			
	M. lingua	*R. rostratum*	*Cx. tarsalis*	*An. freeborni*
Checks 1-2	9.85	0.01[1]	0.60[2]	2.41
Checks 3-8	16.31	10.48	1.52	2.68
Checks 9-18	10.23	19.21	2.94	1.82

[1] 0.01 significantly different from 10.48 and 19.21.
[2] 0.60 significantly different from 1.52 and 2.94.
(No other differences significant. Duncan's multiple range test, $\alpha = .05$).

means were calculated for flatworm and mosquito density measurements, and all data were ranked and compared by means of Spearman's rank correlation coefficients. (This non-parametric technique was chosen for the preliminary analysis because very high week to week sample variability did not allow any assumptions about underlying distributions).

As is immediately apparent from the 1978 data (see Table 3), the densities of *M. lingua* and both mosquito species were more strongly correlated with the age of the rice field than with each other. The fact that *R. rostratum* was not so correlated and that correlations between this flatworm and *An. freeborni* were not consistent between the 1977 and 1978 seasons may be a consequence of the very high sampling variability associated with this species. Part of this variability is due to the location of the sampling station within the rice field. As can be seen in Table 4, *R. rostratum* was practically absent from the two upper checks, even in fields where it was otherwise abundant. However, since the correlation coefficient based on a data set from which upper check measurements have been removed ($r_s = .068$) is not significant, the negative *R. rostratum/An. freeborni* relationship observed in the 1977 data is probably spurious.

Although none of the results contradict the hypothesis that *M. lingua* is an important predator of *Cx. tarsalis* in rice fields, the data may be given an alternative interpretation by assuming a temporal maturation of the rice field ecosystem which favors *M. lingua* and *An. freeborni* and hinders *Cx. tarsalis*. Not only is such an explanation consistent with that aspect of flatworm biology which necessitates a period of recolonization in rice fields that have undergone rotation, but it also accords well with observed differences between the ecological strategies of *Cx. tarsalis* and *An. freeborni* (Bohart and Washino 1978). Such a process has actually been observed in the rice field chironomid community (Clement et al. 1977, Darby 1962). While an important predator/prey interaction is not precluded under such an interpretation, it does indicate the possibility that the significant negative in-field correlation between *Cx. tarsalis* and *M. lingua* may be an artifact of the age correlated changes in the aquatic rice field community.

In summary, this study thus far has produced two important observations which relate directly to the control of mosquitoes in California rice fields: (I) the age correlated change in the structure of the rice field mosquito community, and (II) the probable role of *M. lingua* as an important natural predator of *Cx. tarsalis*. Future work will focus on resolving the relative importance of these two phenomena to determine how they can best be utilized to augment mosquito control efforts.

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BIO-ECOLOGICAL STUDIES OF *CULEX* MOSQUITOES IN A FOCUS OF WESTERN EQUINE AND ST. LOUIS ENCEPHALITIS VIRUS TRANSMISSION (NEW RIVER BASIN, IMPERIAL VALLEY, CALIFORNIA)

II. HOST-SEEKING AND HUMAN BITING CYCLES

L. L. Walters[1], E. W. Gordon[2] and R. E. Fontaine[3]

Although *Culex tarsalis* Coq. is assumed to be the primary vector of both western equine and St. Louis encephalitides (WEE and SLE) in the Imperial Valley, California, *Cx. pipiens quinquefaciatus* Say was recently proposed to be of additional vector importance in the transmission of SLE in this area (Magy et al. 1976, Work 1977). In evaluating the relative importance of these species in endemic transmission cycles of arboviruses to man, affinity for human bait and times of biting in relation to risk of human exposure are critical factors to determine. As host preference of *Culex* mosquitoes can vary both geographically and seasonally (Reeves and Hammon 1944, Reeves et al. 1963, Tempelis et al. 1967), host-seeking and biting patterns of *Cx. tarsalis*, *Cx. p. quinquefaciatus* and *Cx. erythrothorax* Dyar were investigated within the New River basin, an endemic focus of WEE and SLE 7 km southwest of El Centro. A one-night collection of *Culex* mosquitoes from human bait was done previously by Berlin et al. (1976) at the Finney Ramer Wildlife Refuge, 42 km north of the New River site.

METHODS AND MATERIALS.- The collection site was adjacent to the New River, surrounded by salt cedar (*Tamarix* sp.), arrowed (*Pluchea* sp.) and pickleweed (*Salicornia* sp.) vegetation (Figure 1) and located 0.2 km from a productive breeding area of *Cx. tarsalis*, *Cx. p. quinquefaciatus* and *Cx. erythrothorax*.

Human-bait Collections.- - All-night mosquito collections from human bait were made on May 9, May 31, June 16, July 1 and October 14, 1978, these periods associated with seasonally high *Culex* densities. The collection period encompassed the hour before evening civil twilight to the hour after morning civil twilight, corresponding to 11 hr in May and June, 10 hr in July and 13 hr in October. Collections were usually aborted on nights when wind speed became variable with gusts over 10 kts or when all *Culex* sp. were biting at rates of less than 5 per hr. No collections were made during the summer months when densities of *Culex* were low; the October 14 collection monitored the fall population peak of *Cx. tarsalis* in the Imperial Valley (Gordon et al. 1978).

Human bait each night consisted of the first author and one other volunteer. Fully clothed baits sat in chairs (Figure 1) with legs and arms extended in front, lower arms and hands were left exposed to mosquitoes. Head nets were worn at times of intense biting activity of *Psorophora columbiae* Dyar and Knab, otherwise only hats were worn with the face exposed. Baits took turns collecting off one another in 15 min intervals (subsamples) for the first 45 min of each hour. The bait was scanned for mosquitoes every few minutes with a dimmed flashlight and those biting or probing collected bv mouth aspirator. Mosquito subsamples were expelled into separate 150 ml plastic vials and placed on dry ice until laboratory identification the next day.

Animal-bait Collections. - - Various animals (pigeon, goat, rabbit, pig, duck, turkey) were utilized (based on their availability) as an adjunct to human bait collection. A magoon-type stable trap, provided by Dr. W. C. Reeves, University of California, School of Public Health, Berkeley, California, was used to house all animals except pigeons which were isolated individually in lard-can traps (Bellamy and Reeves 1952). Lard-can traps were modified with a net front to allow aspiration of mosquitoes and were placed 1.5 m high in salt cedar vegetation. The proximity of stable and lard-can traps to the human bait station is shown (Figure 1).

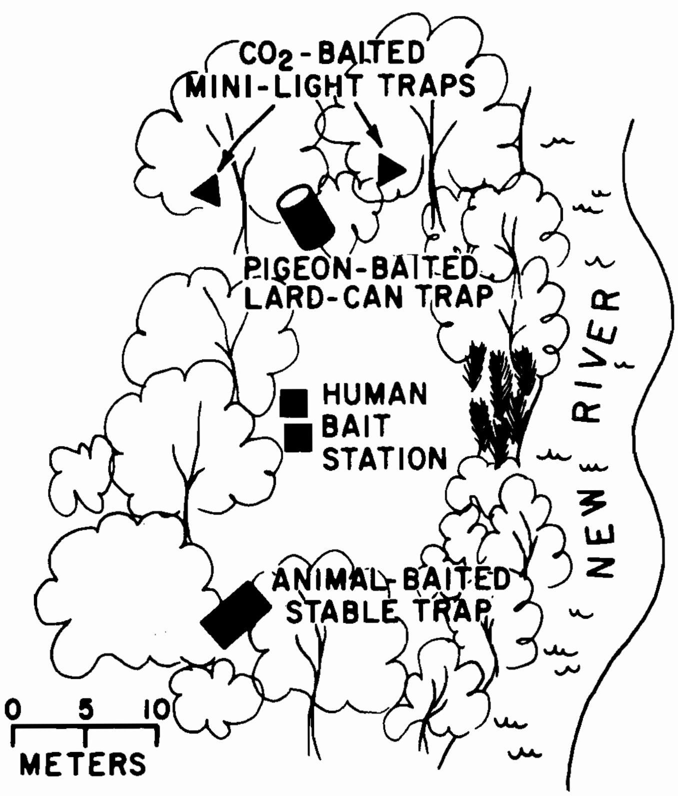

Figure 1.- Proximity of human bait to animal and CO_2-light trap bait stations during all-night mosquito collections at the New River.

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The stable trap was cleared of resting mosquitoes before addition of unrestrained animal bait. Bait was placed in the trap just before human catch began each night and removed along with the total mosquito catch in the morning.

Lard-can traps contained one pigeon, confined in a nylon stocking to prevent destruction of biting mosquitoes. Mosquitoes were aspirated from the can following human catches each hour. As this usually required a 10-15 min collection period, the interval of pigeon exposure to mosquitoes was considered equivalent to that of human bait.

CO^2-baited Light Trap Collections. - - Two miniature light traps (Rohe and Fall 1979) baited with ca. 1 kg dry ice as an attractant for host-seeking mosquitoes were used on each night of human bait collection. Traps, placed 1.5 m high in salt cedar vegetation, were operated on the same schedule as human bait catch without subsampling. Each hour's catch was killed and stored on dry ice.

Parous Condition and Virus Determination. - - Mosquitoes were separately identified on dry ice and abdominal condition noted. Fresh-feds were stored for confirmation of bait-feeding by precipitin test (to be published subsequently). Most unfed specimens of *Cx. tarsalis* and *Cx. p. quinquefaciatus* were dissected for parous condition based on the presence of tracheal skeins on the ovaries (Detinova 1962). In addition, pools of unfed *Cx. tarsalis* from the May 31 and October 14 human bait collections were submitted for virus determination to Dr. T. H. Work, University of California, School of Public Health, Los Angeles, California.

Environmental Factors. - - During each night, air temperature, relative humidity and wind speed were measured hourly between human bait catches. Temperature and relative humidity were determined using a battery-operated psychrometer; wind speed using a hand held air-flow meter.

Crepuscular Units. - - Hourly and subhourly data from each night were standardized by converting catch times to Crepuscular Units (Crep units) (Nielson 1961). Catch times of each sample in relation to sunset were converted according to Nielson's formula $\frac{\text{time of day-time of sunset}}{\text{duration of twilight}}$ and in relation to sunrise, $\frac{\text{time of sunrise-time of day}}{\text{duration of twilight}}$. Sunset and sunrise represent a zero reference with one Crep unit equal to the time between sunset and the end of civil twilight or the beginning of morning civil twilight and sunrise. Positive Crep values refer to periods after sunset and sunrise; negative values to periods before these times. Twilight was determined from tables of the American Ephemeris, 1978. Seasonal variance in catch nights was found to be 1.86-2.20 Crep units in this study (i.e., 1 Crep unit = 27-32 min).

For each species, the number of mosquitoes collected in each 4 Crep unit interval was combined for nights with significant biting activity and converted to a percent of total attraction. *Culex tarsalis* was considered active on all nights except July 1; *Cx. erythrothorax* on the first three nights sampled and *Cx. p. quinquefaciatus* only on two nights, May 31 and June 16. Additionally, hourly mean numbers of mosquitoes attracted to all bait types were determined from subhourly and nightly data.

RESULTS AND DISCUSSION.–Environmental conditions potentially influencing mosquito activity and biting cycles are shown in Table 1. Air temperature each night was highest at sunset and decreased to sunrise as indicated by the range in Table 1. Prevailing temperatures were equivalent on sample nights during May-July with slightly lower mean temperature noted on October 14.

Relative humidity was highest just before sunrise, with considerable nightly variation. However, humidities within this range were reported to have no appreciable effect on *Culex* activity patterns in laboratory studies (Muirhead-Thompson 1938).

Windy nights are known to reduce the numbers of mosquitoes caught biting (Service 1976) and in preliminary catches in the present study, highly variable winds over 10 kts resulted in erratic biting counts with waves of mosquitoes attacking between gusty periods. The calm to light wind conditions observed on sample nights did not appear to affect mosquito activity and biting times (Table 1).

Phases of the moon and duration of moonlight may influence the density of both biting and light trap catches. Enhanced biting during periods of moonlight has been demonstrated with some nocturnal mosquitoes (van Someren and Furlong 1964, Gillies and Furlong 1964), although moonlight had no effect on biting cycles of more strictly crepuscular species (Haddow 1964). Moon phase is reported to reduce mosquito density in light traps, with fewer numbers caught at or near a full moon than at new moon or intermediate illuminations (Provost 1959). Table 1 shows the moon phase (fraction illuminated) and timing of moonlight for each night of biting collections. Conditions ranged from new moon (May 9) to full moon (October 14) with moonlight present during the evening crepuscular period (0- 4 Crep units) on all nights except May 31 when a morning moonlight period occurred. Additionally, moonlight extended into morning June 16 and October 14 from 7.5 and 2.5 Crep units before sunrise, respectively.

Table 1.–Environmental conditions for each night of mosquito collections from human bait along the New River, Imperial Valley, California.

Catch Night (1978)	Air Temperature (C) $\bar{X}$	Air Temperature (C) Range	Relative Humidity (%) $\bar{X}$	Relative Humidity (%) Range	Moonlight[1] Nightly Duration (Crep units)	Moonlight[1] Fraction Illuminated	Wind Speed (kts)
May 9	21	16-29	34	28-50	sunset (0) to + 4.8	0.03	calm, gusts to 7
May 31	22	12-33	47	28-75	-6.8 to sunrise (0)	0.30	calm, gusts to 8
June 16	21	13-31	58	22-82	sunset (0) to + 13.3	0.71	calm
July 1	23	13-30	37	22-60	sunset (0) to + 5.9	0.17	calm, gusts to 8
Oct. 14	16	12-31	80	49-89	sunset (0) to + 25.3	0.94	calm

[1] Moonlight conditions determined from tables of the American Ephemeris, 1978.

Host-seeking and Biting Cycles. - - *Culex tarsalis*. - - *Cx. tarsalis* were slightly more attracted to human bait than to CO_2-baited light traps (Table 2). Moon phase on June 16 and October 14 possibly rendered mosquitoes even less successful in orienting to light traps compared to human bait. Relatively low numbers collected in July reflected the seasonal decline of *Cx. tarsalis* populations.

Culex tarsalis patterns of host-seeking and human biting are shown in relation to sunset and sunrise (Figure 2). Although *Cx. tarsalis* appeared to be attracted equivalently throughout the night to both bait types, a definite evening crepuscular peak in human biting occurred (0-4 Crep units), whereas light trap data was less defined, exhibiting perhaps two peaks (0-4) and 8-12 Crep units). Biting activity decreased through the night after the evening peak of crepuscular activity. Berlin et al. (1976) found *Cx. tarsalis* primarily seeking hosts nearly 3 hr after sunset and biting mosquitoes were uncommon. These results, based on only one night sampled do not appear to accurately represent mosquito behavior and are contradicted by present data.

Analysis of the biting cycle by one-half and by one Crep unit intervals delineated the exact primary peak of *Cx. tarsalis* human biting to be within the first one-half hr after the end of evening civil twilight. Additionally, on two sample nights of greater than 22 Crep units duration, a second lesser morning crepuscular peak was observed between 22-24 Creps (morning civil twilight for these nights).

Culex tarsalis displayed a marked preference for human bait when a choice of baits was presented (Table 2). Although somewhat attracted to pigeon bait on May 9, preference for birds compared to man was not demonstrated as indicated by Berlin et al. (1976), and this species was little attracted to stable-trapped mammals.

Pattern of attraction to pigeon bait differed from human bait (Figure 3). Although the total number of mosquitoes attracted was few (n=30), *Cx. tarsalis* was most attracted to pigeons during 8-12 Crep units, somewhat later than the primary period of attractivity to man, but perhaps coinciding with the second peak exhibited by the total host-seeking population.

Culex p. quinquefaciatus. - - Host-seeking *Cx. p. quinquefaciatus* were mainly attracted to human and CO_2 light trap bait on May 31. Enhanced human biting this night was believed to be a result of moonlight during the early morning hours (Table 1) and seasonal abundance. Absence of moonlight on May 9 may have inhibited biting activity. Peak biting on human bait was 4-8 Crep units before sunrise although biting was observed all night (Figure 3). No crepuscular tendency was demonstrated.

The numbers of mosquitoes attracted to CO_2 light traps and human bait appeared similar (Table 2). In associated research on dispersal, we found *Cx. p. quinquefaciatus* to be under-attracted to these light traps when compared to larval prevalence (Walters et al. 1979). Therefore, equivalent attraction to human bait compared to light traps may indicate no preference for man in these riverine areas. Attraction to

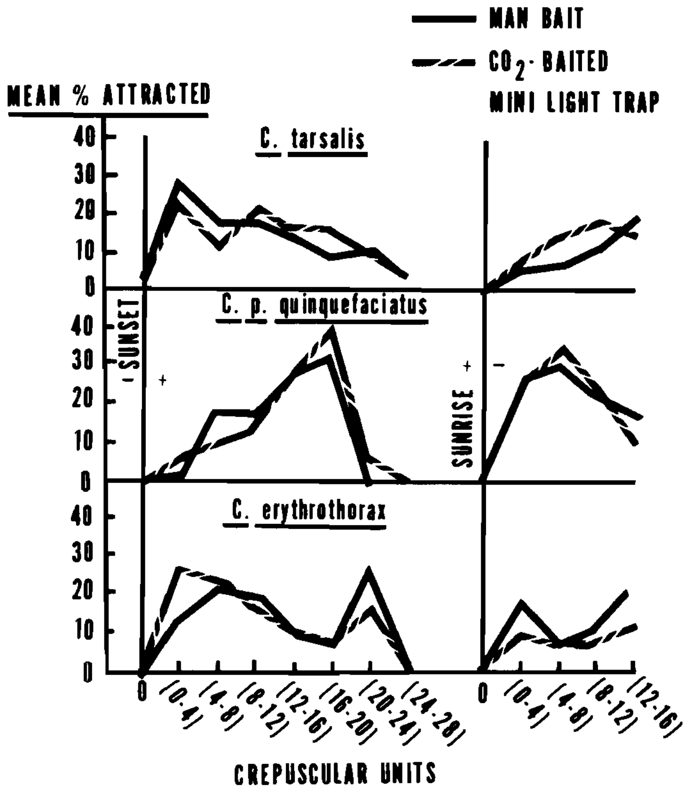

Figure 2.–Host-seeking and human biting cycles of *Culex* mosquitoes within the New River basin during May-June and October (*Cx. tarsalis*) and May-June (*Cx. p. quinquefaciatus* and *Cx. erythrothorax*), 1978.

Table 2.–Relative attaction of *Culex* mosquitoes to selected baits during all-night collections at the New River.

	Mean No. ♀♀ Attracted/Hour														
	Culex tarsalis					*Culex p. quinquefaciatus*					*Culex erythrothorax*				
Bait Type 1978	May 9	May 31	June 16	July 1	Oct. 14	May 9	May 31	June 16	July 1	Oct. 14	May 9	May 31	June 16	July 1	Oct. 14
Human	16.0	15.6	8.3	0.8	10.3	0.0	6.7	0.5	0.1	0.0	22.2	2.6	5.0	0.1	0.4
CO_2/Light	14.4	14.7	5.1	0.7	3.7	0.4	4.0	1.0	0.0	0.2	14.7	1.0	0.6	0.0	0.2
Pigeon	2.4	-	0.3	0.2	0.8	0.2	-	2.1	0.5	0.0	0.1	-	0.1	0.0	0.1
Goat	-	1.1	-	-	-	-	4.0	-	-	-	-	1.0	-	-	-
Pig	-	-	0.0	-	-	-	-	0.5	-	-	-	-	0.0	-	-
Rabbit	-	-	-	0.0	-	-	-	-	0.1	-	-	-	-	0.0	-
Duck/Turkey[1]		0.0					0.3					0.0			

[1] Bait used on July 14.

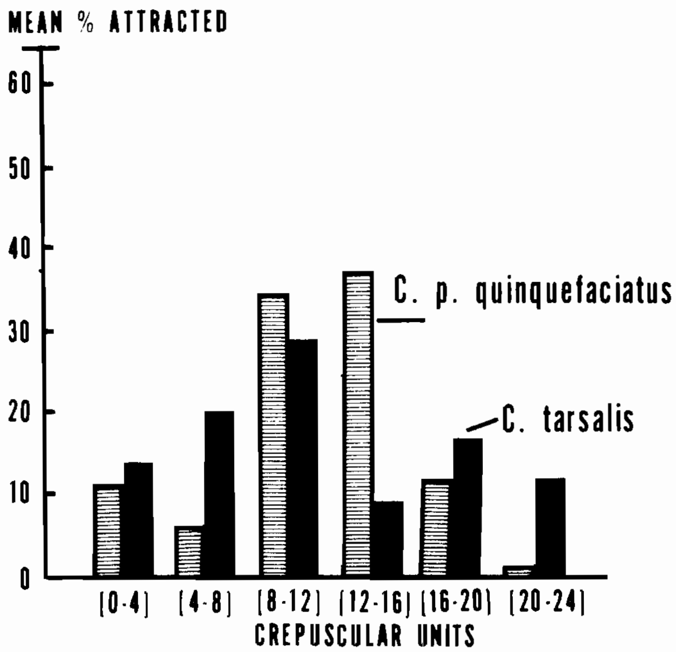

Figure 3.—Pattern of attraction of *Cx. tarsalis* and *Cx. p. quinquefaciatus* to pigeon-baited lard-can traps during May-July, 1978 in the New River basin.

pigeons in this habitat was slightly greater than to human bait on most nights (Table 2). Additionally, *Cx. p. quinquefaciatus* was attracted to duck/turkey bait on July 14, a time of negligible seasonal abundance. Species attraction to the stable-trapped mammals used in this study appeared equal to man.

Biting activity on pigeon bait was similar to that on human bait, however, peak biting occurred somewhat earlier, 8-16 Crep units after sunset (Figure 3). With both this species and *Cx. tarsalis* there perhaps was a correlation between peak biting and maximum quiescense of host birds in the habitat.

Culex erythrothorax. - - *Cx. erythrothorax* was considerably more attracted to human bait than to CO_2-baited light trap or animal bait each sample night (Table 2). Major host-seeking activity observed on May 9 appeared associated with the seasonal peak of abundance of this species.

Culex erythrothorax were actively host-seeking all night attracted to human and CO_2-light in an equivalent cycle (Figure 2). A crepuscular pattern was demonstrated, more pronounced in the morning. Host-seeking behavior in the evening extended several hours beyond the crepuscular period, especially in relation to human hosts.

Attraction of *Cx. erythrothorax* to pigeon bait was observed rarely between 12-16 Crep units after sunset.

Parous Condition. - - The parous/multiparous rate of host-seeking *Cx. tarsalis* averaged over 70%, ranging 62-84% from human bait and 57-89% from CO_2-baited light traps (Table 3). Parous mosquitoes found in lowest proportion in the June collection was attributed to peak numbers of nullipars emerging from the nearby breeding area during this month.

The proportion of parous *Cx. p. quinquefaciatus* seeking hosts was notably lower than that of *Cx. tarsalis* and was no higher than might be expected by chance due to larval proximity. Although numbers of *Cx. p. quinquefaciatus* collected were limited, the consistently low parous rates observed (less than 50%) may indicate low daily survival of this species or relate to greater numbers of nullipars generated within the basin compared to *Cx. tarsalis*.

Epidemiologically, the differences observed between *Cx. tarsalis* and *Cx. p. quinquefaciatus* are significant, especially in respect to human bait collections. We conclude that *Cx. tarsalis* has a greater vector potential because of its higher incidence in human biting collections during late spring and fall (times of arboviral activity) and to the higher percentage of mosquitoes having previously fed, and therefore, potentially able to transmit viruses to man or reservoir hosts. We have not confirmed whether or not an autogenous population of *Cx. tarsalis* is present in the New River basin, although Nelson (1971) reported autogeny of populations in northern Imperial County.

Parous rates of *Cx. tarsalis* attracted to pigeon and goat were appreciably lower compared to human bait. The ability of older individuals to selectively orient to human hosts over other animals would be epidemiologically significant, however, further investigations are needed in this regard. Pigeons appeared to attract a slightly greater proportion of parous *Cx. p. quinquefaciatus* compared to human or other mammal bait.

Virus Isolation. - - A pool of 23 *Cx. tarsalis* tested for virus from the May 31 human bait collection was found positive for western equine encephalitis virus. Seasonally, this was the time of highest WEE activity in *Cx. tarsalis* with ca. 40% pools found positive in concurrent investigations. WEE/SLE viruses were not recovered from the October 14 pools.

Table 3.—Parous rates of *Culex tarsalis* and *Culex p. quinquefaciatus* attracted to selected baits during all-night collections at the New River.

Catch Night	Percent Parous/Bait Type							
	Culex tarsalis				*Culex p. quinquefaciatus*			
(1978)	Human	CO_2-Light	Pigeon	Goat	Human	CO_2-Light	Pigeon	Goat
May 9	72	(89)[1]	(67)	-	-	(50)	(100)	
May 31	84	70	-	(60)	44	48	-	44
June 16	62	57	(50)	-	(33)	54	33	-
July 1	(80)	70	(50)	-	-	-	(50)	-
Oct. 14	73	71	64	-	-	-	-	-
$\bar{X}$	74.2	71.4	57.8		38.5	50.7	61.0	

[1] Parentheses indicate sample size less than 10.

SUMMARY AND CONCLUSIONS.- On the basis of man-vector contact, *Cx. tarsalis* is considered a more important link in arbovirus transmission to humans in riverine areas of the southern Imperial Valley than *Cx. p. quinquefaciatus. Culex tarsalis* fed on human bait in peak numbers during the evening crepuscular period, coinciding with probable period of human exposure. In contrast, *Cx. p. quinquefaciatus* peak feeding occurred several hours before sunrise, the lowest period of human presence in the area. Moreover, *Cx. tarsalis* human biting rates were higher than *Cx. p. quinquefaciatus* each night and biting activity occurred on all nights sampled. Among the two species, *Cx. tarsalis* showed significantly greater mean proportion parous collected from human bait (74.2% compared to 38.5% for *Cx. p. quinquefaciatus*) and finally, *Cx. tarsalis* collected from human bait was found infected with WEE virus one month.

Culex erythrothorax, although never confirmed as a vector of WEE/SLE, was strongly attracted to human bait when seasonally abundant and showing peak human biting rates during the crepuscular period.

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THE UNATTRACTIVENESS AND REPELLENCY OF *AEDES SIERRENSIS* EGGS TO SELECTED PREDATORS

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ABSTRACT

No eggs exposed on pieces of filter paper placed in tree holes and other field sites were contacted or disturbed by such predators as ants, sowbugs, centipedes and carabid and staphylinid beetles. When eggs were treated 7 different ways (including bleaching and sonification) and exposed on pieces of filter paper pinned across a trail of foraging Argentine ants (*Iridomyrmex humilis*), most ants ignored the mosquito eggs. The ants which responded did so by antennulation, biting and removal. About 50% of the bleached and sonicated eggs that were antennulated were bitten, but few such eggs were carried away. Untreated eggs and those immersed in water before exposure were rarely bitten and never removed. Therefore, the hydrophobic plastron on *Aedes sierrensis* eggs which keeps the outer egg shell surface dry, thereby renders the eggs unattractive to potential predators. Also, a repugnant taste factor associated with bitten eggs apparently was responsible for few bitten eggs subsequently being carried off.

In research on the mating competitiveness of normal and sterilized males of *Aedes sierrensis* (Ludlow) released into field tents (Anderson, Asman and Ainsley 1979) two related objectives were to compare the number of eggs laid at different elevations, and the number laid/tent. However, when ants were observed foraging on chunks of fruit provided in containers as a source of carbohydrate, it became important to learn whether ants also might enter the oviposition containers and eat or carry away eggs deposited by the caged mosquitoes.

A visual examination of several tree holes, aided by a flashlight, revealed such known predators as ants, sowbugs, centipedes and carabid and staphylinid beetles, but none of these arthropods appeared to be foraging on mosquito eggs. As Hinton (1968a) has reported that *Culex pipiens* eggs were protected by a drop of natural repellent that discouraged predation by two species of ants and, as a parallel, it seemed possible that eggs of the tree hole mosquito might also possess a substance that protected them from being eaten by predators. Since many *Aedes sierrensis* eggs remain exposed for long periods above the waterline and in totally dried out tree holes, exposed eggs would seem to be vulnerable to predators, unless the immobile eggs were hidden under cracks, camouflaged, or protected by an obnoxious-distasteful repellent or some other mechanism.

In two preliminary tests about 100 eggs obtained from laboratory-reared mosquitoes were placed on filter paper discs and exposed along the sides and bottoms of tree holes harboring ants and the other predators mentioned above. Similar numbers of eggs also were twice exposed along active columns of ants on the ground adjacent to fallen tree trunks. None of the approximately 400 eggs exposed in the preliminary tests were contacted or disturbed by the potential predators, suggesting that the eggs were inherently unattractive or repellent.

Scanning electron photographs revealed a complex outer chorionic layer consisting of a plastron and many small globules that could be the site of a repellent. Accordingly, groups of eggs were next bleached to remove the outer layer of chorion. In two preliminary experiments comparing the response of the Argentine Ant, *Iridomyrmex humilis* (Mayr), to bleached and untreated eggs, only bleached eggs placed in the path of foraging ants were attractive to, bitten by, and sometimes carried off by the ants, about 50% of the bleached eggs were bitten and 10% were carried away. Since there was a possibility that the ants were responding to moisture present on the surface of bleached eggs, rather than being repelled from the untreated eggs, the following experiments were conducted in a riparian habitat on the U. C. Berkeley campus.

The *Aedes sierrensis* eggs used were from an established laboratory colony, and from August 22nd through September 19th, 1978 eggs treated in 7 different ways were exposed to worker Argentine ants. This was done by placing 150-200 eggs of each treatment on a 3 cm^2 filter paper, and then pinning the paper on the ground across an active ant trail which came from a nearby redwood tree. The same ant trail was used for all the experiments, and all observations were made between 10:00 a.m. and 2:00 p.m.

Ant behavior was observed through a 2.5X magnifying lens mounted on a 25 mm high tripod and placed over the paper and eggs. In total, about 1600 eggs were placed on 9 different pieces of filter paper, and each set of eggs was observed for 30-60 minutes.

The 7 different types of eggs presented to the ants were: untreated eggs, eggs immersed in water for 1 hour just prior to the experiment, eggs bleached in a solution of glacial acetic acid plus sodium hypochlorite for 24 hrs. and then rinsed in water, eggs bleached for 24, 7, and 1 hour, then rinsed and allowed to air dry for 7 days, and eggs sonificated for 2 minutes while immersed in xylene, and then rinsed in 95% ethanol and water. The latter treatment removed a thin, clear, amber colored coating which surrounded the entire egg, but this process did not decolorize or desclerotize the eggs as the bleach treatment did.

The preliminary observations of ant behavior with normal and bleached eggs showed them to make 3 responses to the eggs. The first response was always antennulation, ants touching the egg with their antennae. The second response, when it occurred, was biting, an ant grasping an egg with its mandibles. After biting, most ants dropped the egg and departed, but in a few cases the ant carried the egg off the paper and this constituted the third response, removal of an egg.

An individual ant was observed from the time it walked onto the paper until the time it walked off, and if it responded directly to the eggs, its behavior was recorded. An ant which antennulated one egg was scored the same as an ant which antennulated numerous eggs.

Thus the number of responses represented the number of ants performing the act rather than the number of eggs receiving the response. Numbers of responses for each experiment were totalled and the percentage of ants which bit eggs after antennulating them were compared to determine if the ants responded differently to the treated and untreated eggs. From the results of the 7 egg treatments (Table 1) we conclude that there may be two factors associated with *Aedes sierrensis* eggs that repel or discourage predators. First, there appears to be a surface factor associated with the outer chorion, and secondly, there appears to be a repugnant taste factor associated with bitten eggs (because only a few of the bitten eggs were carried off).

In the case of the bleached, moist eggs, the removal response by 14% of the ants which antennulated eggs (Table 1) seemed due primarily to the moisture associated with these bleached eggs. Therefore, a hydrophobic layer that keeps the outer egg shell surface dry may be a significant beneficial factor separate from a repellent factor. Because, if the eggs were naturally wet they could serve as a moisture source in a dry habitat, and therefore actually attract small predators. They might then be carried away only in response to the moisture factor.

The egg shell respiratory system of these mosquito eggs, like those of other species (Hinton 1968a, 1968b, 1969) is a plastron, or physical gill. As Hinton has stated, "The plastron is simply a gas film of constant volume and an extensive water-air interface. Such a film is held in position by a system of water-repellent structures, and it resists wetting at the hydrostatic pressures to which it is normally subjected in nature". The outer chroionic layer of the *Aedes sierrensis* egg is thus an open hydrofuge network, so the eggshell surface is unwettable, or hydrophobic.

As is the case with sessile plants, the chemical defense of the eggs of certain insect species is largely passive. The egg must be bitten or tasted before a predator is repelled. Among a large population of eggs in a tree hole a few bitten, and embryos presumably killed, would seem to be a small price to pay for the repulsion of foraging ants or other predators.

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Table 1.–The response of foraging workers of the Argentine Ant when exposed to eggs of the Western Tree Hole Mosquito.[1]

Eggs	No. ants antennulating eggs	Antennulating Ants which bit eggs		Biting Ants which removed eggs	
		No.	%	No.	%
Untreated	100	2	2	0	0
Water immersed 1 hr	30	0	0	0	0
Bleached 24 hr, moist	100	61	61	14	14
Bleached 24 hr, dry[2]	50	27	54	2	4
Bleached 7 hr, dry[2]	40	27	67.5	0	0
Bleached 1 hr, dry[2]	50	25	50	0	0
Stripped by sonification	50	20	40	0	0

[1] From 150-200 eggs of each treatment were exposed on 3 cm^2 filter papers and pinned on the ground across an active ant trail. There were two replicate exposures for groups one and three.

[2] Air dried for 7 days prior to exposure.

THE PATTERN OF PUPATION AND EMERGENCE OF LABORATORY-REARED *AEDES SIERRENSIS*

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ABSTRACT

Newly-emerged males of the Western Treehole Mosquito, *Aedes sierrensis*, were separated from females by time spent in the larval stage. At 23°C and a 15:9 LD cycle larvae which pupated in the first 2 to 3 days yielded predominantly males. No evidence was found for a strong diurnal rhythm in pupation or emergence of *Ae. sierrensis* under these rearing conditions.

Large-scale production of *Aedes sierrensis* for use in a sterile male population management program will require efficient and economical techniques for separation of male and female mosquitoes. The public will not accept a noticeable proportion of biting females in the release population - - even if they are sterile. For maximum efficiency the producer of sterile male mosquitoes will need to separate the males with a minimum of hand labor as early in the rearing process as possible. Since the first metamorphosed pupae in our laboratory colonies of *Ae. sierrensis* were observed to yield males, the objective of this study was to determine if separation of males at time of pupation, based on the duration of the larval stage, might be easier and more economical than sexing newly-emerged adults.

Ae. sierrensis males must be irradiated between 12 and 24 hours of emergence to render them sterile (Terwedow and Asman 1977). The optimal age for sterilization is from 16 to 20 hours (R. Ainsley, personal communication). If *Ae. sierrensis* showed a strong diurnal rhythm in pupation and emergence, it might be possible to collect and irradiate newly emerged males just once daily. Such a diurnal rhythm has been demonstrated for *Ae. taeniorhynchus* (Nayar 1967, Provost and Lum 1967) and several other mosquito species (Nayar and Sauerman 1970). Mean peaks of pupation occurred at nearly 24-hour intervals and nearly all larvae pupated within 3 to 4 hours. Species such as *Ae. aegypti* and *Ae. triseriatus* (the Eastern Treehole Mosquito), however, do not exhibit a diurnal rhythm of pupation and emergence (Haddow et al. 1959, Nayar and Sauerman 1970).

MATERIALS AND METHODS.--The sequence of pupation and adult emergence was determined during the course of mass-rearing two lots of mosquitoes for use in experiments on the competitiveness of sterilized males (Anderson, Asman and Ainsley 1979). The first mass rearing included 20,000 mosquitoes for which data on larval development and pupation were taken once daily. The second lot included 528 mosquitoes in two containers. In the second lot counts of pupae and adults were made every 4 to 6 hours for 11 days. Eggs used in these experiments were from a colony strain originating from a population of mosquitoes collected near Fresno, California. Mature eggs were preconditioned by storage at 11°C and a 10L:14D photoperiod.

For the first lot of mosquitoes reared, 10 polyethylene pans (3.51 capacity) were filled with 1500 ml deionized water (500 cm^2 surface area). Nitrogen was bubbled through the water for 10 to 15 minutes to lower the dissolved oxygen content. Next, 0.5 g of powdered Bacto nutrient broth (Difco Laboratories, Inc.) and 2000 to 4000 eggs were added to each pan. The next day the newly hatched larvae were divided among 35 pans to yield a density of about 700 larvae per pan. The larvae received 0.6 g of ground rat chow (Ralston Purina Co.) per pan per day. All pans were supplied with an air bubbling system which prevented the water from fouling. The insectary was maintained at 22°-24°C, 70%-80% RH and a photoperiod of 13 hours light preceded and followed by 1 hour "twilight" and 9 hours dark. At the onset of pupation, 11 days after hatching, feeding was terminated. Pupae were hand-skimmed at midday with fiberglass screen scoops, and about 100 pupae were stored in individual pint cups half filled with deionized water. To increase the number of mosquitoes which would emerge on the day chosen for male sterilization newly formed pupae were moved to a refrigerator kept at a 15L:9D photoperiod and 11°C. All pupae were simultaneously returned to the insectary 4 days before adults were needed. Thus all pupae from the first mass rearing spent at least 24 hours at 11°C.

For the second lot of mosquitoes reared approximately 300 eggs were hatched in each of two pans. The feeding ration was scaled to 250 mg per pan per day. The same procedures used for the first mass rearing were followed except that the pupae and adults were removed and counted at 05:00, 09:00, 13:00, 19:00, and 23:00 each day. Twilights turned on at 05:00 and turned off at 20:00.

RESULTS AND DISCUSSION.--The pattern of male and female pupation is shown in Figures 1 and 2. There are three reasons why the pupation curve of the males was much broader in the first mass rearing (Figure 1) than the sharply peaked curve obtained for the second lot of mosquitoes reared (Figure 2). A larger sample size and a higher larval rearing density (which intensified competition) both led to more variability in the chronological appearance of pupae. But probably the most important factor was the asynchronous hatching of many eggs in the first lot of mosquitoes reared. Although most eggs hatched in the first day, between 20 to 30 percent were observed to hatch over the following 3 to 4 days. Synchronous

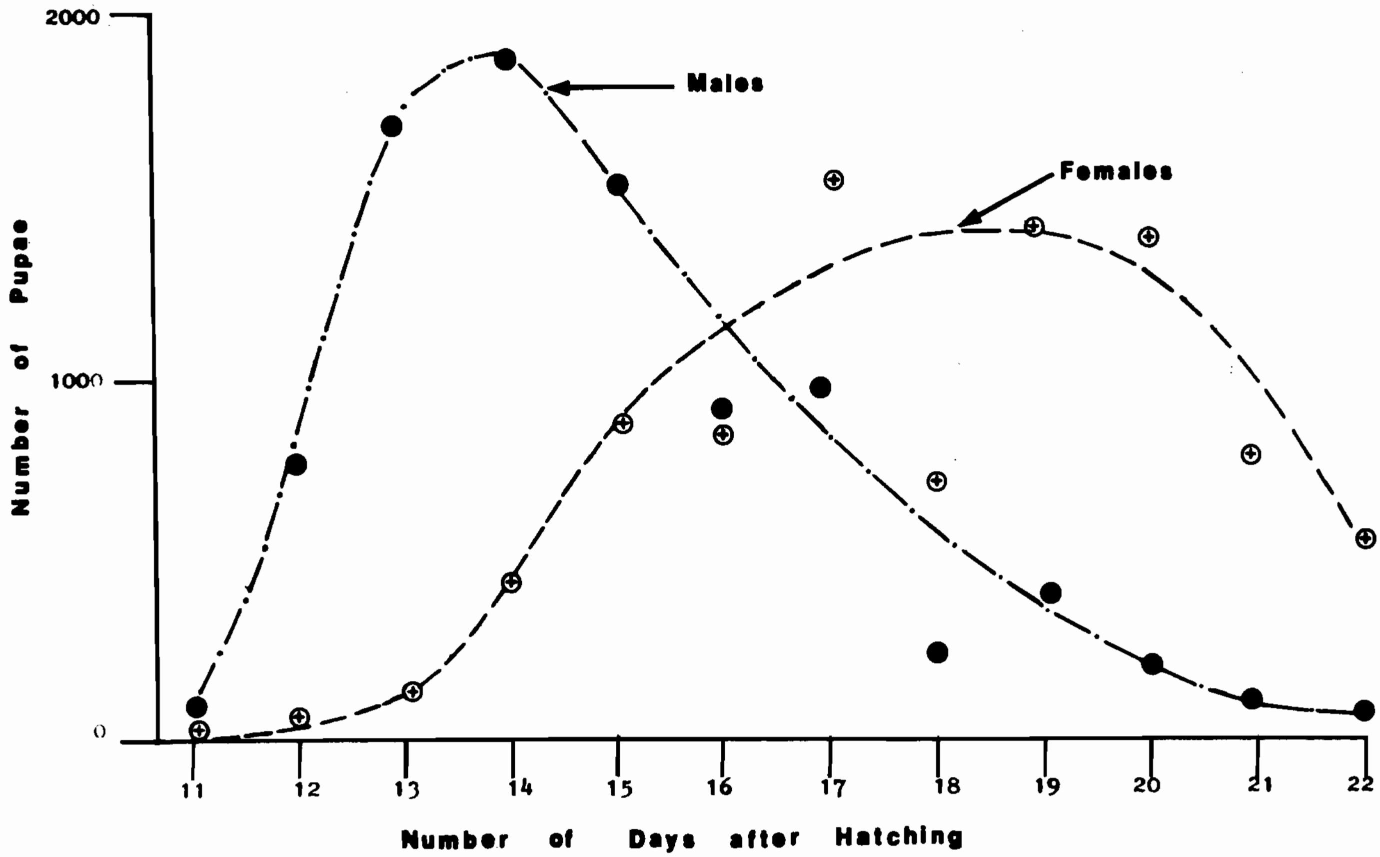

Figure 1.–The distribution of male and female pupation following a 3-4 day asynchronous egg hatch (sample size = 20,000; rearing temperature = 23°C; photoperiod = LD 15:9).

hatching of all eggs was achieved in one of the two pans of the second rearing. This, coupled with a lower rearing density, resulted in more synchronous development and more uniformity in larval and pupal size. These factors thus resulted in a sharp peak in the production of male pupae, with most appearing in the first 2 days.

A sharp peak in the appearance of male pupae allows a relatively clean separation of males from females and an efficient recovery of most of the males produced from the population of eggs hatched. For example, in pan "A" of the second rearing 94% of the mosquitoes pupating in the first two days were males, and these represent 74% of all males hatched (Table 1). A similar purity of males (93%) was achieved after 2 days of pupation in the first lot of mosquitoes reared (Table 2), but, because of the asynchrony in hatching and the higher rearing density, only 28% of the total males were recovered in the first 2 days. Approximately half of the eggs used in the experiments were male. Thus, if 5% is chosen arbitrarily as an acceptable level of female contamination in a sterile male release program, less than 14% of the eggs hatched in the first experiment would have yielded useable males as opposed to 37% in the second experiment.

No strong diurnal rhythm in pupation or emergence was observed under the above rearing conditions. In both experiments new pupae were collected in every sampling period throughout the diel. Once pupation started it continued unin-

Table 1.–Separation of males by day of pupation: synchronous hatch.[1]

Day of Pupation	Efficiency of male recovery = cumulative males pupated x 100% / total males hatched	Per cent female contaminants = cumulative females pupated x 100% / cumulative mosquitoes pupated
1	29%	0%
2	74%	6%
3	87%	28%
4	93%	37%
5	95%	42%

[1]Sample size: 267 pupae; Lot 2, Pan A.

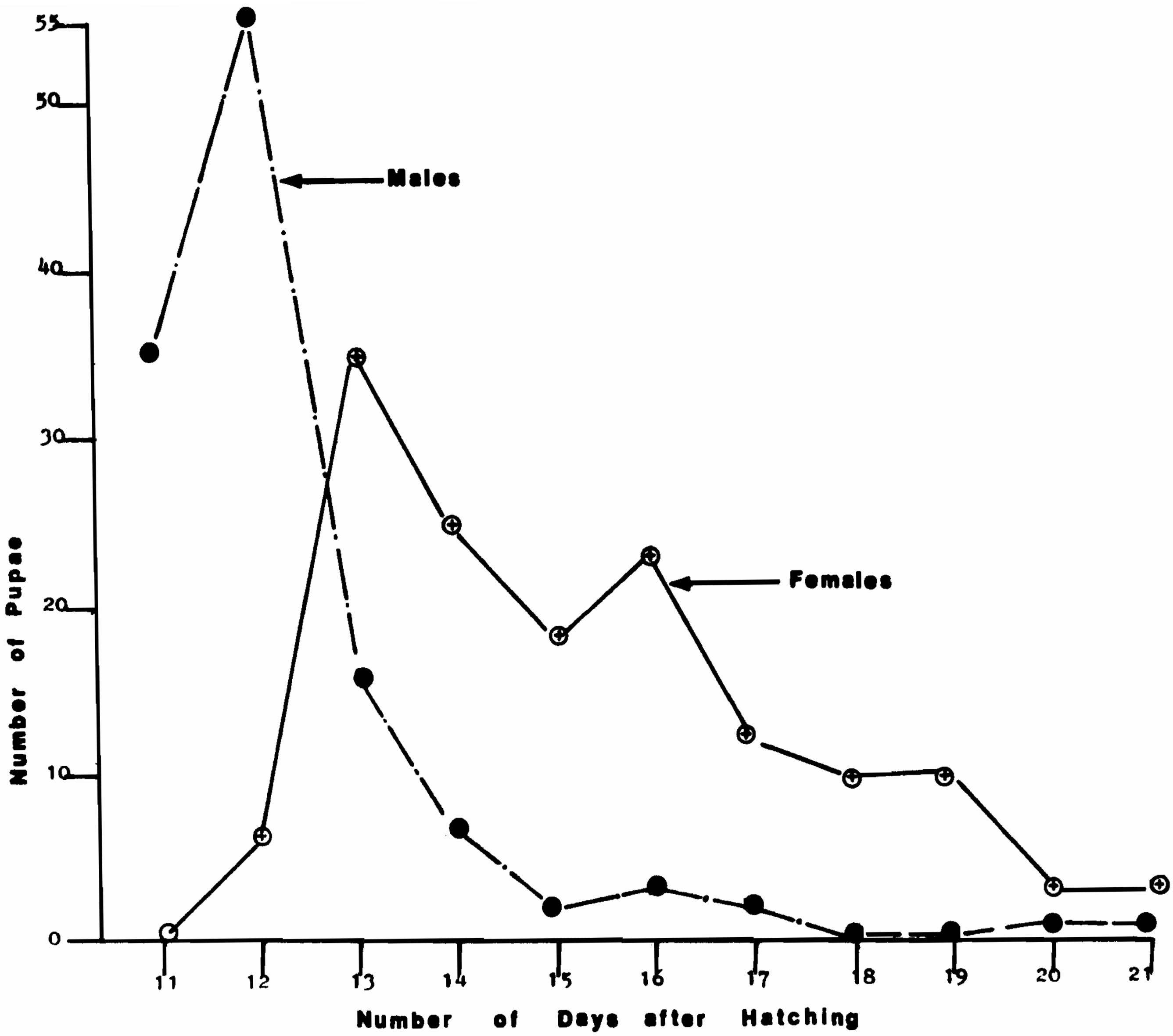

Figure 2.–The distribution of male and female pupation following synchronous hatching (sample size = 267; rearing temperature = 23°C; photoperiod = LD 15:9).

Table 2.–Separation of males by day of pupation: asynchronous hatch.[1]

Day of Pupation	Efficiency of male recovery = cumulative males pupated x 100% / total males hatched	Per cent female contaminants = cumulative females pupated x 100% / cumulative mosquitoes pupated
1	9%	3%
2	28%	7%
3	49%	13%
4	67%	20%
5	77%	26%

[1]Sample size: 20,000 pupae, Lot 1.

terrupted until greater than 95% of the larvae had pupated. In both experiments the peaks of male and female emergence followed the peaks in pupation (Figures 1 and 2) by about 4 days. The emergence curves were similar in shape to the pupation curves. No matter what period of the diel the male mosquitoes pupated they tended to emerge between 78 and 92 hours later (Table 3). Likewise, most females emerged 82-96 hours after pupation. This suggestion that duration of the pupal stage is primarily a function of physiological time, i.e., day-degrees. Once pupae have accumulated sufficient physiological time they commence ecdysis immediately. They do not wait as pharates for a specific period of the diel to emerge.

Table 3.–Duration of pupal stage.[1]

Hours after pupation	Percentage of adults emerged: Males	Percentage of adults emerged: Females
78 - 82	30	8
82 - 86	41	22
86 - 92	22	30
92 - 96	4	33
96 - 100	3	7

[1] Lot 2: average of pans A and B; total = 528 mosquitoes.

The continuous pattern of pupation and emergence in *Ae. sierrensis* will require that males reared for sterile-male release be collected and irradiated more than once a diel to assure sterility.

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RELATIVE EFFICIENCY OF EXPERIMENTAL FAN BLADES OF EVS BATTERY POWERED LIGHT TRAPS

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ABSTRACT

The development of an experimental fan blade to improve the airflow characteristics of the EVS battery powered mosquito light trap is described. Data comparing the physical performance of commercially available and experimental fan blades are also presented. This comparative data concludes that a fan blade three-quarters inch wide and having a pitch angle of 30 degrees provide 82% greater air velocity than commercially available plastic blades with only a small increase of current drain on the batteries. At the same time, the mutilation of mosquitoes caused by the fan blades is reduced by almost 19 times.

INTRODUCTION.--Field experience with EVS battery powered light traps suggested the possibility that for each night's operation the carbon-zinc battery, Mabuchi RE-260-2680 motor, and Cox. No. 247, 3D-1.25P plastic fan blade combination was not fully utilizing the available amp-hour charge of the batteries. It was found (Rohe and Fall 1979) that the plastic blades could be boiled and twisted until cool to achieve a greater angle of pitch (10° as received from supplier twisted to 30°-45°). This accomplished two things. First, the intial velocity of air moving through the trap was increased from approximately 314 fpm to about 485 fpm. Secondly, visual observation indicated that the increased pitch angle did not mutilate as many mosquitoes as did fan blades with 10° pitch angle. Since it was determined that it was not commercially feasible to increase the pitch angle of existing commercially supplied plastic propellers or to have specially designed plastic fan blades manufactured, the decision was made to develop a fan blade of maximal efficiency out of sheet aluminum.

LITERATURE REVIEW.--Almost all of the literature devoted to the evolution of CDC type battery powered light traps has been concerned with modifications designed to make these units less complicated, more portable, more rugged, and more reliable (Nelson and Chamberlain 1955; Sudia and Chamberlain 1962, Newhouse et al. 1966, Smith and Downs, 1966; Johnson et al. 1973, Rohe 1974, and Rohe and Fall 1979). Very little published comparative data exists concerning the catch efficiency of the various forms of these traps. Sudia and Chamberlain (1962) indicated that the first named version of the CDC trap captured from one-fourth to equal the number of adult mosquitoes as the standard New Jersey light trap with 15 watt bulb, but did not provide any comparative data on the air flow characteristics of the two traps. Recently, Smith et al. (1979) published data on the comparative catch efficiency of the newer EVS light trap versus the CDC light trap, but comparative data on the airflow characteristics of the two types of traps was not part of the experimental design. Pfuntner (1979), as part of his publication on an electronic timer for CDC type light traps, developed data concerning total volumes of air sampled. The cross-sectional area of his CDC trap was much larger than the area of the EVS trap making direct comparison of air flow characteristics of the two traps difficult.

Most of the existing CDC and EVS light traps fabricated in California have been limited to the use of similar types of plastic model airplane propellers, available from toy manufacturers, to move air through the traps. It is the performance of these propellers (fan blades) that posed the basic question dealt with in this paper.

OBJECTIVES.--The objectives of this paper are to present data on the development of an experimental fan blade intended to improve the air flow characteristics of the EVS battery powered light trap without sacrificing the operational reliability of the existing trap configuration, and to present data on the comparative physical performance of commercially available and experimental fan blades.

The basic question dealt with in this paper is: "What is the most efficient fan blade configuration, width and pitch angle for this particular trap?"

While this paper presents data comparing the physical performance of commercially available fan blades versus experimental blades, relaible data concerning comparative catch efficiencies of adult mosquitoes must await extensive field testing.

METHODS OF INVESTIGATION. Data was gathered in four phases: First, tests were conducted to determine the most efficient pitch angle of fan blades fabricated from sheet aluminum. Second, were tests to determine the most efficient sheet aluminum fan blade configuration. Third, were tests to determine the most efficient fan blade width. Then, fourth, additional tests were conducted comparing the physical performance of the plastic fan blade with the sheet aluminum fan blade that was found to have the most efficient combination of pitch, configuration, and width.

Measurements of the performance characteristics of the different fan blades during the tests were mainly based on DC voltage, current draw in milliamperes, revolutions per minute (RPM) as measured by a calibrated variable impulse Xenon strobe light, and velocity in feet per minute as measured by a mechanical anemometer.

Bio-Quip Products EVS-CO_2 Mosquito Traps (Cat. No. 2802A) were used for all tests. Before testing was started, new Mabuchi RE-260-2680 motors were installed. Whenever sheet aluminum fan blades were used, they were fabricated by hand and attached to the motor shaft by using a grommet made from short sections of Tygon plastic tubing (1/16" ID x 1/8"

OD). The use of this means of attachment was suggested by Allen Pfuntner, Northwest Mosquito Abatement District (personal communication).

All tests were begun with fresh sets of batteries. Either heavy duty carbon-zinc or alkaline batteries were used as required.

Live adult mosquitoes, *Culex quinquefasciatus*, for one test were obtained from Dr. Mir S. Mulla, Department of Entomology, University of California, Riverside.

RESULTS AND DISCUSSION.—Pitch Angle. - - To determine the most efficient angle of pitch and, secondarily, to provide preliminary information on blade width, a variety of rectangular pieces of sheet aluminum (1/4", 3/8", 1/2", 5/8", and 3/4" wide) were bent to pitch angles of 15°, 30°, 45°, and 60°. Each experimental fan blade was test run in the same trap with one fresh carbon-zinc battery replacing a used battery for each run. Each test run required about three to four minutes to complete the necessary measurements.

When the increase of pitch angle was plotted aganst the increase in velocity (Figure 1), there was very little increase in velocity above 30°. Also, the 3/4" wide blade produced the greatest increase in velocity.

When the percent increase in velocity was plotted against the percent increase in current draw (Figure 2), it was found that, as the pitch angle increased above 30°, the velocity did not increase even though the current draw increased 80%.

This indicated that the most efficient pitch angle was 30°. This was the pitch angle used in all subsequent tests.

Blade Configuration. - - To determine the most efficient fan blade configuration, a variety of differently shaped blades were fabricated. These shapes varied from straight edged blades, curved scimitar shapes, and various tapered shapes, with some narrower at the tip and others wider at the tip. A number of these shapes were formed with the blade surfaces either flat or curved. All of the experimental shapes were tested in the same trap with one fresh carbon-zinc battery replacing a used battery for each run. The most efficient blade configuration was the shape that was found to deliver the highest initial velocity. The configuration producing the highest velocity (Figure 3) was shaped like two flat rectangular paddles on either end of a narrow handle, similar to the paddle used for propelling a kayak.

Blade Width. - - The most efficient fan blade width was determined by making 15 hour runs using blade widths of 1/2", 3/4", 1", and 1 1/4". It was found that the velocity produced by the fans at the start of each run, when the batteries were fresh, increased as the blade width increased (466 fpm for 1/2" to 545 fpm for 1 1/4") (Figure 4). However, at the end of the run the velocity produced by the different blade widths showed very narrow differences with the 3/4" width producing the greatest velocity (318 fpm for 1/2" to 343 fpm for 3/4"). For the purposes of this test, the data values used for blade width determination were the averages of measurements taken at the start and end of each 15 hour run.

When the average revolution per minute (RPM) was plotted against blade width, it was found that the RPM decreased as the blade width increased (Figure 5).

When the average current draw (milliamperes) was plotted against blade width, the current draw increased as the blade width increased.

When the average velocity (feet per minute) was plotted against blade width, the velocity produced by the different fan blades remained relatively constant at 3/4", 1", and 1 1/4" (339.4 fpm to 441.8 fpm).

The data from these tests strongly indicated that the most efficient blade width was 3/4", for it produced the greatest velocity per milliampere (Table 1).

Additional Tests. - - Additional tests were conducted to provide comparative data on the physical performance of the commercially supplied plastic propeller (7/16" x 10°) and the experimental sheet aluminum fan blade developed during these present tests (3/4" x 30°).

When the RPM and the velocity produced by the plastic and sheet aluminum blades were compared (Table 2), it was found that while the 3/4" x 30° blade slowed the motor down by 46%, the velocity of air passing through the trap increased by 82%.

Since the EVS trap is designed to capture live mosquitoes for encephalitis virus testing, significant mutilation of mosquitoes (loss of head or abdomen) as they pass through the fan

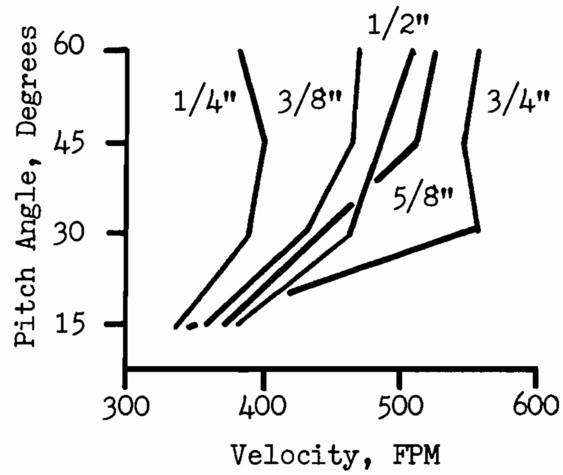

Figure 1.- Fan blade pitch angle vs. velocity.

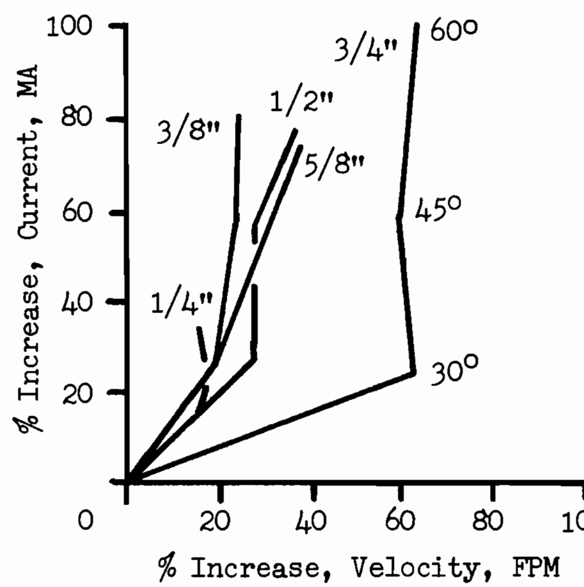

Figure 2.—Percent increase in velocity vs. percent increase in current draw.

Table 1.–Average velocity per milliampere for different fan blade widths, carbon-zinc batteries, 15 hour run.

Blade Width:	½″	¾″	1″	1¼″
FPM/MA:	1.741	1.786	1.696	1.739

Table 2.–Comparison of R.P.M. and velocity for commercially available and experimental fan blades, carbon-zinc batteries.

	Commercial Plastic 7/16″ x 10°	Experimental Aluminum ¾″ x 30°	Percent Difference
RPM:	2050	1400	-46
Velocity: (FPM)	309	562	82

Table 3.–Comparison of mutilation of mosquitoes by commercially available and experimental fan blades.

	Commercial Plastic 7/16″ x 10°	Experimental Aluminum ¾″ x 30°
Number:	192	188
Undamaged:	152	182
Mutilated:	40	2
Percent:	20.8	1.1

blades is detrimental to the purpose for which the trap was designed because it results in death of the mosquito and subsequent loss of viability in viruses that may be present. When live mosquitoes were drawn through the same trap in tests using plastic and sheet aluminum fan blades (Table 3), it was found that the plastic (7/16″ x 10°) blade mutilated 20.8% of the mosquitoes while the aluminum blade mutilated only 1.1%. This meant that the 7/16″ x 10° blade mutilated almost 19 times more mosquitoes than the 3/4″ x 30° blade.

Testes were also conducted which compared the effect of carbon-zinc and alkaline batteries on the performance of the EVS trap using 3/4″ x 30° blades. First, a survey of retail outlets in the San Bernardino area indicated that alkaline batteries were approximately twice as expensive as carbon-zinc batteries. This means that alkaline batteries would have to be used for two runs in order to equal the cost-per-run advantage of the less expensive carbon-zinc batteries. Second, during 15 hour runs, it was found that when velocities were measured each hour, the velocities for both types of batteries drifted downward at fairly constant rates, but that the rate of decrease for the alkaline batteries was slower than that for carbon-zinc batteries (Figure 6). Even though the initial velocities were approximately the same (526 fpm for carbon-zinc and 512 fpm for alkaline), the ending velocities were much further apart. The carbon-zinc batteries showed a 205 fpm decrease while the alkaline batteries showed a 121 fpm decrease. Therefore, the average velocity produced by the

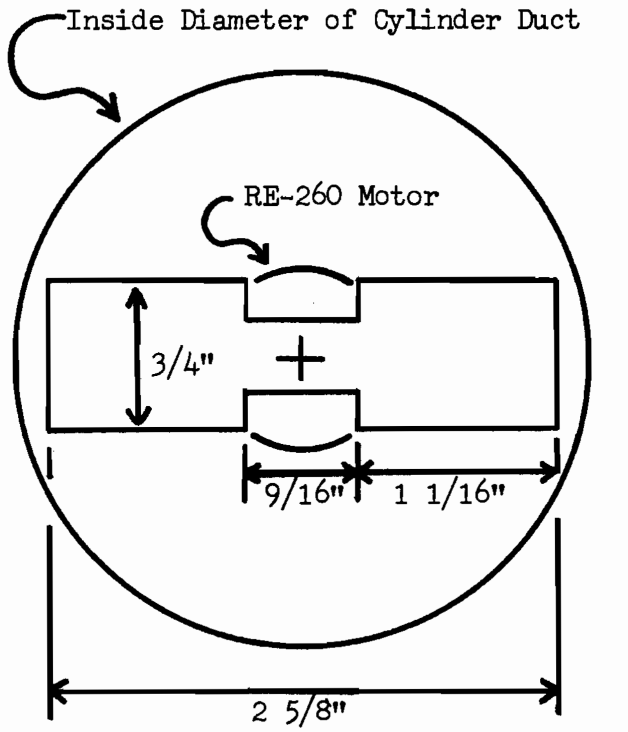

Figure 3.–Dimensions of experimental fan blade producing the greatest initial velocity.

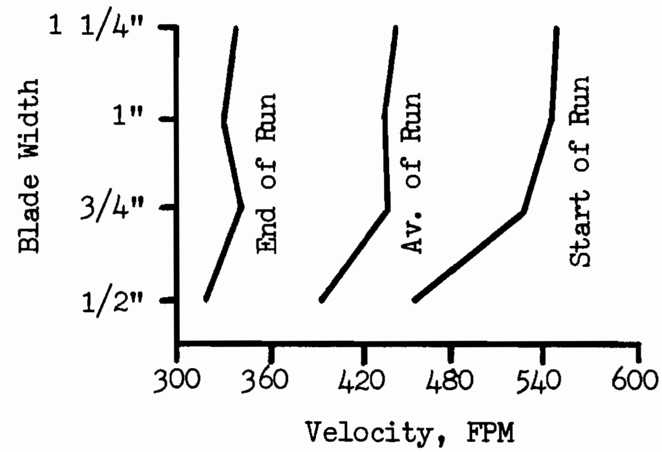

Figure 4.–Velocity change, 15 hour run.

carbon-zinc batteries was 412 fpm while the average velocity produced by the alkaline batteries was 454 fpm. Third, when the same set of alkaline batteries were run for four consecutive nights (15 hours per run), it was found that the decrease in average velocity per run continued to be relatively constant (Figure 7). Even with the decrease in average velocity per run, the alkaline batteries could be used for three runs before they dropped to the average velocity produced by carbon-zinc batteries for one run. The fourth run produced average velocities below that which could be produced by fresh carbon-zinc batteries, but which were still considerably above the velocity that could be produced by commercially supplied plastic

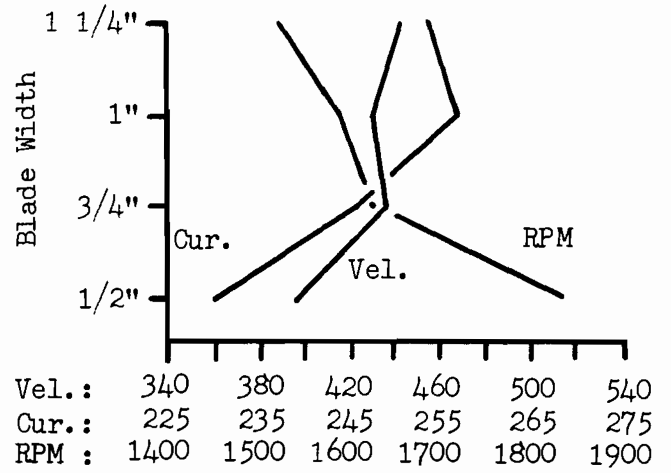

Figure 5.--Average velocity, current draw, and R.P.M. vs. fan blade width, 15 hour run.

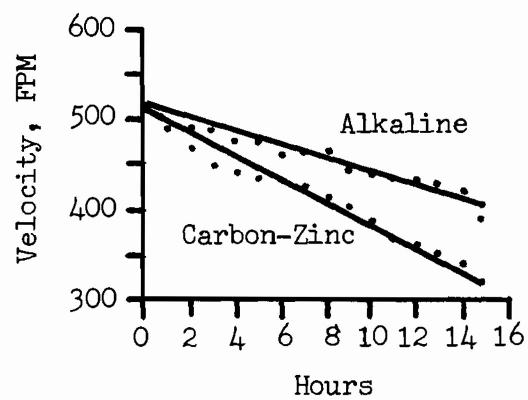

Figure 6.--Comparison of velocity decrease, alkaline vs. carbon-zinc batteries, ¾" x 30° fan blade, 15 hour run.

7/16" x 10° fan blades. Since the number of consecutive night runs made during each field trip differ according to the program needs of each investigator, the data from these battery comparison tests are intended to help individual investigators choose which would best fit their particular needs. However, it should be noted that higher average velocities will always be obtained from fresh, fully charged batteries.

CONCLUSIONS.--Based on the data collected during the tests described in this paper, the following conclusions can be drawn:

1. The most efficient experimental sheet aluminum fan blade tested was 3/4" wide with a pitch angle of 30°. It produced 82% greater air velocity and reduced mutilation to mosquitoes by almost 19 times.

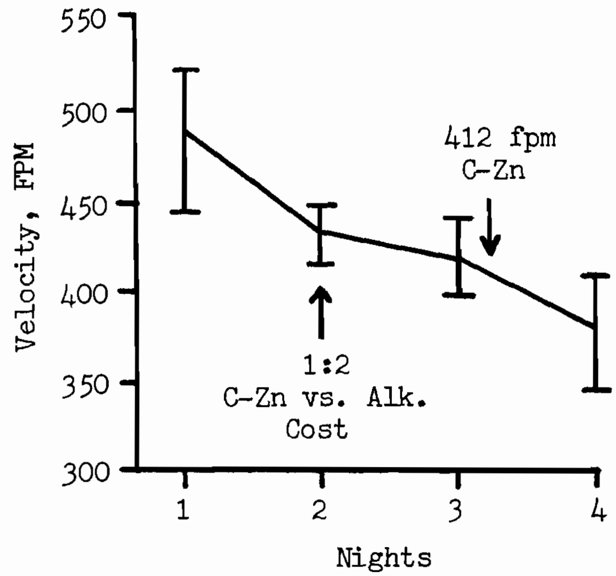

Figure 7.--Average velocity decrease, ¾" x 30° fan blade, simulated four night run, alkaline batteries.

2. Higher average velocities were produced by fresh, fully charged batteries. Even though the choice of the actual type of battery depends upon the program needs of the individual investigator, a set of alkaline batteries used for two or three nights does show some cost advantage over carbon-zinc batteries which should be changed for each night's run.

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USE OF A MATHEMATICAL MODEL TO PREDICT LEVELS OF ADULT *CULEX PIPIENS* IN ALAMEDA COUNTY

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INTRODUCTION.–*Culex pipiens* is one of the important species of pest mosquitoes in Alameda County. The primary source of this species during the summer and early fall is the catch basin and storm drain systems. Because of the high costs associated with physical correction of the sources, the District, until recently, relied heavily upon routine chemical applications to provide control (Dill and Roberts 1975). Organophosphate resistance was detected in *Culex pipiens* in 1974. To reduce insecticide pressure, only selected catch basins and storm drains were treated with oil (GB1313) in the years 1975 through 1978.

TOLERANCE THRESHOLD.–It was expected that the levels of *Culex pipiens* would increase above that of the years prior to the selective treatment program. A "tolerance threshold" was established to be used as a treatment guideline. The tolerance threshold was based upon numbers of service requests by the public. The acceptable level of service requests for any given month was the number of requests that could be properly processed by District staff. The selective treatment program would be conducted as planned unless the mosquitoes reached a level above the established "tolerance threshold" at which time emergency chemical control would be applied to bring about the desired level of control.

The problems with such a program are obvious. One would not know if the tolerance threshold is exceeded until the technicians have more service requests to process than they can complete. These requests would have to be ignored to implement the required emergency control, resulting in poor public relations. Finally, the emergency treatment of catch basin and storm drain systems would not immediately reduce the high levels of adult mosquitoes. Ideally, what would be needed to make the program operate effectively would be an early warning system that would forecast levels of *Culex pipiens* and predict if and when they would approach the tolerance threshold.

MESSAGE OF PROPOSITION 13.–With the passage of Proposition 13, the District's revenue was cut back by 63% in the fiscal year 1978/79. Program decisions were made to emphasize physical and biological methods to control the important mosquito species. In the case of *Culex pipiens*, treatment of all catch basins and storm drains was curtailed until and unless they were determined to actually be the cause of service requests. Due to the relatively low levels of service requests that occurred in the summer and fall of 1978, chemical treatments dropped far below previous years and the levels of adult *Culex pipiens* rose dramatically as evidenced by light trap data. By the end of September, the population of *Culex pipiens* reached levels greater than twice as high as the previous year, and the population curve had followed a classical pattern of exponential growth during the months of June through September (Figure 1).

PREDICTIVE MODEL.–The population curve that was exhibited by the uncontrolled population of *Culex pipiens*, being obviously exponential in nature, strongly suggested that a well known demographic equation might assist in predicting future levels of the population (Clark, Geier, Hughes and Morris 1967). The equation is as follows:

$$N_t = N_o e^{k_t}$$

t =times in months

N_t =the numbers of adult *Culex pipiens* after time t (adults/trap night)

N_o =the numbers of adult *Culex pipiens* at the beginning

k =constant for growth rate (birth rate - death rate)

e =the base of the natural logarithms.

By making the following assumptions, the equation may be used to assist in predicting levels of adult *Culex pipiens*:

1) The light trap samples adequately represent the relative densities of adult *Culex pipiens*.
2) The reproductive rate and movements of the population are relatively consistent from year-to-year.
3) Natural mortality factors remain essentially the same each year.
4) The carrying capacity of the storm drains and catch basins is not exceeded.
5) The greatest preponderance of the population of *Culex pipiens* produced June through October is produced from catch basin and storm drain systems.

UTILITY OF THE EQUATION.–By solving for K during each of the months from June through September 1978 a rate of growth can be established for each month. When a light trap index for *Culex pipiens* is determined in June of 1979, these K values can be used in the formula to predict the approximate levels of the species in the months of July, August and September. If predicted levels exceed acceptable levels (the tolerance threshold) a preventive chemical treatment can be made in advance of high adult emergence. In order to predict whether the forecasted light trap levels exceed the tolerance threshold, correlations between light trap and service request data must be established. The treatment decision, of course, should be made in light of other information such as larval collection data.

The simplicity of the equation, and the advent of relatively inexpensive scientific calculators and micro-computers, makes the manipulation of the equation quite simple. For illustrative purposes, a program has been developed for use in the TRS-80 micro-computer system with video display. The program represents predicted monthly levels of adult *Culex pipiens* by bar graphs and graphically depicts whether or not the predicted levels exceed an established tolerance threshold.

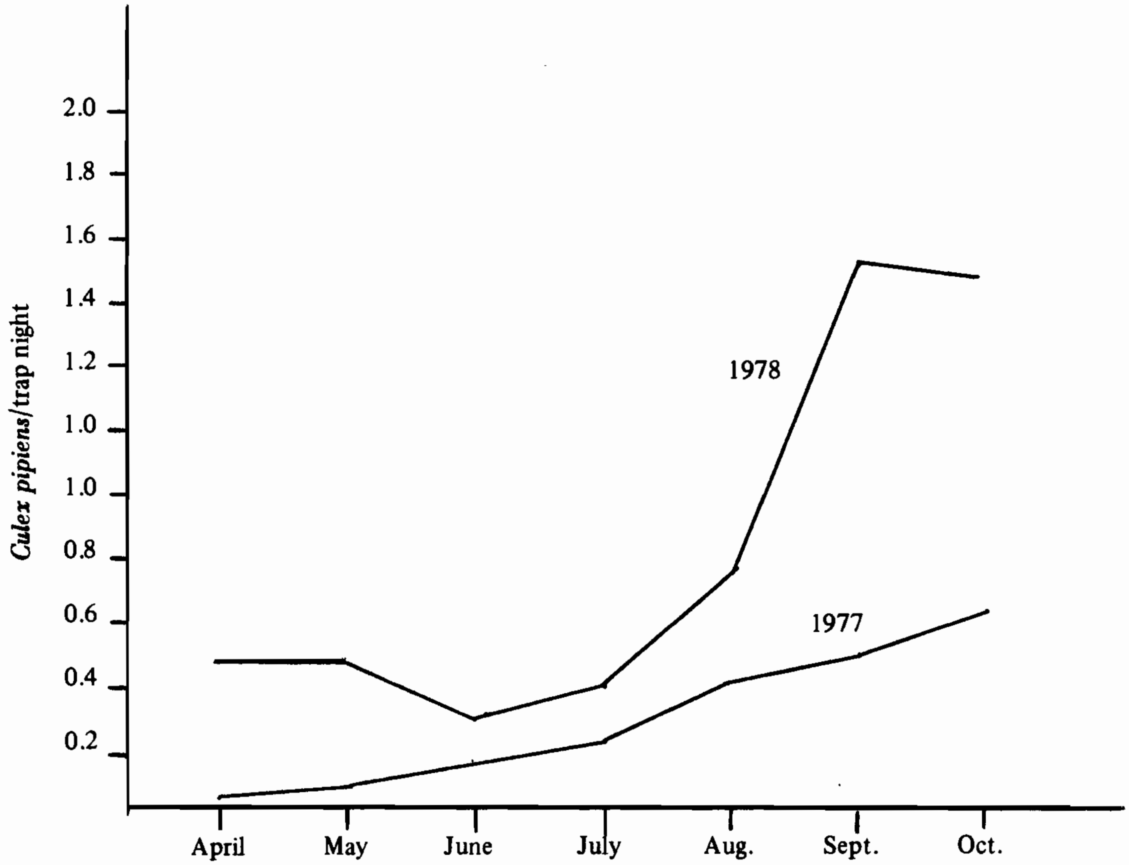

Figure 1.– Light trap data indicating levels of adult *Culex pipiens* in Alameda County Mosquito Abatement District.

The reliability of the equation in this application has not yet been tested. Continued efforts will be made to correlate larval collection data, service requests and light trap data. It is hoped that a similar predictive model can be established with larval data to further aid in making treatment decisions.

SUMMARY AND CONCLUSIONS.–A critical need exists in the Alameda County Mosquito Abatement District to reduce chemical treatments of *Culex pipiens*. The need stems from severe financial constraints and organophosphate resistance. Recent cutbacks in the treatment program have enabled the District to determine the natural growth rates of *Culex pipiens* produced in storm drains and catch basins. A simple mathematical equation will be used to predict levels of adult *Culex pipiens* to aid in treatment decisions. The reliability and utility of the model will be further tested.

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THE RESULTS OF THE JACKSON COUNTY VECTOR CONTROL DISTRICT'S INTEGRATED MOSQUITO CONTROL PROGRAM

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ABSTRACT

The Jackson County Integrated Mosquito Control Program consists of verbal, biological, physical and chemical control with the object of reducing the number and size of mosquito source areas with an emphasis on cost reduction.

Verbal control consists of sending letters and contacting individuals requesting their aid in reducing our control costs through their water management practices.

The Biological control program consists of planting *Gambusia* (mosquitofish) wherever feasible.

The Physical control efforts consist of using a backhoe at no cost, to help individuals with their drainage problems. This ditching program has eliminated and reduced the size of many mosquito source areas making aerial applications impracticable. Ground rigs with high flotation tires have replaced the aircraft.

The Chemical control program consists of using malathion both as a larvacide and adulticide with the oil G.B.1313 as a pupicide. Control operations consist of using the individual's irrigation cycle to indicate when the property should be inspected and of using the mosquito larvae growth chart to time applications. This changes operations from an area wide inspection and application program to inspections and applications on individual parcels of property. This allows applications on each source area when they are at their smallest dimension containing 4th instar larvae or pupae, the results being a reduced use of costly chemicals.

We used this system to determine the feasibility of a pupiciding program using G.B.1313. The experiment was successful and in the future when our ***Aedes nigromaculis*** become resistant to malathion we will incorporate a full time pupiciding program.

The Physical control program has reduced large mosquito producing areas or completely eliminated them. The feasibility of using aircraft on small source areas was not justified so we changed some of our ground equipment to high flotation spray rigs. This approach was very successful. The results at the end of the season were better then anticipated. There was a 90% reduction of aircraft applications and we have enough malathion on hand for two more seasons.

Application and insecticide costs were reduced from $15,309.00 in 1976 to $$11,197.00 or 26.8% in 1977; in 1978 the cost was reduced to $4,875.00, an additional 56%.

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ORIENTAL COCKROACH CONTROL PROGRAM IN SANTA ANA

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During the spring of 1978, the Orange County Vector Control District received numerous complaints of cockroach infestation from citizens in the south central section of Santa Ana. The complaints stemmed from frequent sightings of oriental cockroaches (*Blatta orientalis*) along parkways, water meter boxes, curb sides, and around the homes. The District had previously conducted a survey within that city in 1976. This survey by seasonal help involved a block by block inspection of the water meter boxes in the city. The presence of oriental cockroaches and oothecae in these meter boxes was used as an indicator for cockroach infestation. Data from the survey indicated that an adjacent area in Santa Ana had a meter box infestation rate of 16.3 percent based on 3,000 meter boxes inspected. With this information in hand, we conducted additional survey work in May within the complaint area, which confirmed that water meter boxes within this one square mile area were infested at near double (30 percent) the 1976 infestation rate.

Through the efforts of the residents within this area, the City of Santa Ana was persuaded to seek out and contract with the Orange County Vector Control District for control work on this cockroach problem. Although a number of pest control companies were provided the opportunity to contract for this, all declined. At the request of the city, the Vector Control District agreed to 1) provide informational workshops for the homeowners demonstrating how they could eliminate or minimize oriental cockroaches on their premises, and 2) treat the water meter boxes. The sewer system was to be treated by the City of Santa Ana.

CHOICE OF INSECTICIDE AND EQUIPMENT USED.-- Water meter boxes present numerous problems in the selection of pesticides. The usual damp conditions, porosity of concrete, and frequent inundation by rain or watering compromises the best of insecticides. Ideally, the chlorinated hydrocarbons are best suited for this type of situation but they are either prohibited or objectionable to environmental groups and others. Boric acid was also considered, however, it can be quite phytotoxic at the root zone level or if sprayed on parkway plants. After considerable investigation into which insecticide would provide the best control and allow for expedient application, the choice was Baygon Wettable Powder (70 percent) diluted to a 1.1 percent field formulation. Spraying was accomplished in a manner similar to our mosquito control on community drainage routes. The use of the right hand drive International Scout permitted the operator to approach each meter box with ease and rapidity. It was necessary to remove the meter box lid in order to adequately spray the interior of each box.

SPECIFIC TREATMENT INFORMATION.

Dates treated	11/6 thru 11/9/78
Number of water meter boxes treated	2,126
Number of hours for treatment (1 man)	36 hours
Pounds of insecticide (Baygon 70% Wettable Powder diluted 2 oz per gal)	5 lbs
Evaluation (Pre & Post-Treatment)	7.5 hours
Total cost to City	$576.22
Cost per water meter box	$0.27
Ounces of finished spray per meter box	2.4
Area of each meter box	4 ft^2

EVALUATION OF INSECTICIDE EFFICACY.--A pre-treatment survey was conducted (October 1978) on 10 percent of the 2,126 water meter boxes to be sprayed. This confirmed our previous determination of a 30 percent infestation rate in May. This pre-treatment survey was conducted at random although all water meter boxes inspected were logged by address. The pre-treatment survey was done independently of the personnel used in the insecticide application.

The post-treatment survey was conducted in all water meter boxes (76) known as positive in the pre-treatment survey. The check backs were done at intervals of one week after treatment, one month, and two months. This information follows thusly:

a) Post-Treatment Survey - One Week
Investigation of the positive meter boxes one week after treatment revealed (visually) only dead specimens or physiologically distressed specimens which had apparently just come in contact with the residual.

b) Post-Treatment Survey - One Month
This survey (again visual) indicated the Baygon still had sufficient residual to affect specimens just coming into contact with it. This month also recorded 4.0 inches of rain. One meter box was found with numerous cockroaches in it. Apparently, the box was not sprayed. Our estimations are that perhaps one percent of the meter boxes were missed due to parked cars, overgrowth, and overlooking.

c) Post-Treatment Survey - Two Months
Visual observations at this time were augmented by the use of De-Con Roach Traps left overnight in water meter boxes. The use of these traps in survey work was suggested to us by Ken Hansgen of the California Department of Health Services. The traps demonstrated to us that although we did not observe live cockroaches in the meter boxes (as in the pre-treatment survey), the cockroaches were apparently wandering into the water boxes overnight. The traps do contain food as a lure which may entice. More importantly,

the post-treatment surveys did not show evidence of new oothecae which was evident prior to treatment. In this respect, the presence of the cockroaches was of a transient nature and not true infestation concurrent with breeding. Based on the number of meter boxes previously known to be infested (76) the roach traps indicated we still had 92 percent control after two months.

CONCLUSION. Oriental cockroach control presents numerous problems which must be dealt with to successfully reduce the roach population. The sole reliance on chemicals will only thwart their proliferation temporarily. Full cooperation from a community is necessary to complement the chemical control work. The four informational workshops held in the control area were well attended and well received. The residents of the area demonstrated their concern and followed through with the prescribed recommendations. Some of these efforts are still to be evaluated such as the filling of the meter boxes with sand (so as to alter the environment within the meter box and hopefully reduce deposition of egg cases). Removal of parkway overgrowth and foliage around the home foundation is slow in coming.

The use of Baygon Wettable Powder, formulated as a spray, proved itself successful to our District in the immediate control of Oriental cockroaches with good residual and ease of application. The level of control was better than anticipated in view of the amount of rainfall (7 inches) during the two months of observation.

THE EFFECTS OF PROPOSITION 13 ON MOSQUITO ABATEMENT DISTRICTS IN CALIFORNIA[1]

The passage of Proposition 13 in June 1978 by the voters of California brought about dramatic changes in the posture of governmental agencies seldom thought of by the general public. Mosquito control organizations throughout California have been low profile members of the public community for the better part of their existence. These agencies, concerned about funding for their programs, began to assert themselves publicly. Representatives of these have attempted to apprise the residents of California of the plight of mosquito control.

Budgets for mosquito control agencies in the State of California totalled $17,854,399 during the Fiscal Year 1977-78 and ranged from $28,394 to $1,454,991. The total for Fiscal Year 1978-79 was $14,712,816 with the range from $16,000 to $1,405,890. Proposition 13 reduced the revenues of individual Districts from 1.5% to 69.6% when consideration of receipt of funds from the State of California is included in the calculations.

The following data are presented to demonstrate the anticipated impact upon mosquito control agencies throughout California. The figures were supplied by the agencies in question for the purpose of calculating the dollar loss in revenues to their operations.

District	1977-78 Revenue	1978-79 Revenue	Per Cent Reduction	State Aid
Coastal Region				
Alameda County	$530,381	$200,045	62.3%	$118,419
Contra Costa	586,210	235,000	60.0	?
Diablo Valley	102,735	52,740	60.0	?
Marin-Sonoma	454,789	208,777	54.0	94,042
Napa County	152,277	65,000	57.0	?
No. Salinas Valley	469,331	371,140	20.0	91,026
San Mateo County	875,482	570,415	35.0	?
Santa Clara County Health Dept.	200,940	186,462	7.2	0
Solano County	470,551	325,036	31.0	74,724
Sacramento Valley Region				
Burney Basin	66,431	57,868	12.9	0
Butte County	664,951	286,000	56.9	25,000
Colusa	66,458	51,842	21.9	9,174
Corning	33,495	14,137	57.8	0
El Dorado Service Area III	N.R.	N.R.	N.R.	N.R.
Glenn County	34,440	27,212	20.9	3,600
Lake County	N.R.	N.R.	N.R.	N.R.
Los Molinos	28,000	10,000	64.3	0
Oroville	N.R.	N.R.	N.R.	N.R.
Sacramento-Yolo	N.R.	N.R.	N.R.	N.R.
Shasta	319,449	181,983	43.0	13,970
Sutter-Yuba	633,793	304,000	52.0	?
Tehama	73,000	29,000	60.3	0
No. San Joaquin Valley Region				
Eastside	596,866	306,148	48.7	0
Merced County	639,000	308,000	51.7	0
No. San Joaquin	164,130	77,862	52.5	17,664
San Joaquin	968,000	404,000	58.3	0
Turlock	418,869	216,175	48.4	0

[1]Compiled by Peter B. Ghormley, Burney Basin MAD, Post Office Box 1049, Burney, California 96013.

District	1977-78 Revenue	1978-79 Revenue	Per Cent Reduction	State Aid
*So. San Joaquin Valley				
Coalinga-Huron	32,665	28,772	11.9	?
Consolidated	559,950	558,450	.3	?
Delano	181,851	173,189	4.8	48,000
Delta	606,967	474,271	21.9	?
Fresno	543,350	393,800	27.5	?
Fresno-Westside	406,150	331,300	18.4	?
Kern	897,669	836,906	6.8	?
Kings	414,884	304,425	26.6	?
Madera County	311,818	379,809+21.8		104
Tulare	373,768	289,365	22.6	?
West Side	364,560	226,000	38.0	?
Southern California Region				
Antelope Valley	39,569	13,202	66.6	0
Carpinteria	-	-	-	-
Coachella Valley	664,000	313,000	49.1	25,000
Compton Creek	59,250	18,000	69.6	0
Goleta Valley	65,243	26,218	59.8	0
Inyo County Health Dept.	-	-	-	-
Long Beach, City of	-	-	-	-
Los Angeles County West	187,001	77,834	58.4	0
Moorpark	28,921	11,081	61.7	0
Northwest	285,426	122,325	48.4	25,000
Orange County	715,000	363,000	3.8	325,000
Southeast	592,903	236,000	60.2	0

*The figures for the Southern San Joaquin Valley Region reflect Budget figures rather than Revenues.

These figures present a rather dismal financial picture for the mosquito control agencies in California. It would appear the immediate effects are but a prelude to the possible severe ramifications for the next three or four years. Many agencies have supplemented their revenues by utilization of a portion of existing reserves, anticipating alternative sources of revenue becoming available at some time in the future. This action is obviously only an immediate solution to the problem. When the reserves are consumed, the expenditures of the agencies will have to be reduced to a level equal to the revenues received from various sources.

The mosquito abatement districts of California cover an extremely broad spectrum in size, budget, expertise, and cost-effectiveness. Therefore the direct effect of this initiative will vary substantially relative to the individual agency in question. The insidious effect of Proposition 13 on these operations would appear to be the attrition on mosquito prevention phases of programs and the loss of qualified competent personnel. The continuity of many programs will suffer in the immediate future and it will undoubtedly require years in order to fully recover.